www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; SP-11(7): 3985-3990

www.thepharmajournal.com
Received: 22-04-2022
Accepted: 25-05-2022

Anju Kujur
Ph.D. Scholar, Division of Animal Reproduction, ICAR-Indian Veterinary Research Institute, Bareilly, Uttar Pradesh, India

Nancy Jasrotia
Ph.D. Scholar, Division of Animal Reproduction, ICAR-Indian Veterinary Research Institute, Bareilly, Uttar Pradesh, India

MP Sinha
Ex-University Professor, Department of Gynaecology and Obstetrics, R.V.C, Kanke, Jharkhand, India

Nirmala Minj
Assistant Professor Cum Junior Scientist, Department of livestock Production and Management, R.V.C, Kanke, Jharkhand, India

Manmohan Kumar
Department of Animal Husbandry, TVO, Rupauli, Patna, Bihar, India

Sunita Kumari Murmu
Touring Veterinary Officer, Haldipokhar, East Singhbhum, Jharkhand, India

Corresponding Author
Anju Kujur
Ph.D. Scholar, Division of Animal Reproduction, ICAR-Indian Veterinary Research Institute, Bareilly, Uttar Pradesh, India

Estrus induction response and fertility performance in anestrus cows treated with *Aegle marmelos* (Bael) and *Murraya koenigii* (curry)

Anju Kujur, Nancy Jasrotia, MP Sinha, Nirmala Minj, Manmohan Kumar and Sunita Kumari Murmu

Abstract
The current study uses *Aegal marmelos* (Bael) and *Murraya koenigii* (curry) to induce estrus in anestrus cattle and evaluate fertility performance. Selected cows are divided into 4 groups of 6 animals each, all of whom have smooth, dormant ovaries and no pathological conditions or a history of anestrus. Different doses of prepared curry powder at a dose rate of 400 mg/Kg, bael at a dose rate of 500 mg/Kg, and their combination at 200 mg/Kg of curry and 250 mg/kg of bael supplied orally for nine days. The combination of both Murraya and Aegal showed higher results in estrus induction, estrus grading, and also enhanced biochemical profile which is crucial for the fertility of cattle. It was discovered that combination treatment shows 83.33% of estrus induction followed by the 50% and 66.67% by other groups respectively.

Keywords: Aegal marmelos, *Murraya koenigii*, anestrus, estrus induction, fertility

Introduction
In India, Reproductive issues are anticipated to become a bottleneck for improving animal performance, which would have a substantial impact on the economy of the country. Anestrus is one of the most alarming conditions that has a disastrous effect on sheep and cattle, diminishing their reproductive potential and the profitability of small and marginal farmers, encompasses the dairy sectors. In India, recurrence reported to range among 2.13 to 67.11% and 9.09 to 82.50% in native cattle (Selvaraju *et al*., 2005; Thakor and Patel, 2013) [25, 37] and buffalo (Modi *et al.*, 2011; Kumar *et al.*, 2013; Thakor and Patel, 2013) [22, 15, 37]. Adult cattle and buffalo have a higher anestrus prevalence than heifers do (Bharkad and Markandeya, 2003) [4].

Anestrus is a functional abnormality of the reproductive cycle marked by the absence of an overt indication of estrus, either not expressed or not detected, typically, it is seen in post-pubertal heifers, pregnancy, lactation, and adult animals in the early postpartum period. It is a herd issue brought on by poor nutrition, seasonal stress and also contributing to the issue by poor management techniques, uterine pathology, inadequate nutrition, and environmental stress are the main culprits.

Different therapeutic agents, both hormonal and non-hormonal, are being used to deal with this issue, but most synthetic medications were rejected by dairy farmers owing to its high expenditure and variable results. Today's medical plants have promise for the future in treating a variety of cattle reproductive diseases since they are more affordable, effective, and safe than more expensive hormones (Khade *et. al.*, 2011 & Mehrotra, 2011) [14, 21]. Since ancient times, India has been home to numerous medicinal plants. Numerous phytochemicals found in plants, such as alkaloids, glycosides, terpenes, and tannins (secondary metabolites), have medicinal properties. While many plants are excellent sources of vitamins and minerals, others include estrogenic properties that can help anestrus animals regain their cyclicity.

The Rutaceae family, which is well-known for its medicinal plants, includes *Murraya koenigii* and *Aegal marmelos.* These two plants were each employed separately to increase fertility in laboratory animals (Jhondhale *et al*., 2009; Satheshkumar and Punniamurthy, 2009) [13, 31]. Curry leaves, also known as *Murraya koenigii*, are a genus of plants that are common in India and have a number of alkaloids and free amino acids, mentioned in table 1. *Murraya koenigii* leaves increase fertility and induce estrus leading to an increase in serum calcium and phosphorus levels (Sathes Kumar and punniamurthy, 2005; Jhondale *et al*., 2009) [30, 13].

Either *Murraya koenigii* alone and in combination with a mineral mixture and a minimal dosage of GnRH injection were incredibly useful at promoting fertility in anestrus buffaloes (Umashanker *et al.*, 2006) [39] and boost ovarian function in rats and promote treatment responses in acyclic goats and cattle (Mehrotra, 2002) [20]. *Aegal marmelos* medicinal leaves contains alkaloids and other consituents which greatly advances onset of puberty, increasing the weight of the uterus and the ovary in rats, anestrus goats, and buffaloes (Jhonadale, 2007; Kumar, 2008) [18]. Due to their specialised functions in reproductive tissue and their impact on cellular level metabolism, growth, and maintenance, minerals and vitamins play a significant role in influencing reproduction.

Mineral deficiencies or excess, have been linked to abnormally low fertility and anestrus conditions. The release of gonadotrophic and gonadal hormones depends on macrominerals like calcium and phosphorus and other minerals. GnRH Calcium is required for the stimulation of LH release from pituitary cells, and LH is not released in the absence of a specific calcium concentration. Because phosphorus is an integral part of nucleic acids, nucleotides, and certain proteins, phosphorus deprivation may have an adverse effect on reproduction by impairing phosphorus-dependent metabolic reactions (Hurley and Doane, 1989) [10]. As a precursor to several steroidal hormones, cholesterol can be used to determine the hormone necessary for a proper estrus is enough present in the blood. *Aegle marmelos* and *Murraya koenigii* were noted as having therapeutic properties in several Indian reports like Antioxidants, minerals (Co, Cu, Fe, I, Mn, Se, Zn), and vitamins (vit A, B, C, and E) as per Bhandari, 2012; Janarthanan *et al*., 2012 [3, 11]. Considering the above facts in view the proposed study has been planned to assess the efficacy of herbal plant *Murraya koenigii* and *Aegle marmelos* either alone or in combination on induction of estrus in anestrus cow under farm and field condition.

Table 1: Constituents of leaves of *Murraya koenigii* and *Aegal marmelos*.

Plants	Constituents	Pharmacological uses	References
Murraya koenigii	Protein, carbohydrate, minerals phosphorus, potassium, calcium, magnesium fibre, Vitamin C, Vitamin A, carotene, nicotinic acid, calcium, oxalic acid carbazole alkaloids, koenigin, crystalline glycosides, girinimbin, iso-mahanimbin, koenine, koenidine, koenimbine, triterpenoid alkaloids cyclomahanimbine and tetrahydromahanimbine. Free amino acids such as asparagines, glycine, serine, aspartic acid and glutamic acid	Antibacterial, antifungal, antioxidants, antiinflammatory, antidiabetic, antianxiety and antidepressant, hepatoprotective, cytotoxicity, anticancer (breast, oral), promoting fertility, endometritis, neuroprotective and immunomodulator	Nalli *et al*., 2016; Arun *et al*., 2017; Sharma *et al*., 2017; Tripathi *et al*., 2018; Rautela *et al*., 2018 [24, 1, 34, 38, 29]
Aegal marmelos	Minerals (Co, Cu, Fe, I, Mn, Se, Zn), Skimmianine, Aeglin, Rutin, β-sitosterol, γ-sitosterole, Flavone, Cineol, Citral, Lupeol, O-isopentenyl, Glycoside, Hallordiol, Mameline, Citronellal, Cuuminaldehyd ephenylethylecinnamamides, Euginol, Marmesinin, Aegelin, Glycoside	Antiinflammatory, promoting fertility, endometritis, laxative, asthma, opthalmia and eye affection, expectorant, cold and respiratory infection, backache, abdominal disorder, vomiting, cut and wounds, dropsy, beriberi, weakness of heart, cholera, diarrhoea, cardiac tonic, control blood sugar, nervous disorders, hair tonic, acute bronchitis, veterinary medicine for wound healing, antiworms, stimulation of respiration.	Patel *et al*., 2012

Materials and techniques

The current study was done on cross-breed cows from neighbourhood farms and gaushala in and around Ranchi, Jharkhand. Crossbred cows without any pathological anomalies in their female reproductive systems and with a history of anestrus and postpartum anestrus were chosen. They also had smooth, quiescent ovaries.

Experimental design

The experiments was conducted in two parts.

Part 1: Selection of herbs

The young, green leaves of *Murraya* and *Aegal* were gathered, dried in the shade, powdered in a mixer grinder, and kept at room temperature.

Part2: Each of the four groups, each of which had six animals, was randomly assigned one of the 24 anestrus crossbred cows, and the treatment listed below (table 2) was then carried out on each of the animals in that group.

Table 2: Treatment protocol

Group 1	**6**	**No treatment**	**.....**	**.....**
Group 2	6	*Murraya koenigii*	400mg/kg	9 days or till onset of estrus which ever earlier
Group 3	6	*Aegle marmelos*	500mg/kg	9 days or till onset of estrus which ever earlier
Group 4	6	50% mixture of *Aegle* and 50%of *Murraya*	250mg/kg of the *Aegle* and 200 mg/kg of *Murraya*	9 days or till onset of estrus which ever earlier

Dose extrapolation

Dose of *Murraya koenigii* and *Aegal marmelos* leaves powder for cattle was extrapolated from the rats using the dose equivalent system via km factor described by VAN MIERT, 1986 [40] using km factor table (table 3)

Table 3: Dose of extract for the species

Species	Body wt.(kg)	Surface area (m^2)	km factor	dose equivalent (per kg)
Man adult	60	1.6	37.5	1
Man child	20	0.8	25	1.5
Mouse	0.02	0.0066	3	12.5
Rat	0.15	0.025	6	6.3
Cat	3	0.24	12.5	3
Dog	16	0.65	24.5	1.5
Sheep/goat	50	1.1	45.5	0.8
Pig	75	1.5	50	0.75
Cow	500	5.0	100	0.4
1Pony	280	4.4	63.5	0.6
Horse	650	5.9	110	0.3

Dose of extract for the species was calculated as below

$$\text{Dose of extract to be extrapolated for species} = \frac{\text{Km factor of known sp.}}{\text{Km factor of to be extrapolated sp.}} \text{ X dose of known sp.}$$

The dose of the extract was converted to powder form based on the percent yield using the formula

$$\text{Dose of powder} = \text{dose of extract x } \frac{100}{\text{percent yield}}$$

The percent yield of *Aegle marmelos* and *Murraya koenigii* are 11.27 and 14.44 (Mehrotra *et al*., 2009; Jondhale *et al*., 2009) [23, 13]. Leaf powder dose on per Kg body weight basis was worked out for *Aegle* & *Murraya* separately.

ANOVA was used to examine the data collected for both between and within groups, and the t-test was used to compare the pre-treatment and post-treatment of various treated groups.

Result and Discussion

The responses to estrus induction, the number of days expected for animals to attain estrus, and estrus characteristics in anestrus cows under various treatment regimens mentioned in Table 4. With 83.33% of the animals in group IV entering into estrus, that group outperformed, groups II and III, which each had 50% and 66.67%, respectively. The 83.33% estrus induction response in group IV of anestrus cows is consistent with earlier findings that stated 80% estrus induction in delayed pubertal heifers (Kumawat *et al*., 2014) [19], a marginally higher 92.90% in delayed pubertal buffalo heifers by Das *et al*. (2012b) [7], and 86.70% in delayed pubertal heifers by Das and Mehrotra (2016). Estrus induction of 50% in group II was found, which is consistent with the results from Shankar *et al.* (2006) who reported 50% in anestrus buffaloes, Kumar & Punniamurthy (2009) [16] who reported 60% in anestrus heifers, and Dutt *et al.* (2010) [8] who reported 50% in anestrus goats. The group III's 66.67% estrus induction response is commensurate with Kumar's 2008 [18] study of 57.14% in acyclic goat.

Further the days required for animals coming to estrus in different treatment groups were found to be non-significantly affected by treatment with Group IV (curry + bael) requiring minimum number of days for estrus induction with a mean value of 4.00±1.29 days which was concurrence with interval of 5.07± 3.74 days by Das *et al.*(2102b) [7] and Das *et al*., (2012c) in delayed pubertal buffalo heifers. Group II(curry) showing estrus induction of 4.83 ±2.16 mean number of days but Umashankar *et al.*(2006) who reported a period of 15.25 days for induction of estrus in anestrus buffaloes and longer estrus interval of 18.66±2.18 days in anestrus goats (Dutt *et al.*, 2010) [8] and 28.0±2.56 days (Satheskumar and Punniamurthy, 2009) [31] was however reported in anestrus heifer and Group III (bael) showing most delayed estrus induction out of three Groups with the mean value of 6.67 ± 2.18 days required for estrus induction which is in harmony with the Kumar (2008) [18] who reported 10.00 ± 2.00 days for onset of estrus in anestrus goats (Table-4).

Further the estrus grading based on estrus signs mentioned in (table 5) was found to be highest in Group IV (2.50 ± 0.80).

Table 4: Estrus induction response & estrus characteristics in anestrus cows under different treatment protocols

Treatment	No. of animals treated	No. of animals exhibited estrus	Percentage of animals exhibited Estrus (%)	Last injection to estrus induction interval (days)	Estrus Grading
Group I (control)	6	Nil.	Nil	-	-
Group II (Curry)	6	3	50	4.83 ±2.16	1.67 ± 0.75
Group III (Bael)	6	4	66.67	6.67 ± 2.18	2.33 ± 0.75
Group IV (curry + bael)	6	5	83.33	4.00 ± 1.29	2.50 ± 0.80
F value				0.50	0.32

Table 5: Grading of estrus intensity (1-4 score) was done on the basis of below behavioural signs:

Score	Estrus characteristics
4	Clear, ropy, vaginal discharge, frequent vocalization, motor movement and excitation.
3	All the characteristics of estrus with less intensity
2	Clear vaginal discharge with no other behavioral symptoms.
1	None of the external characteristics of estrus noticed except cervical relaxation and presence of follicles on per rectal examination.

The mean of serum cholesterol level differed significantly from pre to post treatment in all groups except in group I showed in table 6. The post treatment mean value of cholesterol level were obtained to be 112.41±9.13, 170.88±12.36, 183.38±11.42, 192.80±9.06 in group I, II, III and IV. The serum cholesterol level of group II was in partial

harmony with the Asokan *et al.* (2010) who recorded the level as 193.71±20.5 mg/dl. However the cholesterol level obtained in present study are within normal range as reported by various author in cyclic cows and animals (Ramakrishna, 1997; Singh *et al.*,2004) [28, 36].

The plant *Murraya koenigii* has the potential to augment follicular development and steroidogensis thereby effective in inducing estrus in anestrus cows. Cholesterol is precursor of different steroidal hormone and its level can indicate circulating adequacy of the hormone responsible for normal estrus. In an experiment higher level of cholesterol in respective groups represented the better formation of steroids regimes for bringing the animals into fertile estrus.

Table 6: Serum Cholesterol level (mg/dl) in anestrus cows of different treatment Groups

Treatment Group (n = 6)	Pre-treatment (mg/dl)	Post-treatment (mg/dl)	t-value
Group I (control)	112.02± 9.50	112.41 ± 9.13^{a}	0.031^{NS}
Group II (Curry)	118.69 ± 6.97	170.88 ± 12.36^{b}	3.68*
Group III (Bael)	119.95 ± 9.50	183.38 ± 11.42^{b}	4.27*
Group IV(curry+bael)	120.53 ± 12.30	192.80 ± 9.06^{b}	4.73*
F value	0.17^{NS}	11.62**	

*-P< 0.05; **-P < 0.01; NS = Non significant;

Effect of different treatment on Serum Calcium level was found to be significant (P<0.05) post treatment (table 7). The post treatment Calcium level in group II (curry) was found to be 10.35±0.60 mg/dl, which are in partial agreement with Das *et al.* (2011) [5] as they reported serum Calcium level as 11.78±2.41mg/dl in anestrus heifers supplemented with 100g of *Murraya koenigii* (curry) fresh leaves and in group III post treatment serum Calcium level was found to be 9.43±0.64 mg/dl. The mean Calcium level after treatment was highest in the group IV with 10.67±0.67 mg/dl. The present study is in harmony with Kumawat *et al.* (2014) [19] who recorded 11.09 to 11.47 mg/dl serum calcium concentration in delayed pubertal heifers.

Table 7: Serum Calcium level (mg/dl) in anestrus cows of different treatment Groups

Treatment Group (n = 6)	Pre-treatment(mg/dl)	Post treatment (mg/dl)	t-value
Group I (control)	8.44 ± 1.18	7.64 ± 0.89^{a}	0.53^{NS}
Group II (Curry)	7.72± 0.80	10.35± 0.60^{b}	2.61*
Group III (Bael)	6.42 ± 0.71	9.43 ± 0.64^{ab}	3.13*
GroupIV (curry+beal)	6.69 ±0.52	10.67 ±0.67^{b}	5.04**
F value	1.24^{NS}	4.18**	

Effect of different treatment on average serum Phosphorus level was found to be highly significant post treatment (P<0.01) which mentioned in table 8. The mean value of serum phosphorus was found to be 2.67±0.21, 4.57±0.54 and 4.59±0.71 in group I, II and III in post treatment but it was found highest in group IV with 5.62±0.32mg/dl. It was in partial agreement with Das *et al.* (2011) [5] and Sathiskumar (2009) [31] as they reported Phosphorus level as 6.36±1.66mg/dl in anestrus heifers supplemented with fresh curry leaves and Kumawat *et al.* (2014) [19] who recorded 4.86 to 5.31 mg/dl of serum Phosphorus level in delayed pubertal heifers after treatment with a combination of *Aegle marmelos* and *Murraya koenigii*.

Sathiskumar (2005) states that disturbed Ca: P ratio and deficiency of these minerals could be accounted for pituitary and gonadal dysfunction in anestrus heifers. Ca and P also influence the animal's ability to utilize other micro minerals and they have sensitizing action on reproductive hormone through various enzymes system (Dutta *et al.*, 2001) [9]. The curry leaves are rich sources of minerals, especially calcium and phosphorus, iron etc (Shantala and Prakash, 2005) [33]. The leaf extract of *Murraya koenigii* was found to contain 66.3% moisture, 6.1% protein, 1% fat (ether extract), 6.4% carbohydrate and 4.2% mineral matter. It contains 810 mg Calcium, 600 mg Phosphorus, 3.1 mg Iron, 12,600 IU carotene, 2.3 mg nicotinic acid and 4 mg Vit. C/100 g of leaf extracts (Kumar *et al.*, 1999). Thus the supplementation of curry leaves and bael would have contributed for the improved serum Calcium and Phosphorus levels and supported the induction of ovarian activity in anestrus heifers.

Table 8: Serum Phosphorus level (mg/dl) in anestrus cows of different treatment Groups.

Treatment Group (n = 6)	Pre-treatment (mg/dl)	Post-treatment (mg/dl)	t-value
Group I (control)	2.97 ± 0.45	2.67 ± 0.21^{a}	0.59^{NS}
Group II (Curry)	2.25 ± 0.22	4.57 ± 0.54^{b}	3.94*
Group III (Bael)	2.69 ± 0.33	4.59 ± 0.71^{b}	2.39*
Group IV (curry + bael)	3.35 ± 0.27	5.62 ± 0.32^{b}	5.47**
F value	1.96^{NS}	6.27**	

Conclusion

The current study revealed that combining *Murraya koenigii* and *Aegle marmelos* together, as opposed to either one alone, might be effective for enhancing fertility and inducing estrus in anestrus cows. The fertility, productivity, and economy were all improved since it was highly helpful in both farm and field conditions.

References

1. Arun A, Patel OP, Saini D, Yadav PP, Konwar R. Anti-colon cancer activity of *Murraya koenigii* leaves is due to constituent murrayazoline and O-methylmurrayamine A induced mTOR/AKT downregulation and mitochondrial apoptosis. Biomedicine & Pharmacotherapy. 2017;93:510-521.
2. Ashokan SA, Gopi B, Dhasarathan K, Ilayaraja T. Effect of *Murraya koenigii* (Curry leaves) feeding on estrus induction in Anestrus cows under Centre for Ethnoveterinary Herbal Research and Training, VUTRC, TANUVAS, Thanjapur and Foundation for Revitalisation of local Health Tradition, Bangluru, 2010.

3. Bhandari PR. Curry leaf (*Murraya koenigii*) or cure leaf: review of its curative properties. Journal of medical nutrition and nutraceuticals. 2012;1(2):92.
4. Bharkad GP, Markandeya NM. Incidence of bovine anoestrum. Indian Vet. J. 2003;80:190-191.
5. Das AK, Rajkumar V, Dwivedi DK. Antioxidant effect of curry leaf (*Murraya koenigii*) powder on quality of ground and cooked goat meat. International Food Research Journal. 2011;18(2).
6. Das GK, Mehrotra S, Narayanan K, Kumawat BL, De Kumar U, Khan TA. Estrus induction response and fertility performance in delayed pubertal heifers treated with Aegle marmelos and *Murraya koenigii*. Journal of Animal Research. 2016;6(1):151-156.
7. Das GK, Mehrotra S, Kumar B, Jan MH, Pande M, Singh SK, *et al*. Pattern and estrus induction response in delayed pubertal heifers treated with a combined treatment of *Murraya koenigii* and *Aegle marmelos* National symposium and XXVIII Annual convention of ISSAR, Khanapara, Guwahati, India, November 21-23, 2012. Compendium and Souvenir F.I.31(abstract), 2012b, 50.
8. Dutt R, Mehrotra S, Hoque M, Shanker U, Singh G, Agarwal SK, *et al*. Effect of *Murraya koenigii* and *Aegle marmelos* combination on resumption of fertility in anestrous goats. J. Appl. Anim. Res. 2010;38:275-278.
9. Dutta A, Sarmah BC, Baruah KK. Concentration of serum trace elements in cyclic and pregnant heifers in lower Brahmaputra valley of Assam. Ind. Vet. J. 2001b;78(4):300-302.
10. Hurley WL, Doane RM. Recent developments in the role of vitamins and minerals in reproduction. J Dairy Sci. 1989;72:781-801.
11. Janarthanan UK, Varadharajan V, Krishnamurthy V. Phytonutrients in *Aegle Marmelos* (L.) Leaves. World J Pharmacy Pharmaeut. Sci. 2012;1:799-809.
12. Jondhale NS. Effect of *A. marmelos* and *Ficus religiosa* on the ovarian function in rats and follicular dynamics in goat. M.V.Sc. Thesis, Indian Veterinary Research Institute, Izatnagar (Deemed University), Bareilly. India, 2007.
13. Jondhale NS, Umashanker, Mehrotra S, Agarwal SK, Singh SK, Das GK, Deori S. Effect of *Aegle marmelos* and *Ficus religiosa* leaves extracts on the ovarian function in rats. Indian Vet. J. 2009;86:1141-1144
14. Khade NB, Patel DM, Naikoo M, Dhami AJ, Sarvaiya NP, Gohel MM. Estrus induction in pubertal anestrus Gir heifers using different hormone protocols. Indian J Field Vet., 7:4. Khanapara, Guwahati, India, November 21 23, 2012. Compendium, 2011.
15. Kumar PR, Shukla SN, Purkayastha RD. Economical analysis of the estimated cost of management of anestrus buffaloes under field conditions using different hormonal and non-hormonal strategies. J. Anim. Health Prod. 2013;1(4):39-41
16. Kumar S, Punniamurthy N. Estrus induction by supplementation of *Murraya koenigii* in anestrus heifers. Indian J. Anim. Reprod. 2009;30(2).
17. Kumar D, Tripathi HC, Tandan SK, Chandra S, Lal J, Malik JK. Folk-lore herbal veterinary medicines employed in reproductive disorders and for enhancing milk production in India and Nepal-an update, 2012.
18. Kumar R. Effect of *A. marmelos* (Bael) and *Ficus religiosa* (Pipal) on the reproductive performance in anoestrus goat and buffalo. M.V.Sc. Thesis, Indian Veterinary Research Institute, Izatnagar, India], 2008.
19. Kumawat BL. M.V.Sc. Thesis, I.V.R.I (Deemed university), Izatnagar, 2014.
20. Mehrotra S. Studies on ovarian function using certain medicinal plants in rats, goats and cattle. Ph.D. Thesis submitted to Indian veterinary Research Institute, Izatnagar (Deemed university), Bareilly, India, 2002.
21. Mehrotra S. Efficacy of herbal remedies for improvement for improvement of livestock. In: short course on 'recent Advances in Endocrine Control of Livestock Production and Reproduction. Centre Advanced Studies in Veterinary Physiology ,Division of Physiology and Climatology, Feburary 25th- march, 17th 2011, compendium, 2011, 125-128.
22. Modi LC, Patel PA, Patel SP, Patel GG, Joshi AH, Suthar DN. Prevalence of reproductive Problems in buffalo in Mehsana Milk–Shed area of Gujarat. Int. J. Ag. Vet. Med. Sci. 2011;5(4):424-428.
23. Morabad BR, Kerur BR, Lagare MT, Anilkumar S. Heavy metal content in some therapeutically important medicinal plamts. Analyt. Chem. Int. J Anim. Reprod. 2009;30:66 -67.
24. Nalli Y, Khajuria V, Gupta S, Arora P, Riyaz-Ul-Hassan S, Ahmed Z, *et al*. Four new carbazole alkaloids from *Murraya koenigii* that display anti-inflammatory and anti-microbial activities. Org Biomol Chem. 2016;14:3322–3332
25. Patel KP, Sahu J, Sahu L, Dubey BK. Aegal Marmelos: A Review on its Medicinal plants. Int J. Pharm Phytopharmacol Res. 2012;1(5):332-341.
26. Pugh DG, Elmore RG, Hembree TR. A review of the relationship between mineral nutrition and reproduction in cattle. Bovine. Pract. 1985;20:10.
27. Rajkumar R, Srivastava SK, Varshney VP, Mahmood S. Effect of medicinal plants *Saracaasoca* and *Trigonella foenum–graecum* in anoestrus cows. Indian Vet. J. 2008;85(12):1281-1283
28. Ramakrishna KV. Comparative studies on certain bio-chemical constituents of anoestrus crossbred Jersey rural cows. Indian Journal of Animal Reproduction. 1997;18:33-35
29. Rautela R, Das GK, Khan FA, Prasad S, Kumar A, Prasad JK, *et al*. Antibacterial, anti-inflammatory and antioxidant effects of A. marmelos and *M. koenigii* in dairy cows with endometritis. Livestock Sci. 2018;214:142-148.
30. Satheshkumar S, Punniamurthy N. Effect of *M. koenigii* (Curry leaves) supplementation on estrus induction and serum calcium and phosphorus concentrations of anestrus heifers (Abstract), XXI Annual Convention of ISSAR and National Symposium on: Recent Trends and Innovations in Animal Reproduction: Nov. 23rd – 25th, SKAUST, Jammu, 2005, 100.
31. Satheshkumar S, Punniamurthy N. Estrus induction by supplementation of *Murraya koenigii* in anoestrus heifers. Indian J. Anim. Reprod. 2009;30:66-67
32. Selvaraju M, Veerapandian C, Kathiresan D, Chandrahasan C. Incidence of bovine reproductive disorders. Indian Vet. J. 2005;82:556.
33. Shantala M, Prakash J. Acceptability of curry leaf (*Murraya koenigii*) incorporated products and attitude toward consumption. J Food Process. Preser. 2005;29:33-44

34. Sharma S, Handu S, Dubey A, Sharma P, Mediratta P, Ahmed Q. Anti-anxiety and anti depressant like effects of *Murraya koenigii* in experimental models of anxiety and depression. Anc Sci Life. 2017;36:215–219
35. Shemesh MW, Hansel, Strauss JF. Calcium dependent cyclic nucleotide independent steroidogenesis in the bovine placenta. Cell Biology, 1994;81:6403.
36. Singh C, Prabha B, Manowar SAL. Serum cholesterol concentration in crossbred (Friesian x Hariana) cows and calves. Indian Journal of Animal Science. 2004;74(4):400-401
37. Thakor D, Patel D. Incidence of Infertility Problems in Cattle and Buffaloes. Dairy Cattle, 2013. http://en.engormix.com.
38. Tripathi Y, Anjum N, Rana. A Chemical composition and *in vitro* antifungal and antioxidant activities of essential oil from *Murraya koenigii* (L.) Spreng Leaves. Asian J Biomed Pharm Sci. 2018;8:6-13.
39. Umashanker, Agarwal SK, Mehrotra SK, Singh SK, Hoque M. Restoration of fertility in anestrus buffaloes using *M. koenigii* (Curry leaf plant). XXII Annual Convention and National Symposium on Innovative Technologies for Fertility Enhancement in Livestock, Nov. 10-12, MHOW (M.P), 2006, 28-283
40. Van Miert ASJPAM. The use of animals of drug licensed for human use only. In: Comparative Veterinary Pharmacology, Toxicology and Therapy. Eds. Van Miert (A. congress of European Association Vet. Pharmacology and Toxicology, Belgium, MTP press, Limited, Lancaster, 1986, 489-500.