THE CHANGES IN THE UTERUS OF OVAREICTOMIZED MICE IN RESPONSE TO *PLUMERIA ACUMINATA* AIT STEM EXTRACT

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ABSTRACT

Objective*: The primary objective of this study is to assess the effect of methanolic stem extract of Plumeria acuminata Ait. on the uterus and estrogenic/antiestrogenic potency of the plant, which are traditionally used by the Mising tribes of Assam, India for curing different reproductive ailments.*

Method: *Extracts of the plant was prepared and given to ovariectomized mice at two dose level (200mg/kg and 400mg/kg orally). Evaluations were made based on the uterotrophic assay by observing the amount of protein, uterine wet weight, histopathological examination and serum cholesterol label.*

Results*: The extract was found to have significant changes in the wet uterine weight and in the other biochemical parameters. Similarly the histological examination of the uterus also reveals the similar effect of the plant extract though there was non-significant change in the body weight.*

Conclusion: *The results suggest that the stem extract of Plumeria acuminata Ait. have significant estrogenic property suggesting for a possible contraceptive activity which is consistent with the literature report on folk medicine of the plant used by the missing tribe of this region.*

Keywords: *Plumeria acuminata* Ait. Ovariectomy, Estrogenic/ antiestrogenic activity, Cholesterol, uterotrophic assay

INTRODUCTION

Plants and their parts have played a very significant role in maintaining health of human since antiquity. The rural people in most parts of the world have been using different plant parts for curing different types of ailments. Traditional herbal medicines and its healing power have been realized and documented since long back (Bhattacharjya and Borah, 2008). From generation to generation this knowledge on traditional medicine has been transmitted orally and is been continued till date. These plants product now form a major source of raw material in modern day pharmaceutical industry (Chopra et al., 1999). Preclinical investigation on the effect of different plant extract on animal reveals that many of them are found to interfere with the reproductive function of the female. Some are found to enhance the fertility while others are anti-fertility in nature. This interference may be expressed commonly as change in the weight of the animal, disruption in certain stages of the female cycle, increase or decrease in the weight of ovary and uterus (Byers et al., 2012). Among these plant constituents, the phytoestrogens, especially isoflavones, found in many plants, mimic the effect of natural estrogenic compounds due to the similarity in structure of both these compounds (Cordial Reginald et al., 2006). A variety of such plant constituent like the phytoestrogens have already been investigated and were found that these compounds bind strongly to ERβ receptors which induces a estrogen-like action (Davis et al., 1999 ; Nikov et al., 2000). The

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ISSN: 2186-8476, ISSN: 2186-8468 Print
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biological effect of consuming dietary supplement of isoflavone rich compound such as soy, may help in reducing the risk of cardiovascular disease, helpful in alleviating menopausal health symptoms and endrometrial and breast cancer (Adlercreut, 1995). Phytoestrogens are also found to help in preventing the occurrence of hormonal carcinogenesis by preventing genotoxic estrogen metabolites (Dixon and Ferreira, 2002).

Keeping the potential health benefit of the plants in mind a study was conducted to ascertain the effect of the methanolic stem extract of a plant that are used by the mising tribes of upper Assam districts (India). *Plumeria acuminata* Ait. commonly known as "Boga gulonchi, white frangipani, dalan phul or temple tree". It is a deciduous plant species, planted occasionally near temples for ornamental purposes. Sporadically found in different parts of Assam, Meghalaya and Nagaland (NE India), this plant has been used extensively by traditional healers to cure different diseases. This plant is used widely to cure fever, diarrhea, and prevention of heart stroke, elevate from rheumatic pain and the latex is employed for the treatment of itch and inflammation. A lot of experimental works on the different parts of the plants have already been done which showed that the leaves have anti-inflammatory activity (Gupta et al., 2006), antioxidant and free radical scavenging property (Gupta et al., 2007). However, no research has been carried out to assess the potential estrogenic/anti-estrogenic property of the plant. Therefore, the present work waqs undertaken to substantiate scientifically the estrogenic/ant-estrogenic activity in *P. acuminata* Ait by taking ovariectomized mice as a model and exploiting the subsequent constant criterion: uterotrophic assay, biochemical, histopathological and morphometric analysis.

MATERIALS AND METHODS

Collection of Plant material and authentication

The plant samples were collected from Bangalmara region of North Lakhimpur District, Assam, India and herbarium sheets prepared. It was then identified and authenticated by the Department of Botany, Guwahati University, Assam, India. A voucher specimen (Acc. No. - GUBH-17872-10/03/2015) was deposited at the department of Botany, Guwahati University.

Preparation of the plant extract

Preparation of the plant extract was done as per the procedure described by Yakubu et al. (2005). The stem of the plant was pealed of its bark and sliced into small pieces and shade dried for a week. It was then grinded in a mortar into fine powder. The powdered plant material was then dissolved in methanol & run in a Soxhlet extractor for 36 hrs. The filtrate was taken and allowed to evaporate. After complete evaporation of the methanol we get a gummy (paste) concentrate of dark brown colour plant extract. The extract is stored in a refrigerator until use.

Phytochemical screening of the plant

The methanolic extract of the plants was analyzed to identify the presence of various phytoconstituents such as terpenoids, tannins, steroids, phenols, flavonoids, saponins, and cardiac Glycosides by using standard procedures (Kokate, 1986; Harborne, 1998).

Animals

Adult female albino mice (C3H strain) of average body weight (22 ± 3g) were procured from Pasture Institute, Directorate of Health Services, Govt. of Meghalaya, Shillong (India). The mice were housed in groups of five in polypropylene cages and maintained under natural

conditions of photoperiod, temperature (26 ± 3) ˚C and humidity. Standard pellet diet with vitamins and mineral supplements and water was given *ad libitum*.

Duration and route of administration of test solution

Based on the weight of the animal, dosage of methanolic extract of *P. acuminata* Ait in 1% Tween-80 solution was prepared. Tween-80 was used as the vehicle. 17β -estradiol was prepared in 100% alcohol (analytical grade) as 1 mM stock solution and diluted with normal saline (1%) as per the experimental requirement. Vehicle for administration was prepared by mixing Tween-80 with normal saline at the ratio of 1:10 and was used as a vehicle for different experimental groups. The extract was given orally by using feeding tube. The mice received treatment once daily for seven consecutive days.

Ethical consideration

The care and handling of the animals as well as the experiments were conducted in accordance with the internationally accepted standard guidelines for laboratory animal (EEC Directive of 1986; 86/609/EEC). The study was carried out following approval from Institutional Ethical Committee on the Use and Care of Experimental Animals, Guwahati University, Assam, India

Experimental Design

Twenty healthy female mice showing regular estrous cycle were considered for the present study. The animals were exposed to mild anesthesia; Ketamine (50mg/Kg Bw) and Xylazine (10mg/Kg Bw) in the ratio 2:1(Intra-muscular). Ovariectomy (OVX) was done following standard rodent ovariectomy procedure (Kalita et al., 1998). A period of two weeks was allowed for acclimatization and wound healing. The mice were then divided into four groups of five mice in each group.

1. Group I:- Vehicle Control group receiving 1% Tween-80 solution (1%, v/v, 0.2 ml/mouse/day)
2. Group II:- Positive control-1µg /kg bt. Wt./mouse/day of 17β estradiol in 0.2ml 1% Tween-80 solution
3. Group III:- 200mg/kg bt. Wt./mouse /day of the plant extract in 0.2ml 1% Tween-80 solution
4. Group IV:- 400 mg/kg bt. Wt./mouse /day of the plant extract in 0.2ml 1% Tween- 80 solution.

Uterotrophic Assay: Determination of uterine wet weight

Using a balance, mice body weight was measured initially before treatment and then for all other day for the 7 day duration of the treatment. 24hours after the last dose, their body weight was recorded and then sacrificed. The uteri was carefully dissected out, trimmed of fat and connective tissue and their wet weights were measured (OECD Test Guideline 440,2007a). Absolute uterine weights were determined as dividing the wet uterine weight by the body weight and multiplying by 100(Padilla-Blanks et al., 2001).

Biochemical Estimation

Before sacrifice, the blood sample is collected by cardiac puncture method, allowed to clot for 30 minutes at room temperature and then centrifuged at 3000 rpm for about 15 minutes. After separation, the sample was analyzed on the same day to determine concentration of total cholesterol level using a Cholesterol Kit (CHOD/PEP Method). M.L.No.; 1/UA/2012 Lot: RCHO1005. The total protein of the uterus was estimated by the method as described by

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Lowry et al. (1951). Measurements were made using a Thermo Scientific AquaMate Plus (UV-VIS) spectrophotometer.

Preparation of the tissue for Histological examination

A part of the uterine horns were fixed in bouin's fixative for 24 hours and washed thoroughly and then processed through a series of alcohols and xylene, infiltrate and embedded in paraffin. 5µ sections of cross section of the mid-uterine horn were cut in a rotatory microtome and are mounted on slides. They are then stained with eosin and hemotoxylin (Grunert et al., 1987; Tinwell et al., 2000). The sections were studied in the laboratory using a light microscope (Leica, DM 5000B).

Statistical Analysis

All the data are expressed as Mean±SEM and significance was statistically analyzed by using one-way analysis of variance (ANOVA) followed by Dunnett Multiple Comparison Test. The significance level was considered at $P<0.05$.

RESULTS

Preliminary phytochemical analysis

The freshly prepared methanolic extract of *Plumeria acuminata* Ait. shows the presence of following bioactive phytochemical constituent of the plant, such as alkaloids, saponins, flavonoids, carbohydrates, protein and phenols.

Effect of the methanolic extract of *P. acuminate* Ait. on the weight of uterus

Oral administration of methanolic stem extract of *Plumeria acuminata* Ait. (PAE) to OVX mice at 200mg/kg and 400mg/kg (Table-1) caused a significant increase in the weight of the uterus in a dose dependent manner in Group-III($p< 0.05$) and Group-IV($p< 0.01$) when compared with the control. However, the response was significantly less when compared with the 17β estradiol treated mice, indicating lower potency of the extract. Data of the uterine weights were expressed as uterine wet weight and absolute uterine weight. Even though there was a slight increase in the weight of the mice, the dose response increase was insignificant in both the control as well as treated group of mice.

Table 1. Wet uterine weight and body weight of bilaterally ovariectomized mice treated with methanolic stem extract of *Plumeria acuminata* Ait. (PAE), 17β estradiol (EE) and Vehicle control group

Treatment	*No. of mice (n)*	*Initial body weight(mg)*	*Final body weight(g)*	*Uterine wet weight(mg)*	*Absolute uterine weight*
Vehicle control	5	23.61±0.41	23.69±0.40	9.83±0.98	41.49±0.24s
17β estradiol(EE)	5	23.21±0.82	23.57±0.67	69.83±1.31**	296.27±0.20**
200mg/kg PAE	5	22.52±0.61	22.67±0.59	13.02±1.13*	57.43±0.91*
400mg/kg PAE	5	22.26±1.09	22.69±0.93	17.33±2.68**	76.38±1.12**

Value for each group is expressed as Mean±SEM.

Significantly different from the control group ($p<0.05$, ** $p<0.01$), n=5

Effect of the methanolic extract of *P. acuminata* Ait. on the amount of uterine protein

The effect of the plant extract on the uterine protein level is shown in Fig.1. Analysis of the uterine protein reveals that the amount increased significantly in both the treated groups i.e., (35.42±7.68µg/gm and 39.76±4.97µg/mg tissue for 200mg/kg and 400mg/kg body weight of the extract) with that of the control(30.16±3.26 µg/mg). The amount of protein content was maximal (67.45±6.89 µg/mg) in the mice treated with 17-β estradiol.

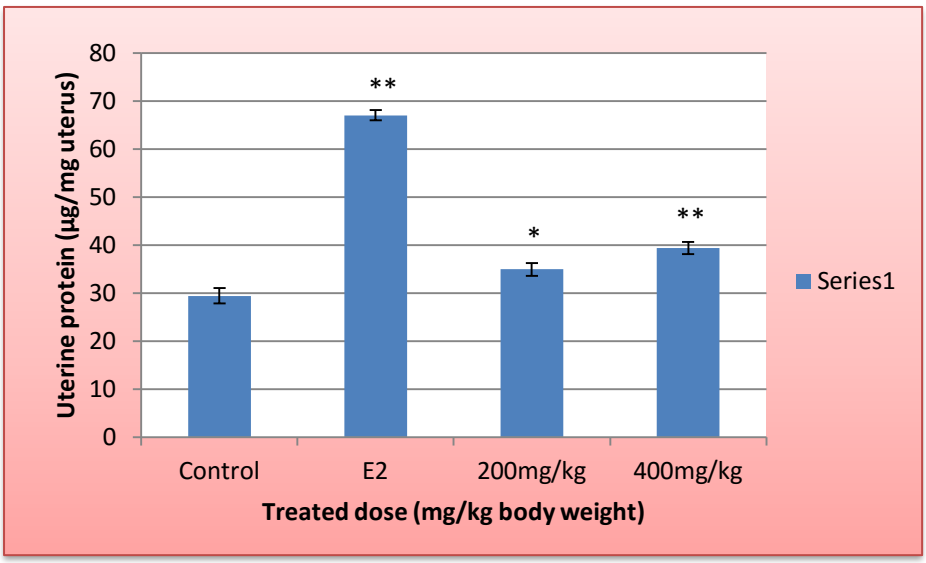

Figure 1: Changes in the amount of protein content of uterus of OVX mice following treatment with PAE

Effect of the methanolic extract of *P. acuminata* Ait. on the level of serum cholesterol level

The effect of the plant extract caused a significant decrease in the total cholesterol level at a dose of 200mg/kg body weight. At a higher dose of 400mg/kg body weight the decrease in total cholesterol level was even greater. However, the hypocholesteromic effect of EE (17β estradiol) was found to be highly significant compared to the control.

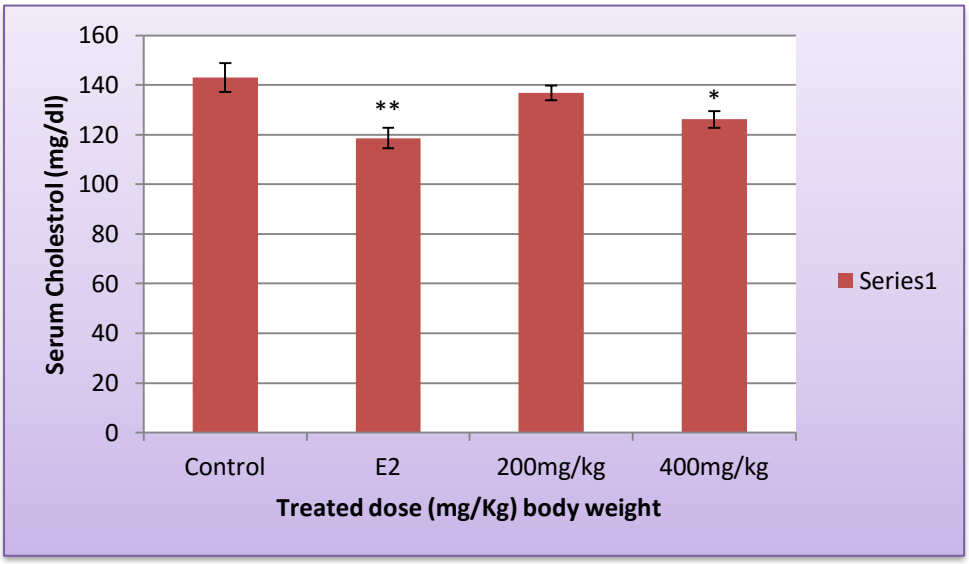

Figure 2: Changes in the amount of total cholesterol content of OVX mice following treatment with PAE.

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Effect of the plant extract on histological parameters in uterus of OVX mice

Analysis of uterine histological sections unconcealed vital atrophy within the uterus of untreated OVX mice as evident by degeneration of the endometrium, epithelial cells of the uterine lumen and secretory glands. Treatment of OVX mice with 17β estradiol considerably restored uterine morphology, as indicated by the thickening of the uterine endometrium, the amplified number of glands and more extended glandular cavities compared with untreated OVX samples. The effects of methanolic stem extract of *Plumeria acuminata* Ait. were found to have a similar ability as 17β estradiol that are found to reverse the atrophy caused by ovariectomy.

Table 2. Effect of PAE on diameter, thickness of endometrium and myometrium and epithelial cell height in ovariectomized female mice

Group	*Epithelial cell height(µm)*	*Thickness of endometrium (µm)*	*Thickness of myometrium (µm)*	*Diameter of uterus (µm)*
I. Vehicle Control	8.45±0.49	50.39±05.14	30.67±1.90	312.37±15.13
II. EE	18.47±1.06**	199.03±17.74**	91.54±8.30**	742.82±41.76**
III. 200mg/	10.29±0.74*	73.36±05.53*	33.36±1.3	419.77±18.01*
IV. 400mg/kg	12.60±0.81*	83.03±01.97*	58.27±4.96**	454.74±27.76

Value for each group is expressed as Mean±SEM.

*Significantly different from the control group (*p<0.05, ** p<0.01), n=5

As in figure 3 and table2, it was observed that the thickness of the myometrium increased with increasing doses of PAE treatment. PAE at 200mg/kg/day and 400 mg/kg/day caused a 1.08 and 1.90 times increase in the myometrial thickness as compared to control. EE treatment resulted in 2.98 times increase in this parameter as compared to control.

Based on histology it was also found that the thickness of the endometrium increased following treatment with PAE in a dose-dependent manner. With a dose of 200mg/kg/day body weight of PAE the endometrium showed a 1.45 times increase as compared to control and 400mg/kg/day body weight of PAE caused a 1.64 times increase in the endometrium as compared to control. However mice fed with EE showed a maximum i.e. 3.94 times increase in the endometrial layer. An increasing response of the uterine epithelial cells was also observed after 7 days treatment of the animal with the stem extract of *Plumeria acuminata* Ait. There was clear evidence of luminal epithelial proliferation in the treated group in response to control group. The uterine luminal epithelial height increased with increasing dose of the extract and reached its maximum in mice treated with 17B estradiole. Treatment at higher doses at 400mg/kg body weight showed a 1.49 fold increase in the height with respect to control. Though there was no significant increase in the height of the cells in Group III to that of Group IV, but when compared to the control group, the increase in cell height was significant. Group II, however showed the highest (2.18 fold) increase in the height of the cells.

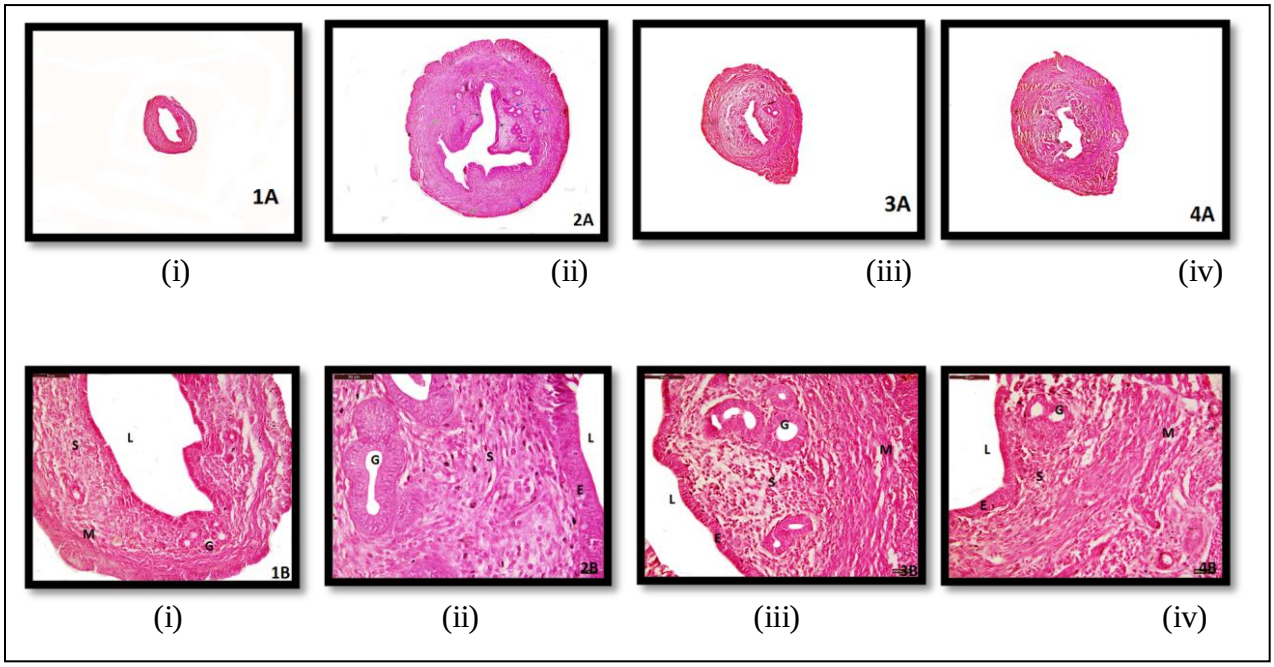

Figure 3. Photomicrographs showing cross-section of hematoxylin and eosin stain uterine horn in the OVX mice. Each treatment groups are shown: (**i**) vehicle control ovariectomized (OVX) mice; (**ii**) OVX mice treated with 17β estradiol; (**iii**) OVX mice treated with PAE at 200mg/kg body weight; (**iv**) OVX mice treated with PAE at 400mg/kg body weight.

Representative photomicrographs taken at 5x (1A, 2A, 3A, 4A) and at 40x (1B, 2B, 3B, 4B) magnification.

L, uterine lumen; **M**, myometrium; **S**, endometrial stroma; **G**, glands of the endometrium; **E**, luminal epithelial cells

As evident from figure 3, the quantity and size of the uterine glands also increased with increasing doses of PAE treatment. Treatment with PAE resulted in a significant increase in the number of glands, average size of the glandular lumen and glandular epithelial cell heights as compared to control. The diameter of the uterus also showed a significant increase in a dose dependent manner.

DISCUSSION

The focus of the present study is to determine the estrogenic or antiestrogenic activity of the plant extract (*Plumeria acuminata* Ait) and its effect on the uterus in sexually matured bilaterally ovariectomized female mice. OVX rats are a decent model for evaluating estrogen activity in female reproductive organs, together with bone and cholesterol connected parameters (Wronski et al., 1991). This model was reported to be an efficient indicator of the changes in low density lipoprotein (LDL) cholesterol and is responsible for monitoring the effects of estrogen on cholesterol (Windler et al., 1980). Further, ovariectomy has the advantage of mimicking exact menopausal condition minimizing the obstruction of endogenous estrogen (Liu and Bachmann, 1998). So in the present study, ovariectomy was performed to reproduce postmenopausal condition. Ovariectomy raises the cholesterol content in serum. The deficiency of estrogen is understood to increase in cholesterol levels, both in humans (Stevenson et al., 1993) and animals (López‐Belmonte et al., 2012; Dodge et al., 1996; Lundeen et al., 1997). Ovariectomy also increased body weights of rodents resulting in overweight. The increase in body weight is considered as an outcome of altered energy metabolism caused by estrogen shortage favoring fat deposition (Arjmandi et al., 1997).

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Contrary to the enhancement in the body weights, uterine weights were found to be reduced. Such a decrease in uterine weight is a direct effect of estrogen deficiency, which is obligatory for the normal maintenance and functioning of the uterus (Stevenson et al., 1993).

One of the established methodologies to work out the estrogenicity of a chemical is the rodent uterotrophic assay that measures a rise within the wet weight of the uterus (Evans et al., 1941). Estrogens are responsible for eliciting growth response in uterine tissue (Grunert et al., 1987), which includes water imbibitions, vascular permeability and cellular infiltration (Rockwell et al., 2002). In developing mice, the development of the reproductive system largely depends on the circulating level of estrogens. In ovariectomized mice, as the ovaries are removed, the circulating level of estrogen is negligible.

In the present study, it was found that, oral administration of the plant extract at 200mg/kg body weight and 400mg/kg body weight to ovariectomized female mice produced significant($p < 0.05$) increase in the uterine wet weight, when compared with the control group. Treatment of 17β-estradiole to the ovariectomized mice however, showed a many fold ($p < 0.01$) increase in uterine wet weight thus confirming to the well known sensitive bioassays of estrogenisity (Jordan et al., 1985). The increase in the weight of the uterus in uterotrophic assay can very well be endorsed to the effect of *Plumeria acuminata* Ait. The estrogenic compounds that are present in the plant extract are found to bind with estrogen receptors in the uterus as an agonist ligand. After binding, this ligand- receptor complex now bind to the response element i.e., the DNA, specific for the estrogen receptor, which in turn down regulates or up regulates the transcription of specific genes. This gene transcription modulation results in a biological response of the target tissue i.e., it leads to uterine cell division and growth. Moreover, an increase in the wet weight of the uterus may well be explain for the effect of the extract on hormonal changes that might have taken place in the ovariectomized mice via the effect of the extract on the uterus. The absence of change in body weight revealed that there is no major negative impact on the general metabolic status of the animals.

Results of the effect of PAE on the uterine protein (Fig.1) revealed that there was a dose dependent increase in uterine protein contents but these effects were of lesser in magnitude than estradiol. The effect of PAE at the two doses shows a significant increase in the weight of the uterus in treated mice as compared to the control (Table-1). This result suggests that the methanolic stem extract of *Plumeria* acuminate Ait. possess estrogenic activity. Similar finding was reported by Dabhadkar et al. (2013), while working on the effect of pod extract of *Plumeria rubra* on the reproductive organs of female rats.

Cholesterol acts as an obligatory precursor for the production of the entire steroid hormone including estrogen, progesterone, cortisol and testosterone in rat, rabbit and bovine luteal tissues has been reported earlier (Wilks et al., 1970). Since cholesterol is the parent molecule, the altered parameters associated with a decrease in the serum cholesterol levels may be indicative of use of cholesterol for steroidal hormone synthesis and thus might also result in increased circulating levels of estrogen hinting at an estrogenic property of the extract.

The findings from the above studies were supported by histological evidences which showed that the height of luminal epithelial cells, myoetrium increased in the mice treated with PAE group on seven day of treatment in comparison to that of the control groups. These is due to increase in the cell number and size and conversely to the increase in cell proliferation. Proliferation of epithelial cells in response to estrogenic compounds was demonstrated in mice and rats (Huet-Hudson et al., 1989), which suggest that it is a common phenomenon which affects the uterus. This study reveals that treatment with PAE at two dose level was

able to make remarkable changes to the uterine morphology in which it caused an increase in the mitotic figures as well as number of glands. These findings indicate that high PAE doses stimulate hyperplastic changes in the uterus. This epithelial hyperplasia could also affect normal endometrial development which could interfere with the implantation process (Nikas and Makrigiannakis, 2003). Changes in endometrial morphology have been proposed to affect fertility as reported in Polycystic Ovarian Disease, which was featured by a prolonged proliferative phase (Rudnicka et al., 2009). We contemplate that hyperplastic changes in the endometrium may restrain its transformation into a receptive state, which may therefore interfere with the processes of implantation. Apart from such estrogenic compounds of plants, other environmental oestrogens for examples p-tert-octylphenol (OCT) and bisphenol-A (BPA), have also been found to induce changes in the uterine morphology thereby affecting normal fertility.

Treatment of the mice with PAE also revealed a significant change in the thickness of the endometrium (Fig: 3). The thickness of the endometrium increased with increase in the dose of the plant extract. Estrogenic substance has an influence on the growth and development of the endometrium. These substances assist in activation of cell genome through its receptor in the nucleus of target cells of the uterus (Horne and Blithe, 2007). Similar findings were revealed by Murray about the uterine endometrial and epithelial cells of the ovariectomized sheep which undergo morphological alternations in protein-synthesizing organelles (Murray, 1992). Our findings have also shown that treatment with PAE resulted in a significant increase in the endometrial layer, the number and size of the glands and the epithelial cell heights as compared to control, which indicate that this plant extract possess estrogenic property.

CONCLUSION

The present study was able to provide evidences on the effect of methanolic stem extract of *P. acuminate* Ait. on the wet weight, biochemical and morphological features in the uterus of OVX mice. In vivo experiments using mice as a model have shown that PAE stimulates the growth and development of the uterus which indicates the estrogenic property of the plant. The chemical nature of phytoestrogens in *P.acuminata* Ait is still unknown and therefore needs further investigation. Further work on the mechanism(s) of action of PAE on fertility/ant fertility in intact mice and on isolation of the active component(s) responsible, are underway.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this work.

ACKNOWLEDGEMENT

The Authors are thankful to Cotton College, Guwahati, Assam (India), RGU Arunachal Pradesh (India) and Guwahati University, Assam (India) for providing laboratory facility to carry out this study.

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ISSN: 2186-8476, ISSN: 2186-8468 Print
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REFERENCES

[1]. Adlercreut, Z. H. (1995). Phytoestrogens: Epidemiology and a possible role in cancer protection. *Environmental Health Perspectives, 103* (7): 103-112.

[2]. Arjmandi, B.H., Khan, D.A., Juma, S.S., & Svanborg, A. (1997). The ovarian hormone deficiency induced hypercholesterolemia is reversed by soy protein and the synthetic isoflavones, ipriflavone. *Nutr Res, 17*: 885-894.

[3]. Bhattacharjya, D.K., & Borah, P.C. (2008). Medicinal weeds of crop fields and role of women in rural health and hygiene in Nalbari district, Assam. *Indian J. of Traditional Knowledge, 7*(3): 501-504.

[4]. Byers, S.L., Wiles, M.V., Dunn S.L., & Taft R.A. (2012). Mouse estrous cycle identification tool and images. *PLoS One*, *7*(4): e35538.

[5]. Chopra, R.N., Nayer, S.L., & Chopra, I.C. (1999). *Glossary of Indian Medicinal plants*. CSIR, New Delhi Publication and Information Directorate.

[6]. Cordial, R. R., et al., (2006). Estrogenic Activity of *Pueraria phaseoloides* Roxb. Benth Evaluated in Ovariectomized Rats. *Philippine Journal of Science*, *135* (1): 39-48.

[7]. Dabhadkar, D., Zade, V., Dawada, S., Dhore, M., & Kodape, M. (2013). Effect of alcoholic pod extract of *Plumeria rubra* on biochemical and haematological parameters of female albino rats. *Int. J. Pharm. Asi. Rev. Res*, *19*(1): 69-74.

[8]. Davis, S.R., Dalais, F.S., Simpson, E.R., & Murkies, A.L. (1999). Phytoestrogens in health and disease. *Rec Prog Hormone Res*., *54*: 185-210.

[9]. Dixon, R.A., & Ferreira, D. (2002). Genistein. *Phytochem., 60*(3): 205-211.

[10]. Dodge, J.A., Glasebrook, A.L., Magee, D.E., Phillips, D.L., Sato, M., Short, L.L., & Bryant, H.U. (1996). Environmental estrogens: Effects on cholesterol lowering and bone in the ovariectomized rat. *J Steroid Biochem Mol Biol*., *59*: 155‑161.

[11]. Evans, J. S., Varney, R. F., & Koch, F. C. (1941). The mouse uterine wet-weight method for the assay of estrogens. *Endocrinol., 28*: 747-752.

[12]. Grunert, G., Neumann, G., Porcia, M., & Tchernitchin, A. N. (1987). The estrogenic response to clomiphene in the different cell types of the rat uterus: Morphometrical evaluation. *Biol. Reprod., 37*: 527–538.

[13]. Gupta, M., Mazumder, U.K., & Gomathi, P. (2007). Evaluation of antioxidant and free radical scavenging activities of *Plumeria acuminata* leaves. *Journal of Biological Sciences, 7*(2): 1361-1367.

[14]. Gupta, M., Mazumder, U.K., Gomathi, P., & Thamil Selvan, V. (2006). Antiinflammatory evaluation of leaves of *Plumeria acuminata*. *BMC Complementary and alternative medicine. 6*(36): 1472-6882.

[15]. Harborne, J.B. (1998). *Phytochemical methods*: *A guide to modern techniques of plant analysis.* 2nd ed. London: Chapman and Hall. 54–84.

[16]. Horne, F.M., & Blithe, D.L. (2007). Progesterone receptor modulators and the endometrium: changes and consequences. *Hum Reprod Update*. *13*: 567-580.

[17]. Huet-Hudson, Y.M., Andrews, G.K., & Dey, S.K. (1989). Cell type-specific localization of c-myc protein in the mouse uterus: modulation by steroid hormones and analysis of the periimplantation period. *Endocrinol., 125*(3): 1683–1690.

[18]. Jordan, V.C., Mittal, S., Gosden, B., Koch, R., & Liberman, M.E. (1985). Structure-activity relationship of estrogen. *Enviro. Health Perspect, 61*: 97-110.

[19]. Kalita, J.C., Milligan, S.R., & Balasubramanian, A.V. (1998). Relative potency of xenobiotics estrogens in an acute in vivo mammalian assay. *Environmental Health Perspective, 106*(I): 23-26 .

[20]. Kokate, C.K. (1986). *Practical Pharmacognosy*, 1st edition, Vallabh Prakashan, New Delhi. 15- 30.

[21]. Liu, D., & Bachmann, K.A. (1998). An investigation of the relationship between estrogen, estrogen metabolites and blood cholesterol levels in ovariectomized rats. *J Pharmacol Exp Ther., 286*: 561‑8.

[22]. López‑Belmonte, J., Nieto, C., Estevez, J., Delgado, J.L., & del Prado, J.M. (2012). Comparative uterine effects on ovariectomized rats after repeated treatment with different vaginal estrogen formulations. *Maturitas, 72*: 353‑358.

[23]. Lowry, D. H., Rosenbrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. *J. Biol. Chem., 193*(1): 265-275.

[24]. Lundeen, S.G., Carver, J.M., McKean, M.L., & Winneker, R.C. (1997). Characterization of the ovariectomized rat model for the evaluation of estrogen effects on plasma cholesterol levels. *Endocrinology, 138*: 1552‑8.

[25]. Murray, M.K. (1992). The effect of estrogen and progesterone on structural changes in the uterine glandular epithelium of the ovariectomized sheep. *Biol Reprod., 47*: 408-417.

[26]. Nikas, G., & Makrigiannakis, A. (2003). Endometrial Pinopodes and Uterine Receptivity. *Annals of the New York Academy of Sciences, 997*(1): 120-123.

[27]. Nikov, G.N., Hopkins, N.E., Bou, E.S., & Alwort, H.W.L. (2000). Interactions of dietary estrogens with human estrogen receptors and the effect on estrogen receptor-estrogen response element complex formation. *Environ Health Perspect, 108*: 867-872.

[28]. Padilla-Blanks, E., Jefferson, W.N., & Newbold, R.R. (2001). The immature mouse is a suitable model for detection of estrogenicity in the uterotropic bioassay. *Environ Health Perspect, 109*: 821-826.

[29]. Rockwell, L.C., Pillai, S., Olson, C.E., & Koos, R.D. (2001). Inhibition of vascular endothelial growth factor/vascular permeability factor action blocks estrogen induced uterine edema and implantation in rodents. *Biol Reprod., 67*: 1804-1810.

[30]. Rudnicka, E., Wierzba, W., & Radowicki, S. (2009). Evaluation of endometrial histologic morphology in patients with polycystic ovary syndrome. *Ginekol Pol., 80*(2): 103-6.

[31]. Stevenson, J.C., Crook, D., & Godsland, I.F. (1993). Influence of age and menopause on serum lipids and lipoproteins in healthy women. *Atherosclerosis, 98*: 83‑90.

Leena and Luna International, Chikusei, Japan.
(株) リナアンドルナインターナショナル, 筑西市,日本
ISSN: 2186-8476, ISSN: 2186-8468 Print
www.ajsc. leena-luna.co.jp

[32]. Tinwell, H., Soames, A.R., Foster, J.R., & Ashby, J. (2000). Estradiol-type activity of coumestrol in mature and immature ovariectomized rat uterotrophic assay. *Environ Health Perspect, 108* (7): 631-634.

[33]. Wilks, J.W., Fuller, G.B., & Hanse, W. (1970). The role of cholesterol as a progestin precursor in rat, rabbit and bovine luteal tissue. *Endocrinology*, *87*(3): 581-587.

[34]. Wronski, T.J., Yen, C.F., Burton, K.W., Mehta, R.C., Newman, P.S., Soltis, E.E., & Deluca, P.P. (1991). Skeletal effects of calcitonin in ovariectomized rats. *Endocrinology, 129*: 2246‑50.

[35]. Windler, E.E., Kovanen, P.T., Chao, Y.S., Brown, M.S., Havel, R.J., & Goldstein, J.L. (1980). The estradiol‑stimulated lipoprotein receptor of rat liver. A binding site that membrane mediates the uptake of rat lipoproteins containing apoproteins B and E. *J Biol Chem.*, *255*: 10464‑10471.

[36]. Yakubu, M.T., Akanji, M.A., & Oladiji, A.T. (2005). Aphrodisiac potentials of the aqueous extract of *Fadogia agrestis* (Schweinf. Ex Hiern) stem in male albino rats. Asian J *Androl*., *7*: 399-404.