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Original Research article

Root of *Lasia Spinosa* used in Traditional Medicine for Female Reproduction Possesses Compound(s) Capable of Cellular Proliferation: An In-vivo Experimental Evidence on Mice Uterine Tissue

Padmini Boruah*, Indira Sarma, Krishnakshi Misra and Hirendra N. Sarma

Department of Zoology, (Center with Potential for Excellence in Biodiversity) Molecular Endocrinology and Reproductive Biology. Centre with Potential for Excellence in Biodiversity, Rajiv Gandhi University, Itanagar- 791112, India.

***Corresponding author:** pgboruah646@gmail.com

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Abstract: The root of *Lasia spinosa* is used to cure dysmenorrhea, a painful cramping sensation occurring just before or during menstruation in female. This practice is prevailed among certain community of Assam and Arunachal Pradesh. A paste of freshly collected root (approximately 5g in weight) is given orally to women suffering from pain on first day of menstruation. Based on the traditional information, it has been hypothesized that the root extract of *Lasia spinosa* may contain phytosteroids having agonistic property with native estrogen and enhances the endometrial proliferation during menstrual cycle. In the present study, methanol extract of the dry root of the plant was prepared and used to observe its efficacy on endometrial cellular proliferation in cyclic mice uterus. It was administered to female albino mice in three doses (250mg/kg, 500mg/kg and 750mg/kg body wt.) through oral route begin with diestrus for 2 consecutive cycles during 7.00 – 9.00 hours. The estrous cycle of the mice was observed subsequently. The rate of cornification duration of estrous phase and histological study of uterine epithelium were studied at the end of treatment. Data were analysed using GraphPad Prism and the significance measured with Tukey's multiple comparison test at $P<0.05$. The results showed increase rate of cornification and longer duration of estrus phase (statistically significant $P<0.05$) in extract treated females. Increased endometrial proliferation was reflected in histological sections in dose dependent manner. The experiments indicate that the root extract of the plant have certain degree of steroidogenic compound which can exert estrogen agonistic effects on mice uterine epithelium.

Key words: Endometrium, estrogenicity, estrous cycle, *Lasia spinosa*, stromal cell

Introduction

The uterus is the vital organ that is responsible for the implantation of embryo in female mammals. The inner layer of uterine epithelium is known as endometrium which undergoes cyclic growth and regeneration under the response of ovarian steroids. Estradiol-17 β is one of the major ovarian steroids, secreted by ovarian follicles that are responsible for endometrial growth and regeneration (Groothuis *et al*., 2007). Before ovulation, the regeneration and growth of endometrium is primarily regulated by estrogen. In primates, the proliferated endometrium sheds off following failure of fertilization. . During the shedding of endometrium, dysmenorrhea or menstrual pain is commonly seen in human female. The cause of dysmenorrhea is known to be due to improper proliferation and detachment of the endometrial cells. In rodents the endometrium is reabsorbed within the uterine cavity in absence of fertilization of gametes and formation of embryo

Along with the endometrium, the vaginal epithelial cells also show variation in response to estrogen (Long and Evans, 1992). The vaginal epithelium undergoing cyclic changes can be monitored by observing stained vaginal smear of mice

(Shorr, 1941; Papanicolaou, 1954). The estrous cycle of mice has four stages viz. Proestrous, Estrous, Metestrous and Diestrous which shows different types of cell accordingly (Long and Evans 1922, Mandl 1951, Feder 1981). The transition of the nucleated epithelial cells to cornified epithelial cells is considered as the marker of effects of native estrogen or an agonist which was shown in in vivo experiments (Montes and Luque, 1988).

Lasia spinosa (Lour.) is a large herbaceous plant that belongs to the family Araceae. It is commonly known as '*Kohila'* or *'Unicorn plant'.* It is a stout, marshy plant with a creeping spiny rhizome that can grow up to 2 m tall; leaves arise from the base and are arranged like a rosette (Kumar *et al*., 2013). It is a perennial herb mostly distributed in Southern Asia and occurs usually in swamps, wet forests, sub-tropical forests, open marshes, muddy streams, ditches, and wetlands or in permanently standing water (Jayaweera., 1981; Shefana *et al*., 2009). In north east India it is found commonly in Assam and low altitudinal areas of Arunachal Pradesh.

In Arunachal Pradesh, root of *L.spinosa* is traditionally used for treatment of painful menstruation. Freshly collected root paste in specific dose (approximately 5g) as recommended by traditional healer is orally taken during first three days of cycle to overcome the painful menstruation. In modern science painful menstruation is treated by ovarian steroid administration. On this background, it has been speculated that root of Lasia spinosa contain compound(s) having steroidogenic property which facilitate the process of menstruation to occur smoothly. The compound(s) may induce proliferation of endometrial cells and detachment of cells during the cycle which relieves the menstrual pain. Thus, in the present study, *L.spinosa* root extract has been used to evaluate its estrogenic property and its effect on endometrial cellular proliferation in female mice. The cyclic mice were administered with different doses for determining the threshold dose for uterine epithelium proliferation.

Materials and methods

Collection and Preparation of plant extract

The plant *Lasia spinosa* (Fig. 1) was collected from Lakhimpur District of Assam. The roots were peeled and shade dried.

Fig. 1. The plant *Lasia spinosa*.

The dried roots were chopped and powdered to 60 mesh size. The dried root powder was soaked in methanol for 72-96 hours at room temperature (25±2ÚC) for cold extraction following the existing method from our laboratory (Das *et al*., 2014). The extract was then filtered and was allowed to dry at room temperature to evaporate the methanol. The semi solid methanolic extract was diluted in distilled water and was orally administered to cyclic female mice.

Experimental Model

Adult cyclic female albino mice (25±3 g) 60-70 days old were used to investigate the effect of methanolic crude root extract of *Lasia spinosa*. The animals were kept under Departmental Animal Facility of Rajiv Gandhi University providing them with proper food and suitable environment. The cyclic mice were divided into four groups i.e. one control and three treated groups. The control and the three groups of treated cyclic mice consist of 6(six) females each.

Study of estrous cycle

The estrous cycles of the mice were monitored following the method of Montes and Luque, 1988. It was done by observing the stained vaginal smear under the microscope for the

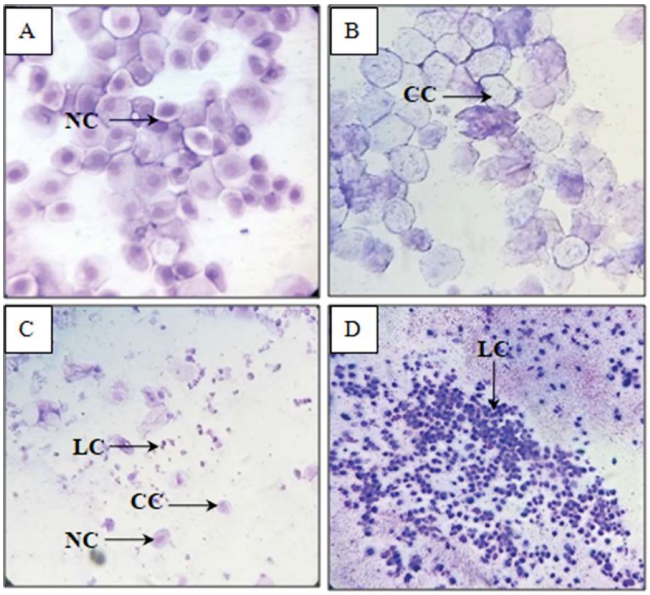

Fig. 2. Photomicrographs of different stages of estrous cycle of control female mice. A. Proestrus, B. Estrus, C. Metestrus, D. Diestrus. and NC- Nucleated cells, CC- Cornified cells, LC- Leucocytes.

identification of different type of vaginal epithelial cells. The vaginal smear was taken prior to treatment every day around 8:00hrs-9:00hrs. The brief concept of taking vaginal smear method is as follows: a little amount of distilled water is taken in glass dropper and is flushed into the vagina of the female mice. The fluid is recollected in the dropper and spread on a glass slide to make vaginal smear. Three numbers of clean slides were taken for each mouse. The vaginal smear was then air dried followed by fixation with add a drop of methanol on the smear. The fixed smear was allowed to air dry followed by staining with Giemsa for 5 minutes. Stained slides were washed with distilled water gently. Different types of cell on the air dried slides were observed and photomicrographed under Leica DM 5000B Microscope (Fig. 2 A-D). The numbers of cornified epithelial cells were counted and represented using GraphPad Prism 8.0 with the mean value ± SEM. Subsequently, the duration of estrous phase was also observed in females of treated and control groups.

Administration of extract and sample collection

The concentrated semi solid methanol extract was weighed and diluted in distilled water to prepare the final dose of the extract on the basis of body weight of individual female. Extract in specific dose was orally administered to cyclic female mice. The mice were divided into 4 groups (n=6) viz. Control group (Group I) treated with vehicle (distilled water) and treated groups (Group II-250 mg/kg body wt./day; Group III-500 mg/kg body wt./day and Group IV-750 mg/kg body wt./day). All the group of mice were treated with the respective doses for 2 consecutive cycles (8days) beginning with proestrous. On completion of 2 cycles mice were sacrificed in between 8:00hrs to 9:00hrs to collect the uterine horns for histological study.

Histological study

Uterine horns were collected and immediately fixed in 10% formaldehyde for 72hrs. Afterwards, the tissues were processed in ascending grade of alcohol (Dehydration) and cleared in xylene followed by paraffin embedding. The blocks were sectioned by using rotary microtome and stretched on Poly-L-Lysine (Sigma Cat. No. P8920) coated slides. The sections were stained using Eosin-Hematolyxin stain method (Culling, 1974) and mounted on DPX. The sections were observed and photomicrographed under Leica DM 5000B. To study the estrogenic property of root extract of *Lasia spinosa*, the parameters that are taken into consideration are endometrial thickness, diameter of uterus, myometrial thickness, perimetrial thickness, numbers of gland, the perimeter of lumen and thickness of luminal epithelium. All these parameters in both the control as well as the treated groups were measured using LAS V4.4 software provided with Leica DM 5000B microscope.

Results

Estrous cycle and rate of cornification

The rate of cornification was studied in both the control and treated groups of mice. The number of cornified epithelial cells was found to be increased in treated groups in comparison to the control group (Fig. 3). The difference between rate of cornification of all the groups (Group-I, II, III, & IV) were statistically analysed using Tukey's multiple comparison

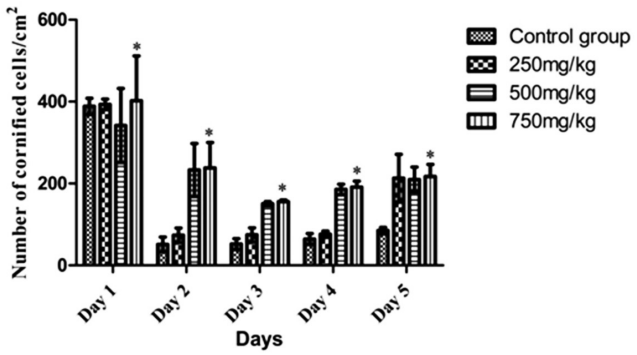

Fig. 3. Rate of vaginal epithelial cornification in control and treated (250mg/kg; 500mg/kg and 750mg/kg) groups. values were presented as Mean ± SE (*p<0.05). The treated (750mg/kg) group shows significant difference than that of the control values.

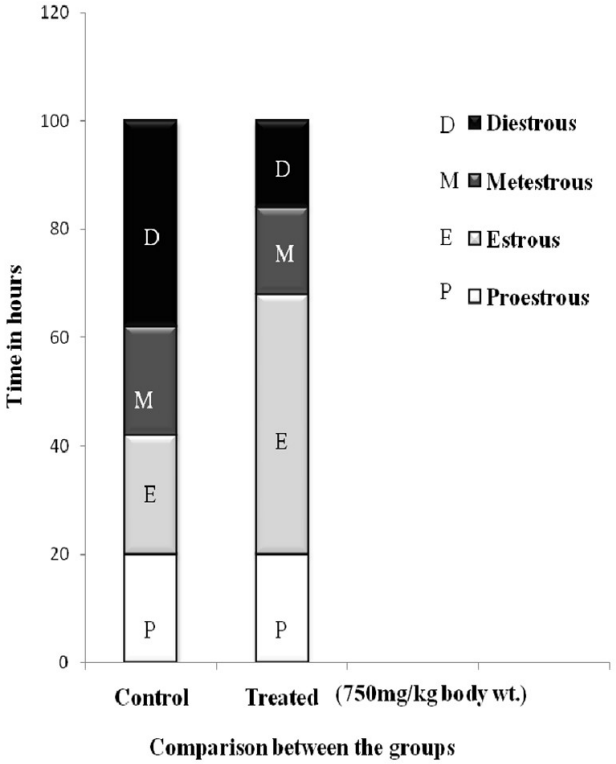

Fig. 4. Graphical presentation of duration of different phases of estrous cycle in control and treated (750mg/kg body wt.) group.

(p<0.05) test which clearly signifies the presence of estrogenic compound(s) in the plant extract.The duration of estrous cycle was observed and seen to have a longer estrous phase in the treated group in comparison to the control group of mice (Fig 4).

Uterine histology

Oral treatment of the extract caused visible changes in the uterine histoarchitecture of the treated group of mice in comparison to that of control. Uterine epithelium is found to have extensive proliferation forming finger like projections by luminal epithelium. Simultaneously, the rate of proliferation of endometrium is found to be increased in the groups with higher doses of plant extract than that of the lower ones.

The uterine histology of control mice showed uniform pattern of all the uterine layers including endometrium when compared to treated groups of mice. The uterine histological structures of the control female is presented in Fig. 5. Mice that were treated with the dose of 250mg/kg body wt. for two consecutive estrous cycles showed higher rate of proliferation of endometrial epithelium (Fig. 6) in comparision to the control group as shown in Fig. 5. The result showed no significant difference between control group (Group-I) and treated group-II (250mg/kg body wt./day). Similarly, the mice that were treated with the dose of 500mg/kg body wt. (Group-III) for two consecutive estrous cycle was also compared with the control group of mice (group-I) to analyse the rate of proliferation of endometrium as shown in Fig. 7. Increase in the thickness of endometrium was seen in extract treated group (500mg/kg body wt.) of mice (group-III) than that of control and 250mg/kg body wt. (group-II). However, no significance was found in the values observed for both the treated groups when compared with the control group. Another group of treated females (750mg/kg/day) showed increase in endometrial thickness. Values showed significant difference (p<0.05) between control group (group-I) and 750mg/kg body wt. (group-IV) as shown in the representative graphs, Fig. 5, Fig. 8 and Fig. 9.

Apparently, the perimetrium and myometrium are also found to have a higher rate of proliferation in the treated group of mice (250mg/kg body wt., 500mg/kg body wt. & 750mg/kg body wt.) compared to the control group as shown in the representative photographs (Fig. 5, 6, 7 and 8). There is relative increase in the width of perimetrium and myometrium in both 250mg /kg body wt./day and 500mg/kg body wt./day

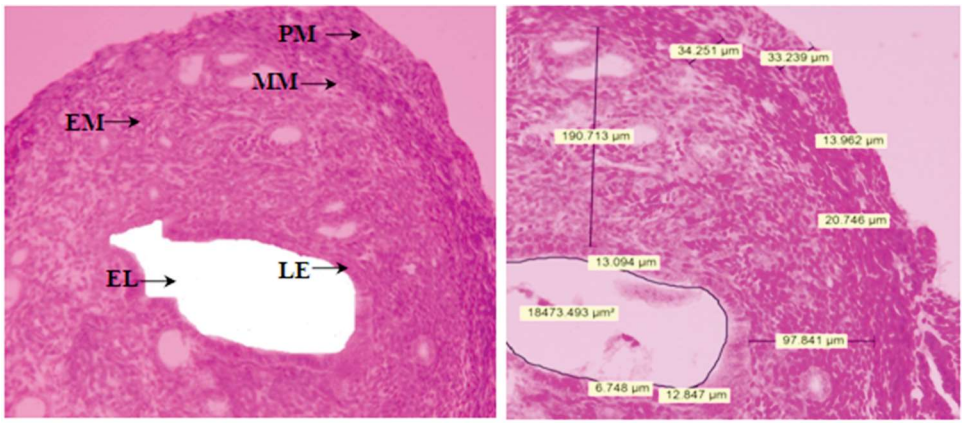

Fig. 5. Photomicrographs of uterine histology of Control female mice (H & E). Figures showed the various tissue layers and the measurements. EM- Endometrium, MM- Myometrium, PM- Perimetrium, EL-Endometrial lumen and LE- luminal Epithelium.

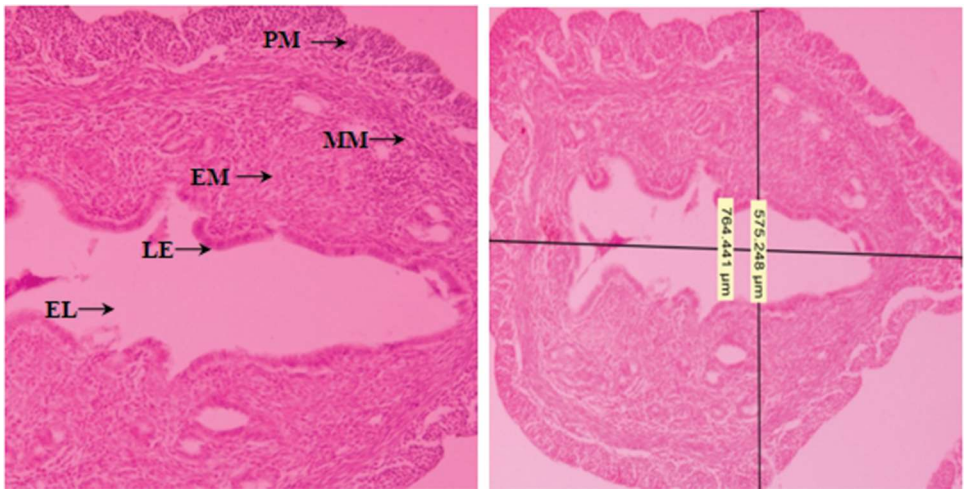

Fig. 6. Photomicrographs of uterine histology of treated female (250mg/kg/day) mice (H & E). Figures showed the various tissue layers and the measurements. EM- Endometrium, MM- Myometrium, PM- Perimetrium, EL-Endometrial lumen and LE- luminal Epithelium.

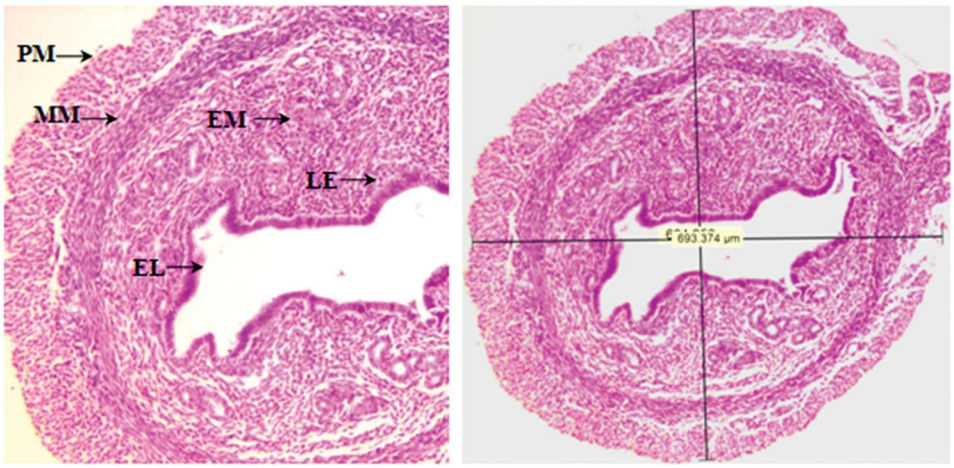

Fig. 7. Photomicrographs of uterine histology of treated (500mg/kg /day) female mice (H & E). Figures showed the various tissue layers and the measurements. EM- Endometrium, MM- Myometrium, PM- Perimetrium, EL-Endometrial lumen and LE- luminal Epithelium.

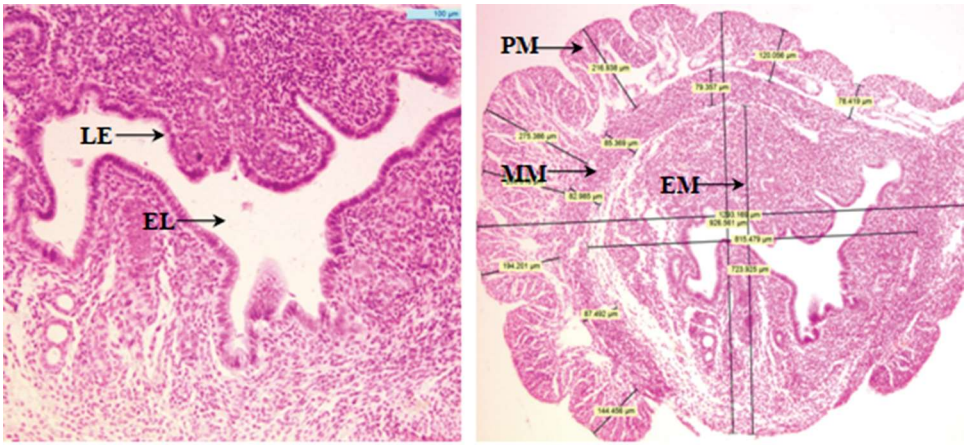

Fig. 8. Photomicrographs of uterine histology of treated (750mg/kg /day) female mice (H & E). Figures showed the various tissue layers and the measurements. EM- Endometrium, MM- Myometrium, PM- Perimetrium, EL-Endometrial lumen and LE- luminal Epithelium.

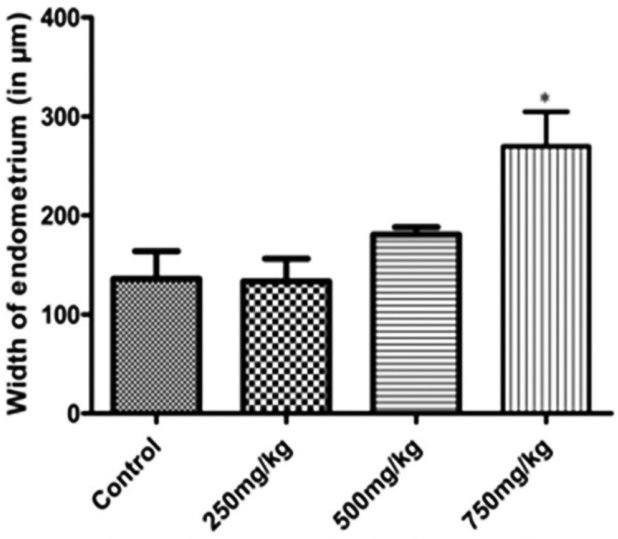

Fig. 9. Changes of thickness of endometrial cell layers in control and treated females. Values are Mean ± SE, (*p<0.05). Treated (750mg/kg) group showed significant increase in endometrial cellular proliferation than that of the control group.

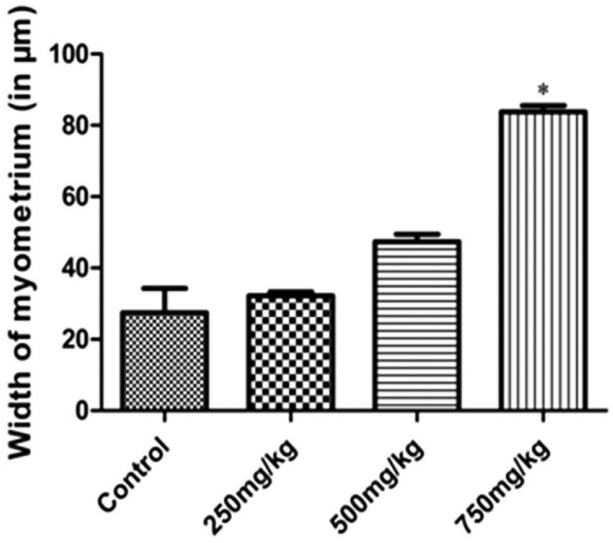

Fig.10. Changes of thickness of myometrium in control and treated females. Values are Mean ± SE, (*p<0.05). Treated (750mg/kg) group showed significant increase in myometrium than that of the control group.

than the vehicle treated (control) group. As evidenced by the histological study and measurement, the measured values of width of perimetrium and myometrium of 750mg/kg body wt./day treated group depicts significant difference with control group when statistically analysed with the help of Tukey's multiple comparison ($p<0.05$) test. The statistical analysis of proliferation of myometrium and perimetrium are represented in Fig. 10 and 11 respectively.

Simultaneously, the number of endometrial glands was also counted under Leica DM 5000B. The endometrial glands count showed that the treated groups i.e. 250mg/kg body wt., 500mg/kg body wt. & 750mg/kg/day developed more number of endometrial glands in compared to control group. The no. of endometrial glands was also found to be increased with the increasing doses. Females treated with maximum dose (750mg/kg body wt.) were found to have higher number of endometrial glands. The result of

endometrial gland count is represented graphically in Fig. 12. The number of glands was found to be increased but no significance was found between the control and the treated group. Along with that, the mice that has been treated with 750mg/kg body wt. is found to have a uterus larger (width) in size in comparison to all the other three groups i.e. control group, treated 250mg/kg group and treated 500mg/kg group. The diameter of the uterus of all the groups presented in Fig. 13. The comparison showed significant difference between the uterus of control group and the treated 750mg/kg body wt./day group.

Along with this, the height of luminal epithelium was also measured in all groups of control and treated females. The result depicts increase in height of luminal epithelium of treated group i.e. 250mg/kg body wt., 500mg/kg body wt. & 750mg/kg body wt. in comparison of the control group (Fig. 14). The result showed the highest proliferation of luminal epithelium in treated 750 mg/kg body wt. group than that of the control females ($P<0.5$). Data were found not significant among other treated (250mg/kg/day and 500mg/kg/day) groups.

The perimeter of the control group, 250mg/kg body wt. group, 500mg/kg body wt. group and 750mg/kg body wt. group were measured (Fig. 15). The result showed that the luminal epithelium of mice that has been treated with 750mg/kg body wt. were tend to have increased perimeter due to formation of finger like projections and it also showed significance difference with the control group of mice. The graphical representation of perimeter of all the groups is shown in Fig. 15.

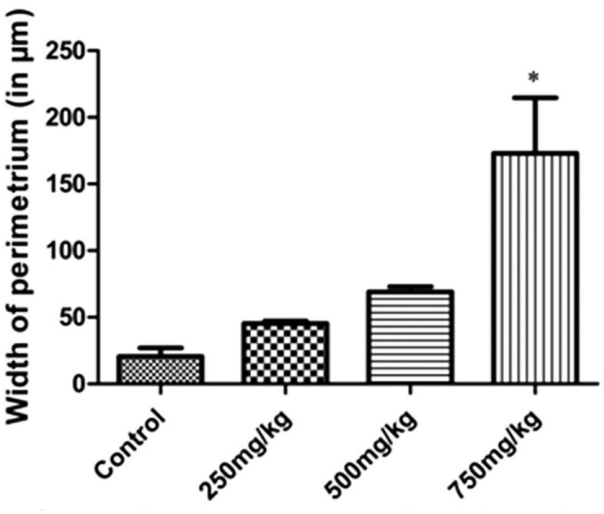

Fig. 11. Changes of thickness of perimetrium in control and treated females. Values are Mean ± SE, (*p<0.05). Treated (750mg/kg) group showed significant increase in perimetrium than that of the control group.

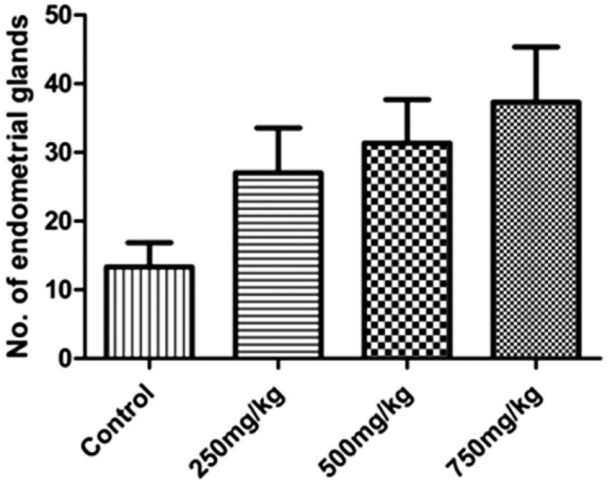

Fig. 12. Graphical presentation of no of endometrial glands of control and treated groups. (250mg/kg body wt/day, 500mg/kg body wt/day and 750mg/kg body wt/day). No significance was found in the comparison.

Discussion

Dysmenorrhea or painful menstruation is prevalent in high numbers of women of different ages and nationality. According to a survey that was carried out in the year 1996, in developed countries estimated that about 45% to 95% women suffer from dysmenorrhea during their menstrual cycle. The second review followed by the first survey was done in 2002 where it was evident that about 25% to 50% adult women and around 75% of adolescents undergo the pain (Latthe *et al.*, 2011). These survey results are the evidence that dysmenorrhea is a very common problem in female during their menstrual cycle.

It is believed that the root of *Lasia spinosa* contain compound(s) which possess estrogen agonistic property on target cells. Traditionally this root has been used by folk women traditional healers to cure the painful menstruation. The first hand information from traditional healer states that paste of freshly collected root is orally taken by suffered women during day 1 to day 3 of menstrual cycle. Three days medication recovers the patient for future cycles. Furthermore, there are many

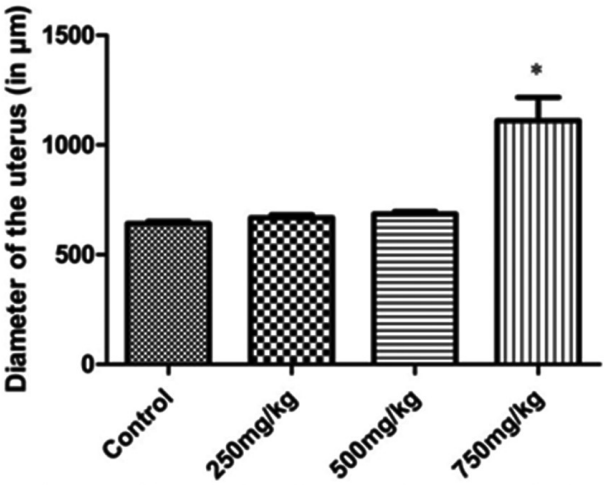

Fig.13. Changes in the diameter of uteri in control and treated females. Values are Mean ± SE, (*p<0.05). Treated (750mg/kg) group showed significant increase than that of the control group.

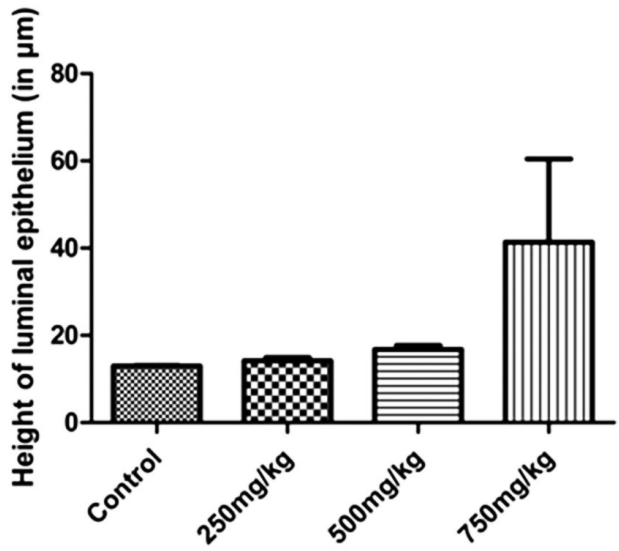

Fig. 14. Graphical presentation of height of Luminal epithelium in control and treated groups of female mice. Values are Mean ± SE., (*p<0.05). No significance was found in comparison.

other phytocompounds that are being used to avoid painful menstruation in female. Herbs such as fennel, ginger, cinnamon, mint extract and rose can be used to manage the pain during dysmenorrhea (Aksu and Ozsoy, 2016). The use of plant extract to cure dysmenorrhea has been reported as the remedy having minimal side effects (Sanogo, 2011).

The ovarian steroids estrogen and progesterone regulates the uterine functions and female reproduction.

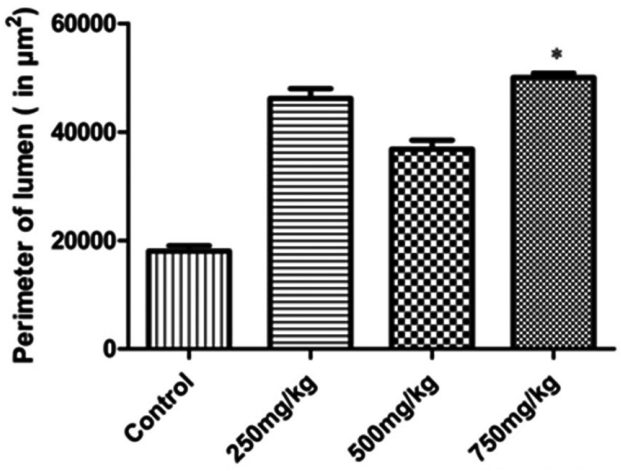

Fig. 15. Changes in perimeter of uterine lumen in control and treated females. Values are Mean ± SE, (*p<0.05). Treated (750mg/kg) group showed significant increase in perimeter of lumen than that of the control group.

During the process of ovulation, uterine epithelial cells regeneration and proliferation is regulated by the ovarian steroids (Rodriguez and Guzman, 1996). Estrogen synthesized by the ovarian follicles targets the uterine tissue during reproductive cycle. Estrogen enhances the rate of cell proliferation in uterus and induces cornification of vaginal surface epithelium. This phenomenon is reflected in the study of estrous cycle. In the present study on the effect of root extract of *Lasia spinosa*, females treated with doses of the Methanolic extract showed increased rate of cornification in comparison to that of control females. The increase in the uterine height of luminal epithelial, myometrium and perimetrium are the key marker of presences of estrogenic property in a plant extract (Lemini *et al.,* 2004).

According to the traditional knowledge prevailing among the khamti tribe of Arunachal Pradesh, the root of *Lasia spinosa* is reported to be used for curing dysmenorrhea. The paste of the root is taken for three consecutive days during menstrual cycle by the women and found to have relieved from the prevailing dysmennorrhea. Though this practice is prevalent traditionally, but it has not been experimentally established. So, the present investigation is the first of its kind where the presence of estrogenic compounds (phytoestrogens) is reported through uterotrophic study using mice as an experimental model.

For determination of threshold dose of a plant extract for its effects on reproductive organs has been defined as the minimum dose at which the effect is significant (Sarma *et al*., 2019). In the present study, efforts have been made to determine the threshold dose of crude methanol extract of *Lasia spinosa* based on the experiments on the vaginal smear, rate of cornification, histological study on the height of luminal epithelium, myometrium and perimetrium. From the above observations it has been found that the treated (group IV - 750mg/kg body wt.) group of mice are showing significant difference with the control group of mice in all studied parameters except endometrial gland formation. Therefore, the dose 750mg/kg body wt. has been determined as the threshold dose for female cyclic albino mice.

According to Aritonang *et al.,* 2017 the uterine endometrial epithelium shows higher rate of proliferation under the influence of estradiol 17 β . The result of histological analysis of treated group of present study also depicts higher rate of endometrial cellular proliferation in uterus of female mice. The impact of plant extract was also seen in other uterine layers i.e. myometrium, perimetrium and luminal epithelium. Thus, it can be concluded that the root extract have some compound(s) having estrogenic property which facilitates the endometrial and other cell type's proliferation. Aritonang *et al.,* 2017 also proved the effect of estradiol 17 β on the estrous cycle i.e. estradiol 17 β enhances the number of cornified vaginal epithelial cells during the estrous phase. On this basis, it has been speculated that the root extract of *Lasia spinosa* contains certain estrogenic compound that increase the rate of cornification and duration of estrous phase during the cyclic regeneration in female mice. Hence, the compound that is responsible for endometrial cellular proliferation and increase in rate of cornification can be synonymising as phytoestrogen because of its plant origin.

The present study helps in understanding of the changes imparted on the uterus of cyclic female albino mice by the *Lasia spinosa* plant extract. Furthermore, future studies on the constituents present in the extract can help us understand the pathways of its action. With promising techniques like GCMS (Gas chromatography-Mass spectroscopy) (Ezhilan and Neelamegam, 2012) and in-silico molecular docking (Shivakumar *et al*., 2018) the nature of the constituent compounds and be lucidly understood. By studying and understanding the mechanism of action of the compounds present in the extract the plant Lasia spinosa can be projected as the promising herbal medicine for the treatment of dysmennorhea in near future.

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References

Aksu H, Ozsoy S. 2016. Primary Dysmenorrhea and Herbals. J. Health. Commun. 1-3.

Aritonang TR, Rahayu S, Sirait LI, Karo MB, Simanjuntak TP, Natzir R, Sinrang AW, Massi MN, Hatta M, Kamelia E. 2017. The role of FSH, LH, Estradiol and Progesterone Hormone on Estrus Cycle of female Rats. Int. J. Sci. Basic and Appl. Res. 35: 92-100.

Colgan TJ and McLachlin CM. 2008. Endometrial Lessions, Unusual Tumours and Extrauterine Cance. Comprehensive Cytopathology. 3rd Edition. Elsevier. Amsterdam.

Culling CFA. 1974. Handbook of Histopathological and Histochemical Techniques. 3rd edition. Butterworths. London.

Das M, Saikia PJ, Sarma HN. 2013 . Crude bark extract of Dysozylum alliarium induces alteration in histological structures and VEGF-C expression in uterus during days 4-7 of gestation in albino rat. Reprod. Med. Biol. 12: 85-98.

Deb D, Dev S, Das AK, Khanam D, Banu H, Shahriar M, Ashraf A, Choudhuri MS, Basher SA. 2010. Antinociceptive, anti-inflammatory and anti-diarrheal activities of the hydroalcoholic extract of *Lasia spinosa* Linn. (Araceae) roots. Latin Am. J. Pharmacy. 2010: 29.

Ezhilan BP, Neelamegam R. 2012. GC-MS analysis of phytocomponents in the ethanol extract of Polygonum. Chinese L. Pharmacognosy Res. 4(1): 11-14.

Feder HH. 1981. Estrous cyclicity in mammals. In: Neuroendocrinology of Reproduction, Adler NT, Ed. Plenum Press, New York. Pp: 279/348.

Groothuis PG, Dassen HHNM, Romano A, Punyadeera C. 2007. Estrogen and the endometrium: lessons learned from gene expression profiling in rodents and human. Human reproduction update. 13(4): 405-17.

Hasan CM, Alam F, Haque M, Sohrab MH, Monsur MA, Ahmed N. 2011. Antimicrobial and Cytotoxic Activity from Lasia spinosa and Isolated Lignan. Lat. Am. J. Pharm. 30(3): 550-3.

Hasan MN, Munshi M, Rahman MH, Alam SN, Hirashima A. 2011. Evaluation of Antihyperglycemic Activity of *Lasia spinosa* Leaf Extracts in Swiss Albino Mice. World J. Pharma. Pharmaceut. Sci. 3(10): 118-124.

Jayaweera DMA. 1981 . Medicinal plants (indigenous and exotic) used in Ceylon. Part I. Colombo: National Science Council of Sri Lanka. 134-135.

Kumar M, Mondal PR, Borah SU, Mahato KA. 2013. Physico-chemical evaluation, preliminary phytochemical investigation, fluorescence and TLC analysis of leaves of the plant *Lasia spinosa* (Lour) Thwaites. Int. J. Pharm. Pharm. Sci. 5(2): 306-10.

Latthe PM, Champaneria R, Khan KS. 2011. Dysmenorrhea. BMJ. Clin. Evid. 2011: 0813.

Lemini C, Herna'ndeza A, Jaimeza R, Francoa Y, Avilaa ME and Castellb A. 2004. Morphometric analysis of mice uteri treated with preservatives methyl, ethyl, propyl, and butyl paraben. Toxicol. indust. health. 20: 123-132.

Long JA, Evans HM. 1922. The oestrous cycle in the rat and its associated phenomena. Mem. Univ. California. 6: 1/148.

Mahmood SB, Atif M, Ali SR, Ahmed MI, Rahman SA. 2015. Evaluation of Antihyperlipidemic Activity of Methanolic Leaves Extract of *Lasia spinosa* and Its Role in Prevention of Hyperlipidemia Induced Pancreatitis in Rats. Int. J. Pharmaceut. Sci. Res. 6(4): 1502.

Mandl AM. 1951. The phases of the oestrous cycle in the adult white rat. J. Exp. Biol. 28: 576-584.

Montes GS, Luque EH. 1988. Effects of ovarian steroids on vaginal smears in the rat. Acta Anat. 133: 192-199.

Navot D, Bergh P. 1991. Preparation of the Human Endometrium for Implantation. The New York Acad. Sci. 622 (1).

Papanicolaou GN. 1954. Atlas of exfoliative cytology (Harvard University Press, Cambridge).

Rahmatullah, 2010. Medicinal plants used by folk medicinal practitioners in three villages of Natore and Rajshahi Districts, Bangladesh. Am. Eurasian J. Sustain. Agric. 4(2): 211-218.

Rodriguez AC, Guzman BLA. 1996. Regulation of uterine cellular proliferation with estrogens and growth factors. Ginecol Obstet Mex. 64: 406-10.

Sanogo R. 2011. Medicinal Plants Traditionally Used In Mali For Dysmenorrhea. Sanogo Afr. J. Trad. Complement. Altern. Med. 8(S): 90-96.

Sarma I, wangsa K, Sarma HN. 2019. Threshold Dose Determination of Estrogenic Property using Morphometric Analysis of Mice Uterine Epithelial Proliferation and Study of Hepatic Toxicity of *Scoparia dulcis* L. Extract. J. Bioresources. 6(1): 46-55.

Shefana AG, Ekanayake S. 2009. Some nutritional aspects of *Lasia spinosa* (kohila). University of Sri Jayewardenepura; Vidyodaya J. Sci. 14: 59-64.

Shivakumar R, Venkatarangaiah K, Shastri S, Nagaraja RB, Sheshagiri A. 2018. Antibacterial Property and Molecular Docking Studies of Leaf Calli Phytochemicals of Bridelia scandens Wild. Pharmacogn J. 10(6): 1221-1229.

Shorr E. 1941. A new technique for staining vaginal smears. III. A single differential stain. Science. 94: 545-546.

Sultana S, Rahman RB, Alam SS. 2006. Karyotype analysis in three morphological forms of *Lasia spinosa* (L.) Thwaites (Araceae). Cytologia. 71(4): 359-64.