Original Research Article

Secondary Root Extract Of *Piper Betleoides* Causes Changes In Structural Organization Of Graafian Follicle And Uterine Epithelium In Rat During Estrus And Early Gestational Period.

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Abstract: *Piper betleoides* is a creeper grown in wild habitat of the tropical rain forests in eastern Himalayan biodiversity hot spots. The secondary root of this creeper is traditionally being used for termination of unintended pregnancy by certain group of ethnic people of this region of India. The secondary root extract (SRE) prepared in methanol was tested in albino rats for its effects on ovary and uterus. The main aim of the present study was to determine the possible effects of secondary root extract of *Piper betleoides* on Graafian follicle structure and also to determine whether SRE induces structural aberration of the uterine histoarchitecture during early gestation period resulting in failure of receptivity to embryo. The extract in a dose of 250 mg/kg body weight was administered daily through oral route to normal cyclic female rats for four consecutive days begin with proestrus. Similar dose was tested in ovariectomised females and during day1 to day 5 of gestation the ovaries and the uteri of all the experimental female rats were collected at the end of SRE administration. Tissues were histologically studied following routine hematoxylene-eosin staining method. The results obtained from the present study revealed that the structure of Graafian follicles was affected by the secondary root extract causing degeneration of oocyte. Uterine luminal epithelium fail to proliferate rather, desquamated from the supporting stroma. During gestation, SRE treated females failed to achieve the decidual cell reaction (DCR). The present *in vivo* study showed that secondary roots of *Piper betleoides* contains potential compound(s) which can induce structural aberration of Graafian follicles and uterine epithelium causing failure of gestation in rat.

Key words: *Piper betleoides*, Graafian follicle, DCR, Phytoestrogen

Introduction

Search for alternative compounds of gonadal steroids for human reproduction regulation is going on in different laboratories across India and other countries of the world. India being rich in medicinal plant has been looking for natural products for fertility regulation while, many other countries of the world are investigating the effects of dietary estrogens on male and female fertility (West, 2007). Phytoestrogens are well known as potential steroid hormone receptor ligand (Usui, 2006) which led to the development of conceptual idea of reproduction regulations using these natural products. Accordingly a number of plants or parts there off have been tested for reproduction regulatory properties *in vivo* especially in laboratory model system. The information on the plants having reproduction regulation is very often borrowed from the native people who traditionally being use the plants for reproductive health. Arunachal Pradesh is one of the North Eastern provinces of India situated on the eastern Himalayan region. The population of this state comprises indigenous tribal

dominated society belongs to different ethnic group. They have an age old tradition of using herbal preparation, e.g. the secondary root powder of *Piper betleoides* for fertility control. The plant *Piper betleoides* is a creeper grown wild in the tropical rain forest of Arunachal Pradesh. A large number of Piper species is available in India while, the *Piper betleoides* is found in this eastern Himalayan biodiversity hot spot. In India, 86 species of *Piper* have been reported from two distributional centers: viz., (i) the north-east India comprising the eastern Himalayans and (ii) the Western Ghats of the southern India. The greater diversity of the plant is available in Northeast where 55 species have been reported wherein, 27 *Piper* species reported so far in Arunachal Pradesh (Gajurel *et al.,* 2008).

During the last few decades a number of *Piper* species has drawn attention of scientific community for its various properties. Some of these are reported for its effects on reproduction. The fruit of *Piper longum* was reported for its effects on implantation sites (Kholkute *et al.,* 1979). *Piper betle* (Petiole) is known for its antiestrogenic effects on female reproductive tissue (Sharma *et al.,* 2007) while, another species *Piper nigrum* has been reported for antispermatogenic and antifertility effects in mice (Mishra and Singh, 2009). Development of an effective lead from these herbs for fertility control, a deep insight on histology and biochemical profile of reproductive organs is unavoidable. In the present investigation therefore, the secondary root extract of *Piper betleoides* is tested for its effects on reproductive organs in cyclic and ovareiectomised female rats and during early gestation. Tradition prevails among certain group of tribal people of Arunachal Pradesh of using the dry secondary root powder of this creeper during post coital period to prevent unintended pregnancy.

Materials and methods

Preparation of plant materials: Plant material collection and preparation of secondary root extract (SRE)

The taxonomic identification of the plant was confirmed following the monograph and books available and the recent literature on taxonomy of *Piper* species (Gajurel *et al.,* 2008).

Fig. 1. Photograph of the plant *Piper betleoides.* Arrow shows the secondary root of the creeper used in the present investigation.

The plant was collected from East Siang district of Arunachal Pradesh and brought to the laboratory. The secondary roots of the plant (Fig.1) were separated by cutting from the nodes of the plant, washed in water and dried under low temperature. Dry roots were grinded mechanically for making crude powder in the mixer grinder. The crude powder of secondary roots was stored in normal room temperature until use for extract preparation. The prepared crude powder of the secondary roots of *Piper betleoides* were then subjected to extraction of potential chemical constituent in non polar solvent. The extraction was done in methanol. The secondary root powder was soaked in methanol at room temperature for 72-96 hrs for percolation of the solvent in the secondary root powder of *Piper betleoides* and extraction of the chemical constituent. The extract has been filtered and concentrated by evaporation under $25^{O}C \pm 2^{O}C$ temperature until a semi solid mass appeared. A brown colored semisolid content was the end product of the extract preparation of the secondary roots of *Piper betleoides* which was stored in $4^{O}C$ until use.

Experimental animals

Adult female Sprague Dawley rats maintained in animal maintenance and research facility of Rajiv Gandhi University was used for the present study. All the experimental animals were used for breeding and housed in standard animal poly cages. The animals were maintained under environmentally controlled room provided with natural light and dark cycle

for each 24h period at an ambient temperature in between 25^{O}C to 18^{O}C. They were fed on normal diet (Bengal gram, corn and pea) and tap water *ad libitum*. The estrous cycle of the adult females was studied by observation of cell types in the vaginal smear prior to the experiment following standard method (Montes and Luque, 1988). The females showing normal estrous cycle repeated approximately in four days (96-105 h) were selected for the in vivo studies. The experiments were carried out *in-vivo* using two animal models (i) adult ovariectomized (OVX) female rats and (ii) adult cyclic ovary intact females during estrus phase and during day 2 to day 5 of gestation. The work was carried out following the institution's rule under the animal care and maintenance for research.

Ovariectomy

Bilateral ovariectomy of the adult cyclic females was performed following the standard procedure (Hogan *et al.* 1986). Briefly, adult cyclic females were anesthetized by ketamine hydrochloride. Ovariectomy was done by two dorso-lateral incision approximately 1 cm long above the ovaries. With the use of sharp dissecting scissors, the skin was cut with the dorsal muscles and the peritoneal cavity 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. During this period the animals were kept under observation especially for their healthy diet and tap water '*ad libitum*'. To prevent wound infection, antiseptic powder was applied over the surface of the wound.

Mating and detection of pregnancy

The females with normal estrous cycle were allowed to mate with male of proven fertility. The male rat was added to the females' cage on the evening of proestrus in ration of 2 : 1 (female : male). Vaginal smear was monitored from the following morning onward in between 6.00 – 7.00 hrs; the day on which sperms were observed in the smear was considered as the day1 of gestation.

Animal treatment: administration of SRE to female rats

The crude secondary root extract was administered to the tested female rats through oral route. The root extract (SRE) was suspended in distilled water and administered the threshold dose (500mg/kg body weight/day) to the females during the whole experiments. The threshold dose was determined by giving three different doses (250mg, 500mg, 750mg/kg/day) to the female rats (n=8 in each dose) for four days of an estrous cycle. The minimum amount which was sufficient to induce histological changes in ovary and uterus was considered as the threshold dose. Thus, the cyclic ovary intact females received 04 days (one estrous cycles) oral administration of the SRE beginning with the onset of proestrus. Similarly, the SRE was orally administered to the ovariectomized (OVX) females for four consecutive days. The control females (both the cyclic ovary intact and OVX) received the vehicle only in the similar manner and for the same duration. Administration of both the SRE and vehicle was carried out in between 7.00 – 9.00 hrs.

The females both SRE treated and control were sacrificed on day four in between 17.00 - 19.00 hrs. The uterus and ovary of cyclic females were removed and fixed in 10% formaldehyde for histological studies. Similarly, only the uterine horns of the OVX females were collected and fixed in 10% formaldehyde for histological studies

The secondary root extract (SRE) was administered to the pregnant females from day1 onwards to day 5 of gestation. Each day (from day 2 to day 5 of gestation), one group of animal (n=5) was sacrificed in between 17.00-19.00hrs. The control females received the vehicle only in similar manner and were sacrificed accordingly to collect the tissue sample.

Administration of estradiol-17β to ovariectomized rats

Estradiol-17β (Lancaster, Cat No. # 3801) was used as reference drug to study the steroidogenic effect (if any) of SRE of *Piper betleoides* on uterus. Subcutaneous administration of Estradiol-17β (E2) in a dose of 0.1µg/ 0.5ml sesame oil was made to the OVX rats for 3 consecutive days.

A group control females (n=5) were treated with the vehicle similar to the E2 injection for the same duration (three consecutive days). The rats were sacrificed on 4th day of treatment in between 7.00 – 9.00 hrs and the uterine horns were collected for histological study.

Histological procedures

The ovary and uterus of the control and SRE treated female rats was studied histologically following routine haematoxylin-eosin (H&E) staining following standard histological method (Gamble and Wilson, 2002). The tissues (ovary, uterus and liver) were removed immediately following sacrifice and fixed in 10% formaldehyde solution for 48-72 hours. The fixed tissues were washed and dehydrated following routine histological procedure and then embedded in paraffin at 58-60°C. The paraffin embedded tissues were cut in 5-7 μm thick sections and stretched on poly L- lysine (Sigma Cat No# P8920) coated glass slides. Dehydration and staining of the tissue sections were carried out following standard histological staining (H & E). Alternate section was observed and appropriate areas were photomicrographed (Axio star Plus, Carl Zeiss 426126 microscope and Cannon power shot A80 Digital camara) to collate the most significant results in the histoarchitecture of ovary and uterus of control and SRE treated females.

Results

Effects on Graafian follicle

The histological structures of adult cyclic rat's ovary showed presence of follicles at different stages of development (Fig.2A). The follicular growth takes place from primary follicles which were recruited from the primordial pool. The normal cyclic females during the estrus phase exhibited multiple numbers of follicles and the corpora lutea. The preantral follicles including primary follicles and secondary follicles having centrally located oocyte showed the presence of layers of granulosa cells (Fig.2 A1). The Graafian follicle which is the final stage of follicular development showed the oocyte guarded by single layer cumulus cells in the fluid filled antrum. The granulosa cells are arranged in multiple layers at the periphery of the fluid filled antral cavity of the mature Graafian follicle. The Graafian follicle showed a full grown round oocyte having

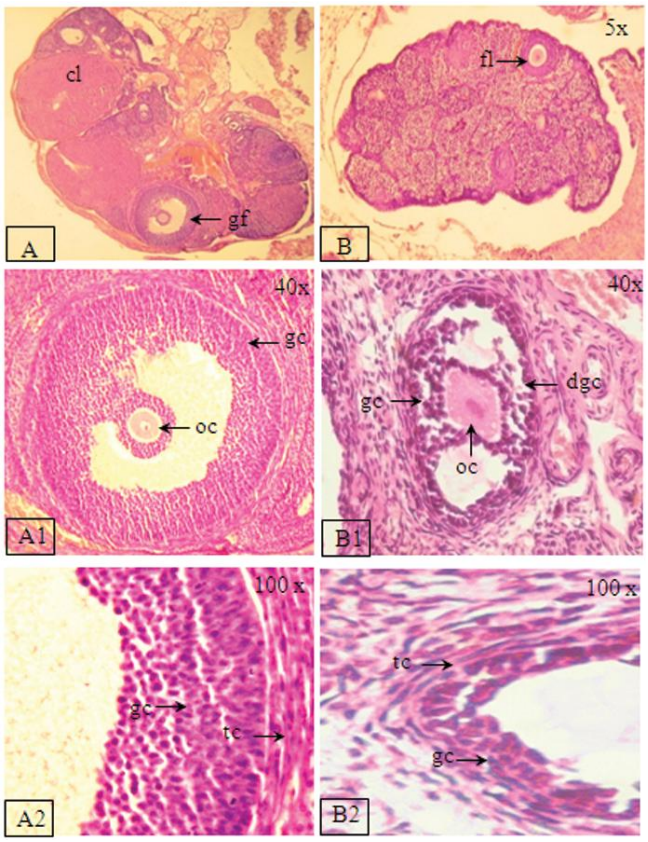

Fig. 2. The control female (A, A1 & A2) showed follicular development at various stages of follicles (fl) and corpora lutea (cl). The Graafian follicle (gf) showed oocytes (oc) and uniformly layered compact granulosa cells.The antrum is filled with antral fluid while, the theca cells (tc) is uniformly layered surrounding the granulosa cells. Administration of SRE (B, B1 & B2) altered the cellular organization of Graafian follicle resulting in degeneration of granulosa cells (dgc) and oocytes. The theca cells becomes disorganized as shown following SRE administration.

the germinal vesicle surrounded by the zona pellucida. The Granulosa cell layers are encapsulated by the thecal cells. The round theca interna cell layer which is the originator of granulosa cells is surrounded by outermost layers of spindle shaped theca externa cells (Fig.2 A2).

Administration of SRE for four consecutive days resulted in structural aberration of the ovarian follicles especially the Graafian follicles' cellular organization (Fig. 2 B, B1 & B2). The oocyte appeared irregular shaped and abnormal structure loosing its round shape. The granulosa cells lost its regular systematic circular arrangement surrounding the antrum and showed irregular cord and detached from one another. Number of granulosa cell layers was lesser in the

SRE treated rat's Graafian follicle than that of the control females. The granulosa cells detached from each other were found degenerating characterized by nuclear pyknosis and floating in the antral fluid. Many of the Graafian follicles exhibited loss of healthy oocytes with shrinkage of the whole structure with few degenerating layers of theca cells at the periphery of the follicle.

Effects on uterine epithelium

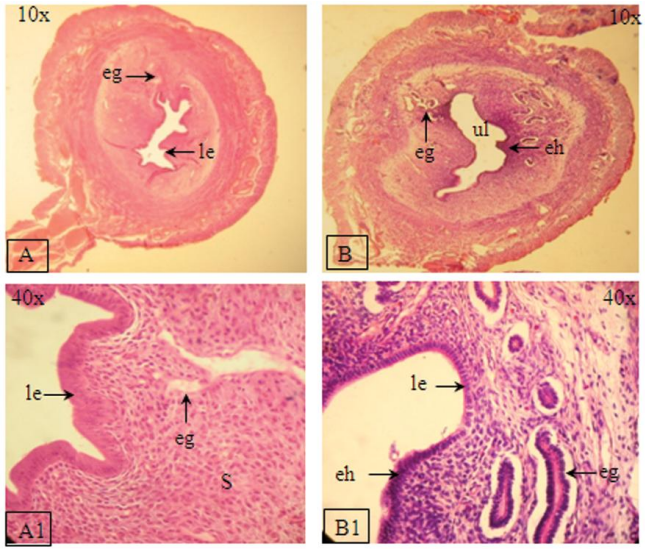

Fig. 3. The uterine sections (H & E) of cyclic ovary intact control (A & A1) and SRE treated (B & B1) females. The control females showed a narrow uterine lumen uniformly lines with columnar epithelial cells. SRE administration results in hyperplasia (eh) at certain points of luminal epithelium. Cells of luminal epithelium remain cuboidal. The epithelial layer of endometrial glands (eg) was desquamated from the supporting stroma.

The adult cyclic female rats showed proliferated, compact and thick uterine histoarchitecture during the estrus phase (Fig. 3 A & A1). The endometrial surface epithelium attained the columnar structure and proliferated increasing uterine luminal surface area. The epithelium showed finger like projections invading the stromal tissue below the luminal epithelial layer. The endometrial stromal tissue showed structurally compact and well developed endometrial glands scattered in between the luminal epithelium and myometrium. Oral administration of crude SRE to cyclic ovary intact females induced structural alteration of the uterine epithelium. SRE treated females in presence of ovary in situ showed a wide uterine lumen lined with a layer of uterine cuboidal epithelial cells (Fig.3 B & B1). The luminal epithelial cell layer at certain places showed endometrial hyperplasia with proliferation of cells. These proliferated areas of luminal epithelium appeared as multinucleated cellular structure wherein the nuclei of the epithelial cells appeared pyknotic and very often fragmented. Baring the hyperplasic region, the uterine luminal epithelium of the SRE treated females exhibited very thin cuboidal epithelial cell lining. Similar to the endometrial surface epithelium, the endometrial glands showed very thin glandular epithelium. It was observed that the glandular epithelium showed desquamation from the supporting stromal tissue resulting in obliteration of the lumen of the gland. The nuclei of the endometrial glandular epithelium and the hyperplasic region of the uterine luminal epithelial cells exhibited strong basophilic staining properties following SRE administration as presented in.

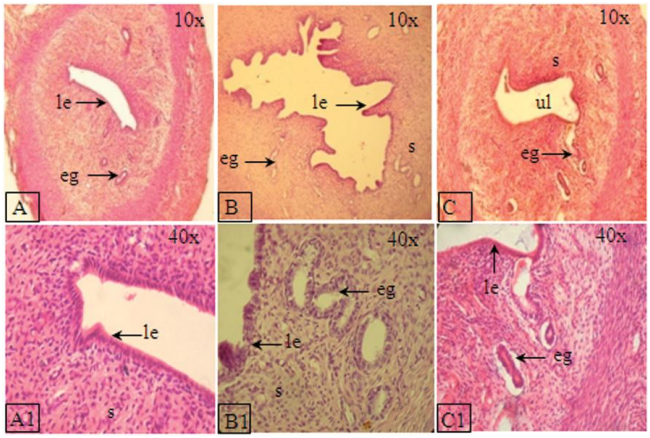

Fig. 4. Uterine sections (H & E) of control ovariectomised (A & A1) females showed unproliferated uterine luminal epithelium (le) and a few endometrial glands (eg). Administration of exogenous estradiol-17β induces proliferation of luminal epithelium and multiplication of endometrial glands (B, B1). Administration of SRE to ovariectomised females (C, C1). Induces the proliferation of the luminal epithelium and the formation of endometrial glands. However, the luminal epithelium remains cuboidal and the glandular epithelium becomes desquamated from the supporting stroma.

Effects of SRE and estradiol-17β on uterine epithelium of ovariectomized rats

The uterine histoarchitechture of control ovariectomized female rats reflected the absence of ovarian estrogen in its structural organization (Fig. 4 A & A1). The endometrial luminal

epithelium appeared unproliferated resulting in absence of endometrial glands embedded in stromal tissue. The uterine lumen was narrow lined with flat cuboidal epithelial cells. The stromal tissue became thinner with least number of endometrial glands in between the uterine luminal epithelium and the myometrial tissue. A few endometrial glands appeared in the stroma with smaller in size and very narrow lumen indicating poor development of the gland. Similar to the cells of endometrial surface epithelium, the glandular epithelial cells appeared thinner and cuboidal in shape. Subcutaneous administration of estradial-17β (E2) stimulates the proliferation of endometrial luminal epithelium (Fig.4 B & B1). The luminal epithelium formed the finger like projections indicating proliferation in response to exogenous E2. The characteristic features of estrogen stimulation were observed in the epithelium of uterine luminal surface and the endometrial glands. Development of endometrial glands and proliferations of epithelium with columnar cell structure resulted in the OVX females following E2 administration. Administration of SRE to OVX females (Fig. 4 C & C1) induced changes in histoarchitechture of uterus than that of the vehicle treated control and exogenous E2 treated females. The cells of the luminal epithelium at certain places of SRE treated females' uteri showed deeply stained nuclei and mitosis indicating occurrence of hyperplasia. The uterine lumen became wider than that of the control females, but with lesser proliferation in comparison to the exogenous E2 administration. The degree of luminal epithelial proliferation was lesser in SRE treated OVX rats than that of the exogenous E2 treated females. The secondary root extract induced proliferation of uterine luminal epithelium and formation of endometrial glands. However, the endometrial glands exhibited aberrant structural organization, getting the epithelium desquamated from the stromal tissues.

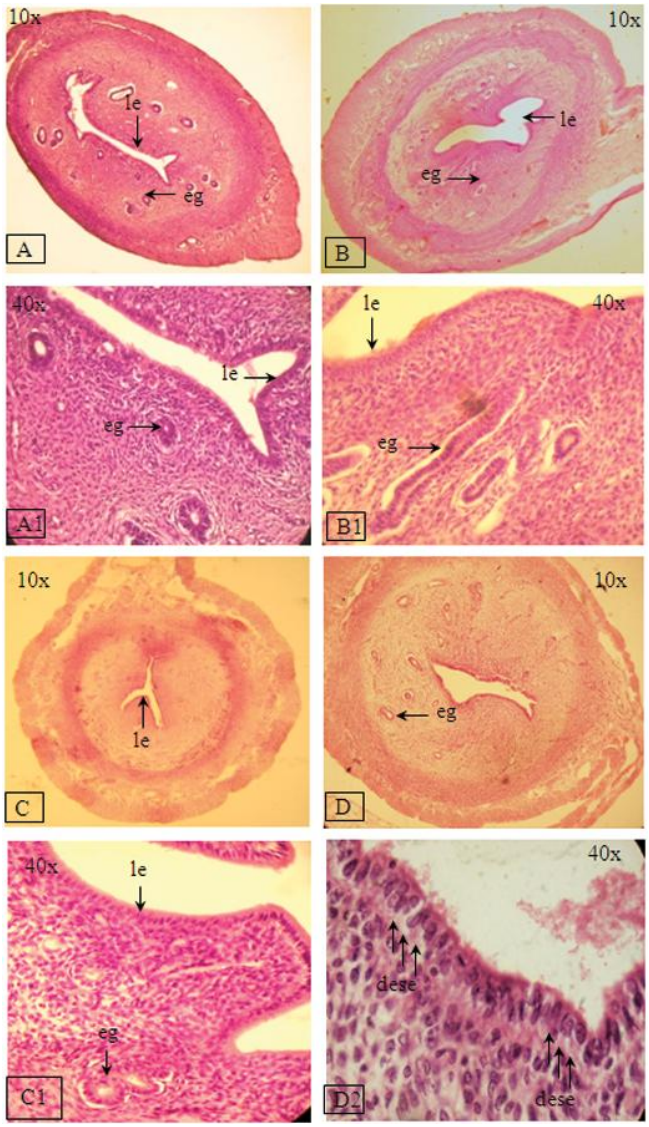

Fig. 5. The normal pregnant females on day2 of gestation (A,A1) showed a narrow uterine lumen, proliferated endometrial epithelium (le) and multiple numbers of endometrial glands(eg). Uteri of SRE treated females on day2 of gestation (B, B1) failed to proliferate resulting in a wide lumen and lesser of endometrial glands. The uteri of control females on day3 of gestation (C, C1) showed higher rate of proliferation making the uterine lumen narrower than earlier with a compact stroma. The uteri following SRE administration on day3 of gestation (D, D1) showed a wide lumen and beginning of desquamation of the luminal epithelium (dese) from the supporting stroma.

Effects of SRE on uterine epithelium during gestational period

The uterine histoarchitecture of the control females during early period of gestation appeared with folds and peaks showing opposing uterine epithelium closed down upon each other obliterating the lumen. This appearance is seen when a uterine epithelial cell opposes a blastocyst (Marphy, 2004). The epithelium showed the presence of tall columnar cells with distinct nucleus. Multiple numbers of endometrial glands are also observed in the uterine stroma. The endometrial glands were well developed in the slightly edematous stroma

with clearly stained glandular epithelial cell nucleus. These gestational tissue remodeling is noticeable from day 2 of gestation (Fig. 5 A & A1) onward in normal pregnant female rats. The degree of change of tissue remodeling becomes more on day3 of pregnancy of normal rats (Fig. 5 C & C1). The stroma becomes more edematous making the uterine lumen a slit like structure. Oral administration of SRE in threshold dose from day1 of gestation reflects its effects on uterine tissue from day2 of gestation onward. SRE reduces the cellular proliferation of endometrial surface epithelial cells and stromal cells leaving the uterine lumen wide on day2 of gestation. Uterine luminal epithelium showed hyperplasic characteristics while, the glandular epithelium showed desquamation from the supporting stromal cells (Fig. 5 B & B1). On day3 of gestation, SRE targeted the uterine luminal epithelial cells causing detachment from the stromal tissue (Fig. 5 D & D1). Uterine lumen remains wide along with few endometrial glands scattered in the deeper region of stroma.

The normal pregnant females on day4 of gestation prepare the uterus receptive for the blastocyst. Characteristically, the uterus develops a very narrow lumen lined with well developed columnar epithelial cells and supported by the compact thick stromal tissue (Fig.6 A & A1). Oral administration of SRE induces drastic structural changes of the luminal surface epithelium. The uterine lumen remains wide while the luminal epithelium develops hyperplasia at certain places. A gradual increase rate of desquamation of the luminal epithelium from the subepithelial stromal tisuue was observed following SRE administration. Number of endometrial glands embedded in stroma was lesser than that of the control females. Few endometrial glands in its state of degeneration were observed in the deeper region of the stromal tissue (Fig.6 B & B1). The control females on day5 of gestation showed the development of decidual cells in the subepithelial region (Fig.6 C & C1). Decidual cell reaction of the srtomal cells is the characteristics feature of the receptive uterus and primary requirement for the embryo implantation. The uterine luminal epithelium of these control females remain strongly attached with the subepithelial decidual zone. Oral

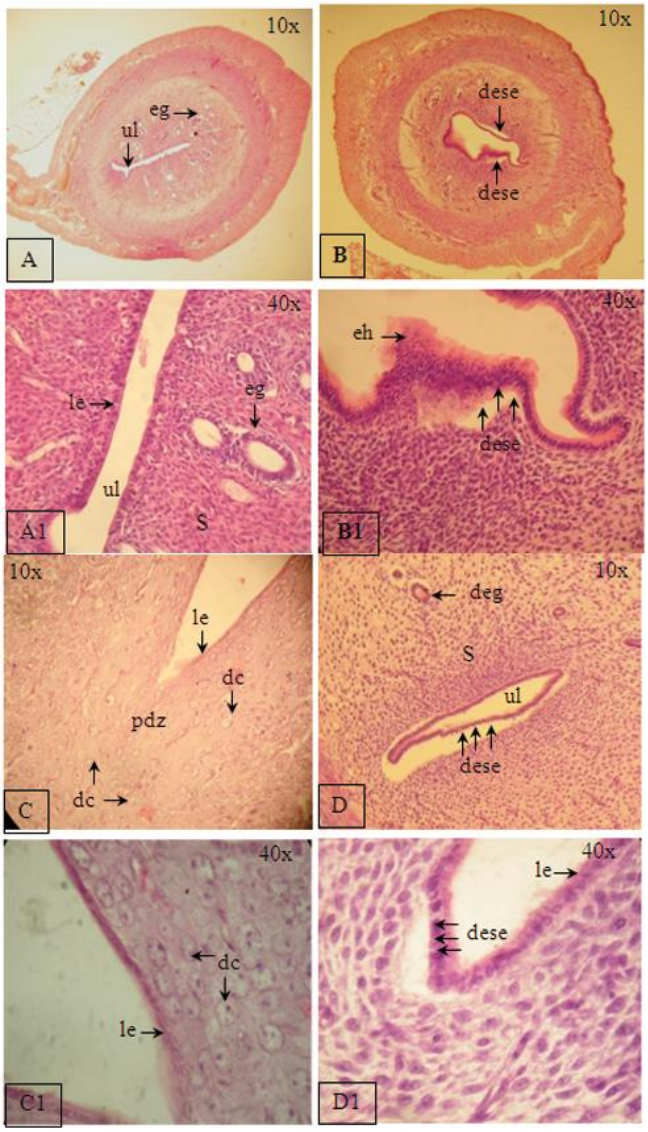

Fig.6. The Uteri of normal pregnant females on day4 of gestation (A, A1) appeared with a very narrow uterine lumen (ul) compact stroma and a number of endometrial glands(eg). The females treated with SRE on day4 of gestation (B, B1) showed the effects of SRE on luminal epithelium which appeared increased rate of desquamation (dese) of the hyperplasic (eh) sites of the uterus. On day5 of gestation, the control females (C, C1) exhibited the development of decidual cell (dc) reaction. Round decidual cells indicated the formation of primary decidual zone (pdz). The SRE treated females on day5 of gestation (D, D1) failed to develop the decidualization process wherein the rate of desquamation was higher than the earlier associated with degeneration of endometrial glands (deg).

administration of SRE retarded the process of decidual cell reaction and transformation of stromal cells to decidual cells on day 5 of gestation. Failure of the physical and biochemical process is accompanied with withdrawal of luminal epithelial

cell lining from the stromal tissue (Fig.6 D & D1). The desquamated luminal epithelial cells showed shrinkage in structure with deeply stained nuclei.

Discussion

The antifertility properties of certain other species of *Piper viz., Piper longum* (fruit), *Piper betle* (stem and root) have been reported during later part of 20th century in India. Some finding showed the contraceptive effect of roots of *Piper betle* mixed with black pepper traditionally used by women. The roots of *Piper betle* contain a phenanthrene alkaloid which possesses encouraging antifertility effects (Khosa and Singh, 1972; Prakash and Mathur, 1976; Lakshmi *et al.,* 2006). These species of *Piper* are cultivated in India for human use either as spice or mouth freshener. The *Piper betleiodes* is a wildly grown species neither uses as spice nor in other domestic purpose. The fertility regulatory property of the secondary roots of this wild species is hitherto unexplored. The present histological studies on ovary and uterus of SRE treated females showed its effects on Graafian follicle and the uterine histoarchitecture of cyclic and ovariectomised females and during early gestation. Oral administration of SRE in threshold dose induced structural aberration of granulosa cell layers leading to the subsequent degeneration of oocyte. Invariably the nature of the phytocompound(s) present in the secondary root extract, it is understood that the later exerts its adverse effects on the development of Graafian follicle. The effects of SRE are not only on the cellular organization but also in the functional aspect of follicular cells. Ovary is the source of estrogen and progesterone which are the prerequisite for development of uterine receptivity and subsequent process of implantation and progress of gestation. Abnormal development and function of Graafian follicle leads to the failure of desired uterine endometrial cell proliferation during all reproductive stages of cyclic or gestational period. Endometrial epithelial cell proliferation is estrogen dependent. The present study on cyclic and ovariectomised females showed that uterine endometrial epithelium failed to proliferate following SRE administration either in presence and absence of ovary *in situ*. This result in cyclic females attributed either to the malfunctioning of the granulosa cells for estrogen biosynthesis or effects of SRE directly on uterine epithelium. The SRE exerts unfavorable effects on endometrial cell proliferation suggesting its estrogen antagonist effects on uterus. In the ovariectomised females, exogenous estradiol-17β restored epithelial cell proliferation in endometrial tissue while, SRE administration results in failure in desired proliferation of the epithelium. In ovariectomized rats the rapid growth of the uterus treated with estradiol-17β has been thoroughly studied and well standardized. The estrogenic effects involve hypertrophy, hyperplasia, and increased tissue hydration of uterus (Sheldon, 1979). The phytoestrogens bind with the steroid hormone receptors in uterus and modulate the physiological function regulating mRNA and protein level in cells (Branham *et al*., 2002; Naragoni *et al.,* 2009). The compound(s) present in SRE may exert either estrogenic or antiestrogenic effects on rat uterine epithelium and alter the cellular structural organization and biochemical milieu resulting in functional disorder. While the phytoestrogens have been shown to induce both estrogenic and antiestecrogenic effects, their biological relevance and potency have not been well characterized (Stark and Madar, 2002).

During early gestation, the process of endometrial receptivity begins with an increase rate of cellular proliferation and transformation of stromal cells to decidual cells. This endometrial reprogramming is ovarian steroid dependent during the very beginning of gestation. Ovarian progesterone exerts effects on enstrogen primed uterus for decidual cell reaction. The endometrial stromal cells of receptive uterus undergo morphological and physiological transdifferentiation and convert itself to the large polyploidy decidual cells with epitheloid appearance (Vallejo *et al.,* 2010). The uterine cells during this phase are reported to be the estrogen source required for all physiological activities to support the ovarian luteal function and embryonic development (Das *et al.,* 2009). In the present investigation, it is not known if the SRE exerts effects on corpora lutea when administered from day1 of gestation. The adverse effect was prominent in the uterus during day1 to day5 of gestation characterized by total absence

of decidual cell reaction. Administration of SRE resulted in endometrial hyperplasia at certain areas in both the cyclic and pregnant rats' uteri. In cyclic females endometrial glandular epithelium was desquamated from supporting tissue resulting in failure of required glandular secretion. During early gestation, hyperplasic epithelium of SRE treated rats gradually desquamated from supporting stroma. This abrogated endometrial phenomenon is either due to ovarian failure or uterine cellular dysfunction induced by SRE of *Piper betleoides*. The mechanism of action of phytocompound(s) present in SRE on ovarian cells and uterine epithelium remain to be unfolded. However, it is well established that many of the phytoestrogens can bind with estrogen receptors and modulates the reproductive performance in animals (Burton & Wells, 2002). The present findings showed that SRE treated females failed to undergo decidualization and thus, decides the fate of fertilized zygote. Successful implantation is the result of synchrony between the implantation of competent blastocyst(s) and receptive uterus. This reciprocal interaction involves an intricate modulation of genetic and cellular activities. The differentiation of the endometrial stromal cells into decidual cells is characterized by development of numerous intracellular organelles, extensive cell to cell contact and junctional complexes (Dey *et al.,* 2004, Wang and Dey, 2006). The results of the present study suggest that the SRE of *Piper betleoides* contains phytocompounds having steroid hormone antagonistic property in uterus and modulate the structural organization of reproductive organs in females. The SRE can modulate the genetic and epigenetic factors of both ovary and uterus resulting in undesired morphological and physiological condition of the uterus for gestation. The nature of the active compound(s) present in secondary roots of *Piper betleoides* and its cell signaling mechanism remain to be established.

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