RESEARCH ARTICLE Indian Journal of Animal Research, Volume 57 Issue 12: 1620-1626 (December 2023)

Estrogenic Effect of *Bambusa arundinacea, Trichosanthes dioica* and *Punica granatum* on Rats

Akanksha Awasthi[1], Mamta F. Singh[2], Saurabh Sharma[3]

10.18805/IJAR.B-4760

ABSTRACT

Background: Phytoestrogens have recently become a hot topic among scientists. Phytoestrogens' estrogen-like properties have led to their widespread use in the reproductive system. The aim of this research was to see whether the ethanolic extract of *Bambusa arundinaceae, Trichosanthes dioica* and *Punica granatum* had any estrogenic activity in female wistar rats.

Methods: In female wistar rats, the estrogenic effect was studied using a uterotropic assay, vaginal cytology and vaginal opening. In ovariectomized immature and mature female wistar rats, a 400 mg/kg body weight (b.w.) dose of ethanolic extract of *Bambusa arundinaceae*, *Trichosanthes dioica* and *Punica granatum* was given.

Result: When compared to ovariectomized control rats, the uterine wet weight increased significantly. The estrogen-treated rats had only cornified epithelial cells, indicating the existence of oestrogen, as well as 100% vaginal opening. At 400 mg/kg b.w., the ethanolic extract of *Bambusa arundinaceae*, *Trichosanthes dioica* and *Punica granatum* demonstrated promising estrogenic activity, as evidenced by uterotropic assays, vaginal opening measurements and histopathological changes. As a result of this research, it's possible to infer that the ethanolic extract of *Bambusa arundinaceae*, *Trichosanthes dioica* and *Punica granatum* play an important role in estrogenic activity in female rats.

Key words: Ethanolic extract, Histopathology, Phytoestrogen, Uterotropic assay, Vaginal cytology, Vaginal opening.

INTRODUCTION

Many plants have been discovered to have estrogenic activity, implying that they contain estrogenic compounds. They contain phytoestrogens, which have biological activity similar to estrogen. The importance of phytoestrogens in human health has been revealed in numerous studies. Phytoestrogens bind to estrogenic receptors in a competitive manner, causing estrogen-responsive genes to be induced (Procházková *et al.,* 2017). 'Bans' is the common name for *Bambusa arundinacea* (Poaceae). It has anthelmintic, antidiabetic and wound healing effects, as well as anti-inflammatory, antiulcer and antifertility properties. Its use in strangury and urinary discharge has been reported in a number of studies (Tripathi *et al.,* 2013; Kumar *et al.,* 2012; Wardhani *et al.,* 2016). Almost every part of the plant has been shown to have medicinal value, such as the root (burnt root) for bleeding gums, the bark for skin eruptions and the leaf as an emmenagogue. *Trichosanthes dioica* Roxb is an Asian pacific tropical herb. Fruit contains the majority of the medicinal properties (Kumar *et al.,* 2012). It's been used for centuries to treat jaundice and hepatic diseases, as well as swollen liver and spleen (Khatua *et al.,* 2016). In addition, it works as an antipyretic, diuretic, cardiotonic, laxative and antiulcer agent. Experiments have shown that it is successful in promoting fertility (Khan *et al.,* 2020). The pomegranate, *Punica granatum*, of the Punicae family, has been found to contain estrone, a sex steroid (Peters *et al.,* 1980). Many pharmacological properties, such as anti-microbial, anti-parasitic, anti-viral and anti-cancer activity, have been discovered in studies (Mertens-Talcott *et al.,* 2006; Sreeja *et al.,* 2012). Pomegranate juice has been found to contain flavonoids such as anthocyanins and phenols (Devi *et al.,* 2017). The effects on fertility and estrogen-like effects have also been discovered in experiments (Bekoe *et al.,* 2020).

Therefore, the current research used an uterotropic assay, vaginal cytology and vaginal opening calculation to assess the estrogenic effects of alcoholic extract of *Bambusa arundinacea*, *Trichosanthes dioica* and *Punica granatum* on wistar rats.

[1]Uttarakhand Technical University, Sudhowala, Dehradun-248 161, Uttarakhand, India.
[2]Sardar Bhagwan Singh University, Balawala, Dehradun-248 161, Uttarakhand, India.
[3]Vivek College of Technical Education, Bijnor-246 701, Uttar Pradesh, India.

Corresponding Author: Akanksha Awasthi, Vivek College of Technical Education, Bijnor-246 701, Uttar Pradesh, India.
Email: akankashaawasthi333@gmail.com

How to cite this article: Awasthi, A., Singh, M.F. and Sharma, S. (2023). Estrogenic Effect of *Bambusa arundinacea, Trichosanthes dioica* and *Punica granatum* on Rats. Indian Journal of Animal Research. 57(12): 1620-1626. doi: 10.18805/IJAR.B-4760.

Submitted: 25-08-2021 **Accepted:** 27-11-2021 **Online:** 11-01-2022

MATERIALS AND METHODS

Study area

This experiment was conducted at Vivek College of Technical Education, Bijnor, Uttar Pradesh during June 2015 to December 2016.

Experimental animals

Impuberal female albino wistar rats, 21-day-old, with body weight of 45-50 g, were used. Animals were housed four per cage, in a multiple rat rack. Temperature (21±2°C) and humidity (55±15%) were controlled and a 12 h light/dark cycle was maintained. Water and food were ad libitum. The experiment protocol was approved by the Institutional Animal Ethical Committee (IAEC) according to the regulation of committee for the purpose of control and supervision of experiments on animals (CPCSEA) and ethical norms was strictly followed during all experimental procedure (Ref. No. VCTE/07/2016 CPCSEA). All animals had been acclimatized for three days in the animal room prior the first treatment (Parhizkarsup *et al.,* 2011).

Dose selection

Doses were selected on the basis of previous toxicity studies carried out for *Bambusa arundinaceae* (Nilsson *et al.,* 2001) (Table 1), *Trichosanthes dioica* (Sharangouda *et al.,* 2008) (Table 2) and for *Punica granatum* (Valadares *et al.,* 2010) (Table 3). The doses selected were 200 mg/kg, 300 mg/kg and 400 mg/kg, p.o.

Preparation of doses

The plant extracts were prepared in distilled water using carboxymethylcellulose (CMC) as a suspending agent (1%). The standard drug was also prepared in CMC and given as suspension to the animals. Control group (Negative control group/Olive oil control group) here through with the help of orogadtric tube 0.2 ml of olive oil was given orally once in a days in between experimental time. Conjugated Equine Estrogen (CEE 0.2 mg/kg) was purchased from Pharmacy shop, Bijnor, India. CEE (Wyeth Montreal, Canada), prepared in a dosage (p.o.) of 0.2 mg/kg by dissolving it in distilled water (Malaivijitnond *et al.,* 2006) and was used as a positive control for comparing with the Test groups. The Plant extracts of *Bambusa arundinaceae, Trichosanthes dioica* and *Punica granatum* in different doses (200 mg/kg, 300 mg/kg and 400 mg/kg body weight, orally) was dissolve in 0.2 ml of Olive oil and with the help of Orogastric tube was given by oral route (Goodman and Parker, 2008).

Treatment protocol

Immature Uterotrophic animals were divided into five groups each containing six animals and one group of normal immature rats. Animals were fasted 18 hrs prior to dosing and 3-4 hours after administration of the plant extracts. The plant extracts were given at a dose of 200 mg/kg, 300 mg/kg and 400 mg/kg oral routes to the immature female animals for a period of 14 days.

Induction of uterotrophic activity in rats

Bilaterally ovariectomized, female immature female albino and wistar rats were randomly allotted to different groups. All test substances were administered for 14 days. The animals will be examined twice a day on 4th and 5th day for the presence of the vaginal opening. After noticing the vaginal opening, the vaginal cytology will be observed to examine the cornification of epithelium. Body weight will be recorded on daily basis. On presence of vaginal opening

Table 1: Treatment protocol for estrogenic activity of *Bambusa arundinacea* using immature uterotropic model.

Group	Treatment
Group I	Animal received normal saline (1 ml/kg, p.o.)
Group II	Animal received ethinyl estradiol as suspension in distilled water (0.2 mg/kg, body weight orally)
Group III	Animal received ethanolic extract of *Bambusa arundinaceae* leaves (200 mg/kg, p.o.)
Group IV	Animal received ethanolic extract of *Bambusa arundinaceae* leaves (300 mg/kg, p.o.)
Group V	Animal received ethanolic extract of *Bambusa arundinaceae* leaves (400 mg/kg, p.o.)

Table 2: Treatment protocol for estrogenic activity of *Trichosanthes diocio* fruit using immature uterotropic model.

Group	Treatment
Group I	Animal received normal saline (1 ml/kg, p.o.)
Group II	Animal received ethinyl estradiol as suspension in distilled water (0.2 mg/kg, body weight orally)
Group III	Animal received ethanolic extract of *Trichosanthes diocio* fruit (200 mg/kg, p.o.)
Group IV	Animal received ethanolic extract of *Trichosanthes diocio* fruit (300 mg/kg, p.o.)
Group V	Animal received ethanolic extract of *Trichosanthes diocio* fruit (400 mg/kg, p.o.)

Table 3: Treatment protocol for estrogenic activity of *Punica granatum* seed using immature uterotropic model.

Group	Treatment
Group I	Animal received normal saline (1 ml/kg, p.o.)
Group II	Animal received ethinyl estradiol as suspension in distilled water (0.2 mg/kg, body weight Orally)
Group III	Animal received ethanolic extract of *Punica granatum* seed (200 mg/kg, p.o.)
Group IV	Animal received ethanolic extract of *Punica granatum* seed (300 mg/kg, p.o.)
Group V	Animal received ethanolic extract of *Punica granatum* seed (400 mg/kg, p.o.)

14th day the animals will be sacrificed by over anesthesia. The uterus was collected and weighed. The percentage of vaginal opening, cornification and relative organ weight were calculated (Nazreen *et al.,* 2011).

Table 4: Weight of uterus of immature female rats treated with ethanolic leaves extract of *Bambusa arundinaceae.*

Treatment	Uterine weight (Mean±SEM)
Control	10.825±0.27
Standard (0.2 mg/kg Ethinyl estradiol)	17.01±0.29***
Test (200 mg/kg)	12.17±0.25*
Test (300 mg/kg)	13.81±0.44*
Test (400 mg/kg)	14.72±0.15***

Note: Means ± Standard error mean (SEM); * Significant at p<0.05, *** Significant at p<0.01.

Table 5: Weight of uterus of immature female rats treated with ethanolic fruits extract of *Trichosanthes dioica.*

Treatment	Uterine weight (Mean±SEM)
Control	10.825±0.276
Standard (0.2 mg/kg CEE)	16.683±0.481***
Test (200 mg/kg)	11.661±0.401^{ns}
Test (300 mg/kg)	13.425±0.454*
Test (400 mg/kg)	12.715±0.45*

Note: Means ±Standard error mean (SEM); * Significant at p<0.05, *** Significant at p<0.01.

Table 6: Weight of uterus of immature female rats treated with ethanolic seed extract of *Punica granatum.*

Treatment	Uterine weight (Mean±SEM)
Control	10.825±0.27
Standard (0.2 mg/kg CEE)	17.01±0.29***
Test (200 mg/kg)	12.55±0.44*
Test (300 mg/kg)	13.815±0.445*
Test (400 mg/kg)	12.496±0.552*

Note: Means±Standard error mean (SEM); * Significant at p<0.05, *** Significant at p<0.01.

RESULTS AND DISCUSSION

The ethanolic extracts of the *Bambusa arundinaceae, Punica granatum and Trichosanthes dioica* respectively caused increase in the weight of uterus. The results (Table 4-6) showed the estrogenic effect of *B. arundinaceae, Punica granatum* and *Trichosanthes dioica* when tested in immature ovariectomized rats. In the control animals weight of uterus was changed while the administration of standard dose of ethinyl estradiol) (0.2 mg/kg) resulted in the significant increase in weights of uterus. In comparison to the control group all the 3 plants extracts showed promising results Fig (1-3).

Vaginal opening in female rats

On analysis of the vaginal opening of the female rats it was found that with increasing days of treatment the vaginal opening dose dependently decreased (Table 7). In the standard group the opening was to the max at day 7 *i.e.* 80%. The treatment of the extracts concised the muscles and resulted decrease in the vaginal opening (Fig 4a-8b).

Histopathology studies

The histopathology studies of *Bambusa arundinaceae* revealed promising results. In vaginal smear of ovariectomized rat from control group treated with normal saline for 7 days. A great number of leukocytes were observed (Fig 9). The Vaginal smear of immature rat treated with ethinyl estradiol (0.2 mg/kg) for 7 days cornified and nucleated epithelial cells were observed (Fig 10). The vaginal smear of immature rat treated with low dose (200 mg/kg) of *Bambusa arundinaceae* cornified, nucleated epithelial cells and leukocytes were observed (Fig 11). Vaginal smear of immature rat treated with medium dose (300 mg/kg) of *Bambusa arundinaceae* showed cornified, nucleated epithelial cells and leukocytes (Fig 12). The vaginal smear of ovariectomized rat treated with medium dose (400 mg/kg) of *Bambusa arundinaceae* cornified, nucleated epithelial cells and leukocytes are observed (Fig 13).

Administration of *B. arundinaceae*, *Punica granatum* and *Trichosanthes dioica* extract on immature ovariectomized

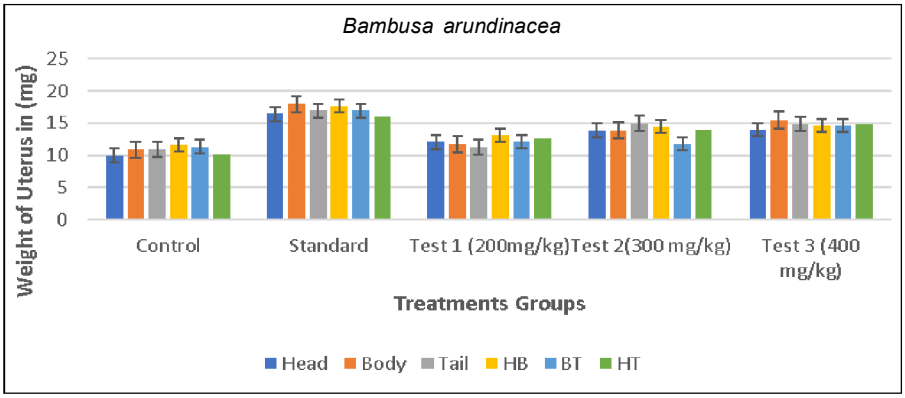

Fig 1: Effects of *Bambusa arundinacea* leaves ethanolic extract on immature female rats. The statistical significance of difference between means was calculated by Analysis of variance (ANOVA) followed by post hoc test for paired comparison.

rats results a significant increase of uterine weight. This might be due to the activation of estrogen responsiveness in the uterine wall. Another study found that uterine knockout mice showed significant results in terms of increasing of uterus after the application of estrogen due to estrogenic response in the immature uterus (Weihua *et al*., 2000). The bioactive molecules of the applied extract may have Estradiol-17β (E_2) like flavonoides which are responsible for the significant effect on the vaginal opening, development of cornified epithelial cells and an increase of uterine wet

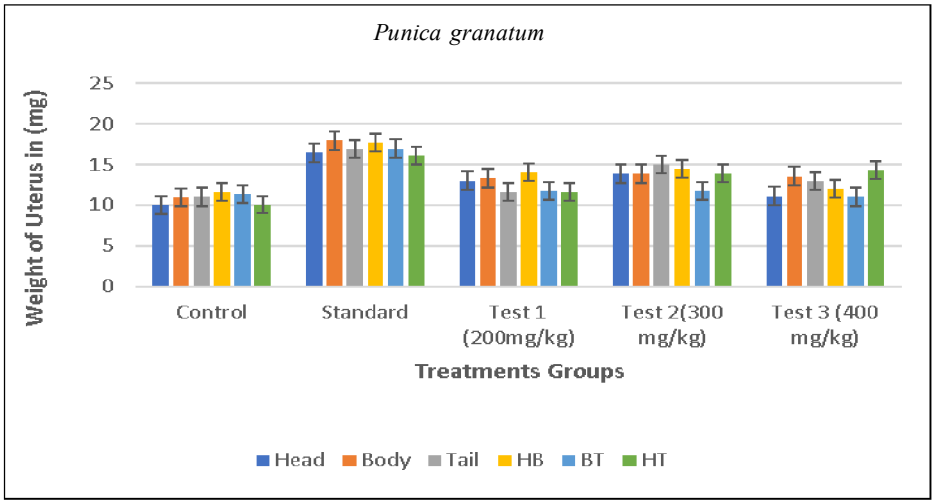

Fig 2: Effects of *Punica granatum* ethanolic extract on female rats. The statistical significance of difference between means was calculated by Analysis of variance (ANOVA) followed by post hoc test for paired comparison.

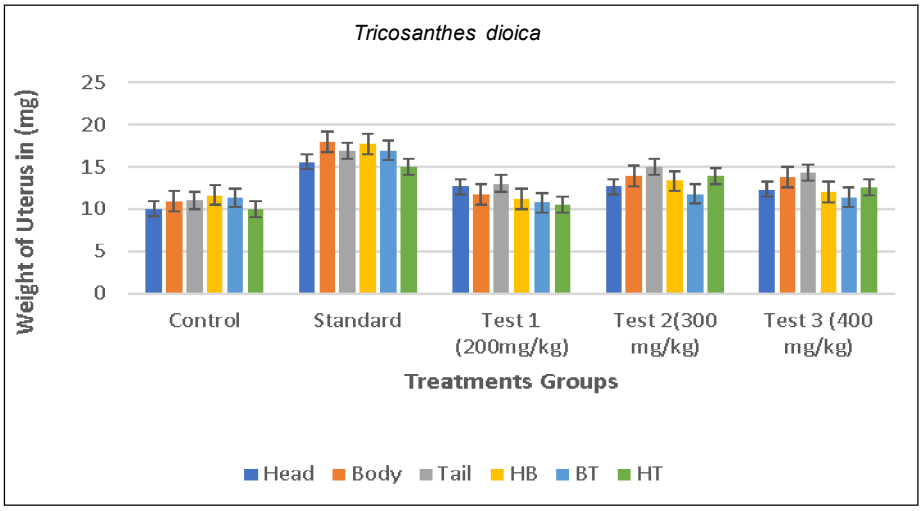

Fig 3: Effects of *Tricosanthes dioica* ethanolic extract on female rats. The statistical significance of difference between means was calculated by Analysis of variance (ANOVA) followed by post hoc test for paired comparison.

Table 7: Vaginal openings in the female rats.

Group	Treatment	Dose (mg/kg)	Vaginal opening (%)						
			Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Control (cmc)	00	00	00	00	00	00	00	00	00
Standard 0.2 mg/kg	0.2	00	00	00	00	70	90	80	80
Bambusa arundinaceae	300	00	00	00	00	60	85	70	65
Punica granatum	300	00	00	00	00	40	55	60	45
Trichosanthes dioica	300	00	00	00	00	35	49	30	25

Control group (cmc 10 mg/kg)

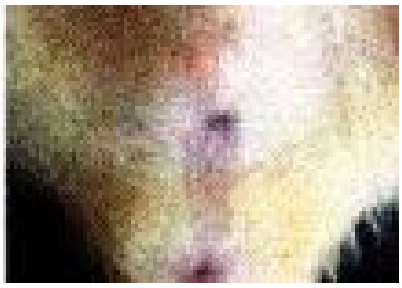

Fig 4a

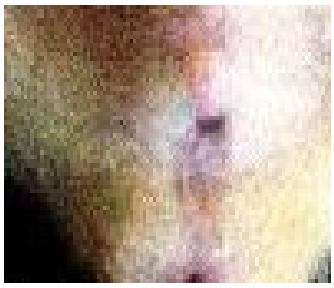

Fig 4b

Standard group (Ethinyl estradiol 0.2 mg/kg)

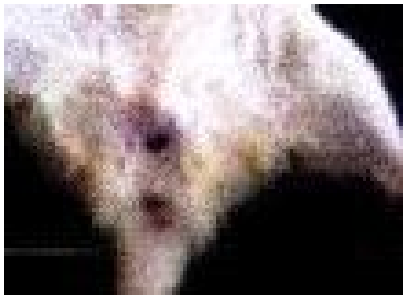

Fig 5a



Fig 5b

Bambusa arundinaceae (300 mg/kg)



Fig 6a



Fig 6b

Punica granatum (300 mg/kg)

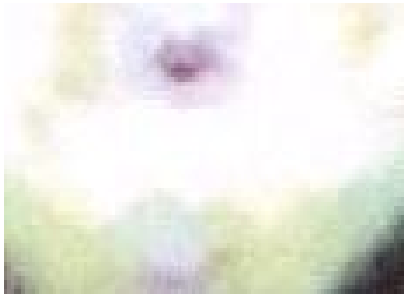

Fig 7a



Fig 7b

*Trichosanthes dioica (*300 mg/kg)



Fig 8a



Fig 8b

Fig (4a-8b): The treatment of the extracts concised the muscles and resulted decrease in the vaginal opening.

weight (Galand *et al.,* 1971; Mäkelä *et al.,* 1999). Therefore, these are the most probable reason for various effects of *B. arundinaceae*, *Punica granatum* and *Trichosanthes dioica* extract as phytoestrogens on reproductive organs of rats.

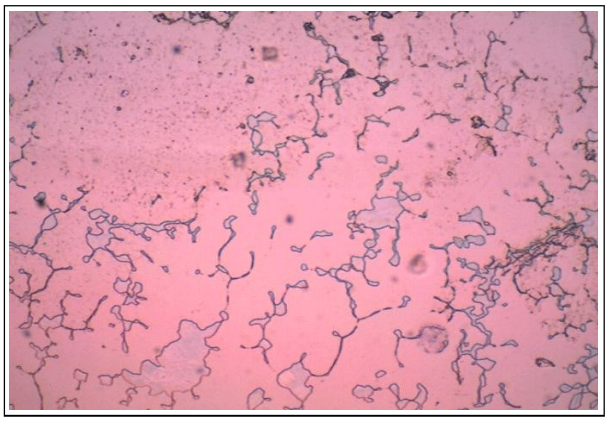

Fig 9: Vaginal smear of ovariectomized rat from control group treated with normal saline for 7 days. A great number of leukocytes were observed.

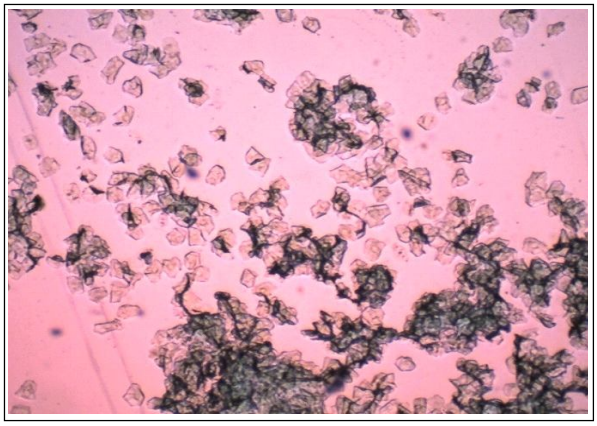

Fig 10: Vaginal smear of Immatured rat treated with Ethinyl Estradiol (0.2 mg/kg) for 7 days. Cornified and nucleated epithelial cells are observed.

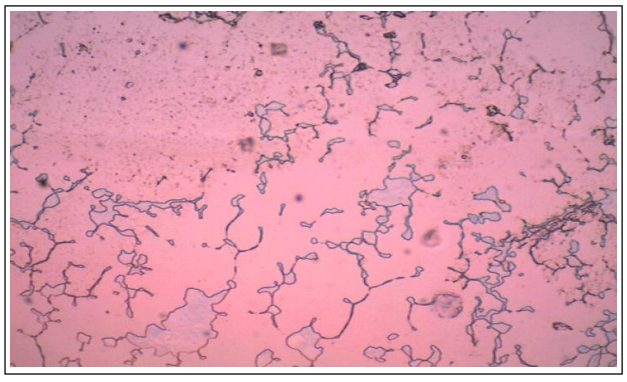

Fig 11: Vaginal smear of immature rat treated with low dose (200 mg/kg) of *Bambusa arundinaceae* cornified, nucleated epithelial cells and leukocytes are observed.

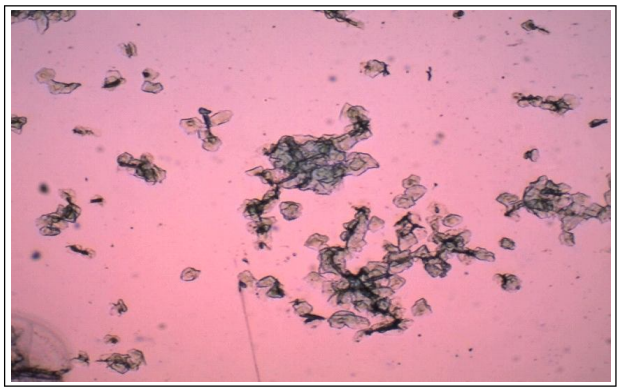

Fig 12: Vaginal smear of immature rat treated with medium dose (300 mg/kg) of *Bambusa arundinaceae* cornified, nucleated epithelial cells and leukocytes are observed.

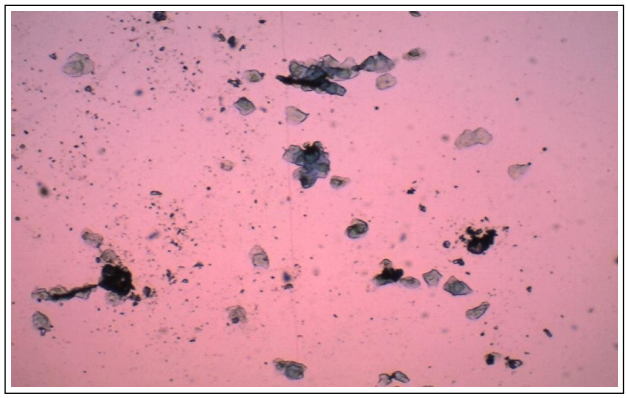

Fig 13: Vaginal smear of ovariectomized rat treated with medium dose (400 mg/kg) of *Bambusa arundinaceae* Cornified, nucleated epithelial cells and leukocytes are observed.

CONCLUSION

In this study, it was aimed to evaluate the estrogenic activity of ethanolic extract of *Bambusa arundinaceae, Punica granatum* and *Trichosanthes dioica,* respectively. The uterotropic assay showed the dose-related increase in uterine wet weight after administration of 200 mg/kg, 300 mg/kg and 400 mg/kg b.w., p.o. both in immature and mature ovariectomized rats. The ethanolic extract of *Bambusa arundinaceae, Punica granatum* and *Trichosanthes dioica,* respectively at higher dose (400 mg/kg b.w., p.o.) showed only cornified epithelial cells. The vaginal opening also showed that EBA (400 mg/kg b.w., p.o.) was having significant estrogenic activity. Thus, it can be concluded that the ethanolic extract of *Bambusa arundinaceae*, *Trichosanthes dioica* and *Punica granatum* play an important role in estrogenic activity in female rats.

ACKNOWLEDGEMENT

First author is highly grateful to Dr. Nisha Dutta and Dr. Avilekh Naryal, Department of Science and Pharmacology, for sharing their technical expertise. We are highly thankful to management of Vivek college of Technical Education, Bijnor, for the financial support to carry out this research work.

Conflict of interest

All authors declare that they have no conflict of interest.

REFERENCES

Bekoe, E.O., Kitcher, C., Debrah, P., Amoateng, P., Donkor, P.O. and Martinson, S. (2020). A study on phyllanthus amarus; Pharmacognostic, mycobactericidal and mutagenic Properties. Pharmacognosy Journal. 12(6s). https://doi.org/10.5530/pj.2020.12.235.

Devi, S., Rashid, R. and Kumar, M. (2017). Phytochemistry and pharmacological properties of *Phyllanthus amarus* Schum: A review. Pharma. Innov. Journal. 6(12): 169-172.

Galand, P., Leroy, F. and Chretien, J. (1971). Effect of oestradiol on cell proliferation and histological changes in the uterus and vagina of mice. Journal of Endocrinology. 49(2): 243-252. https://doi.org/10.1677/joe.0.0490243.

Goodman, L.S. and Parker, K. (2008). Goodman and Gilman's Manual of Pharmacology and Therapeutics. Mcgraw Hill Professional.

Khan, S., Rahman, M.M., Kabir, F., Nahar, K., Mamun, F., Lasker, S. and Haque, M.A. (2020). *Trichosanthes dioica* Roxb. prevents hepatic inflammation and fibrosis in CCl4-induced ovariectomized rats. Clinical Nutrition Experimental. 33: 1-17. https://doi.org/10.1016/j.yclnex.2020.07.001.

Khatua, S., Pandey, A. and Biswas, S.J. (2016). Phytochemical evaluation and antimicrobial properties of *Trichosanthes dioica* root extract. Journal of Pharmacognosy and Phytochemistry. 5(5): 410-413.

Kumar, H.K., Raju, M.B.V., Dinda, S.C. and Sahu, S. (2012). Evaluation of anthelmintic activity of *Bambusa arundinaceae*. Asian Journal of Pharmacy and Technology. 2(2): 62-63.

Kumar, N., Singh, S. and Manvi, R.G. (2012). Trichosanthes dioica Roxb. An overview. Pharmacognosy Reviews. 6(11): 61. https://doi.org/10.4103/0973-7847.95886

Mäkelä, S., Savolainen, H., Aavik, E., Myllärniemi, M., Strauss, L., Taskinen, E. and Häyry, P. (1999). Differentiation between vasculoprotective and uterotrophic effects of ligands with different binding affinities to estrogen receptors α and β. Proceedings of the National Academy of Sciences. 96(12): 7077-7082. https://doi.org/10.1073/pnas. 96.12.7077.

Malaivijitnond, S., Chansri, K., Kijkuokul, P., Urasopon, N. and Cherdshewasart, W. (2006). Using vaginal cytology to assess the estrogenic activity of phytoestrogen-rich herb. Journal of Ethnopharmacology. 107(3): 354-360. https://doi.org/10.1016/j.jep.2006.03.026

Mertens-Talcott, S.U., Jilma-Stohlawetz, P., Rios, J., Hingorani, L. and Derendorf, H. (2006). Absorption, metabolism and antioxidant effects of pomegranate (*Punica granatum* L.) polyphenols after ingestion of a standardized extract in healthy human volunteers. Journal of Agricultural and Food Chemistry. 54(23): 8956-8961. https://doi.org/10.1021/jf061674h.

Nazreen, S., Alam, M.S., Hamid, H., Kaur, G., Alam, M.M., Haider, S. and Shafi, S. (2011). Phytochemical investigation of Bam *Bambusa arundinaceae* Retz. Internationl Journal of Natura l Product Science. 3: 1-7.

Nilsson, S., Makela, S., Treuter, E., Tujague, M., Thomsen, J. Andersson, G. *et al.* (2001). Mechanisms of estrogen action. Physiological Reviews. 81(4): 1535-1565. https://doi.org/10.1152/physrev.2001.81.4.1535.

Parhizkarsup, S., Latiffsup, L.A., Rahmansup, S.A., Dollahsup, M.A. and Parichehr, H. (2011). Assessing estrogenic activity of *Nigella sativa* in ovariectomized rats using vaginal cornification assay. African Journal of Pharmacy and Pharmacology. 5(2): 137-142. https://doi.org/10.5897/ajpp10.276.

Peters, H. and McNatty, K.P. (1980). The Ovary: A Correlation of Structure and Function in Mammals. Univ of California Press.

Procházková, T., Sychrová, E., Javùrková, B., Veèerková, J., Kohoutek, J., Lepšová-Skácelová, O. *et al.* (2017). Phytoestrogens and sterols in waters with cyanobacterial blooms-Analytical methods and estrogenic potencies. Chemosphere. 170: 104-112. https://doi.org/10.1016/j. chemosphere.2016. 12.006.

Sharangouda, S. and Patil, S.B. (2008). Estrogenic activity of petroleum ether extract of seeds of *Citrus medica* on immature albino rats. International Journal of Green Pharmacy. 2(2). https://doi.org/10.4103/0973-8258.41178.

Sreeja, S., Kumar, T.R.S., Lakshmi, B.S. and Sreeja, S. (2012). Pomegranate extract demonstrate a selective estrogen receptor modulator profile in human tumor cell lines and *in vivo* models of estrogen deprivation. The Journal of Nutritional Biochemistry. 23(7): 725-732. https://doi.org/10.1016/j.jnutbio.2011.03.015.

Tripathi, Y.C., Kaushik, P.K. and Bhuyan, T.C. (2013). Sustainable development of quality bamboo resource for employment generation and socio-economic development in NE India. Jorhat: Rain Forest Research Institute. 11-13.

Valadares, M.C., Pereira, E.R.T., Benfica, P.L. and Paula, J.R. (2010). Assessment of mutagenic and antimutagenic effects of *Punica granatum* in mice. Brazilian Journal of Pharmaceutical Sciences. 46: 121-127. https://doi.org/10.1590/S1984-82502010000100014.

Wardhani, D.H., Yuliana, A.E. and Dewi, A.S. (2016). Natrium metabisulfit sebagai Anti-browning agent pada pencoklatan enzimatik rebung ori (*Bambusa arundinaceae*). Journal Aplikasi Teknologi Pangan. 5(4). doi: 10.17728/jatp.202.

Weihua, Z., Saji, S., Mäkinen, S., Cheng, G., Jensen, E.V., Warner, M. and Gustafsson, J.A. (2000). Estrogen receptor (ER) beta, a modulator of ERalpha in the uterus. Proceedings of the National Academy of Sciences of the United States of America. 97(11): 5936-5941. https://doi.org/10.1073/pnas.97.11.5936