

# ajava

Asian Journal of Animal and Veterinary Advances



Academic  
Journals Inc.

[www.academicjournals.com](http://www.academicjournals.com)

## ***Mimosa pudica* Seeds Produce $\beta$ -adrenoceptor Mediated Relaxation of Buffalo Myometrium**

R. Rathore, A. Rahal, R. Mandil and A. Prakash

Department of Pharmacology and Toxicology, UP Pt Deen Dayal Upadhyay, Pashu Chikitsa Vishwavidyalaya, Evam Gau Anusandhan Sansthan (DUVASU), Mathura, 281001, Uttar Pradesh, India

*Corresponding Author: Anu Rahal, Division of Pharmacology and Toxicology, Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh, 243122, India*

### **ABSTRACT**

*Mimosa pudica* is an indigenous antifertility herb. Its use has been advocated against urinary complaints, hypertension, pain relief and menorrhagia but the use has not been scientifically validated till date. Present study was undertaken to delineate the molecular mechanism of tocolytic activity of Hot Methanolic Extract of *Mimosa pudica* Seeds (HMEMPS) on isolated uterine strips of pregnant buffaloes. Myometrial strips were prepared, mounted in an organ bath containing Ringer Lock's solution maintained at 37°C and responses recorded using a physiograph in the presence and absence of different antagonists. *M. pudica* seeds extract produced concentration-dependent inhibitory effect on buffalo myometrium with an EC<sub>50</sub> value of 222.4  $\mu\text{g mL}^{-1}$  perhaps through inhibitory  $\beta$  receptors. Following propranolol ( $10^{-6}$  M) pretreatment, there was an apparent and marked increase in amplitude of spikes of normal spontaneity. An apparent increase in amplitude of spikes upto 1000  $\mu\text{g mL}^{-1}$  followed by a decrease with further increase in concentration of HMEMPS was also recorded. Verapamil ( $10^{-12}$  M), a Ca<sup>2+</sup>-channel blocker, apparently could not significantly alter the HMEMPS-induced inhibition on buffalo myometrium. Calcium channels did not seem to regulate tocolytic effect of *Mimosa pudica* seeds extract. Further studies are indicated to explore the involvement of Ca<sup>2+</sup> and K<sup>+</sup> channels, NO and other signaling mechanisms including second messengers.

**Key words:** *Mimosa pudica* seeds, buffalo myometrium, tocolysis, verapamil, propranolol

### **INTRODUCTION**

Animal health and productivity plays an important role in the national economy and socio-economic development of rural masses. Among various health related disorders, reproductive disorders are considered to be one of major threat and responsible for reduced productivity and profitability from livestock sector. Common animal reproductive disorders are uterine infections, anestrus, cervicitis, repeat breeding, vaginal prolapse, dystocia and retention of placenta. A wide range of herbal remedies are advocated in folklore medicine for female reproductive disorders including hormonal imbalances, addressing endometriosis and other fertility disorders. This has raised a hope among scientists about the use of indigenous products for the scientific management of the reproductive disorders (Das *et al.*, 1999; Rahal *et al.*, 2013, 2014; Rathore *et al.*, 2012a, b; Kumar *et al.*, 2013).

*Mimosa pudica* is a herb used as an antifertility agent (Ganguly *et al.*, 2007) in Indian folklore medicine. Its use has been advocated against urinary complaints, hypertension, pain relief and menorrhagia. In traditional system of medicine, *Mimosa pudica* root (touch me not) has been employed to treat numerous disease conditions like jaundice, dysentery, vaginal and uterine complaints, inflammation of various tissues, fatigue, asthma, leucoderma etc. Literature also cites the use of *M. pudica* as an antispasmodic, muscle relaxant, anti-inflammatory, mild diuretic and promoter of nerve regeneration. The juice of Mimosa roots has been applied to external fistulas and poultice of leaves is use in case of glandular swelling. The whole Mimosa plant is beneficial for vesicle calculi and odema, rheumatism, myalgia and uterine tumors (Sharma *et al.*, 2001). Pharmacological studies have shown *M. pudica* to alter the gonadotropin release as well as prolong the length of estrous in mice after oral administration (Ganguly *et al.*, 2007).

Rat uterus is a traditional experimental model to evaluate the stimulant/relaxant effect of any formulation on myometrium. The patterns of myogenic contractions in buffalo uterus are quite different from that of guinea pig and rats (Coleman *et al.*, 2000; Asokan *et al.*, 2002) but are comparable to human myometrium (Bradley *et al.*, 1998). Moreover, the isolated buffalo uterine strips show a high degree of sensitivity to different spasmogens, oxytocic and tocolytic agents and it exhibits very consistent responses (Narayan *et al.*, 1993). Therefore, isolated uterine strips from pregnant buffaloes were used in the present study in order to evaluate tocolytic activity of hot alcoholic extracts of seed of *M. pudica*.

## MATERIALS AND METHODS

**Collection and extraction of raw plant material:** Authenticated seeds of *Mimosa pudica* were procured from a commercial herb supplier and coarsely ground with the help of an electric grinder. The coarse seed powder was placed in a porous cellulose thimble and extracted with methanol (1:5) in soxhlet extractor at a temperature  $40\pm 5^{\circ}\text{C}$  continuously for 20-22 cycles. The extract (HMEMPS) was dried at moderate temperature ( $37^{\circ}\text{C}$ ) to obtain the residual material.

**Collection of uterine tissues:** Complete uterine along with the ovaries of adult nondescript buffaloes were collected from local abattoir, Mathura. Uterine tissue were collected from mid cornual region in case of early stage of pregnancy while in case of mid or late stage of pregnancy, complete corneal part of uterine tissue was collected. The gestation stage was determined by measuring Curved-crown Versus Rump (CVR) length of foetus by applying the equation (Soliman, 1970).

$$\begin{aligned} \text{When CVR length was below 20 cm, } Y &= 28.66+4.498x \\ \text{When CVR length was 20 cm or above, } Y &= 73.544+2.256x \end{aligned}$$

where, Y is days of gestation and X is curve crown rump length in centimeters.

Uterine tissues were transported to laboratory in a thermos flask containing chilled ( $4\pm 0.5^{\circ}\text{C}$ ) Ringier-Locke solution (RLS, pH 7.4) (Singh, 2006).

**Solutions of chemicals/antagonist:** Solutions of the chemicals, propranolol ( $10^{-8}$  M; Sigma Aldrich) and verapamil ( $10^{-9}$  M; Sigma Aldrich) used for evaluation of uterotonic activity in the present study, were prepared as stock solutions in triple distilled water and stored at  $4^{\circ}\text{C}$ . Further dilutions of the required concentration were made in freshly prepared RLS on the day of use.

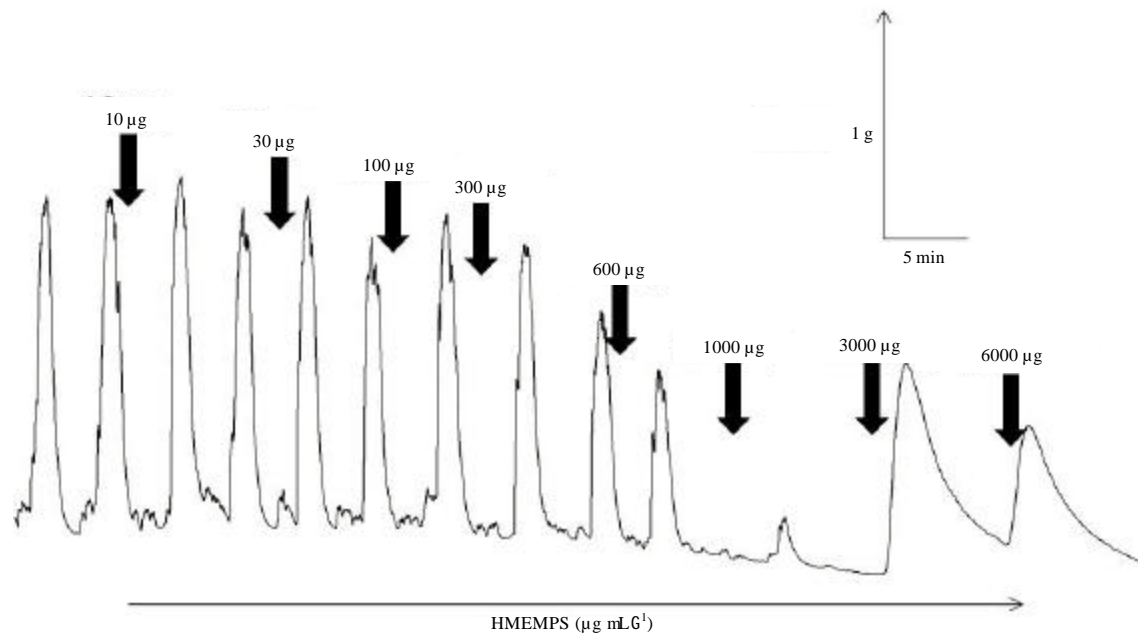


Fig. 1: Representative physiographic recording of the effect of cumulative concentrations of HMEMPS (10-6000 µg) on myometrial strip of pregnant buffalo

### Uterotonic/oxytocic studies

**Preparation and mounting of uterine tissue:** Uterine strips were dissected out from the midcornual region and a perimetrial strip of about 3.0×0.5 cm was prepared by carefully removing the endometrial and myometrial tissues. Both the ends of strip were threaded and mounted in a thermostatically controlled ( $37.0\pm 0.5^{\circ}\text{C}$ ) organ bath of 10 mL capacity containing continuously aerated RLS.

**Calibration of physiograph and recording of responses:** The change in tension of tissue was measured using a high sensitivity isometric force transducer and recorded in a PC using Chart V5.4.1 software programme (Powerlab, AD Instruments, Australia). Sensitivity of the instrument was set as required and the sampling rate was adjusted to 5 samples  $\text{sec}^{-1}$ . The bridge amplifier was calibrated at two points-point 1:0 mV = 0 g and Point 2:1 mV = 1 g and signal range was kept at 10 mV.

After calibration of the equipment, myometrial strip was set under an initial constant tension of 2 g and allowed to equilibrate for 90-120 min till the tissue developed regular phasic contractions (Fig. 1) before recording isometric muscle tension or response to plant extracts or drugs. During the equilibration period, the bath fluid was changed every 10-15 min.

**Qualitative studies on the effect of *M. pudica* root extract on buffalo uterus:** Working solutions of *M. pudica* extracts (10, 30, 100, 300, 600, 1000, 3000, 6000  $\mu\text{g mL}^{-1}$ ) were prepared a fresh on the day of experiment. Different concentrations of *M. pudica* seed extracts were added in organ bath to record the responses of uterine tissues and also to record the minimum threshold concentration required for producing uterotonic effect.

**Contact period of different plants extracts:** Different concentrations of extracts of *M. pudica* seeds (10, 30, 100, 300, 600, 1000, 3000, 6000  $\mu\text{g mL}^{-1}$ ) were added to the tissue bath in an increasing manner. Each concentration was allowed an initial contact period of 5-10 min and the consecutive concentration was added only after the maximum effect of the previous concentration was evident. Once the maximal possible response was achieved, the tissue was washed. A minimum washout period of 30 min was allowed between two successive recordings. Then, the uterine strips were incubated with different antagonist (Propranolol or Verapamil) in the organ bath for 10 min and again the different concentrations of the extract were added. After recording the tissue response(s), bathing fluid in organ bath was changed every 10 min till the base line was achieved.

**Statistical analysis:** Statistically analysis of data was performed using SPSS 10 software applying Duncan's test. Mean of various parameters for contractions between different treatments groups were compared using ANOVA with Duncan Multiple Range Test. A value of  $p < 0.05$  was considered as statically significant.

## RESULTS

HMEMPS in the concentration range of 10-1000  $\mu\text{g mL}^{-1}$  produced concentration-dependent inhibitory effect on amplitude and frequency of spontaneous rhythmic contractions (Fig. 1 and 2). It relaxed the uterine strip completely with an  $\text{EC}_{50}$  of 222.4  $\mu\text{g mL}^{-1}$ . However, at higher concentrations (3000 and 6000  $\mu\text{g mL}^{-1}$ ), the base line was somewhat elevated.

Following propranolol ( $10^{-6}$  M) pretreatment, there was an apparent and marked increase in amplitude of spikes of normal spontaneity. An apparent increase in amplitude of spikes up to 1000  $\mu\text{g mL}^{-1}$  followed by decrease with further increase in concentration of HMEMPS was also recorded (Fig. 3a). The tension following exposure to HMEMPS was less in propranolol-treated tissues compared to that in its absence. There was parallel shift to left of the dose-response curve, thereby suggesting the inhibitory effect of propranolol on HMEMPS-induced myometrial relaxation. Verapamil apparently failed to markedly alter the HMEMPS induced inhibitory effect on buffalo myometrium (Fig. 3b).

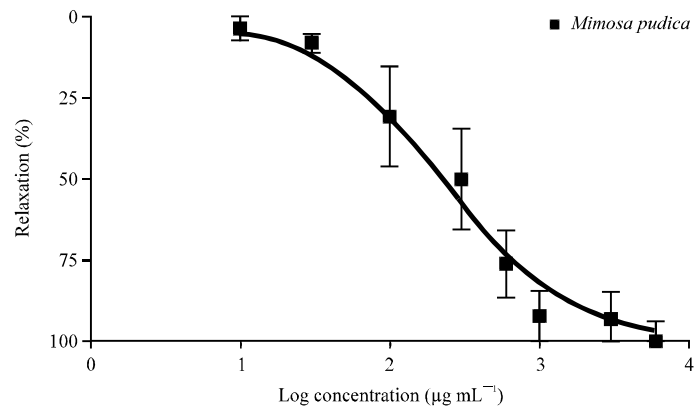


Fig. 2: Cumulative concentrations response curve of HMEMPS on isolated myometrial strips of pregnant buffaloes (n = 5)

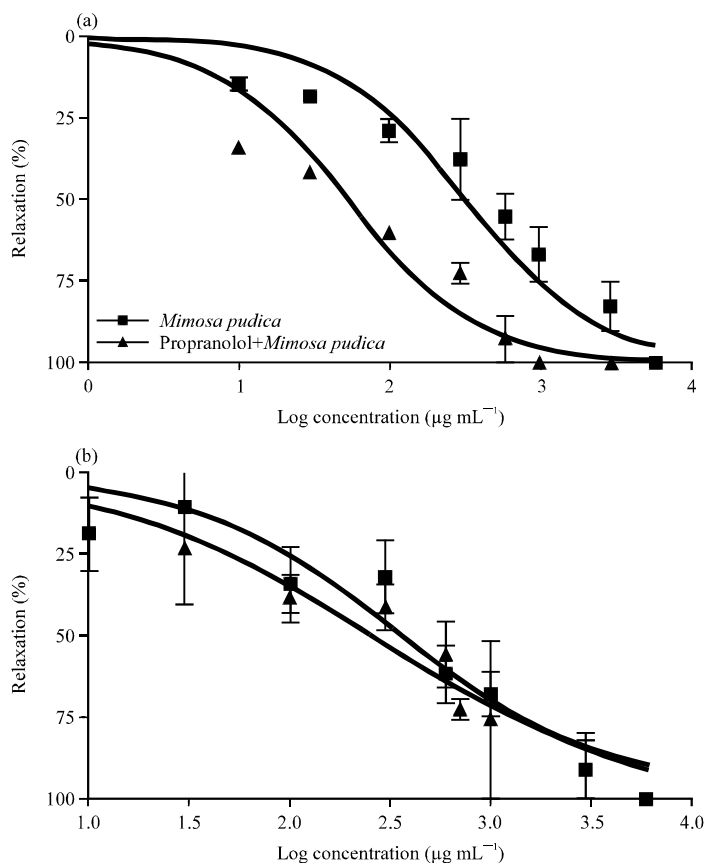


Fig. 3(a-b): Cumulative concentration response curves of HMEMPS alone and in the presence of an antagonist (a) Propranolol ( $10^{-6}$  M) and (b) Verapamil ( $10^{-12}$  M) on pregnant buffaloes isolated myometrial strips (n = 4)

## DISCUSSION

Role of ethnoveterinary medicine in livestock development is beyond dispute (Martin *et al.*, 2001). Because of their holistic nature, traditional remedies more often offer better efficacy combined with safety and economy than single cosmopolitan/conventional drugs (Varier, 1996). *Mimosa pudica* has been used for stomachache, womb cleaning, to stop menstruation and for gonorrhoea (Lans, 2006). In the present study, an attempt had been made to work out the possible mechanism(s) of tocolytic effect of *Mimosa pudica* seeds extract using isolated uterine strips of pregnant buffaloes.

HMEMPS produced consistent, rapid and sharp concentration-dependent inhibitory and excitatory effect in some of the tissues i.e., biphasic effect. The uterine contractibility is determined by increase in intracellular free calcium ion concentration in the myometrial cells and calcium channel blocker have been reported to inhibit uterine contraction by decreasing  $\text{Ca}^{2+}$ -influx (Longo *et al.*, 2003). The bovine myometrial tone and spontaneity has previously been reported to be extra-cellular  $\text{Ca}^{2+}$ -dependent (Singh, 2006).

Pretreatment of uterine tissue with propranolol ( $10^{-6}$  M), the cumulative concentration response curve of HMEMPS shifted leftwards indicating the blockade of relaxation produced by the extract. These observations suggested that HMEMPS-induced myometrial effects are possibly mediated

through  $\beta$ -adrenergic receptors. To explore the possibility of involvement of other inhibitory mechanisms including ion-channels, NO-pathways, second messengers etc., further studies are required to be conducted.

Verapamil ( $10^{-12}$  M), a  $\text{Ca}^{+2}$ -channel blocker, failed to produce marked decrease in tissue tension or the dose response curve was not shifted either to left or to the right. These observations are in contrast to earlier suggestions from this laboratory (Singh, 2006) that the normal spontaneity of buffalo myometrium is  $\text{Ca}^{+2}$  dependent. Verapamil has also been reported to have inhibitory effect on many other smooth muscles including uterus (Forman *et al.*, 1982; Janis and Triggle, 1986) preferentially by inhibiting  $\text{Ca}^{+2}$ -influx through potential-dependent  $\text{Ca}^{+2}$ -channel (Fleckenstein, 1977; Bolton, 1979; Janis and Triggle, 1986). Several studies show the presence of NOS in the uterus from animal species and the human with differences with respect to the predominant form and  $\text{Ca}^{+2}$  dependence (Sladek *et al.*, 1993; Ramsay *et al.*, 1996; Sladek and Roberts, 1997). In pregnant rat myometrium, the activity of NOS has been reported to be quite insensitive to  $\text{Ca}^{+2}$  (Sladek and Roberts, 1997).

*M. pudica* total plant extract is reported to produce depressant effect on isolated rabbit duodenum and the percent decrease in either amplitude or frequency of duodenal contraction was found to be only marginally different from that found after a similar dose of atropine sulphone. The observations apparently suggest that HMEMPS-induced myometrial relaxation was regulated through  $\text{Ca}^{+2}$ -channels-independent mechanism; however, further studies are indicated.

From the results of the present study, it may be inferred that *M. pudica* seeds extracts produced concentration-dependent inhibitory effect on buffalo myometrium and it seemed to be mediated through inhibitory  $\beta$  receptors. Calcium channels did not seem to regulate tocolytic effect of *Mimosa pudica* seeds extracts. Further studies need to be done on to explore the involvement of  $\text{Ca}^{+2}$  and  $\text{K}^{+}$  channels, NO and other signaling mechanisms including involvement of second messengers.

#### ACKNOWLEDGMENT

The authors are grateful to Hon'ble Vice Chancellor of the University (DUVASU) for providing all necessary facilities.

#### REFERENCES

- Asokan, K.T., S.N. Sarkar, S.K. Mishra and V. Raviprakash, 2002. Effects of mibefradil on uterine contractility. *Eur. J. Pharmacol.*, 455: 65-71.
- Bolton, T.B., 1979. Mechanisms of action of transmitters and other substances on smooth muscle. *Physiol. Rev.*, 59: 606-718.
- Bradley, K.K., I.L.O. Buxton, J.E. Barber, T. McGraw and M.E. Bradley, 1998. Nitric oxide relaxes human myometrium by a cGMP-independent mechanism. *Am. L. Physiol.*, 44: C1668-C1673.
- Coleman, H.A., J.D.E. Hart, M.A.Tonta and H.C. Parkinton, 2000. Changes in the mechanisms involved in uterine contractions during pregnancy in guinea-pigs. *J. Physiol.*, 523: 785-798.
- Das, S., S. Pal, A. Mujib and S. Dey, 1999. *Biotechnology of Medicinal Plants-Recent Advances and Potential*. 1st Edn., UK992 Publications, Hyderabad, India, pp: 126-139.
- Fleckenstein, A., 1977. Specific pharmacology of calcium in myocardium, cardiac pacemakers and vascular smooth muscle. *Annu. Rev. Pharmacol. Toxicol.*, 17: 149-166.
- Forman, A., P. Gandrup, K.E. Andersson and U. Ulmsten, 1982. Effects of nifedipine on spontaneous and methylergometrine-induced activity post partum. *Am. J. Obstet. Gynecol.*, 144: 442-448.

- Ganguly, M., N. Devi, R. Mahanta and M.K. Borthakur, 2007. Effect of *Mimosa pudica* root extract on vaginal estrous and serum hormones for screening of antifertility activity in albino mice. *Contraception*, 76: 482-485.
- Janis, R.A. and D.J. Triggler, 1986. Effect of Calcium Channel Antagonists on the Myometrium. In: *The Physiology and Biochemistry of the Uterus in Pregnancy and Labor*, Huszar. G., (Ed.), CRC Press, Boca-Roton, CA., USA., pp: 201-223.
- Kumar, A., A. Rahal, S. Chakraborty, R. Tiwari, S.K. Latheef and K. Dhama, 2013. *Ocimum sanctum* (Tulsi): A miracle herb and boon to medical science-A review. *Int. J. Agron. Plant Prod.*, 4: 1580-1589.
- Lans, C.A., 2006. Ethnomedicines used in trinidad and tabago for urinary for urinary problems and diabetes mellitus. *J. Ethnobiol. Ethnomed.*, Vol. 2, 10.1186/1746-4269-2-45
- Longo, M., V. Jain, Y.P. Vedernikov, G.R. Saade and L. Goodrum *et al.*, 2003. Effect of nitric oxide and carbon monoxide on uterine contractility during human and rat pregnancy. *Am. J. Obstet. Gynecol.*, 181: 981-988.
- Martin, M., M.C. McCorkle and E. Mathias, 2001. *Ethnoveterinary Medicine*. ITDG Publishing, London, UK., ISBN: 1853395226, Pages: 611.
- Narayan, P., S.K. Garg, M.A.A. Shah and M. Sabir, 1993. Nature of adrenoceptors in buffalo uterus. *Indian J. Pharmacol.*, 25: 40-40.
- Rahal, A., A. Kumar, S. Chakraborty, R. Tiwari, S.K. Latheef and K. Dhama, 2013. *Cimicifuga*: A revisiting indigenous herb with multi-utility benefits for safeguarding human health: A review. *Int. J. Agron. Plant Prod.*, 4: 1590-1601.
- Rahal, A., Mahima, A.K. Verma, A. Kumar and R. Tiwari *et al.*, 2014. Phytonutrients and nutraceuticals in vegetables and their multi-dimensional medicinal and health benefits for humans and their companion animals: A review. *J. Biol. Sci.*, 14: 1-19.
- Ramsay, B., S.R. Sooranna and M.R. Johnson, 1996. Nitric oxide synthase activities in human myometrium and villous trophoblast throughout pregnancy. *Obstet. Gynecol.*, 87: 249-253.
- Rathore, R., A. Rahal and R. Mandil, 2012a. *Cimicifuga racemosa* potentiates antimuscarinic, anti adrenergic and antihistaminic mediated tocolysis of buffalo myometrium. *Asian J. Anim. Vet. Adv.*, 6: 300-308.
- Rathore, R., A. Rahal, R. Mandil, A. Prakash and S.K. Garg, 2012b. Comparative anti-inflammatory activity of *Cimicifuga racemosa* and *Mimosa pudica*. *Aust. Vet. Practitioner*, 42: 274-278.
- Sharma, P.C., M.B. Yelne and T.J. Dennis, 2001. *Database on Medicinal Plants Used in Ayurveda*. Central Council for Research in Ayurveda and Siddha. 1st Edn., Department of Indian System of Medicine, Govt. of India, New Delhi, India.
- Singh, D., 2006. Studies on phytochemistry and pharmacodynamics of uterotonic effect of *Moringa oleifera* flowers extract on buffalo uterus. M.Sc. Thesis, DUVASU, Mathura, India.
- Sladek, S.M., A.C. Regenstein, D. Lykins and J.M. Roberts, 1993. Nitric oxide synthase activity in pregnant rabbit uterus decreases on the last day of pregnancy. *Am. J. Obstet. Gynecol.*, 169: 1285-1291.
- Sladek, S.M. and J.M. Roberts, 1997. Nitric oxide synthase activity in the gravid rat uterus decreases a day before the onset of parturition. *Am. J. Obstet. Gynecol.*, 175: 1661-1667.
- Soliman, M.K., 1970. Studies on the physiological chemistry of the allantoic, amniotic fluids of buffaloes at the various periods of pregnancy. *Indian Vet. J.*, 52: 106-112.
- Varier, N.V.K., 1996. Repossession of our ancient wisdom. Proceedings of the Symposium on Traditional Veterinary Medicine, May 15, 1996, Malabar Regional Co-operative Milk Producers Union, Kerala, India.