

## Comparative studies on different herbal medicines for induction of estrus in anoestrus buffalo heifers

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### ABSTRACT

**Objectives:** The present study was designed to assess the therapeutic efficacy of *Randia dumetorum*, *Tinospora cordifolia* and commercial herbal medicine (Prajana HS, Indian Herbs Overseas) for induction of cyclicity in anoestrus buffalo heifers.

**Materials and Methods:** Forty eight anoestrus buffalo heifers were examined and redistributed into six groups (G<sub>0</sub>, G<sub>1</sub>, G<sub>2</sub>, G<sub>3</sub>, G<sub>4</sub> and G<sub>5</sub>) and each comprising of eight (n=8) animals. Untreated anoestrus animals (G<sub>0</sub>) and cyclic animals (G<sub>6</sub>) were kept as an anoestrus control and cyclic control, respectively. Group G<sub>1</sub> was supplemented with the mineral mixture, while G<sub>2</sub>, G<sub>3</sub>, G<sub>4</sub>, and G<sub>5</sub> groups were treated with Prajana HS, *Randia dumetorum*, *Tinospora cordifolia*, and the combination of *Randia dumetorum* and *Tinospora cordifolia*, respectively along with supplementation of mineral mixture.

**Results:** Overall estrus induction and conception rate were recorded as 50 % and 75 % in mineral mixture (G<sub>1</sub>), 75 and 83.33 % in Prajana HS (G<sub>2</sub>), 87.50 % and 85.71 % in *Randia dumetorum* (G<sub>3</sub>), 62.5% and 80% in *Tinospora cordifolia* (G<sub>4</sub>), and 87.5% and 85.71% in *Randia dumetorum* and *Tinospora cordifolia* combination (G<sub>5</sub>) whereas none of heifers were exhibited estrus symptom in untreated anoestrus control group.

**Discussion:** On the basis of Overall estrus induction, conception rate and hematological parameters concluded that *Randia dumetorum* and *Tinospora cordifolia* combination have an effective roll in the treatment of anoestrus buffalo heifers. Ethnoveterinary medicines can be utilized as low-cost therapeutic management for control of anoestrus buffalo heifers.

**Keywords:** Anoestrus, Ethnoveterinary Medicine, Hematology, Estrus Induction, Conception Rate.

## INTRODUCTION

India has 108.7 million (approximately 21.2% of the total world) buffalo population. Total milk production of our country was 155.5 million tons (49.6% contribution by buffalo) with an annual growth of 6.27% (DAHDF, 2016). However, milk production efficiency is sub-optimum due to several reproductive problems such as delayed puberty, silent estrus, summer anoestrus, post-partum anoestrus and repeat breeding; buffalo is still considered as a difficult breeder and therefore leads to huge economic losses to the farmers. In India, the incidence of anoestrus has been reported between 9.18-82.50% (Khan *et al.*, 2009; Kumar *et al.*, 2013). Various hormonal and non-hormonal treatments have been tried for the management of anoestrus (Xu *et al.*, 1997; Mwaanga *et al.*, 2004). Hormonal therapy has good success, but its use is limited due to the high cost of treatment, milk disposal and several side effects (Morrow 1980; Dhaliwal *et al.*, 2001). The supplementation of minerals has been found to improve anoestrus in buffaloes. The non-hormonal commercial herbal heat inducer (Prajana HS, Indian Herbs) used in buffalo (Mav and Bahga, 2005; Bisla *et al.*, 2006; Hussain *et al.*, 2009). Prajana HS is a non-hormonal commercial herbal preparation containing high concentration of trienic fatty acids, which act as precursors for biosynthesis of prostaglandin (PG), follicular stimulating hormone (FSH), leutinising hormone (LH) and other hormones that helps in optimization of ovarian functions and thus produce timely estrus, ovulation and conception (Tanwar *et al.*, 2015). Kabir *et al.* (2001) reported 50% estrus response in anoestrus buffaloes using mixture of *Abroma augusta* (root) and *Nigella sativa* (seed) in 2:1 ratio. Rajkumar *et al.* (2008) reported success rate in anoestrus cattle is 83.33 and 66.66% using Methi seed and bark of Ashoka tree, respectively. *Tinospora cordifolia* is used in Ayurvedic, "Rasayanas" to recover the immune system and the body resistance against infections (Singh *et al.*, 2003). *Randia dumetorum* pulp of the fruit is used by many practitioners to also have anthelmintic properties and also used as an abortifacient as folklore remedy (Agarwal *et al.*, 1999). Study of different hematological constituents of blood is of great importance during disease conditions, stress, immunity etc. They also have direct clinical application for diagnostic purposes. A low level of Hemoglobin influences tissue oxygenation of the reproductive tract, which in turn could affect the cyclicity (Ramakrishna, 1997). Estrus induction and conception rate are the most important parameter for the evaluation of the anoestrus buffalo heifers (Meenakshi *et al.*, 2014; Tanwar *et al.*, 2015).

Thus, we need to improve the ethno-veterinary products for estrus induction by different novel

combinations of herbal medicine and the study was planned to see the effects of different herbal products to recovery of anoestrus buffalo heifers to estrus with compare to the commercially available estrus inducers and cyclic animals.

## MATERIALS AND METHODS

The anoestrus buffalo heifers were taken treated with dewormer, those were presented at the veterinary hospital and villages lying adjacent to NDUAT University Kumarganj, Faizabad- India. The anoestrus animal was confirmed by history and two successive rectal examinations at 10 days interval. The animal having smooth ovaries with no palpable structure and having no clinically detectable abnormalities in their genital tract was categorized as anoestrus animal.

Prajana HS (Indian Herbs) was bought from the local market. *Randia dumetorum* dry fruit was pulverized by a mechanical mixer grinder to obtain a fine powder, which was stored in a sterilized, clean, wide mouthed, air tight bottle at roomed temperature. The stem of *Tinospora cordifolia* was crushed and soaked in water. The small crushed pieces of *Tinospora cordifolia* were macerated in the water with hand. This helps to release the starchy material of *Tinospora cordifolia* into the water. Then the fibrous and woody parts were removed from the vessels and the liquid was placed undisturbed to allow the starchy particles to settle down at the bottom. The stable water above was carefully decanted out without disturbing the starch deposited. The starch deposit was washed with a small quantity of water, dried in the shade absolutely and collected. It was stored in moist resistant airtight glass containers. The resultant white powder was around 5% of the original quantity of the herb.

The selected (n=48) anoestrus buffalo heifers were divided into six groups, comprising of eight (n=8) animals in each group and eight (n=8) normal cyclic animals were also taken for study. The blood samples were collected from control anoestrus buffalo heifers and before and after supplementation of mineral mixture (Chelated Agrimin Forte-Virbac, India) and herbal therapy from anoestrusbuffalo heifers and the blood samples were used immediately for analysis of hematological parameters (Table 1.1).

The hemoglobin concentration of blood was estimated by the method using Sahli's hemoglobinometer (Samuel, 1986). The Packed Cell Volume (PCV) was determined by using microhaematocrit tube and Erythrocyte sedimentation rate (ESR) was estimated by Wintrobe method. Leucocytes were counted by the method of Samuel, (1986) using Haemocytometer. The Differential Leucocytes Count (DLC) was

performed according to Coles, (1986) on the basis of morphological and staining characteristics.

After treatment, all the animals were observed for expression of estrus. The induction of estrus was detected by careful visual observation of animals in morning, afternoon and evening hours, at least for 30 minutes. Further, confirmation was done by per rectal examination with the help of Ultrasonography (Toshiba SSA-320, Japan). After detection of estrus, animals were inseminated two times at an interval of 12 hours.

Pregnancy diagnosis was carried out after forty five days of the second artificial insemination by per rectal palpation of the uterine content. Ultrasound was used for the confirmation of pregnancy in buffalo heifers.

Data were analyzed by using one-way ANOVA through Graph Pad Prism 5 for hematological parameters. Data are presented as mean± SE and considered significant at P<0.05.

Group	Treatment protocols
G <sub>0</sub>	Control (Anoestrus buffalo heifers)
G <sub>1</sub>	Mineral mixture supplementation for 10 days (1-10 days) @ 50g. P.O.
G <sub>2</sub>	Mineral mixture supplementation for 10 days (1-10 days) @ 50g. P.O. and Prajana HS @ 3 capsule daily for 2 days followed by 3 capsules P.O. for two days on 11 <sup>th</sup> day of study
G <sub>3</sub>	Mineral mixture supplementation for 10 days (1-10 days) @ 50g. P.O. and <i>Randia dumetorum</i> @ 15g. P.O. daily for 4 days of study
G <sub>4</sub>	Mineral mixture supplementation for 10 days (1-10 days) @ 50g. P.O. and <i>Tinospora cordifolia</i> sat @ 25g. P.O. daily for 10 days of study
G <sub>5</sub>	Mineral mixture supplementation for 10 days (1-10 days) @ 50g. P.O., <i>Randia dumetorum</i> @ 15g. Orally and <i>Tinospora cordifolia</i> sat @ 25g. P.O. daily for 10 days of study
G <sub>6</sub>	Normal Cyclic buffalo heifers

Table 1.1 Dose regimens used in various formulated groups

## RESULTS

The mean value of the hemoglobin (Hb), packed cell volume (PCV), erythrocyte sedimentation rate (ESR), total leucocytes count (TLC) and differential leucocytes count (DLC) in anoestrus buffalo heifers before treatment and after treatment are summarized (Table 1.2). The mean value of hemoglobin (Fig. 1.1) in the *Randia dumetorum*, *Tinospora cordifolia* sat, combination of *Randia dumetorum* and *Tinospora*

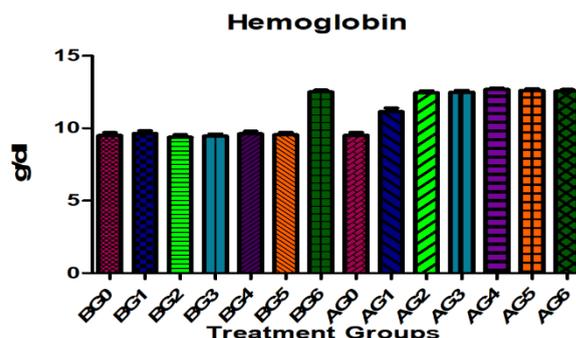


Figure 1.1 Graphical representation of Effect of mineral mixture, commercial herbal drug (Prajana HS) and herbal drug (*Randia dumetorum* and *Tinospora cordifolia* and its combination) on Hemoglobin constituent (mean±SE) in anoestrus buffalo heifers

*cordifolia* sat treated animals had a significantly (P<0.05) higher concentration than anoestrus buffalo heifers. The mean value of Packed cell volume (Fig. 1.2) in the mineral mixture, Prajana HS, *Randia dumetorum*, *Tinospora cordifolia*, Combination of *Randia dumetorum* and *Tinospora cordifolia* treated groups were significantly (P<0.05) higher than anoestrus buffalo heifers. The mean value of Erythrocyte sedimentation rate (Fig. 1.3) in Prajana HS, *Randia dumetorum*, *Tinospora cordifolia*, Combination of *Randia dumetorum* and *Tinospora cordifolia*, were significantly (P<0.05) higher, as compared with anoestrus buffalo heifers. The mean values of Total leucocytes count concentration before treatment groups and after treatment groups were non

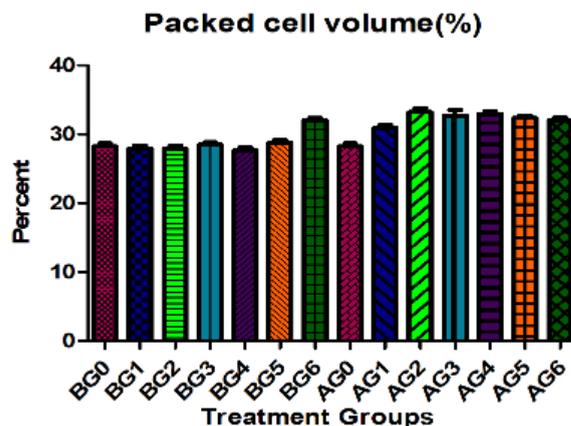


Figure 1.2 Graphical representation of Effect of mineral mixture, commercial herbal drug (Prajana HS) and herbal drug (*Randia dumetorum* and *Tinospora cordifolia* and its combination) on Packed cell volume constituent (mean±SE) in anoestrus buffalo heifers

significantly (P<0.05) difference in mean value between anoestrus and normal cyclic buffalo heifers. The mean value of Neutrophil percent in before treatment and after treatment groups were non significantly (P<0.05) difference. The mean value of Lymphocyte before treatment and after treatment in

anoestrus and normal cyclic buffalo heifers were non significantly ( $P < 0.05$ ) difference.

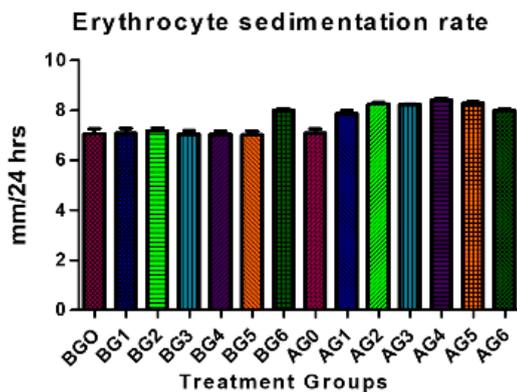


Figure 1.3 Graphical representation of Effect of mineral mixture, commercial herbal drug (Prajana HS) and herbal drug (*Randia dumetorum* and *Tinospora cordifolia* and its combination) on Erythrocyte sedimentation rate constituent (mean $\pm$ SE) in anoestrus buffalo heifers.

All the induced estrus buffalo heifers were confirmed by ultrasonography and palpation of uterine horn and ovary. Results of study (Table 1.3) revealed that eight buffalo heifers in the group (G<sub>1</sub>) treated with mineral mixture showed the symptoms of heat on an average of 50% respond to minerals mixture treatment for the onset of estrus with an interval of 35 days from initiation of treatment. Out of four estrus buffalo heifers, two were conceived after the first insemination and one heifer was conceived after second insemination and total conception rate was recorded as 75%. In Prajana HS treated group (G<sub>2</sub>), 75% buffalo heifers were responded to commercial herbal preparation i.e. Prajana HS treatment for the onset of estrus with an interval of 15 days from initiation of treatment and out of six estrus buffalo heifers, four heifers were conceived at first insemination (66.66%) and one heifer conceived at second insemination (16.67%) and total conception rate in experimental buffalo heifers were reported as 83.33%. In *Randia dumetorum* (G<sub>3</sub>) treated group, 87.5% heifers were exhibited sign of estrous within ten days interval from initiation of treatment and out of seven estrus heifers, four were conceived at first insemination (57.14%) and rests two were conceived at second insemination (28.57%) and total conception rate in experimental buffalo heifers were reported as 85.71%. In *Tinospora cordifolia* sat (G<sub>4</sub>) treated group, 62.5% buffalo heifers were expressed estrus sign with an interval of 35 days from initiation of treatment, all responded heifers were conceived and out of five estrus buffalo heifers, four were conceived at first insemination and remaining was conceived at second insemination and total conception rate in

experimental buffalo heifers were reported as 80%. In combination group of *Randia dumetorum* and *Tinospora cordifolia* (G<sub>5</sub>), 87.5% buffalo heifers were responded to treatment of combination of *Randia dumetorum* and *Tinospora cordifolia* for induction of cyclicity in interval of 10 days. All the induce estrus were ovulatory estrus. Out of seven estrus buffalo heifers four conceived at first insemination (57.14%) and two conceived at second insemination (28.57%) and total conception rate in experimental buffalo heifers were reported as 85.71%.

## DISCUSSION

The present findings of hemoglobin concentrations are in accordance with the finding of Dhoble and Gupta (1981); Kumar *et al.*, (1991) and Rameez Ali *et al.*, (2012). The low value of hemoglobin in anoestrus buffaloes, may be due to poor nutrition and anemic condition of the buffaloes while the higher value in normal cycling buffaloes might be a sign of their body condition and nutritional condition as the animals included in the study were reared under different field conditions. The lower hemoglobin might be due to anemia caused by a gastrointestinal parasite or nutritional deficiency of micro minerals (Mc Dowell, 1992; Srivastava and Khariche, 1986). Though the importance of the hemoglobin level has not been directly implicated in reproductive disorders, yet a decrease in hemoglobin value is indicative of certain systemic disorders that could indirectly affect the functional activity of the reproductive organs. A low level of hemoglobin influences tissue oxygenation of the reproductive tract, which in turn could affect the cyclicity (Ramakrishna, 1997). *Randia dumetorum* and *Tinospora cordifolia* has been increasing the hemoglobin level due to its hepatoprotective function. These findings are supported by Sulong *et al.*, (1980), they reported higher values during summer. The packed cell volume was lower during young age and it increased as age increased. Kumar *et al.*, (1990) observed that hematocrit value showed a marked decrease in one month old calves as compared to day old and other ages. Patil *et al.*, (1992) find the similar result and concluded that the higher erythrocyte value responsible for improved PCV value in growing buffalo heifers as compared to adult buffaloes. This may be due to high basal metabolic rate, which improved the rate of erythropoiesis resulting in higher number of erythrocytes. *Randia dumetorum* and *Tinospora cordifolia* has been increasing the Packed cell volume level due to its hepatoprotective function. Rizvi (1973) reported the higher value of Erythrocyte sedimentation rate during winter compared to other seasons. Age of animals also affected the Erythrocyte

Parameter	Status	Treatment						
		G <sub>0</sub>	G <sub>1</sub>	G <sub>2</sub>	G <sub>3</sub>	G <sub>4</sub>	G <sub>5</sub>	G <sub>6</sub>
Hemoglobin	Before	9.50±0.20 <sup>Y</sup>	9.64±0.19	9.39±0.17 <sup>Y</sup>	9.46±0.15 <sup>Y</sup>	9.61±0.18	9.55±0.17 <sup>Y</sup>	12.51±0.13 <sup>X</sup>
	After	9.53±0.18 <sup>a, Y</sup>	11.15±0.24 <sup>A, ab</sup>	12.44±0.14 <sup>A, bc</sup>	12.49±0.13 <sup>A, bc, d</sup>	12.68±0.11 <sup>A, cde</sup>	12.61±0.12 <sup>A, cd, ef</sup>	12.56±0.11 <sup>X</sup>
Total Leucocytes	Before	8.13±0.10 <sup>Y</sup>	8.21±0.07 <sup>Y</sup>	8.10±0.12 <sup>Y</sup>	8.28±0.08 <sup>Y</sup>	8.09±0.09 <sup>Y</sup>	8.05±0.08	7.25±0.04 <sup>X</sup>
	After	8.11±0.10 <sup>a, Y</sup>	7.45±0.07 <sup>A, ab</sup>	7.33±0.04 <sup>A, bc</sup>	7.29±0.04 <sup>A, bcd</sup>	7.28±0.03 <sup>A, bcde</sup>	7.25±0.03 <sup>A, bcde, ef</sup>	7.28±0.37 <sup>X</sup>
Packed Cell Volume	Before	28.20±0.47 <sup>Y</sup>	27.93±0.51 <sup>Y</sup>	27.91±0.50 <sup>Y</sup>	28.47±0.36 <sup>Y</sup>	27.66±0.40 <sup>Y</sup>	28.82±0.36	32.00±0.40 <sup>X</sup>
	After	28.23±0.45 <sup>a, Y</sup>	30.95±0.44 <sup>A, ab</sup>	33.21±0.61 <sup>A, bc</sup>	32.73±0.80 <sup>A, bc, d</sup>	32.96±0.38 <sup>A, bcde</sup>	32.38±0.34 <sup>A, bc, def</sup>	32.06±0.38 <sup>X</sup>
Erythrocyte Sedimentation Rate	Before	7.07±0.91 <sup>Y</sup>	7.08±0.21 <sup>Y</sup>	7.17±0.14	7.06±0.14 <sup>Y</sup>	7.04±0.13 <sup>Y</sup>	7.03±0.13 <sup>Y</sup>	7.98±0.08 <sup>X</sup>
	After	7.10±0.18 <sup>a</sup>	7.86±0.86 <sup>A, ab</sup>	8.26±0.07 <sup>A, bc</sup>	8.23±0.03 <sup>A, bcd</sup>	8.41±0.08 <sup>A, bcde</sup>	8.28±0.07 <sup>A, bcde, ef</sup>	8.0±0.08 <sup>X</sup>
Neutrophil	Before	26.50±0.42	26.75±0.37	25.88±0.40	26.63±0.46	26.88±0.40	27.13±0.30	26±0.38 <sup>X</sup>
	After	26.63±0.46 <sup>a</sup>	27.50±0.42 <sup>ab, c</sup>	27.88±0.35 <sup>A, ab, c</sup>	27.88±0.35 <sup>abc, d</sup>	27.63±0.32 <sup>bcde</sup>	28.38±0.42 <sup>A, ab, cde, f, Y</sup>	26.50±0.33 <sup>X</sup>
Lymphocyte	Before	67.88±0.44	67.75±0.49	68.38±0.32 <sup>Y</sup>	67.75±0.52	67.63±0.46	67.38±0.50	66.13±0.23 <sup>X</sup>
	After	68.13±0.35 <sup>a</sup>	66.13±0.35 <sup>A, ab</sup>	65.63±0.26 <sup>A, bc</sup>	65.13±0.40 <sup>A, bc, d</sup>	65.50±0.33 <sup>A, bcde</sup>	64.38±0.46 <sup>A, bc, def</sup>	65.75±0.25 <sup>X</sup>
Monocyte	Before	2.50±0.19	2.50±0.19	2.88±0.30 <sup>Y</sup>	2.38±0.18	2.50±0.19	2.63±0.18	3.50±0.28 <sup>X</sup>
	After	2.63±0.18 <sup>a</sup>	3.75±0.31 <sup>A, ab</sup>	4.13±0.23 <sup>A, bc</sup>	4.13±0.23 <sup>A, bcd</sup>	4.13±0.30 <sup>A, bcde</sup>	4.13±0.23 <sup>A, bcde, ef</sup>	3.75±0.16 <sup>X</sup>
Eosinophil	Before	3.13±0.30	3.13±0.30	3.25±0.25	3.13±0.23	3.00±0.23	3.00±0.33	2.75±0.25 <sup>X</sup>
	After	3.0±0.38 <sup>a</sup>	2.38±0.18 <sup>ab</sup>	2.38±0.18 <sup>A, abc, d</sup>	2.25±0.16 <sup>A, abc, d</sup>	2.75±0.25 <sup>abcde, f</sup>	2.50±0.19 <sup>abcde, f</sup>	2.75±0.25 <sup>X</sup>
Basophil	Before	0.50±0.19	0.50±0.19	0.50±0.19	0.50±0.19	0.50±0.19	0.50±0.19	0.38±0.18 <sup>X</sup>
	After	0.50±0.19 <sup>a</sup>	0.50±0.18 <sup>ab</sup>	0.38±0.18 <sup>abc</sup>	0.38±0.18 <sup>abcd</sup>	0.38±0.18 <sup>abcde</sup>	0.38±0.18 <sup>abcde, f</sup>	0.38±0.18 <sup>Y</sup>

1Table 1.2 Effect of mineral mixture, commercial herbal drug (Prajana HS) and herbal drug (Randia dumetorum and Tinospora cordifolia and its combination) on hematological constituent (mean±se) in anoestrus buffalo heifers Mean bearing different superscript in the column (A, B) and in a row (a, b, c, d, e, and f) significantly differed repeatedly for each attributes

sedimentation rate value in heifers. There was observed an increase in ESR values after the achievement of puberty. Patil *et al.*, (1992) declared that erythrocyte sedimentation rate in newborn buffaloes was low but later on it enhanced as the age advances. These results are in accordance with the report of Patil *et al.*, (1992); Horadagoda *et al.*, (2002) and Rameez Ali *et al.*, (2012). The erythrocyte parameter and differential leucocytes counts are affected by various physiological parameters (Klinkon *et al.*, 1994), as well as factors from the environment (Zadnik, 1991). Ahmad *et al.*, (2003) observed non-significant difference in TLC value in cyclic and non cyclic cows. *Tinospora cordifolia* has been suggested to have the direct antibacterial effect it induces leucocytosis and activates macrophages. Similar finding were also reported by Brar *et al.*, (2002) and Ali *et al.*, (2012). It indicates that animals in the anoestrus group were suffering from neutrophilia indicating infection as the infection promotes the release of neutrophils from bone marrow through leucocytosis inducing factor in the plasma. The cause of subclinical infection may be stress and nutritional

deficiency that leads to compromised immune response. *Tinospora cordifolia* enhances antibody production by the proliferation of B-cells that increase the neutrophil production. Lymphocytes are essential in combating infections, they display powerful effector mechanism and their activity must be regulated at all times to avoid self-tissue or cell destruction. The function of lymphocytes and its products range from the neutralization of pathogen, with specific antibodies to the activation of macrophages and to direct cytotoxic activity. The present finding TLC, eosinophils, lymphocyte, monocyte, basophil counts and eosinophils (%), monocyte (%) and basophil (%) did not show any significant differences. The mean neutrophil, lymphocyte, monocyte, eosinophil and basophile count in anoestrus and normal cyclic buffaloes were non-significant. Similar findings were also reported by Brar *et al.*, (2002). The present finding in respect to the effect of mineral supplementation on anoestrus animal was lesser than the findings of Lall *et al.*, (2000); Pander *et al.*, (2003),

Treatment Groups	No. Of Anoestrus Animal	No. Of Animal Exhibited Estrus Sign	Pregnancy After Artificial Insemination			Total Animal Conceive
			I <sup>st</sup>	II <sup>nd</sup>	III <sup>rd</sup>	
G <sub>0</sub>	8	0	-	-	-	-
G <sub>1</sub>	8	4/8	2(50%)	1(25%)	0	3(75%)
G <sub>2</sub>	8	6/8	4(66.66%)	1(16.67%)	0	5(83.33%)
G <sub>3</sub>	8	7/8	4(57.14%)	2(28.57%)	0	6(85.71%)
G <sub>4</sub>	8	5/8	3(60%)	1(20%)	0	4(80%)
G <sub>5</sub>	8	7/8	4(57.14%)	2(28.57%)	0	6(85.71%)

Table: 1.3 Effect of mineral mixture, commercial herbal drug (Prajana HS) and herbal drug (Randia dumetorum and *Tinospora cordifolia* and its combination) on anoestrus buffalo heifers percent estrus induction and conception rate

may be due to different environmental and feeding practices. Lall *et al.*, (2000), reported that 70% of buffaloes showed estrus and conceived within period of 2-4 weeks, whereas Pander *et al.*, (2003) had observed that 72.13% of buffaloes came in heat after 12.9 days of mineral mixture supplementation and the overall conception of field condition was observed as 59.02%. Finding with respect to the effect of Prajana HS on anoestrus animal was equal to the report of Samsad and Hasan (1984), who observed that 75% of buffaloes in Bangladesh showed estrus and 73.3% conceived. However Tanwar *et al.*, (2015) conducted estrus induction study in semi-arid region of India and reported 36.67% of buffaloes came in estrus after 18 days and 20% conceived after treated with Prajana HS. Prajana HS is a non-hormonal herbal preparation containing high concentration of trienic Fatty acids, which act as precursors for biosynthesis of prostaglandin (PG), follicular stimulating hormone (FSH), luteinizing hormone (LH) and other hormones that help is optimization of ovarian function and thus produce timely estrus, ovulation and conception.

The present results with *Randia dumetorum* were higher in comparison to the observations of Akhtar *et al.*, (2010) and Sachan (2014). Akhtar *et al.*, (2010) reported 66.66% estrus induction in buffaloes suffering from true anoestrus after treatment with 15 gm of *Randia dumetorum* nut for 4 days. Sachan (2014) also recorded efficacy in cattle as 71.42% estrus induction. The estrus induction may be due to the presence of phytosterols, flavonoids or other phytochemicals present in the emetic nut (Noorani and Kale, 2012). These isoflavones can act as steroids mimics by filling the stereochemical space that could be occupied by the oestrogenic compound.

*Tinospora cordifolia* is one of the constituents of several ayurvedic preparations used in general debility, dyspepsia, fever and urinary diseases. The aqueous extract of the stem antagonizes the effect of

an agonist such as 5-hydroxytryptamine, histamine, bradykinin and prostaglandins on the rabbit smooth muscles and inhibits the constrictor response to histamines and acetylcholine on smooth muscles. The hepatoprotective action of *Tinospora cordifolia* was reported in one of the experiments in which goats treated with *Tinospora cordifolia* have shown significant clinical and hemato-biochemical improvement in CCL<sub>4</sub> induced hepatopathy. *Tinospora cordifolia* has been suggested to have a direct antibacterial effect (Direkbusarakom *et al.*, 1998). It induces leucocytosis (Thatte *et al.*, 1989) and activates macrophages (Prince and Menon, 1999). Further *Tinospora cordifolia* enhances antibody production by the proliferation of B-cell (Sainis *et al.*, 1997). The reduction in bacterial population in the present study may be due to this immune-stimulatory activity of *Tinospora cordifolia*. The activated phagocyte cells are more component in phagocytizing invading bacteria.

On the basis of Overall estrus induction, conception rate and hematological parameters concluded that *Randia dumetorum* and *Tinospora cordifolia* combination have an effective roll in the treatment of anoestrus buffalo heifers. Ethnoveterinary medicines can be utilized as low-cost therapeutic management for control of anoestrus buffalo heifers. The exact principle involved in the estrus induction should be investigated in future research. Further *In vivo* and *In vitro* nutritional studies and processing is indicated in near future for increased acceptability at large scale. *In vivo* investigations are required to know various pharmacological and physiological effects on the animals and efficacy of *Randia dumetorum* and *Tinospora cordifolia* might be compared with various antibiotics, herbal and ayurvedic medicines in appropriate animal models for various reproductive problems in the domestic animals.

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