COMPARATIVE EFFICACY OF DIFFERENT HERBAL EXTRACT ON SUBCLINICAL ENDOMETRITIS IN POSTPARTUM COWS

T H E S I S

Submitted
In partial fulfillment of the requirements for the Degree of

MASTER OF VETERINARY SCIENCE
IN
ANIMAL REPRODUCTION, GYNAECOLOGY AND OBSTETRICS

BY
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DECLARATION OF STUDENT

I hereby declare that the experimental research work and interpretation of the thesis entitled **"COMPARATIVE EFFICACY OF DIFFERENT HERBAL EXTRACT ON SUBCLINICAL ENDOMETRITIS IN POSTPARTUM COWS"** or part thereof has not been submitted for any other degree or diploma of any University, nor the data have been derived from any thesis/publication of any University or scientific organization. The sources of materials used and all assistance received during the course of investigation have been duly acknowledged.

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Place: Akola **(Nikhade Chaitali Tilakrao)**

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LIST OF ABBREVIATIONS

Abbreviation		Full form
%	-	Per cent
°C	-	Degree Celsius
µg	-	Microgram
µg	-	Microgram
µl	-	Micro liter
AI	-	Artificial Insemination
AI	-	*Azadirachta indica*
ALT	-	Alanine Aminotransferase
AST	-	Aspartate Aminotransferase
BCS	-	Body Condition Score
BUN	-	Blood Urea Nitrogen
cAMP	-	Cyclic Adenosine Monophosphate
CL	-	Corpus Luteum
DIM	-	Days in Milk
DLC	-	Differential Leucocyte Count
DPP	-	Days Post Partum
EC	-	Endometrial cytology
et al.	-	et alia (and others)
Fig.	-	Figure
g	-	Gram
g/dl	-	Gram per deciliter
g/l	-	Gram per liter
Hb	-	Haemoglobin
HPF	-	High Power Field
HPMN	-	High Poly Morpho Nuclear
hrs	-	Hours
i.e.	-	that is/ id est
IL	-	Inter Leukins
IU/dl	-	International unit per deciliter
IU/L	-	International unit per liter
LD_{50}	-	Lethal dose 50
LIF	-	Leukocytosis Inducing Factor

M	-	meter
mg	-	Mili gram
mg/kg	-	Mili gram / kilogram
ml	-	milli liter
mm	-	milli meter
MNC	-	Mono Nuclear Cell
MCV	-	Mean Corpuscular Volume
MCH	-	Mean Corpuscular Hemoglobin
MCHC	-	Mean Corpuscular Hemoglobin Concentration
NS	-	Non significant
PAMP	-	Pathogen Associated Molecular Pattern
PDKV	-	Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola
Pg	-	Picogram
$PGF_2\alpha$	-	Prostaglandin $F_2\alpha$
PMNL	-	Polymorpho Nuclear Leucocytes
RBC	-	Red Blood Corpuscles/Cells
ROC	-	Receiver Operating Curve
RP	-	Retention of Placenta
SCE	-	Subclinical endometritis
TLRs	-	Toll like receptor
TNFα	-	Tumour Necrosis Factor α
U/L	-	Unit per liter
Vs	-	Verses
WBC	-	White Blood Corpuscles/Cells

CHAPTER I

INTRODUCTION

Uterine contamination during parturition is common in all mammalian species including cattle (Sheldon *et al*., 2008). Bacterial contamination of the uterus is common in cows (80-100%) within two weeks postpartum. Within 2-6 weeks of parturition normal cows are able to clear bacterial contaminants with innate defense mechanisms (Hussain *et al*., 1990). It is found that the uterus of approximately 40% of cows is still contaminated beyond three weeks postpartum (Sheldon *et al*., 2008).

In uterine defense mechanism the immune system plays a major role, however our understanding of this role is still greatly limited. Uterine leukocytes provide cellular defense against bacterial contaminants (Stossel, 1975). Receptors on endometrial cells and macrophages, called Toll-like receptors (TLRs), recognize highly conserved molecular patterns present on bacteria called pathogen-associated molecular patterns (PAMPs). Toll-like receptor binding of these PAMPs stimulates cells to produce and release pro-inflammatory cytokines and chemokines, including TNF-α, IL-6 and IL-8 (Beutler *et al.,* 2003). Interleukin-6 and TNF-α stimulate the production of anti-microbial peptides which assist in the elimination of pathogenic bacteria from the tissues. Fischer *et al*. (2010) reported that pro-inflammatory cytokines such as IL-6, IL-8 and TNF-α may accelerate PMN infiltration into the endometrium of cows following infection.

Recently, endometrities has been sub-divided into clinical and subclinical categories (Sheldon *et al*., 2006). Clinical endometrities is defined as purulent or mucopurulent uterine discharge present after 21 or 26 days postpartum, respectively. Subclinical endometritis is defined as the presence of >18% polymorphonuclear (PMN) cells in uterine cytology samples collected 21-33 days postpartum, or >10% PMNs in samples collected at days 34-47. Cows with subclinical endometritis do not have uterine discharge; however, the severity of the disease is still considered sufficient to impair reproductive performance (Sheldon *et al*., 2006).

Subclinical endometritis was first described as cytological endometritis considering the presence of PMNL in the endometrial lumen (Gilbert *et al*., 1998), and then standardized by Kasimanickam *et al*. (2004) based on its negative effects on reproductive performance.

Subclinical endometritis is the common cause of infertility and subfertility in high producing dairy cattle delaying the onset of ovarian cyclic activity after parturition, extending luteal phase and reducing conception rate is (Sheldon *et al*., 2009). Uterine cytology or ultrasonography can be used for diagnosis of subclinical endometritis. Lavage technique (Barlund *et al*., 2008; Gilbert *et al*., 2005 and Kasimanickam *et al*., 2005) or cytobrush method (Barlund *et al*., 2008; Kasimanickam *et al*., 2004 and Kasimanickam *et al*., 2005) can be used for collection of samples for uterine cytology. Cytological smears are evaluated for their proportion of polymorphonuclear cells (PMN) by endometrial cells present in a sample. An increased proportion of PMN is prognostic for impaired subsequent reproductive performance (Barlund *et al*., 2008; Gilbert *et al*., 2005 and Kasimanickam *et al*., 2004). Four to eighteen percent of PMN cells (Barlund *et al*., 2008; Galvao *et al*., 2009a; Gilbert *et al*., 2005; Kasimanickam *et al*., 2004) is the threshold value that define subclinical endometritis. Cows with ≥5% polymorphonuclear cells (PMN) in the cytological sample regarded as affected by subclinical endometritis (Gilbert *et al*., 2005; Plontzke *et al*. 2010). In previous studies, cows diagnosed with subclinical endometritis had prolonged days open and a reduced probability of conception at first artificial insemination (A.I.) compared with cows without subclinical endometritis (Barlund *et al*., 2008; Gilbert *et al*., 2005 and Kasimanickam *et al*., 2004). By the use of 150-day pregnancy status as the outcome Barlund *et al*. (2008) concluded that the sensitivity of endometrial cytobrush cytology was only 12.9%, while the specificity was 89.9% in cows sampled 28 to 41 days postpartum.

The prevalence of subclinical endometritis decreases with increasing days post partum (Gilbert *et al*., 2005) and ranges from 11% to 53% (Barlund *et al*., 2008; Gilbert *et al*., 2005 and Kasimanickam *et al*., 2004). With endometrial cytology the overall prevalence of subclinical endometritis with >5 neutrophills was 38% (Plontzke *et al*., 2010). The

prevalence of subclinical endometritis in pregnant heifers, cows with poor hygienic condition, cows used artificial insemination, cows having good body condition score and cows in small scale farms were 100%, 71.4%, 69.8%, 75% and 86.7%, respectively without statistically significant difference ($p>0.05$) among parity, hygienic condition, body condition score, method of service and farm scales, respectively (Moges and Jebar, 2012).

Neem (*Azadirachta indica*) has been extensively used in India as traditional ayurvedic and folklore medicine for the treatment of various diseases (Bandyopadhyay *et al*., 2002). It has been demonstrated to exhibit immunomodulatory, antiinflammatory, antifungal, antibacterial, antiviral, and antioxidant properties (Subapriya and Nagini, 2005).

Due to its better antibiotic and immunomodulator property of hydro alcoholic extract of neem oil it can be used as a therapy for endometritis in repeat breeding cows and also observed as a better clinical recovery and conception rate with hydro alcoholic extract of neem (Harendra kumar *et al*., 2013).

Achyranthes aspera (Aghada) is distributed as weed throughout India, tropical Asia and other parts of the world. The plant is reported to be used as oestrogenic, antimicrobial, immunostimulant, anti-inflammatory, antioxidant, diuretic, cardiac stimulant, antispasmodic and hepatoprotective (Dey 2011; Sharma *et al.,* 2013; Khan *et al.,* 2009). Immunomodulatory activity of the plant on elicitation of antigen-specific murine antibody response has been reported (Vasudeva *et al*. 2002).The estrogenic and uterotrophic activity of the extracts have successfully detected in mice (Vasudeva and Sharma, 2007 & Shibeshi *et al*., 2006 (a). Achyranthes aspera a potent immunostimulating plant for traditional medicine (Srivastava, 2014).

Keeping in view the economic losses due to subclinical endometritis faced by farming community & lack of cost effective treatment for subclinical endometritis the present study was designed with the following objective.

OBJECTIVES

1. To study the prevalence of subclinical endometritis in post partum dairy cows in and around Akola city.
2. To evaluate the therapeutic efficacy of *Azadirachta indica* and *Achyranthes aspera* in Subclinical endometritic cows.
3. To study the efficacy of *Azadirachta indica* and *Achyranthes aspera* on conception rate in subclinical endometritic cows.
4. To study the hematological and biochemical parameters in subclinical endometritic cows.

CHAPTER II

REVIEW OF LITERATURE

2.1 Subclinical Endometritis

Subclinical endometritis is inflammation of the endometrium, which is characterised by an elevated population of PMN cells (Sheldon *et al.*, 2009) with no systemic signs of illness or clinical infection (Barlund *et al.*, 2008). Subclinical endometritis and clinical endometritis may actually represent different manifestations of reproductive tract disease and inflammation, rather than a difference in severity of the same disease (Dubuc *et al.*, 2010).

Polymorphonuclear cells (PMN) are predominant inflammatory cells found in intrauterine fluid accumulations and they are a good indicator of the stage and the degree of uterine inflammation hence they are used to diagnose subclinical endometritis (Barlund *et al.*, 2008). These cells are a first line of defence against invading pathogenic organisms and are the part of the body's innate immune. Thus, when uterine infection or damage occurs, there is an influx of PMN into the uterine lumen (Kasimanickam *et al.*, 2004).

Uterine cytology is nothing but the collection and counting of cells obtained from the uterus and has become the standard to which other techniques are compared (Barlund *et al.*, 2008; Dubuc *et al.*, 2010). The cytobrush method and the uterine flushing/lavage method are two cytological techniques commonly used to diagnose subclinical endometritis (Kasimanickam *et al.*, 2005). Briefly, in cytobrush method cytobrush is passed through the cervix and rolling against endometrium is performed; then the cytobrush is rolled on a slide for microscopic evaluation to determine the proportion of cells that are PMN within the sample (Kasimanickam *et al.*, 2004; Barlund *et al.*, 2008 and McDougall *et al.*, 2009). In uterine flushing method, with the help of saline soloution the uterine lumen is flushed which is recovered and examined by microscopy for the proportion of cells that are PMN within the sample (Gilbert *et al.*,

2005). Kasimanickam *et al.* (2005) from his study concluded that the cytobrush technique is a more consistent and reliable technique than uterine lavage.

Kasimanickam *et al.* (2004) with the help endometrial cytology found subclinical endometritis by in clinically healthy dairy cows and described a prevalence of 35% and 34% for 20 to 33 day postpartum and 34 to 47 day postpartum, respectively.

Kasimanickam *et al.* (2005) performed comparision between cytobrush and lavage techniques in clinically normal postpartum dairy cows for the assessment of endometrial cytology (EC). The EC samples were collected from Holstein cows (n = 35) during visit 1 (V1) at 20 to 33 day in milk (DIM) and 2 week later during visit 2 (V2) at 34 to 47 DIM by using both techniques.100 (minimum) neutrophils (% PMN) cells were counted to determine the percentage. The mean %PMN was significantly different between the techniques at V1, but not at V2. The overall % PMN cells decreased with time postpartum, but not within V1. The mean %PMN was not influenced by the volume of fluid recovered in successful attempts, but 17% (12/70) of attempts yielded no fluid. From this study they concluded that the cytobrush technique is a consistent and reliable method for obtaining endometrial samples for cytologic examination from postpartum dairy cows.

Ahmadi *et al.* (2006) reported comparative cervical mucus cytology on days 25 to 30 and days 55 to 60 postpartum by gentle suction pipette and 50 ml syringe. By staining with geimsa stain differential cellular count carried out. They revealed that there were no singnificant difference between cell percentages in cows at 25 to 30 days post-parturition and 50 to 60 days post-parturition.

Sheldon *et al.* (2006) observed that the cow with subclinical endometritis had >18 % neutrophils in uterine cytology which was collected on 20-33 days postpartum, or >10 % neutrophils at 34-47 days, or sonographic image revaled the mixed echogenicity fluid within the uterine lumen after 21 days postpartum in the absence of clinical endometritis.

Barlund *et al.* (2008) conducted trial on two hundred and twenty one Holstein cows from eight commercial dairy herds for endometritis between 28 and 48 days postpartum using five diagnostic techniques:(1) ultrasonographic assessment of uterine fluid volume; (2) vaginoscopy; (3) ultrasonographic assessment of endometrial thickness; (4) endometrial cytology corrected by cytobrush and (5) endometrial cytology corrected by uterine lavage. Correlation was used to evaluate the reliability of cytobrush and lavage cytology. Cytobrush cytology was found to have the greatest intraobserver repeatability and was chosen as the reference diagnostic test. They also reported mean days to first service in endometritic positive cows was 77.77 days and in endometritic negative cows was 79.6 days.

Galvao *et al.* (2009a) studied a subclinical endometritis ≥5% PMN cells in endometrial cytology by low volume uterine lavage method.

Galvao *et al.* (2009b) performed endometrial cytology by using uterine lavage technique for diagnosis of subclinical endometritis (SCE) at 21, 35 and 49 days in milk (DIM) in total 406 Holstein cows from 5 herds and reported that the percent PMN cells were ≥8.5%, ≥6.5% and ≥4.0% and also stated that there was increase in interval for pregnancy at 35 days in milk and 49 days in milk as compared to 21 Days in milk in SCE cows.

Hasan *et al.* (2009) stated the comparision between the diagnostic value of the vaginoscopy, cytobrush technique, and transrectal ultrasonography methods for the diagnosis of postpartum endometritis in Brown swiss cows. He examined cows in 45-180 days of postpartum for the endometrial cytology (EC) examination, the presence of ≥5% PMN cells were regarded as positive for the diagnosis of endometritis with cytobrush technique. The slides were prepared for cytological examination by rolling the cytobrush onto a clean glass slide, air dried and it was fixed in methanol for 2 minutes followed staining with giemsa stain. Then 100 cells were counted at 1000x magnification, cytological assesment was done to determine percentage of neutrophils. This study concluded that endometrial cytology could be used in cows safely and effectively for the diagnosis of the infertility perticularly in case of subclinical endometritis. They also

stated that there was no significant difference between clinical diagnoistic method and cytobrush technique (p= 0.658).

Yavari *et al.* (2009) performed the comparative study between cervical and uterine cytology between different classifications (clear mucus with flakes of pus, mucopurulent discharge and purulent discharge with or without palpable contents in uterus) of postpartum endometritis. Total 402 postpartum dairy cows from 13 commercial dairy herds were examined once between 21 and 35 days postpartum and 86 cows with postpartum endometrtis were sampled. The cytology done by cervical aspiration and stained with modified giemsa method. With three classifications they couldn't found any significant differences between neutrophils percentages of cervical mucosa and uterine fluid smear in cows.

Burke *et al.* (2010) proved relationships between endometritis during the calving, transition and early lactation periods. A subset of mixed age and breed dairy cows (n = 78) from a seasonal, pasture-grazed herd of 389 cows were examined. The selected cows were grouped as having endometritis at day 42 postpartum or being unaffected by endometritis. Endometritis was defined as >6% (upper quartile) of uterine nucleated cells being polymorphonuclear cells (High-PMN; n = 38); unaffected by endometritis was defined as ≤1% of nucleated cells being polymorphonuclear (Low-PMN; n = 40).

Dubuc *et al.* (2010) compared diagnostic criteria for postpartum endometritis in dairy cows. Total 1,044 Holstein cows (6 herds) enrolled in a randomized clinical trial were used. Cows were examined for endometritis at 35 ± 3 day (exam 1) and 56 ± 3 day (exam 2) after parturition, using cytobrush technique, vaginal discharge scoring and cervical diameter measurement .Reproductive data were recorded until 200 day after parturition. At exam 1, diagnostic criteria were ≥6% polymorphonuclear cells and mucopurulent or worse (purulent or foul) vaginal discharge for cytological and clinical endometritis, respectively. At exam 2, diagnostic criteria were ≥4% polymorphonuclear cells and mucopurulent or worse vaginal discharge for cytological and clinical endometritis, respectively. Cows were classified as having cytological endometritis only, clinical endometritis only, or both cytological and clinical

endometritis. The detrimental effects of cytological and clinical endometritis on reproductive performance were additive. Among cows with clinical endometritis, only 38 and 36% had cytological endometritis at exam 1 and exam 2, respectively. Overall, these results suggested that cytological and clinical endometritis may represent different manifestations of reproductive tract disease.

Dourey *et al.* (2011) observed the relationships between endometrial cytology and interval to first ovulation, and a pregnancy in primiparous and multiparous postpartum dairy cows in a single herd. On day 25 postpartum, 39 dairy cows were grouped based on endometrial cytology, as having low (≤8%) or high (>8%) polymorphonuclear cells (PMN), and the quantity of uterine fluid was assessed by ultrasound. They reported that the interval from calving to first ovulation was shorter in low, than in high PMN cows (32 vs. 45 day). A greater proportion of cows with uterine fluid had high PMN (64% vs. 21%), and the PMN increased from 14% to 34% as the quantity of uterine fluid increased. The longest mean interval from calving to ovulation was in primiparous cows with high PMN (49 day) compared to that of primiparous and multiparous cows with low PMN (28 and 29 day, respectively).

Deguillaume *et al.* (2012) found the effect of endocervical inflammation on reproductive performance. The specimens of endocervical and endometrial cytological were collected from 168 Holstein cows between 21 and 60 days in milk (DIM) to investigate the prevalence of endocervical inflammation and effect on days to conception. Statistical analyses were stratified based on DIM at examination (<35 vs. ≥35 DIM). Endocervical inflammation with ≥5% neutrophils before 35 DIM (disregarding the level of endometrial inflammation) was associated with decreased hazard of pregnancy within 300 DIM (adjusted hazard ratio, 0.4; 95% confidence interval: 0.3–0.8). A decrease in hazard of pregnancy was observed when >6% neutrophils were counted in endometrial smears (adjusted hazard ratio, 0.4; 95% confidence interval: 0.2–0.7). The study suggested an additive effect of combined endocervical and endometrial inflammation on the hazard of pregnancy within 300 DIM. Using the thresholds of 5% neutrophils for the cervix and 6% neutrophils for the uterus, 11% of the cows examined before 35 DIM presented cervicitis only,

13% were affected by endometritis only, and 32% suffered from both endometrial and endocervical inflammation. This study showed that in addition to uterine inflammation, endocervical inflammation in early lactation affects conception.

Moges and Jebar (2012) observed the prevalence of subclinical endometritis in clinically normal postpartum dairy cows and measured the effect of subclinical endometritis on pregnancy. By rectal palpation the reproductive tracts of selected cows were examined and subclinical endometritis was diagnosed by endometrial cytology on postpartum dairy cows from 30-60 days. With 5% neutrophil on endometrial cytology was taken as positive for SCE.

Prieto *et al.* (2012) determined that the validation of a simple method for the interpretation of uterine cytology in cows. 260 endometrial samples were taken from Holstein cows at different postpartum stages using an adapted cytobrush. Smears were air-dried for fixing and stained with a Romanowsky-type procedure. To evaluate the counting method, the percentage of PMN in 150 cells was calculated as well as the average number of PMN in 10 fields at 1000x. A pearson correlation between both procedures was carried out. The cut-off points corresponding to 5% PMN counts for subclinical endometritis diagnosis were determined using the receiver operating characteristic (ROC) curve. For 5% cut-off value 0.52 PMN/field and area under curve was 0.99. Sensitivity and specificity were 100% and 95.3%, respectively. These results show that this simple method could be used to determine the percentage of PMN in endometrial cytological samples and to diagnose endometritis in cows.

Madoz *et al.* (2013) studied the effect of the stage of estrous cycle on the percentage of (PMN) cells obtained by cytobrush to determine cut off values for the diagnosis of subclinical endometritis under pastoral conditions and to measure the prevalence of subclinical endometritis 21 to 62 days in milk (DIM) also to evaluate the effect of subclinical endometritis on reproductive performance in grazing dairy cows. They observed that the cut off values for the diagnosis of subclinical endometritis in grazing dairy cows are 8% PMN for 21 to 33 DIM, 6% PMN for 34 to 47 DIM, 4% PMN for 48 to 62 DIM, and overall 5% PMN for 21 to 62 DIM. Similarly they

reported that the percent of PMN cell did not vary with the day 0, 4, 11 and 18 of oestrus cycle.

Senosy and Osawa (2013) compared the relationship between metabolic traits and occurrence of subclinical endometritis in 65 multiparous dairy cattle's in cold temperate and hot season. SCE was diagnosed by brush cytology on day 40 ± 2 of lactation and cows having polymorphonuclear cell percentage ≥5 were considered subclinical endometritis positive. Cytological evaluation (brush cytology) was used to estimate the polymorphonuclear cell percentage (PMN %) by counting a minimum of 200 PMN and endometrial cells at 400x magnification for quantitative assessment of endometrial inflammation. Mononuclear cells (lymphocytes and macrophages) were not included in counting. Cows having ≥5% PMN were considered to have subclinical endometritis, whereas cows with <5% PMN were considered to have a healthy uterus. They also stated that the percent of PMN tended to be greater in cows with subclinical endometritis during the temperate season than during the hot season.

Carneiro *et al.* (2014) stated the incidence of subclinical endometritis from 32 to 70 days in milk (DIM) and its effects on the reproductive performance of crossbred dairy cows. Total 172 lactating cows (Holstein/Gir) with no history of retained placenta, without clinical signs of uterine infection were used. The endometrial cytobrush technique was used for diagnosis of subclinical endometritis. The samples were collected stained with giemsa technique and examined microscopically; positive cases for subclinical endometritis were considered with the presence of ≥5 % of neutrophils.

Madoz *et al.* (2014) followed uterine biopsy and endometrial cytology for the diagnosis of subclinical endometritis in grazing dairy cows at 35 to 59 days in milk. 200 cells per smear were counted to determine the percentage of polymorphonuclear neutrophilic leukocytes (PMNL). Cut-off values used were ≥8% PMNL at ≤33 DIM, ≥6% PMNL at 34 to 47 DIM, and ≥4% PMNL at ≥48 DIM. Similarly also stated that cows having clinical endometritis were negative for subclinical endometritis according to cytological evaluation.

Melcher *et al.* (2014) consider the degree of variation in the percentage of endometrial cells and PMN cells determined by six different counting techniques and also evaluated the inter observer reproducibility of the cell counting by two different examiners. 100 samples were examined by the different counting techniques. The applied methods counted a total of 100, 300 or 500 cells (C100, C300, C500), respectively. In addition, they counted 100 and 300 cells in 10 high-power fields (HPF) per slide. Finally, one method estimated (EST) the percentage of PMN by screening the slide under the microscope. The inter observer reproducibility between two examiners was analyzed for method C300. The comparison between the six different methods showed a strong compliance (r ¼ 0.77–0.90) with greatest correlation coefficient between C100 and C300. The results of the inter observer reproducibility showed good correlation and agreement (r ¼ 0.86, k ¼ 0.79). They concluded all methods were suitable for the cytological evaluation of PMN, with method C100 showing lowest agreement with the other methods. A threshold of 5% PMN seems to be useful when C300 and HPF100 are used, whereas counting 100 cells or estimating the percentage of PMN seems to overestimate or underestimate the prevalence of SE, respectively. Lastly they revealed that method C300 and HPF100 can be recommended as methods of choice for evaluating the percentage of PMN in endometrial samples to diagnose subclinical endometritis.

Barrio *et al.* (2015) investigated the influence of subclinical endometritis (SCE) on the reproductive performance of dairy cows. Total 94 dairy cows of parity 1 to 8, distributed in 25 herds, were examined once between 30 and 45 DIM for endometrial cytology by using cytobrush. If percentage of polymorphonuclear neutrophils was superior to 5% of all cells present in the smear except erythrocytes, endometrial cytology was considered indicative of SCE. They reported that the calving to first insemination interval was 77(64.17- 89.83) in subclinical endometritis.

Moscuzza *et al.* (2015) performed endometrial cytology as a diagnostic tool for subclinical endometritis in beef heifers with assisted calving. For endometrial cytology cytobrush was used. They indicated that the use of a cut-off point higher than 5.5 % PMNs was 95.83% specificity and 78.57% sensitivity for the detection of subclinical endometritis. They

also stated that endometrial cytology is a non invasive technique with high specificity and repeatability for the diagnosis of subclinical endometritis postpartum in dystocic beef heifers.

Singh *et al.* (2016) considered the diagnostic efficacy of uterine cytobrush technique for subclinical endometritis in crossbred dairy cattle. For that a total of 170 repeat breeding crossbred cattle at oestrus were examined for the status of genitalia (through rectal palpation) and cervico-vaginal discharge. For confirmation of subclinical endometritis the cows with clear discharge were further subjected to uterine cytobrush technique i.e. on the basis of presence of ≥ 4 % polymorphnuclear (PMN) cells. They concluded that cytobrush technique is an efficient method and can be considered as cow side test for diagnosis of subclinical endometritis.

2.2 Prevalance of Subclinical Endometritis

Leblanc (2008) found the causes, impact, treatment and prevention of metritis, retained placenta (RP), and endometritis in dairy cows. The occurrence of each of these diseases largely depends on immune function in the transition period. 5–10% of calvings is affected by retained placenta and increases the risk of metritis and endometritis. Clinical endometritis affects 15–20% of cows at 4–6 weeks postpartum; an additional 30–35% has subclinical endometritis between 4 and 9 weeks postpartum.

Green *et al.* (2009) examined the prevalence and identification of systemic markers of subclinical endometritis in post partum pasture fed dairy cows. In these expriement they used two groups of population i.e. G1 43 and G2 46 cows. The uterine cytology performed on day 21 and day 42 respectively. They confiremed subclinical endometritis if the PMN cell is greater than 18 % in endometrial cytology and also they revealed that 16/43 cows (37%) and 3/46 cows (6.5%) were positive for subclinical endometritis on D21 and D42, respectively.

Galvao *et al.* (2009b) studied the prevalence of subclinical endometrites and fertility of dairy cows. A total 406 Holstein cows (167 primiparous and 239 multiparous) from 5 herds were used. Uterine lavage

for diagnosis of SCE, PGF2α treatment, evaluation of body condition scores and collection of blood samples for estrous cyclicity determination were performed at 21, 35, and 46 DIM.Polymorphoneculear cells (PMN) were quantified and thresholds for diagnosing SCE were selected by receiver operating characterstics analysis.

Dubuc *et al.* (2010) studied total 1044 cows for endometritis at 35 ± 3 day (exam 1) at threshold level ≥6% and 56 ± 3 day (exam 2) at threshold level ≥4% after parturition, by endometrial cytology (cytobrush technique), vaginal discharge scoring (Metricheck device), and cervical diameter measurement (transrectal palpation). According to their study they revealed that the overall prevalence of endometritis (cytological and clinical together) at exam 1 and exam 2 were 28.7 and 21.5%, respectively. Prevalence was classified as cytological endometritis only, clinical endometritis only, both cytological and clinical endometritis, which were 13.5, 9.4 and 5.8% for diagnosis at exam 1 and 9.6, 7.7 and 4.2% at exam 2, respectively.

Kaufmann *et al.* (2010) studied in multiparous and primiparous 209 cows for the occurrence of clinical endometritis (CE) and subclinical endometritis (SCE). They applied two cut off limits, 5% polymorphonucliocytes (PMN) and 18% PMN for diagnosis of SCE and reported overall prevalence of CE and SCE was 18.7% and 12.4%. on the basis of 18% PMN prevalence of CE and SCE at 5 week in post-partum primiparous cows were 23.4% and 7.8%, respectively and in multiparous cows 15.9% and 15.2%, respectively.

Plontzke *et al.* (2010) studied the overall prevalence of subclinical endometritis with >5 neutrophil on endometrial cytology was 38%. The prevalence of subclinical endometritis in pregnant heifers, cows with poor hygienic condition, cows used artificial insemination, cows having good body condition score and cows in small scale farms was 100%, 71.4%, 69.8%, 75% and 86.7%, respectively without statistically significant difference ($p>0.05$) among parity, hygienic condition, body condition score, method of service and farm scales, respectively (Moges and Jebar 2012).

Cheong *et al.* (2011) examined on the basis of cow-level and herd-level risk factors for subclinical endometritis in primiparous and multiparous lactating Holstein cows. To obtain prevalence estimates for subclinical endometritis (SCE), determine cow-level and herd-level risk factors and evaluate the reproductive consequences of SCE were the objectives of their study. With low-volume uterine lavage of Lactating Holstein cows (779) between 40 and 60 days in milk samples were taken and SCE status was evaluated by cytology. The cow-level prevalence of SCE was 25.9%. The prevalence of SCE in primiparous cows (20.1%) was similar to that in multiparous cows (26.4%). Herd-level risk factors identified were housing early postpartum cows on bedded packs (herd-level SCE = 36.1%), which increased herd prevalence of SCE by 16.7% (SE 5.58) compared with early postpartum cows housed in freestalls (herd-level SCE = 19.4%) and straw bedding in the calving pen which decreased herd prevalence of SCE by 10.7% (SE 3.59) compared with herds that used other bedding material.

Dourey *et al.* (2011) performed endometrial cytology in 39 cows (16 primiparous and 23 multiparous cows) on day 25 postpartum by cytobrush technique to obtain samples. The cows were distributed equally between the low (≤8%) and high PMN (>8%) groups and a large majority of the primiparous cows had high PMN counts relative to multiparous cows (75% vs. 30%). Study revealed the overall prevalence of subclinical endometritis (based on high PMN) within the study population was 49% (19 of 39) and PMN were completely absent in three (8%) of the 39 cows.

Deguillaume *et al.* (2012) with the threshold of 5% PMN stated, the prevalence of endocervical inflammation was 42% in cows examined before 35 DIM. Endometrial inflammation (SCE) was 45% above threshold of 6% PMN the prevalence. They reported that 71% of cows with endometritis were affected by cervicitis.

Heidarpour *et al.* (2012) calculated the overall incidence of subclinical endometritis and clinical endometritis in the cows were 13.5% and 11.83%, respectively.

Moges and Jebar (2012) confirmed that their was overall prevalence of subclinical endometritis with 5% neutrophil on endometrial cytology with 68.3%. The prevalence of subclinical endometritis in pregnant heifers, cows with poor hygienic condition, cows used artificial insemination, cows having good body condition score and cows in small scale farms was 100%, 71.4%, 69.8%, 75% and 86.7%, respectively without statistically significant difference ($p>0.05$) among parity, hygienic condition, body condition score, method of service and farm scales, respectively.

Lima *et al.* (2013) with the help of endometrial cytology reported the prevalence of subclinical endometritis defined by ≥5% PMN was 29.5%.

Madoz *et al.* (2013) found the prevalence of subclinical endometritis in cow was 21.5% from 21 to 33 DIM at 8% PMN cells, 16% from 34 to 47 DIM at 6% PMN cells, 16 % from 48 to 61 DIM at 4% PMN cells and an overall prevalence of 17% for the period from 21 to 62 DIM.

Ribeiro *et al.* (2013) perform the study on prevalence of periparturient diseases and effects on fertility of calving grazing dairy cows supplemented with concentrates. They use cytobrush technique for diagnosis of SCE at >5 % threshold level of PMN cells and concluded 13.4 % prevalence of subclinical endometritis in dairy cows.

Senosy and Osawa (2013) calculated the prevalence of a cytological diagnosis of endometritis were 7/21 (33.3%), 7/23 (30.4%) and 9/21 (42.9%) during the cold, temperate and hot seasons, respectively. Polymorphonuclear cell percentage (PMN %) tended ($P < 0.1$) to be greater in cows with subclinical endometritis that calved during the temperate season (18.1 ± 15.9%) when compared to those that calved during the hot season (6.3 ± 0.9%).

Carneiro *et al.* (2014) survey the incidence of subclinical endometritis from 32 to 70 days in milk (DIM) and its effects on the reproductive performance of crossbred dairy cows. Lactating cows (Holstein/Gir; n=172), without clinical signs of uterine infection, with no history of retained placenta were used. The presence of ≥5 % of neutrophils was considered as positive cases for subclinical endometritis. Without the

influence of season of calving, presence of corpus luteum, DIM and parity they recorded the incidence of subclinical endometritis in the herd was 26%. They also reported cows with a BCS ≤2.50 had a higher incidence of subclinical endometritis.

Madoz *et al.* (2014) delibrate the overall prevalence of subclinical endometritis on 35 to 59 days in milk with cut off value used were ≥8% PMNL at ≤33 DIM, ≥6% PMNL at 34 to 47 DIM, and ≥4% PMNL at ≥48 DIM and found the overall prevalence of subclinical endometritis was 14%.

Melcher *et al.* (2014) calculated the impact of the different methods on the resulting prevalence of subclinical endometritis. The applied methods counted a total of 100, 300 or 500 cells (C100, C300, C500), respectively. In addition, they counted 100 and 300 cells in 10 high-power fields (HPF) per slide with the greatest prevalence determined by counting 100 cells i.e. C100 (33.0%) and the least by high power field i.e. HPF300 (10.0%).

Barrio *et al.* (2015) plotted the influence of subclinical endometritis on the reproductive performance of dairy cows. Examination was done once between 30 and 45 DIM for endometrial cytology by using cytobrush, in total 94 dairy cows of parity 1 to 8, distributed in 25 herds. If percentage of polymorphonuclear neutrophils was superior to 5% of all cells present in the smear, except erythrocytes endometrial cytology was considered indicative of SE. They observed the prevalence of subclinical endometritis between 30 and 45 DIM was 14.9%.

Dini *et al.* (2015) from 2 commercial dairy herds (farm 1 and farm 2) studied the prevalence of subclinical endometritis in postpartum Holstein cows (N=150) that had calved without any difficulty and had passed a normal puerperal period were sampled for endometrial cytology. A threshold level of 18% was set to diagnose subclinical endometritis. They recorded the mean prevalence of subclinical endometritis at day 30 was 38.5%, while in farm 1 the prevalence was 27% and in farm 2, the prevalence was 47%. They concluded that higher prevalence of uterine inflammation in clinically normal cows in two dairy herds.

Moscuzza *et al.* (2015) prepared the prevalence for subclinical endometritis in beef heifers with assisted calving at 5.5% PMN cells with the help of endometrial cytology by cytobrush technique and observed that 38.9% prevalence of subclinical endometritis among dystocic heifers.

Pothmann *et al.* (2015) consider the prevalence of subclinical endometritis in repeat breeder cows. A total of 121 cows with no clinical signs of disease were enrolled. Intrauterine samples were collected to determine the prevalence of subclinical endometritis with the help of cytobrush technique. There was prevalence of 12.7% in subclinical endometritis in repeat breeder cows.

Ricci *et al.* (2015) calculated subclinical endometritis and consequences on fertility in 97 cows. In the interval from 28 to 68 days post-partum cows were sampled for endometrial cytology once. By uterine lavage method uterine cytology was performed and observed that 31% of the cows were diagnosed as being positive for SEM and as having an 8% neutrophil (PMN) presence on the slide which is considered as the best cut-off to diagnose the pathology.

Bajaj *et al.* (2016) reported incidence of endometritis has been to vary from 2.4 to 20 per cent and 4.5 to 25 per cent in an abattoir study in buffaloes. Studies on clinical and sub-clinical endometritis reported the prevalence of these diseases ranging from 18 to 37 per cent and 12 to 94 per cent respectively. Endometritis delays ovarian rebound and uterine involution, increase days open and accordingly extend the calving interval. It not only causes infertility but also results in sub fertility even after successful clinical resolution of the disease.

Singh *et al.* (2016) considered 170 repeat breeder crossbred dairy cattle at estrus they were examined with the help of cytobrush technique for subclinical and clinical endometritis on the basis of presence of ≥ 4 % polymorph nuclear (PMN) cells. They recorded the incidence of clinical and subclinical endometritis was 21.7 % (37/170) and 29.4 % (50/170), respectively.

Syed Anwar (2016) examined total 83 postpartum cows at threshold level of 5>PMN cells using endometrial cytology (cytobrush technique). He reveled that the overall prevalence of subclinical endometritis was 36.14% and 30 out of 83 cases was positive for subclinical endomertitis.

2.3 *Azadirachta indica*

2.3.1 History and origin

Azadirachta indica is a fast growing evergreen popular tree found commonly in India, Africa and America .It has been used in ayurveda medicine for more than 4000 years due to its medicinal properties. Neem is called arista in Sanskrit a word that means perfect, complete and imperishable. Arishtha is the Sanskrit name of the neem tree meaning 'reliever of sickness' and hence is considered as 'Sarbarogaribarini' .The tree is recognized as the 'village dispensary' in India. The importance of neem tree has been recognized by the US National Academy of Science, which publish a report in 1992 entitled 'Neem – a tree for solving global problems'. (Sharma *et al.*, 2011)

Medicinal plants have received growing attention in the drug discovery process for various human disorders, including cancer. A medicinal plant has been defined by Park and Pezzuto as "a plant that has pharmacological activity to treat disease, as compared with an edible plant that is used in daily life as a food". *Azadirachta indica* A Juss commonly known as neem, has been well known in the Indian subcontinent for more than 2000 years, as one of the most versatile medicinal plants possessing a wide spectrum of biological activities. (Subapriya and Nagini, 2005)

2.3.2 General information

Plant description

Leaves: pinnate 20–40 centimeters (7.9–15.7 in) long, with 20 to 31 medium to dark green leaflets about 3–8 centimeters (1.2–3.1 in) long. The terminal leaflet often is missing. The petioles are short.

Flowers: They (white and fragrant) are arranged in more-or-less drooping axillary panicles which are up to 25 centimeters (9.8 in) long. The inflorescences, which branch up to the third degree, bear from 150 to 250 flowers. An individual flower is 5–6 millimeters (0.20–0.24 in) long and 8–11 millimeters (0.31–0.43 in) wide. Protandrous, bisexual flowers and male flowers exist on the same individual tree.

Fruit: smooth (glabrous), olive-like drupe which varies in shape from elongate oval to nearly roundish, and when ripe is 1.4–2.8 centimeters (0.55–1.10 in) by 1.0–1.5 centimeters (0.39–0.59 in). The fruit skin (exocarp) is thin and the bitter-sweet pulp (mesocarp) is yellowish-white and very fibrous. The mesocarp is 0.3–0.5 centimeters (0.12–0.20 in) thick. The white, hard inner shell (endocarp) of the fruit encloses one, rarely two, or three, elongated seeds (kernels) having a brown seed coat.

2.3.3 Scientific taxonomic classification:

Kingdom	:	Planate
Subkingdom	:	Tracheobionta
Superdivision	:	Spermatophyta
Division	:	Maonoliophyta
Class	:	Magnoliopsida
Subclass	:	Rosidae
Order	:	Sapindales
Family	:	Meliaceae
Genus	:	Azadirachta
Species	:	indicia

2.3.4 Common names

Hindi	:	Neem
Bengali	:	Nim, Nimgachh
Konkani	:	Beva-rooku
Marathi	:	Kadunimb
Gujarati	:	Leemdo
Tamil	:	Vembu, Vempu

Punjabi	:	Nimb
Malayalam	:	Veppu, Aryaveppu, Aruveppu, Kaippan, Veppu,
Oriya	:	Nimo
Telegu	:	Vepa
Kannada	:	Bevinmar, Kahibevu
English	:	Margosa, Neem
Indian Lilac French	:	Azarirae d'lnde, Margousier
German	:	Indischer Zadrach
Persian	:	Azade Darakhte
Latin	:	Azadirachta indica A. Juss or Melia azadirachta
Singapore	:	Kohumba, nimba
Indonesia	:	Mimba
Nigeria	:	Don goyaro
Spanish	:	Margosa
Nepal	:	Nim
Portuguese	:	Margosa, Nimbo

2.3.5 <u>Active principle /compound:</u> azadichtrin, nimbin, gedunin, Gallic, catechin, NB- II peptidoglycan

Bandyopadhyay *et al.* (2002) studied the antisecretory and antiulcer effects of aqueous extract of Neem (Azadirachta indica) bark along with its mechanism of action, standardisation and safety evaluation. The extract can dose dependently inhibit pylorus-ligation and drug (mercaptomethylimidazole)-induced acid secretion with ED50 value of 2.7 and 2 mg Kg 1 b.w. respectively. It is highly potent in dose-dependently blocking gastric ulcer induced by restraint-cold stress and indomethacin with ED50 value of 1.5 and 1.25 mg Kg_ 1 b.w. respectively. When compared, bark extract is equipotent to ranitidine but more potent than omeprazole in inhibiting pylorus-ligation induced acid secretion. In a stress ulcer model, it is more effective than ranitidine but almost equipotent to omeprazole. Bark extract inhibits H+- K+-ATPase activity in vitro in a concentration dependent manner similar to omeprazole. It offers gastroprotection against stress ulcer by significantly preventing adhered

mucus and endogenous glutathione depletion. It prevents oxidative damage of the gastric mucosa by significantly blocking lipid peroxidation and by scavenging the endogenous hydroxyl radical (SOH)-the major causative factor for ulcer. The S OH-mediated oxidative damage of human gastric mucosal DNA is also protected by the extract in vitro. Bark extract is more effective than melatonin, vitamin E, desferrioxamine and a-phenyl N-tert butylnitrone, the known antioxidants having antiulcer effect. Standardisation of the bioactive extract by high pressure liquid chromatography indicates that peak 1 of the chromatogram coincides with the major bioactive compound, a phenolic glycoside, isolated from the extract. The pharmacological effects of the bark extract are attributed to a phenolic glycoside which is apparently homogeneous by HPLC and which represents 10% of the raw bark extract. A single dose of 1g of raw extract per kg b.w. (mice) given in one day and application of 0.6g raw extract per kg b.w. per day by oral route over 15 days to a cumulative.

Subapriya and Nagini (2005) evaluated more than 140 compounds from different parts of neem. All parts of the neem tree- leaves, flowers, seeds, fruits, roots and bark have been used traditionally for the treatment of inflammation, infections, fever, skin diseases and dental disorders. The medicinal utilities have been described especially for neem leaf. Neem leaf and its constituents have been demonstrated to exhibit immunomodulatory, anti-inflammatory, antihyperglycaemic, antiulcer, antimalarial, antifungal, antibacterial, antiviral, antioxidant, antimutagenic and anticarcinogenic properties. This review summarises the wide range of pharmacological activities of neem leaf.

Rathod *et al.* (2012) proved to access the susceptibilities from aqueous and ethanol extracts of Neem (leaves and bark) and Tulsi (leaves) against some clinically significant bacterial species. Antimicrobial activity has been done by disc diffusion method against two Gram positive bacteria (Bacillus subtilis and Staphylococcus aureus) and two Gram negative bacteria (Klebsiella pneumoniae and Escherichia coli). The ethanolic extract of Neem bark showed more significant activity at 250 µg concentrations than Neem leaves at all the studied concentrations. E. coli was highly susceptible to ethanol extract of Tulsi leaves at 250 µg concentration. Most bacterial strains were resistant at lowest

concentrations (5 µg) of various extracts of both plants. The study indicates that Neem bark was found to possess more significant antibacterial activity than Neem leaves and Tulsi leaves.

Amit Kumar *et al.* (2013) conducted an experiment to investigate the immunomodulatory and therapeutic efficacy of neem on endometritis in repeat breeding crossbred cows. Twenty four cows were selected on the basis of history, breeding records, transrectal examination, white side test and randomly divided into 3 groups (Group A: 30 ml normal saline; Group B: 30 ml hydro-alcoholic neem bark; Group C: 30 ml hydro-acetonic neem bark). Treatments were given intrauterine beginning on the day of estrus for seven days in each group. Cervical mucus samples were collected on the day of estrus before treatment and at subsequent estrus after treatment and tested for appearance, pH, white side test and bacterial load. Blood samples were collected on the day of treatment and 24 hr after treatment and analyzed for glucose, Hb, PCV, TLC and DLC to know health status of the experimental animals. Uterine flushings were collected on the day of estrus before treatment and again on eighth day of first collection i.e. 24 hr after last treatment. These flushings were used for the estimation of total protein, immunoglobulin, TLC and polymorphonuclear cells (PMNs). At subsequent standing estrus following treatment, all cows were artificially inseminated twice using frozen semen 12 h apart. Pregnancy was confirmed transrectally 45-60 days after insemination. Significant ($p<0.05$) decline in pH and bacterial load was observed in cervical mucus of the groups after treatment. Hemoglobin, neutrophils, lymphocytes and WBC were increased significantly ($p<0.05$) in both hydro-alcoholic and hydro-acetonic extract treated groups. Moreover, RBCs and glucose were increased in hydro alcoholic extract treated group. Significant ($p<0.05$) rise was found in TLC, PMN and immunoglobulin concentration in both the treated groups. Although both the extracts led to improved clinical recovery and conception rates, the hydro-alcoholic extract was more effective. Based on these results, it may be concluded that hydro-alcoholic extract of the neem has a better antibacterial and immuno-modulation and can be used as a therapy for endometritis in repeat breeding crossbred cows.

Harendra Kumar *et al.* (2013) studied the effects of intrauterine infusion of methanol fraction of neem oil and neem seed powder in treatment of endometritis in cows. A total of 46 crossbred cows with endometritis were randomly divided into three groups. Animals of group 1 (n=14) received 25 ml methanolic fraction of neem oil diluted with groundnut oil (1:1 ratio) intrauterine twice at 24 hours interval after onset of estrus. Likewise, the animal of group ll (n=16) were administered with methanolic extract of neem seed powder diluted with groundnut oil (1:1 ratio)following similar dose and schedule. Whereas, the control cows (III) were administered with groundnut oil at similar times. Efficacy of both neem preparations was assessed by white side test (color reaction to cervico–vaginal mucus) and bacterial load at subsequent estrus. It was observed that the administration of neem preparations retrieved the cows from endometritis; majority (100 % in I and 62.5% in II) showed negative to whiteside test. There was a significant decrease in bacterial load in animal treated with neem–oil (96.02±2.02%) and seed–powder fraction (98.70±0.46%) compared to control (24.97±29.64 %).

Raut *et al.* (2014) evualated antimicrobial activity of Azadirachta indica was against gram negative pathogenic bacteria (Escherichia coli, Salmonella typhi and Vibrio cholerae) and gram positive bacteria (Bacillus subtilis). Azadirachta indica leaves and barks were collected from the fields of Botanical Garden in Padmashri Vikhe Patil College, Pravaranagar and pure cultures of the test organisms used for antimicrobial study were obtained from the Dept. of Microbiology, Pravara Institute of Medical Sciences, Loni. All the test organisms were screened for their antibacterial activity against leaf and bark extract of A. indica by agar well diffusion method. Leaf and bark extract of Azadirachta indica showed more inhibition zone against Vibrio cholerae and Bacillus subtilis, while E. coli and S. typhi are less susceptible to neem extract.

2.4 *Achyranthes aspera*

2.4.1 History and Origin

According to Ayurvedic and Chinese medicines, red and white are the two varieties of A. aspera mentioned. In Sanskrit Achyranthes aspera is described as as a rough flowered stalk. It is described in 'Nighantas' as pungent, purgative, digestive and a remedy for inflammation of the internal organs, itch, piles, abdominal enlargements and enlarged cervical glands. The diuretic property of the plant was well known to the natives of India and European physicians. Various plant parts form ingredients in many native prescriptions were used in combination with more active remedies.

The plant is available globally as a medicinal weed in Baluchistan, Ceylon, Tropical Asia, Africa, Australia and America. It is reported as an invasive alien species in northern Bangladesh. It is also found to be the most prevalent weed in Himachal Pradesh, Shivbari and an exotic medicinal herb of district, India and Lalitpur (Uttar Pradesh). Throughout India A. aspera is found in field boundaries, road sides and waste places as a medicinal herb (Sharma and Chaudhary 2015).

2.4.2 General Information

Plant description:

Growth form: perennial hair herb up to 1.2 m tall.

Stem: Stems are 4 sided and covered in short hairs.

Foliage: Green, papery leaves (1.5-7 cm long, 0.4-4 cm wide) are broadly obovate (egg-shaped) or elliptic-oblong (oval- elongated). They are hairy on both sides.

Fruits: Dry, indehiscent fruit known as a utricle is bladder- like and covered by loose, papery tissue. Each egg-shaped fruit (2.5-3mm long) contains 1 brown, egg-shaped seed (2mm long).

Flowers: Flowers are arranged in a 10-30 cm long spike inflorescence which is initially erect, but later bends backwards after the flowers bloom.

2.4.3 Scientific taxonomic classification

Kingdom	:	Plantae
Subkingdom	:	Tracheobinota
Super Division	:	Spermatophyta
Division	:	Mangoliophyta
Class	:	Mangoliophsida
Subclass	:	Caryophyllidae
Order	:	Caryophyllales
Family	:	Amaranthaceae
Genus	:	Achyranthes
Species	:	aspera

2.4.4 Common names of *Achyranthes aspera*

English	:	Prickly chaff flower, Hawai chaff flower.
Hindi	:	Latjira, Chirchira, Lamchichra, Sonpur, Onga.
Marathi	:	Aghada, Pandhara-aghada.
Sanskrit	:	Aghata, Apamargah, Mayoorah, Markatapippalee,
Arabian	:	Atkumah, Na´eem, No´eim, Mahout, Wazer (Yemen)
Ayurvedic	:	Apamarga, Chirchita, Shikhari, Shaikharika
Bengali	:	Apaang.
French	:	Achyranth a feuilles rudes, Collant, Gendarme
Gujarati	:	Safad Aghedo, Anghadi, Andhedi, Agado.
Indonesia	:	Jarong.
Kannad	:	Uttaranee, Utame.
Latin	:	A. aspera

Gambhir *et al.* (1965) executed pharmacological study of Achyranthes aspera linn. They used alcoholic and aqueous extracts of pulverised roots of the plant. The aqueous extract was concentrated to contain 2.0 gm. of the crude drug in 1.0 ml of the final extract. The soloution was labelled as A. By shaking with petroleum ether the alcoholic extract was defatted and water soluble fraction of the alcoholic extract was

prepared. Each ml of the water soluble fraction of the alcoholic extract contained the active principle present in 1.0 gm. of crude drug. It was labelled as solution 'B'. Effect of the drug was observed in isolated preparations of rabbit's intestine and gravid and non gravid uteri of albino rats, guinea-pigs and rabbits and observed that the solution A and B increased the tone and amplitude of contractions in gravid and non-gravid uteri of albino rats, guinea-pigs and rabbits. Gravid uterus was found to be more sensitive to these drugs than the non gravid uterus. The onset of action was usually after a latent period of few minutes, but the action was prolonged lasting for 10 to 55 minutes. Atropine could not block the ecbolic action of these drugs. LD50 of solution A as calculated by Karber's (1937) method was found to be 7.16 ml/kg.

Vasudeva *et al.* (2002) observed that the extract of Achyranthes aspera was found to enhance the induction of ovalbumin (OVA) specific humoral antibody response in mice, on intraperitoneal injection of extract along with OVA. By passive cutaneous anaphylaxis (PCA) and ELISA for IgE and other classes or subclasses of antibodies, respectively the antibody response was evaluated. A significant elevation of IgM, IgG1 and IgG3 antibodies was observed ($p < 0.01$); however, the anti-OVA PCA titers were suppressed. The adjuvant property of the extract was further examined in different strains of mice and they found a significant elevation of the OVA-specific IgG antibody response in all strains. When the extracts of different parts of the herb were tested, the seed and root extracts appeared to exhibit relatively higher activity. On the basis of results they confirmed the immunostimulatory properties of Achyranthes aspera.

Gathogo (2006) noted significant oxytocic activity with the methanol extracts of Achyranthes aspera on the uterus of guinea pigs.

Naidu *et al.* (2006) inspected the successive extracts of Achyranthes aspera herb parts for invitro antimicrobial activity against Staphylococcus aureus, Bacillus subtilis, Escherichia coli and Salmonella typhi by disc diffusion method. For extraction different solvents such as methanol, acetonitrile, chloroform and n-hexane were selected. As standard drugs, Ciprofloxacin and Clotrimaxazole 1000 µg/ ml were used. The growth of Staphylococcus aureus, Salmonella typhi and Bacillus subtilis was inhibited by methanol extracts of Achyranthes aspera powder

with inhibition zone diameter of 9 mm, 7 mm and 9 mm, respectively. Chloroform extract of Achyranthes aspera plant parts was found to inhibit S. aureus, S. typhi with zone diameter of 6 mm (leaves), and 6 mm (inflorescence), respectively. N-hexane extract showed the growth inhibition against S. aureus, E. coli and B. subtilis with 6 mm (stem), 8 mm (inflorescence) and 6 mm (whole plant), respectively.

Shibeshi *et al.* (2006a) observed phytochemical, contraceptive efficacy on some indicators for anti-fertility activities and safety evaluations of crude extracts of Achyranthes aspera. The anti-fertility activity of the methanolic extract of the leaves of Achyranthes aspera was determined by the number of implantation sites in both horns of uterus and the number of litters after completion of one gestation period in rats. The effect of the extract on the length of oestrous cycle and the weights of ovary and uterus/100 gm of body weight of the animal was also evaluated. Phytochemical screening revealed the presence of known anti-fertility principles such as phytosteroids, polyphenols and saponins. The methanolic leaf extract reduced significantly ($p< 0.05$) the number of litters and implantation sites in rats. The extract prolonged oestrous cycle, oestrous and met oestrous phases ($p < 0.05$) of rats. The weight of ovary was reduced, but that of uterus was increased ($p < 0.05$). The oral LD50 of the extract was found to be 9.7 gm/kg in mice.

Shibeshi *et al.* (2006b) studied the effect of methanolic leaves extract of Achyranthes aspera on some indicators for anti-fertility activities such as abortifacient, estrogenesity, pituitary weight, ovarian hormone level and lipids profile in female rats. The extract showed significant ($p<0.05$) abortifacient activity and increased pituitary and uterine wet weights in ovarectimized rats. The extract, however, did not significantly influence serum concentration of the ovarian hormones and various lipids except lowering HDL at doses tested.

Vasudeva and Sharma (2007) reported the estrogenic and pregnancy interceptory effect of Achyranthes aspera root for screening of antifertility activity in female albino rats. For that four successive solvent extracts of the root were used in albino rats. The chloroform and ethanol extracts exhibited 100% anti-implantation activity and estrogenic activity when given orally at 200 mg/kg body weight. Histological study confirmed

the estrogenic study. This study concluded that the anti-implantation activity may be due to estrogenic activity, causing the expulsion of ova from the tube.

Kumar *et al.* (2009) give the oral administration (50, 100, and 200 mg/kg) the alcoholic extract of Achyranthes aspera of in wistar rats and investigated the anti-inflammatory potential of the plant. This was done using the carrageenan-induced paw edema method (acute inflammatory model) and cotton pellet granuloma test (chronic inflammatory model). The alcohol extract showed significant suppressed granuloma formation. They observed promising anti-inflammatory activity against both acute and chronic inflammation.

Amrutia *et al.* (2011) calculated the potency of anti-inflammatory activity of different fractions of ethanolic extract of Achyranthes aspera leaves. For screening Carrageenan induced rat paw oedema method was used. Ethanolic, ethyl acetate and hexane fraction was screened among which ethyl acetate fraction was found to be most potent one with percentage inhibition of 50, 74, 84, 86% at 1st to 4th hour, respectively. By this experiment it seems that leaves of Achyranthes aspera can be used for the treatment of acute inflammation.

Dey (2011) stated that Achyranthes aspera L. (Amaranthaceae) has long been used in different systems of medicine in the treatment of cancer, leprosy, asthma, fistula, piles, arthritis, wound, insect and snake bite, dandruff, hepatitis, renal disorders, dermatological disorders, gynecological disorders, gonorrhea, malaria, fever, cough, diabetes, pyorrhea, dysentery, opthalmia, rabies, hysteria, toothache etc.It has also been used as antimicrobial, immunostimulant, hypoglycemic, hypolipidemic, anti-inflammatory, antioxidant, diuretic, cardiac stimulant, antihypertensive, anti-anasacra, analgesic, antipyretic, antinoiceptive, prothyrodic, antispasmodic and hepatoprotective.

Hossain *et al.* (2013) investigated antibacterial and anti-oxidant activity of an important medicinal plant Achyranthes aspera methanolic leaf extract. By disc diffusion method anti-bacterial activity was performed. Significant susceptibility was observed against gram positive bacteria than gram negative strains. Anti-oxidant activity was studied in

terms of 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging potential with ascorbic acid as standard. Highest DPPH scavenging activity for A. aspera and ascorbic acid was 59.21% and 92.41%, respectively. IC50 value was 472.93 µg/ml for A. aspera and 1.4965 µg/ml for ascorbic acid.

Sharma *et al.* (2013) calculated minimum inhibitory concentration values of inflorescence and leaves extracts of A. aspera against various pathogenic strains of bacteria i.e. Escherichia coli, Bacillus cereus, Staphylococcus epidermis, Shigella flexineri and Pseudomonas aeruginosa. Increasing the dilution from the stock solution i.e. 100 µg/ml, in order to 2 times i.e. 50 µg/ml, 4 times i.e. 25 µg/ml and 8 times i.e. 12.5 µg/ml, the concentration of active antimicrobial compound was found to be decreased. Decreased absorbance with varying concentrations of plant extracts indicated that minimum concentration of various plant extract is enough to inhibit the growth of bacteria. For both plant parts, all the extracts have MIC values ranges from 12.5-100 µg/ml for various pathogenic strains.

Reddy and Kamble (2014) considered the acute and sub acute toxicity studies for the Achyranthes aspera L methanol extract as per OECD guideline in Swiss mice. The dose of 100, 200, 250 mg/kg body weight methanol extract by administering drug intraperitoneally and recorded the growth, body weight, organ weight, general symptoms, morphological and physiological behavior, mortality. These parameters on various organs system in mice were studied. It was considered necessary to assess its potential health hazard in man and to find the safe and effective dose. The observations of changes in body weight, food and water intake as well as cage side observations were reported. There was no abnormality observed in all groups. The whole plant powder methanol extract of Achyranthes Aspera L were found to be nontoxic.

Syed Anwar (2016) studied the effect of methanolic leaves extract of Achyranthes Aspera of subclinical endometrities cows. He use the extract for three consiqutive days in SCE cows .the therapeutic effaacy he found was 66.66%

2.5 Conception Rate

Barlund *et al.* (2008) evaluated a comparison of diagnostic techniques for postpartum endometritis in dairy cattle and reported endometritis-positive cows had a 17.9 percentage point decrease in probability of pregnancy at first insemination, and had a tendency to require more services per conception than endometritic negative cows. Time to first service was not significantly different between endometritis-positive cows (77.7 DIM) and endometritis-negative cows (79.6 DIM). Cows with endometritis were one-third as likely to be pregnant at first service as cows negative for endometritis on cytology. They also reported that the probability of pregnancy at first insemination was 14.2% in endometritis positive cows 32.1 in endometritis negative cows.

Sheldon *et al.* (2009) found that subclinically disease animals have more days open therefore they take longer time to conceive, have lower conception rates and are culled more than normal animals. Conception rates are half that of normal animals.

Burke *et al.* (2010) observed relationships between endometritis and metabolic state during the calving transition and early lactation periods. 389 cows were examined. The selected cows were grouped as having endometritis at day 42 postpartum or being unaffected by endometritis. Endometritis is nothing but >6% of uterine nucleated cells being polymorphonuclear cells (H-PMN i.e. High-PMN; n = 38); unaffected by endometritis was defined as ≤1% of nucleated cells being polymorphonuclear (L-PMN i.e. Low-PMN; n = 40). According to their study first service conception rate was 31% in endometritic cows (H-PMN) and 54% in unaffected cows i.e. (L-PMN).

Dourey *et al.* (2011) observed no significant difference between low (≤ 8%) and high PMN (>8%) groups in the first service conception rates (35% vs. 37% respectively) or in the interval from calving to pregnancy (177.6 ± 14.2 vs. 176 ± 15.1 day) in low (≤ 8%) and high PMN (>8%) groups, respectively.

Deguillaume *et al.* (2012) studied the effect of endocervical inflammation on reproductive performance and confirmed that healthy cows had a significantly higher pregnancy rate compared with cows affected by combined endometrial and endocervical inflammation.

Moges and Jebar (2012) stated that cows positive in subclinical endometritis had a relative pregnancy rate of 21.4% with highly significant association compared to cows free of subclinical endometritis and also stated that SCE diagnosed by endometrial cytology was associated with reduced rate of cytology

Amit Kumar *et al.* (2013) performed experiment to investigate the immunomodulatory and therapeutic efficacy of neem on endometritis in repeat breeding crossbred cows. Twenty four cows were selected on the basis of history, breeding records, transrectal examination, white side test and randomly divided into 3 groups (Group A: 30 ml normal saline; Group B: 30 ml hydro-alcoholic neem bark; Group C: 30 ml hydro-acetonic neem bark). At subsequent standing estrus following treatment, all cows were artificially inseminated twice using deep frozen semen 12 hours apart. Although both the extracts led to improved clinical recovery and conception rates, the hydro-alcoholic extract was more effective. The clinical recovery and conception rates in Groups A, B, and C were 25.00% and 0.00%, 87.50 and 62.50%, and 75.00% and 50.00%, respectively. Based on these results, it may be concluded that hydro-alcoholic extract of the neem has a better antibacterial and immuno-modulation and can be used as a therapy for endometritis in repeat breeding crossbred cows.

Biswal *et al.* (2013) studied immunomodulatory effect of oyster glycogen on endometritic crossbred cows and found 60% conception rate after first post treatment insemination as compared to 70% in normal cows.

Madoz *et al.* (2013) evaluated the relationship between endometrial cytology during estrus cycle and cut-off points for the diagnosis of subclinical endometritis in dairy cows. They reported the pregnancy rate at first insemination in subclinical endometritis was 29%.

Carneiro *et al.* (2014) recorded the conception rate to first insemination and pregnancy rate at 150 days postpartum were not influenced by the presence of subclinical endometritis in crossbred dairy cows.

Barrio *et al.* (2015) stuided the influence of subclinical endometritis on the reproductive performance in 94 dairy cows. According to their study 80% of healthy cows were pregnant within 200 days postpartum whereas only 60% of cows with subclinical endometritis were pregnant.

Dini *et al.* (2015) stated that cows diagnosed as a subclinical endometritis showed an increase in calving to first service (73 vs. 66 days; P=0.097) and calving to conception interval (118 vs. 105 days; P=0.3, positive vs. negative, respectively). Results showed the negative impacts on fertility by the increase in the time to first service and the time to conception.

Ricci *et al.* (2015) evaluated subclinical endometritis and consequences on fertility in 97 beef cows and reported that only 13% of the cows positive for SCE were pregnant within 130 day post partum. Subclinical endometritis has a detrimental effect on fertility, causing an increase in partum to conception and a decrease in the rate of cows who become pregnant within 130 day postpartum, particularly for those cows housed in a tie stall.

Syed Anwar (2016) stuied the effacy of Tinospora cordifolia, Achyranthes aspera and closprostenol sodium and last group was untreated control group for the treatment of subclinical endometriries cows. 24 cows were divided into 4 equal groups (T1, T2, T3 and T4) T1 was given Tinospora cordifolia T2 group was given Achyranthes aspera, T3 group was given closprostenol sodium and T4 group left as a control. All the animals from different groups were inseminated after completion of treatment with frozen semen. He reported conception rate of 33.33%, 50.00%, 50.00%, and 16.67% in *Tinospora cordifolia, Achyranthes aspera*, Closprostenol sodium and control group, respectively.

2.6 Hematological Parameters

Roberts (1971) reported that anemia (reduced Hb) in repeat breeding cows may be associated with reproductive disorders.

Sastry (1989) observed that the release of neutrophills from the bone marrow promotes through leukocytosis-inducing-factor (LIF) of the plasma, as a result of infection. In bacterial diseases the concentration of LIF is increased in by bacterial products; hence leukocytosis (neutrophilia) occurs in bacterial disease.

Upadhyay *et al.* (1990) stated that the animal treated with neem oil showed a significant leukocytic infiltration in the uterine epithelium between days 3 and 5 post coitum, i.e.during the pre-implantation period. Cellular infiltration was obsereved the day after the oil administration, this infiltration consisted mainly of macrophages and neutrophils .The cells phagocytosed the oil and migrated back into the uterine epithelium no trace of neem oil droplets was noted in the uterine lumen after 4-5 days of administration of neem oil.

Thrall (2004) reported that in inflammatory disease, erythropoietin is diminished presumably because of inflammatory cytokines leading to lowered erythropoiesis and ultimately lowered PCV in blood

Amin *et al.* (2008) stated that neem has been found to increase the Hemoglobin content in cattle.

Nazifi *et al.* (2008) had initiated study on haematological changes of dairy cows in postpartum period and early pregnancy. The cows were checked on days 25 to 30 and days 50 to 60 postparturition. They found the mean packed cell volume (L/L), haemoglobin (g/l), red blood cell (× 1012/l) and white blood cells (× 109/l) level in subclinical endometritic (SCE) cows were 0.19 ± 0.03, 63.86 ± 7.31, 4.81 ± 0.099 and 5.21 ± 1.14, respectively on days 25-30 and 0.20 ± 0.03, 66.33 ± 5.51, 4.13 ± 0.23 and 5.00 ± 0.79, respectively on days 55-60 post partum. In differential leucocytes count they reported the mean neutrophil (%), lymphocyte (%), monocytes (%) and eosinophils (%) level in SCE cows were 47.86 ± 16.79, 48.43 ± 14.48, 2.29 ± 3.04 and 1.43 ± 1.40, respectively on days 25-30 and 35.67 ± 14.15, 60.00 ± 13.00, 3.33 ± 1.53 and 1.00 ± 1.00, respectively on

55-60 days postpartum. By the subclinical endometritis in 25– 30 days after parturition there were significant differences in segmented neutrophils in the clinically healthy and affected cows. The percentage and absolute number of segmented neutrophils were significantly higher in cows with subclinical endometritis than in the clinically healthy cows.

Green *et al.* (2009) evaluated hematological parameters in subclinical endometritis (SCE) cows. According to this study all the hematological parameters were within normal ranges. They reported the mean red blood cells (× 1012/L) count in SCE cows was 6.1 ± 0.2. The mean haemoglobin (g/l) level was 10.9 ± 4. The mean heamatocrit level was 0.29 ± 0.01 (L/L). They found white blood cells (× 109 /L) in the range of 7.0 ± 0.5. The mean percentage of neutrophils, lymphocytes, monocytes and eosinophils were 39.2 ± 2.9, 49.3 ± 2.8, 5.0 ± 0.7 and 5.7 ± 1.0, respectively.They found that was increased in plasma neutrophil and monocyte concentrations.

Belic *et al.* (2012) observed periparturient haematological finding in 20 dairy cows with uterine inflammation. They collected blood samples in the 7-10 days before and 7-10 days after parturition. They found the mean erythrocyte count ×1012 was 6.22 in prepartum metritis and 5.23 in postpartum metritis, respectively. The mean haemoglobin (g/l) level was 10.0 in prepartum metritis and 7.4 in postpartum metritis respectively. The mean leukocytes ×109 count was 9.2 in prepartum metritis and 8.7 in postpartum metritis, respectively. The lymphocytes% calculated was 51 in prepartum metritis and 47 in postpartum metritis, respectively. The monocytes recorded % was 1.94 in prepartum metritis and 0.93 in postpartum metritis, respectively. The mean neutrophills % was 49 in prepartum metritis and 53 in postpartum metritis, respectively. 3.3 in prepartum metritis and 2.1 in postpartum metritis, respectively was eosinophills % calculated.

Mondal and Paul (2012) investigated TEC (106/µl), HB (g/dl) and PCV (%) values was highest ($P<0.05$) in normally cyclic cows compared to repeat breeding and post partum anestrous cows. The mean values of ESR were significantly higher ($P<0.05$) in problem groups than in control group. The TEC (103/µl) differ ($P<0.05$) amongst the group and highest value was noted in repeat breeder cows. Lower Hb indicates

anaemia and its values are significantly low in all problem groups compared to cyclic cows.

Amit kumar *et al.* (2013) collected blood and uterine flushing before and after treatment with hydroalcoholic and hydroacetonic neem bark to study hemoglobin, neutrophils, lymphocytes and WBC count .It was observed that hemoglobin, neutrophils, lymphocytes and WBC were increased significantly ($p<0.05$) in both hydro-alcoholic and hydro-acetonic extract treated groups. Moreover RBCs and glucose were increased in hydroalcoholic extract treated group. Significant ($p<0.05$) rise was found in TLC, PMN and immunoglobulin concentration in both the treated groups. The significant increase in neutrophil, lymphocyte and WBC in the neem treated groups suggests an effective immunomodulatory role of the neem. In hydro-alcoholic extract treated groups; blood glucose values became normal indicating that the treatment improved the general body condition also. Uterine flushing parameters (TLC, PMNs, Protein and Total Immunoglobulin) increased after treatment with both hydro-alcoholic and hydro-acetonic extracts of neem indicating a positive effect on the uterine defense mechanism.

Harendra Kumar *et al.* (2013) observed that there was increases phagocytosis or elimination of pathogens due to stimulation of uterine defense mechanism. The reduction in bacterial count is due to antibacterial action of neem hence there is reduction in neutrophils.

Ndodo *et al*. (2013) studied the effects of water and methanol extracts of Neem (*Azadirachta indica*) leaves were studied in male Wister rats following treatment with 20 % w/w and 30% w/w equivalent of water and methanol extracts of Neem leaves incorporated into rat diet and administered orally for 90 days. The control group had no1rmal diet and water ad libitum. The result showed that PCV of the control group was significantly lower than that of the treated groups at $p<0.001$. 30% w/w equivalent of neem leaves (C2) significantly increased the PCV ($p<0.001$) of treated rats. This is consistent with the little or no toxicity observed in Neem extracts. There was no significant variation between the mean TLC of the control and the neem treated groups at $P > 0.05$. The findings suggest relative safety of the Neem extracts.

Duvel *et al.* (2014) calculated peripheral blood leucocytes of cows with subclinical endometritis (SCE). They found that SCE cows showed significantly higher blood leucocytes number with significantly increased mononuclear cells (MNC) and polymorphonuclear cells (PMN) numbers. They concluded that elevated number of selected mononuclear subpopulations in peripheral blood and enhanced expression of distinct genes encoding for inflammatory mediators in blood leukocytes is due to the subclinical uterine inflammatory process in cows.

Heidarpour *et al.* (2014) studied haematological changes before and after treatment in dairy cows affected with subclinical endometritis (SCE). The animals treated with 500 mg of cloprostenol intramuscular on day 1 or 14 and 500 mg of benzathine cephapirin in 19.6 g ointment base iu and then 500 mg of cloprostenol im 7 days after benzathine injection. They compared the level of haematological parameters in subclinical cows at the time of diagnosis (before treatment) and after seven day of treatment. The level of heamatocrit (L/L) was 0.25 ± 0.02 before treatment and 0.26 ± 0.03 after treatment. The haemoglobin (g/l) level was 84.5 ± 8.2 before treatment and 86.2 ± 10.4 after treatment. The red blood cells (× 1012) level was 5.46 ± 0.89 before treatment and 5.60 ± 1.17 after treatment. The white blood cells (× 109) level was 15.46 ± 4.98 before treatment and 7.02 ± 1.33 after treatment. The mean neutrophil (× 109) level was 9.17 ± 4.98 before treatment and 2.73 ± 0.75 after treatment. The level of lymphocyte (× 109) was 5.85 ± 1.42 before treatment and 4.18 ± 1.12 after treatment. The level of monocytes (× 109) was 0.35 ± 0.24 before treatment and 0.12 ± 0.14 after treatment. The level of eosinophils (× 109) was 0.09 ± 0.13 before treatment and 0.05 ± 0.08 after treatment. They revealed that the cows of subclinical endmetritis (SCE) group presented a significant decrease in PCV and Red blood cell (RBC) values, when compared to healthy groups. Significant increases in white blood cell (WBC), neutrophil, and lymphocyte, counts in SCE groups were observed when compared to healthy cows. WBC, neutrophil, lymphocyte and monocyte counts decreased significantly after treatment with 500 mg of cloprostenol intramuscular on day 1 or 14 and 500 mg of benzathine cephapirin in 19.6 g ointment base iu and then 500 mg of cloprostenol im 7 days after benzathine injection.

Islam *et al.* (2014) inspected the leucocyte profile of periparturient cows 30 days in milk with or without postpartum reproductive disease. They reported the total leucocyte count level was 8.55 ± 1.54 in clinical endometritic cows. The neutrophil and lymphocyte percentage were 23.75 ± 3.38 and 73.00 ± 3.11 in clinical endometritic cows, respectively on 30 days postpartum.

Patil *et al.* (2015) conducted study under field condition on 40 surti buffaloes with postpartum metritis divided into five groups of eight animals each. The study included use of four treatment lines and one control group, viz., Group I- Metronidazole (500 mg) I/U for 5 days; Group II- Ciprofloxacin (1500 mg) and Tinidazole (1800 mg) I/U for 5 days; Group III- Ciprofloxacin (1500 mg) and Tinidazole (1800 mg) I/U for 5 days plus $PGF_2\alpha$ 25 mg i/m once on first day; Group IV- Ceftiofur (long acting) @ 6.6 mg/kg body weight subcutaneously once and Group V (control)- Normal saline I/U for 5 days. The effects of these therapeutic regimes were evaluated by comparing the clinical and haematological alterations before and 6 days after treatment. The mean values of Hb, PCV and TEC after four treatments were significantly ($P<0.05$) higher than the corresponding pre-treatment values, however no significant differences were observed in pre- and post-treatment values of control group. Similarly, mean TLCs on day 6 post-treatment were significantly ($P<0.01$) lower than the pretreatment TLC values in all four treatment groups. The mean neutrophil count was found to be significantly ($P<0.01$) decreased and the lymphocyte count increased in groups II, III and IV on day 6 post-treatment, whereas in group I, both the values were found to vary significantly ($P<0.05$). No significant differences were observed in pre- and post treatment values of monocytes and eosinophils in the treatment groups, and control group. The changes in haematological profile were related to the degree of improvement of the clinical conditions of the animals. They also stated that the decrease in haemoglobin level may be due to disintegration of erythrocytes and effect of some haemolytic type of microbes and their endotoxins on the blood cells and haemopoitic system.

Ahmad Ijaz *et al.* (2016) Blood samples were collected from 75 crossbred cows belonging to one of the three experimental groups i.e. cyclic, noncyclic and endometritic, with 25 animals in each group. The

samples were analyzed for haematological parameters including red blood cell count (RBC), total leukocytic count (TLC), differential leukocytic count, haemoglobin concentration (Hb), packed cell volume (PCV), erythrocyte sedimentation rate (ESR), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC). The results revealed that the values of Hb, ESR, MCHC and TLC were significantly lower ($P<0.05$) in the non-cyclic cows as compared to cyclic or endometritic animals. The differences in these parameters among cows of the latter two groups were non significant. The RBC counts (106/µL) were significantly higher in the endometritic cows (9.32 ± 0.46) than the cyclic (7.23 ± 0.31) or non cyclic (6.89 ± 0.20) crossbred cows. MCH (Pg) was higher in cyclic cows (17.84 ± 0.95) than non-cyclic (13.61 ± 0.55) or endometritic (14.78 ± 0.59) cows. Among leukocytes, neutrophils (%) were higher in endometritic (30.64 ± 3.10) than non-cyclic (23.28 ± 1.79) cows. Eosinophils (%) were higher in non-cyclic (12.0 ± 1.20) cows than cyclic (7.12 ± 0.89) or endometritic (5.52 ± 0.65) animals. The difference among cow of latter two groups was non significant. However, PCV, MCV, lymphocyte, monocyte and basophil percentages did not differ among cows of the three groups. It was concluded that lower erythrocytic indices could be attributed to non-cyclic condition in crossbred cows included in the study.

Syed Anwar (2016) studied haematological changes before and after treatment in diary cows affected with subclinical endometritis. The animal were treated with *Achyranthes aspera* (T_1), *Tinospora Cordifolia* (T_2) and Cloprostenol sodium (T_3) for 3 consecutive days and untreated groups(T_4). He compared the level of haematological parameters in subclinical endometritic cow at the time of diagnosis and after 7 days of treatment. The level of haemoglobin before treatment in T_1, T_2, T_3 and T_4 were 7.33 ± 0.14, 7.36 ± 0.21, 7.95 ± 0.09 and 7.35 ± 0.24, respectively and after treatment in T_1, T_2, T_3 and T_4 were 7.40 ± 0.22, 7.41 ± 0.14, 8.03 ± 0.06 and 7.31 ± 0.10, respectively. The level of PCV before treatment in T_1, T_2, T_3 and T_4 were 26.52 ± 0.56, 26.66 ± 0.49, 27.91 ± 0.27 and 26.58 ± 0.49, respectively and after treatment in T_1, T_2, T_3 and T_4 were 26.66 ± 0.88, 26.79 ± 0.30, 28.03 ± 0.29 and 26.66 ± 0.21, respectively. The level of TLC before treatment in T_1, T_2, T_3 and T_4 were 12.51 ± 0.79, 12.61 ± 0.74, 12.46

± 0.47 and 12.38 ± 0.26, respectively and after treatment in T_1, T_2, T_3 and T_4 were 9.95 ± 0.21, 9.81 ± 0.33, 11.40 ± 0.36 and 11.89 ± 0.21, respectively. The level of TEC before treatment in T_1, T_2, T_3 and T_4 were 5.01 ± 0.30, 5.02 ± 0.24, 5.58 ± 0.17 and 5.02 ± 0.30, respectively and and after treatment in T_1, T_2, T_3 and T_4 were 5.25 ± 0.33, 5.20 ± 0.21, 6.15 ± 0.13 and 5.13 ± 0.09, respectively. The level of neutrophil before treatment in T_1, T_2, T_3 and T_4 were 47.50 ± 0.76, 45.66 ± 0.91, 44.16 ± 1.01 and 42.83 ± 1.10, respectively and after treatment in T_1, T_2, T_3 and T_4 were 34.16 ± 1.62, 33.50 ± 1.23, 33.16 ± 0.87 and 43.00 ± 1.46, respectively. The level of lymphocyte before treatment in T_1, T_2, T_3 and T_4 were 48.83 ± 0.74, 50.66 ± 1.02, 53.00 ± 1.15 and 53.66 ± 1.35, respectively and after treatment were 61.83 ± 1.53, 62.66 ± 1.33, 64.00 ± 0.73 and 53.66 ± 1.64, respectively. The mean values of blood eosinophils count (%) in T_1, T_2, T_3 and T_4 groups were 1.66 ± 0.21, 1.66 ± 0.21, 1.16 ± 0.16 and 1.66 ± 0.21, respectively before treatment and 1.83 ± 0.16, 1.66 ± 0.21, 1.33 ± 0.21 and 1.50 ± 0.22, respectively after treatment. The mean level of monocytes count (%) in T_1, T_2, T_3 and T_4 groups were 2.00 ± 0.63, 2.00 ± 0.63, 1.50 ± 0.83 and 1.83 ± 0.75, respectively before treatment and 2.16 ± 0.40, 2.16 ± 0.75, 1.50 ± 0.54 and 1.66 ± 0.51, respectively after treatment on 7th day.

2.7 Biochemical Parameters

Kale *et al*. (2003) conducted study to assess the hepatoprotective activity of *Azadirachta indica* (AI) aqueous leaf extract on antitubercular drugs-induced hepatotoxicity in albino rats. Hepatotoxicity was induced in rats by combination of isoniazid, rifampicin and pyrazinamide given orally as suspension for 30 days. Treatment groups received AI aqueous leaf extract along with antitubercular drugs. In the second phase of study the effect of AI aqueous leaf extract on established hepatotoxicity was studied by giving the extract for 20 days after withdrawal of antitubercular drugs. Liver damage was assessed by biochemical and histological parameters. Results observed was AI aqueous leaf extract significantly prevented changes in the serum levels of bilirubin, protein, alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase. Similarly it significantly prevented the histological changes as compared to the group receiving antitubercular drugs. It also significantly

reversed the biochemical and histological changes. He conclued Al aqueous leaf extract significantly prevents and reverses the hepatotoxic damage induced by antitubercular drugs in rats.

Ahmad *et al.* (2004) observed total protein level was highest in endometritic cows (19.16 ± 1.00) followed by non cyclic (15.23 ± 0.89) and lowest in cyclic cows (9.19 ± 0.45). Serum level of urea did not differ in cyclic (30.88 ± 2.42), non cyclic (33.80 ± 3.43) and endometritic (37.12 ± 3.45) animals, respectively. Although highest value was recorded in endometritic cows.

Subhapriya and Nagini (2005) studied the levels of marker enzymes such as aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) were elevated 24 hours after paracetamol treatment, whereas rats fed with neem extract prior to paracetamol treatment showed much lower enzyme activities. Kale *et al.* reported the protective effects of neem leaves on hepatotoxicity induced by antitubercular drugs in rats. Aqueous neem leaf extract significantly prevented changes in serum bilirubin, protein, AST, ALT and ALP induced by antitubercular drugs such as isoniazid, rifampicin and pyrazinamide. Oomacham reported neem as one of the plants used for the treatment of jaundice.

Green *et al.* (2009) considered biochemical parameters in subclinical endometritis (SCE) affected cows. They found the mean total protein (g/l), albumin (g/l) and globulin (g/l) level in subclinical endometritic cows were 80 ± 1, 35 ± 1 and 45 ± 1, respectively. According to this study all biochemical parameters were within normal range. They stated that the greater concentration of globulin is an indicative of an activation of adaptive immune response or also indicative of acute inflammation process in the uterus.

Burke *et al.* (2010) considered relationships between endometritis and metabolic state during the calving transition and early lactation periods. Blood samples collected on day 14 before calvin, 0 (day of calving), 4, 7, 14, 28 and 42 day after calving were analyzed for indicators of energy status (nonesterified fatty acids, glucose and urea) and liver function (albumin, globulin, glutamate dehydrogenase and aspartate

aminotransferase). Plasma concentrations of albumin and the albumin: globulin ratio was consistently lower in High-PMN cows. Whereas glutamate dehydrogenase and aspartate aminotransferase were higher, in High-PMN cows during early lactation compared with Low-PMN cows. Circulating metabolites indicative of energy status (nonesterified fatty acids, glucose, and urea) were not different between low and high polymorphonuclear groups. Ruginosu *et al.* (2011) initiated study on the biochemical profile in cows with reproductive disorders reported that the serum total protein (g/dl) in cows with puerperal genital infection was 6.74 ± 0.15. The albumin (%) level was 38.30 ± 0.70. The globulin (%) level was 62.70 ± 0.50. The urea (mg/dl) level was 28.50 ± 1.50. The ALT level was 45.50 ± 0.20 U/L. The AST level was 30.10 ± 1.50 U/L.

Sharma *et al.* (2011) observed neem helps to protect the liver from damage, which in turns helps to cleanse the blood. Neem leaf minimizes, chemically induced liver damage by stabilizing levels of serum markers enzymes and boosting levels of antioxidants, like those found in vitamin C and E and in natural carotenoids, which neutralize free radicals and prevent damage.

Ruginosu *et al.* (2011) performer study on the biochemical profile in cows with reproductive disorders and observed the biochemical profile in cows with reproductive disorders and reported that the serum total protein (g/dl) in cows with puerperal genital infection was 6.74 ± 0.15. The albumin (%) level was 38.30 ± 0.70. The globulin (%) level was 62.70 ± 0.50. The urea (mg/dl) level was 28.50 ± 1.50. The ALT level was 45.50 ± 0.20 U/L. The AST level was 30.10 ± 1.50 U/L.

Heidarpour *et al.* (2012) organized study on biochemical changes before and after treatment in dairy cows. The animals treated with 500 mg of cloprostenol intramuscular on day 1 or 14 and 500 mg of benzathine cephapirin in 19.6 g ointment base iu and then 500 mg of cloprostenol im 7 days after benzathine injection. They found the mean AST (IU/L) level was 64.13 ± 23.10 before treatment and 48.06 ± 15.12 after treatment. The albumin (mg/dl) level was 3.46 ± 0.34 before treatment and 3.61 ± 0.38 after treatment. They observed mean AST levels were significantly decreased after treatment in subclinical endometritis cows and non significant variation were detected in mean albumin level.

Mondal and Paul (2012) concluded, the mean concentration of serum glucose (mg/dL) was higher (P<0.05) in normally cyclic cows compared to problem cows but the values were comparable among the problem groups. Serum total protein (g/dL), albumin (g/dL) and globulin (g/dL) were highest (P<0.05) in normally cyclic cows than other two groups but the albumin: globulin ratio was unaffected. There was significant low (P<0.05) concentration of plasma total protein in the repeat breeding cows in comparison with the normally cycling cows.

Reddy *et al.* (2012) collected blood samples from all the experimental animals, serum was separated and stored at -20°C until assayed for glucose (g/dl), total protein (g/dl), urea (mg/dl) and cholesterol (mg/dl). The results revealed significantly (P<0.05) higher values of glucose (58.08 ± 2.59) and cholesterol (290.72 ± 15.95) in endometritic cows as compared to cyclic (50.72 ± 1.12, 199.12 ± 9.38) and non-cyclic cows (50.56 ± 1.12, 202.96 ± 14.84). Total protein level differed significantly (P<0.05) among cows of all the three groups, being highest in endometritic (19.16 ± 1.00), followed by non cyclic (15.23 ± 0.89) and lowest in cyclic (9.19 ± 0.45) cows. However, serum level of urea did not differ in cyclic (30.88 ± 2.42), non cyclic (33.80 ± 3.43) and endometritic (37.12 ± 3.45) animals, although highest value was recorded in endometritic animals.

Biswal *et al.* (2013) prepared the immunomodulatory effect of oyster glycogen in endometritic crossbred cows. There was no significant alteration in total protein levels between pre and post treated cows were 7.24 ± 0.18 vs. 8.04 ± 0.35 and in case of control it was 8.30 ± 0.34. There was no significant variation in serum albumin among control, pre and post treatment (4.23 ± 0.10 and 3.86 ± 0.18). The serum globulin concentration between pre and post treatment group showed significant difference while it didn't vary in control and pretreatment. The serum globulin concentration was 3.00 ± 0.07, 4.19 ± 0.19 and 3.53 ± 0.19 for pretreatment, post treatment and control cows respectively. The serum concentration of AST for pre-treated endometritis cows was significantly much higher than post treatment (78.28 ± 2.66 vs 47.98 ± 2.95) while for unaffected it was (39.87 ± 1.66). There was no significant difference in ALT values in pre and post treatment (32.58 ± 2.26 vs. 28.34 ± 2.37). They confirmed that the elevation in serum AST concentration during endometritis might be due to damage to

uterine tissue and increased plasma membrane permeability. The significant reduction of this enzyme in the serum after treatment may be due recovery following immunomodulatory therapy.

Senosy and Osawa (2013) investigated association among calving season and measures of energy status, resumption of ovulation and subclinical endometritis in early lactating dairy cows. They recorded that the concentration of blood urea nitrogen (BUN) was higher in cows that calved during the temperate season than cows that calved during the hot season at week 2 ($P < 0.05$), 3 ($P < 0.01$), 4 ($P < 0.01$), 5 ($P < 0.01$), 6 ($P < 0.01$) and 7 ($P < 0.01$) postpartum. Furthermore, BUN concentration in cows that calved during the cold season was higher ($P < 0.05$) at weeks 4 and 6 postpartum when compared with those that calved during the hot season. BUN concentration in cows that calved during the cold season was lower ($P < 0.05$) than of cows that calved during the temperate season.

Akbar *et al.* (2014) calculated total protein, albumin, globulin and aspartate aminotransferase in subclinical endometritic (SCE) cows and healthy cows. They reported that mean level of serum total protein (g/l), albumin (g/l), globulin (g/l) and AST (IU/L) in SCE cows were 82.1, 36.8, 45.3 and 78.5, respectively. And in healthy cows were 77.1, 37.5, 39.6 and 87.5, respectively. They revealed that there was no effect of health status on the concentration of blood biomarkers measured also the milk production, blood metabolites and disease biomarkers (Albumin, AST) did not differ greatly between healthy and SCE cows. Animal's humoral immune status or response can provide an indication of Serum globulin concentration. High concentration of globulin is suggestive of lymphocyte proliferation and greater level of circulating antibodies. The numerically greater concentration of globulin is indicative of an activation of adaptive response.

Krause *et al.* (2014) outlined that cows that resumed ovarian activity early during the postpartum period had higher serum albumin concentrations and lower counts of uterine PMN cells, which were not associated with higher subclinical endometrtis incidence.

Sanshez *et al.* (2014) observed endometrial cytology and metabolic profiles for selection of donor cows in embryo transfer programmes. At the start of the superovulation procedure (Day 0), blood and endometrial samples were obtained to determine metabolic and uterine status, respectively. They analysed albumin and found the mean level of albumin (g/l) was 38.01 ± 0.62. The level of total protein (g/l) was 64.99 ± 0.70 and the mean level of urea (mg/dl) was 33.07 ± 1.36. They stated that they did not find any variation in albumin or total protein results probably because the cows used for the study had calved for several months.

Barrio *et al.* (2015) organised study on influence of subclinical endometritis on the reproductive performance of dairy cows and studied the effect of total protein, albumin, ALT, AST and urea from calving interval to conception. They recorded that the albumin had significant effects on the interval calving to conception according to the Cox model. Increased level of albumin or reduced level of triglycerides was associated to shorter calving to conception interval. They found there was no variable difference in urea. No other metabolic variables had relationship with the interval calving to conception.

Walker *et al.* (2015) regulated study on modulation of the immune system during postpartum uterine inflammation. They reported the mean level of total protein, albumin, globulin and aspartate aminotransferase in subclinical endometritic (SCE) cows were 82.17 g/l, 36.83 g/l, 45.33 g/l and 78.50 IU/L, respectively. In metabolites measured, their was no difference.

Syed Anwar (2016) conducted study on biochemical changes before and after treatment with in dairy cows. The animals were treated with *Achyranthes aspera*, *Tinospora cordifolia*, Cloprostenol sodium and the last was untreated i.e. control group. Blood biochemical study was carried out twice, first on the day of diagnosis and 2nd after 7 days after treatment. The mean level of total serum protein in (g/dl) in T_1, T_2, T_3 and T_4 groups were 6.93 ± 0.16, 6.93 ± 0.06, 6.83 ± 0.12 and 6.93 ± 0.08, respectively before treatment and 6.88 ± 0.17, 6.95 ± 0.05, 6.81 ± 0.12 and 6.95 ± 0.09, respectively after treatment on 7th day. The mean serum albumin level in (g/dl) in T_1, T_2, T_3 and T_4 groups were 3.13 ± 0.02, 3.26 ± 0.05, 3.15 ± 0.06 and 3.26 ± 0.04, respectively before treatment and 3.18 ±

0.10, 3.28 ± 0.06, 3.16 ± 0.08 and 3.30 ± 0.06, respectively after treatment on 7th day. The mean serum AST level (IU/L) in T_1, T_2, T_3 and T_4 groups were 76.43 ± 2.87, 72.95 ± 2.48, 78.58 ± 6.06 and 72.73 ± 2.64, respectively before treatment and 52.90 ± 2.68, 53.55 ± 2.70, 65.33 ± 4.34 and 73.30 ± 2.48, respectively after treatment on 7th day. The mean serum ALT level (IU/L) in T_1, T_2, T_3 and T_4 groups were 25.33 ± 4.44, 24.83 ± 2.90, 23.75 ± 2.07 and 24.55 ± 1.75, respectively before treatment and 25.70 ± 1.98, 24.91 ± 2.20, 22.75 ± 3.33 and 24.21 ± 1.90, respectively after treatment on 7th day. The mean serum BUN level (mg/dl) in T_1, T_2, T_3 and T_4 groups were 15.07 ± 3.75, 15.77 ± 1.02, 15.41 ± 1.37 and 16.38 ± 1.04, respectively before treatment and 15.90 ± 2.68, 15.89 ± 1.26, 15.65 ± 1.38 and 16.35 ± 2.07, respectively after treatment on 7th day.

CHAPTER III

MATERIALS AND METHODS

The present research work entitled “Comparative Efficacy of Different Herbal Extract on Subclinical Endometritis in Postpartum Cows” was carried out in the Department of Animal Reproduction Gynecology and Obstetrics, Department of Pharmacology and Toxicology and Department of Veterinary Medicine. These trials were conducted at Instructional Livestock Farm Complex (ILFP), Dr Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Shri. Gorakshan Sanstha Akola, Rashtrisala, Umbri, Nimbi Shetki Sala Akola, Guddi, Akola and Gow Sanstha dabki road, Akola.

3.1 Health and Managemental Practices

3.1.1 Instructional Livestock Farm Complex (I.L.F.C)

The characteristics of the managemental practices at Instructional Livestock Farm Complex, Dr. PDKV, Akola were as under.

The byres were constructed in north-south direction in which walled were half cement shed. The floor was kept under normal hygienic conditions and was paved with cement. Cows were fed a balanced concentrates ration at the rate of 1.5 kg for maintenance and 50 percent of milk yield for production and was regularly supplied with 8 to 10 kg green and dry fodder.

The cows were milked twice a day in morning from 4.30 to 6 a.m. and at afternoon from 3.30 to 5.30 p.m. The calves were allowed to suckle their dams. The heat was detected by vasectomised bull which was left loose in the open paddock. In the evening it was paraded in the cattle shed at 6 to 7 pm. On rectal examination estrus were confirmed and they were artificially inseminated with frozen semen.

3.1.2 Shri Gorakshan Sanstha Akola, Rashtrisala, Umbri, Nimbi Shetki Sala Akola, Guldhi, Akola and Gow Sanstha dabki road, Akola

The byres were constructed in north-south direction in which walled were half cement shed. The floor was kept under normal hygienic conditions. Cows were fed a balanced concentrates ration containing cotton-seed-cake and Turchuni at the rate of 1.5 kg for maintenance and 50 percent of milk yield for production. In addition chaffed fodder kadabi or grass, or green fodder about 10 kg was also regularly fed.

All cows were milked twice a day in morning from 6 to 7.00 a.m. and at evening from 5.30 to 6.30 p.m. The calves were allowed to suckle their dams. The heat was detected by vasectomised bull which was left loose in the open paddock. On rectal examination estrus were confirmed and they were artificially inseminated with frozen semen.

3.2 Selection of Animals

For the current study post partum cows between 30 to 60 days in milk were selected from ILFC, PDKV, Akola and surrounding Gorakshans of Akola city and were screened for subclinical endometritis.

3.2.1 Clinical examination

Examination was carried on selected animals for the signs of clinical endometritis and those cows without signs of clinical endometritis were selected. By rectal palpation of uterus and ovaries reproductive tract of all selected cows were examined. On the first day of examination the post partum cow's uterus was examined for evidence of subclinical endometritis.

3.2.2 Detection of subclinical endometritis by using PMN cells

With the help of endometrial cytology, all cows were determined for subclinical endometritis before the treatment and after treatment on subsequent oestrus. Thereafter cytobrush technique was used for cytological study.

3.2.3 Cytobrush technique

The cytobrush was made by inserting the standard used in human gynecology into the plunger of an insemination rod. The plunger was then placed inside the metal sleeve of the gun and finally the entire device was covered with a plastic sheath. After threading through the cervix and once into the uterus, the cytobrush was exposed. The sample was obtained by moving the cytobrush clockwise around its longitudinal axis. For protection the cytobrush was subsequently retracted into the plastic sheath.

The mucus which was collected was taken on slide for detection of PMN cells and was stained with leishman stain and Giemsa. More than 5 % PMN cells in stained smear was taken as the positive cases for postpartum subclinical endometritis. (Lima *et al*., 2013 and Senosy and Osawa, 2013).

3.2.4 Endometrial cytology

By rolling the cytobrush on a clean glass, cytology slides were prepared and fixed with methanol (Madoz *et al.,* 2013). With the help modified Giemsa stain slides were stained within 2 hour after bringing to the laboratory (Kasimanickam *et al.,* 2004, Ahmadi *et al*., 2006, Barlund *et al*., 2008 and Dourey *et al*., 2011). By counting a minimum of 100 cells at 400x magnification and at 1000x magnification cytological assessment of percent neutrophils (% PMN cells) was determined in order to provide a quantitative assessment of endometrial inflammation. The endometrial cytology slides were checked twice by the investigating clinician and once by a technician, who was blinded regarding sampling.(Senosy and Osawa, 2013, Melcher *et al*., 2014, Barrio *et al*., 2015).

3.3 Blood Collection

A total 7ml of blood was collected aseptically from jugular vein by using 16 gauge hypodermic needle, for hematological and biochemical parameters before treatment on '0' day and after treatment on 7^{th} day. Out of that 5 ml of blood was taken for biochemical estimation in serum vacutainer and 2 ml of blood was taken for hematological study in EDTA vial.

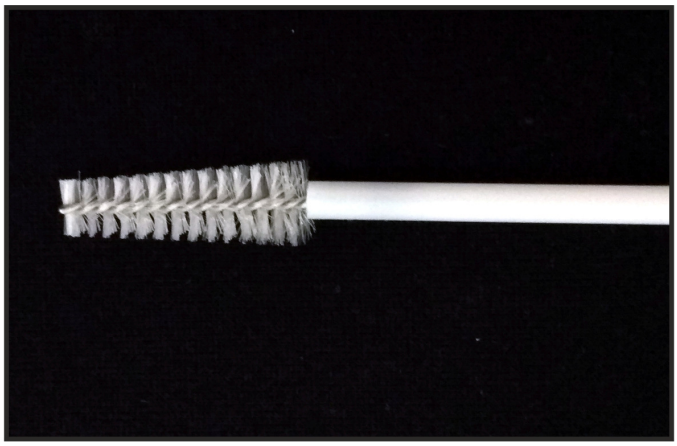


Plate 1. Cytobrush for Endometrial cytology

Plate 2. Collection of Endometrial mucous for cytological examination

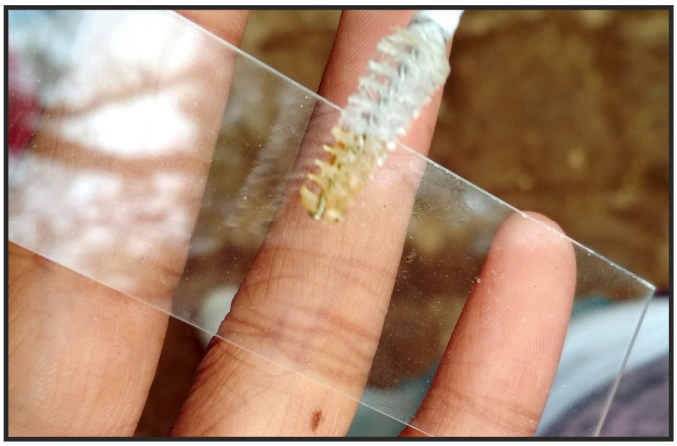


Plate 3. Preparation of Endometrial mucous smear for cytology

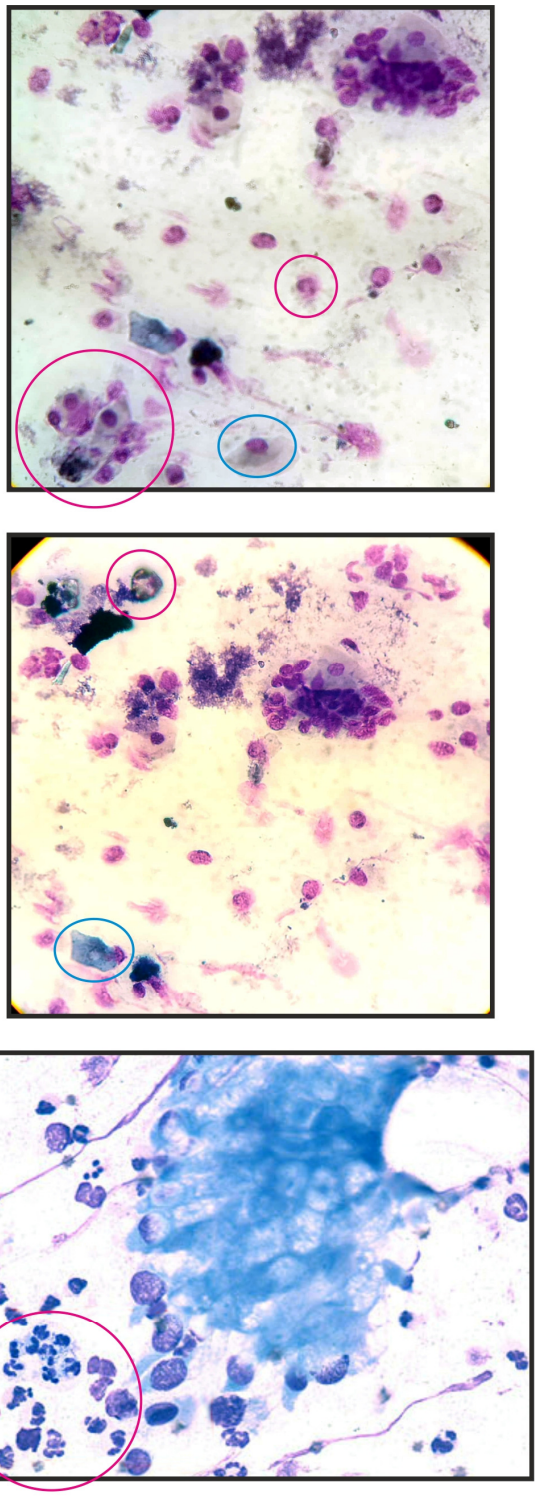


Plate 4. Endometrial cytological observation for positive animals

PMN cells Endometrial cells

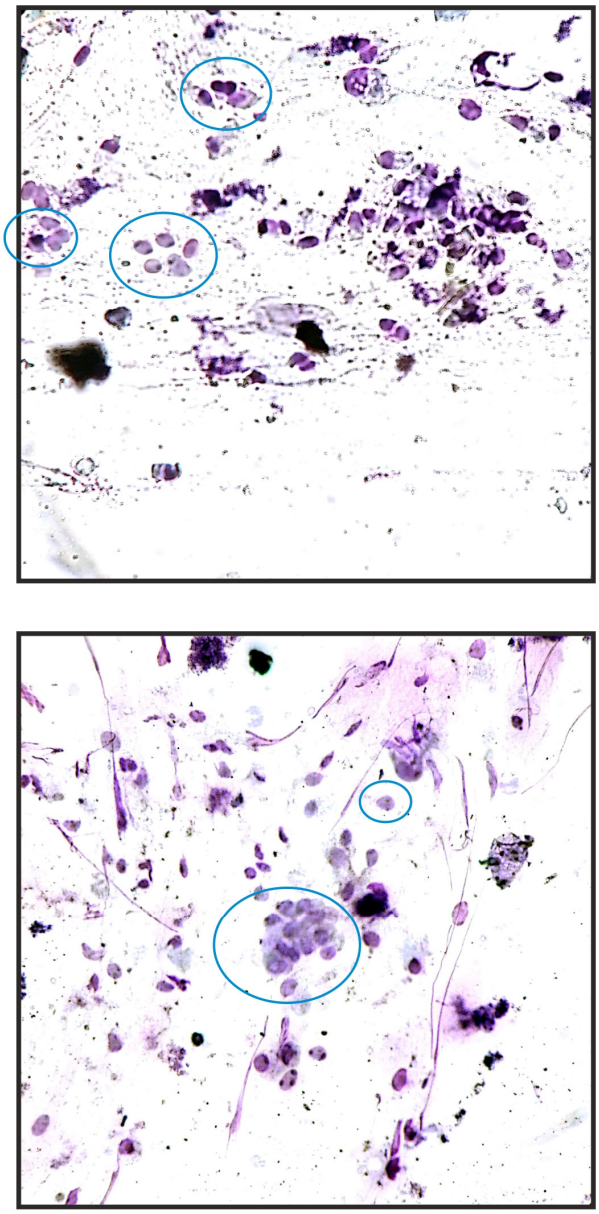


Plate 5. Endometrial cytological observation for negative animals

Endometrial cells

3.3.1 Preparation of Blood smears

Thin blood smears were prepared by taking a drop of blood on a clean grease free glass slide. Before fixing with methanol, the animal number was marked on glass slides for identification of smear. Blood smears were dried and fixed with methanol for 2-5 min. Duplicate blood smears were made for every sample.

Before biochemical estimation centrifugation at 2000 rpm for 10 minutes was done and serum was separated and stored in oven dried ependorf tube under refrigeration at -20^0 C until used for biochemical analysis.

The blood biochemical parameters viz. Serum total protein, Albumin, AST, ALT, BUN, Creatinine and Haematological parameters viz. Hemoglobin, Packed Cell Volume, Total Erythrocyte Count, Total Leucocyte Count and Differential Leucocyte Count parameters were carried out on 'O' day (before treatment) and on 7th day of post treatment.

3.4 Haematological Studies

Total leucocyte count (TLC) (X 10^3/cu.mm.) was done with improved Neubar's chamber using W.B.C. diluting fluid as described by Benjamin (2001).

Differential leucocyte count was carried out as per standard method by using Leishman's stain and observed under oil immersion as described by Benjamin (2001). Blood smear slides were also prepared simultaneously on clean and dried glass slide while collecting blood and stained with Leishman's stain. After drying, the slides were examined under oil immersion and 100 cells were counted (Benjamin, 2001) to know the following constituents.

1. Neutrophils (%)
2. Lymphocytes (%)
3. Eosinophils (%)
4. Monocytes (%)
5. Basophils (%)

Total erythrocyte count was estimated with improved Neubar's chamber using R.B.C. diluting fluid as described by Benjamin (2001).

Hemoglobin was estimated by means of Sahlis haemoglobinometer as described by Benjamin (2001).

Packed cell volume was estimated by macrohaematocrit method as described by Benjamin (2001).

3.5 Biochemical Estimation

Biochemical estimations were carried out with kits supplied by AGD Biomedicals (P) Ltd. Baddi-Nalagarh Road, Himachal Pradesh, India.

Serum total protein (gm/dl) was estimated by using Biuret and Dye Binding method (Annonimus, 1976) with Span Diagnostic kit. Serum Albumin (gm/dl) was estimated by modified Biuret and Dumas method.

Aspartate Aminotransferase (AST) (U/L) and Alanine Aminotransferase (ALT) (U/L) were estimated by Modified UV (IFCC), Kinetic assay method by using kit supplied by AGD Biomedical (P) Ltd. Baddi-Nalagarh Road, Himachal Pradesh, India.

Blood Urea Nitrogen (BUN) (gm/dl) was estimated by NED-dye, Initial Rate Assay supplied by AGD Biomedical (P) Ltd. Baddi-Nalagarh Road, Himachal Pradesh, India. Creatinine was estimatated by Jaffe kinetic Method supplied by AGD Biomedical (P) Ltd. Baddi-Nalagarh Road, H.P., India.

3.6 *Achyranthes aspera*

For the investigation leaves of the plant *Achyranthes aspera* (Aghada) were used.

3.6.1 Selection of plant

In the month of January-February the mature green leaves of *Achyranthes aspera* were collected from the campus of Post Graduate

Institute of Veterinary and Animal Sciences, Akola and surrounding area of Akola city. Leaves were cut into small pieces and subjected for shade driying in the Department of Pharmacology and Toxicology, PGIVAS, Akola. With the help of pulverizing machine the shade dried leaves of *Achyranthes aspera* were processed to get fine powder. Freshly prepared powder was subjected to hydro-methanolic extraction. The extract thus obtained was used for further studies.

3.6.2 Preparation of extract and determination of per cent extractability

In hydro-methanolic solution (40% distilled water + 60 % methanol), the freshly prepared powder of leaves (25 g) was immersed in a flask stoppered tightly with cotton plug and was kept at room temperature for 48 hours at 150 rpm in an orbital shaker. With the help of muslin cloth the contents of the flask were filtered. The residue left in the flask was rinsed with little quantity of hydro-alcoholic solvent and filtered through the muslin cloth. The filtrate thus obtained was filtered through Whatman No. 1 filter paper. Final filtrate, so obtained was transferred to previously weighed large petri dish and was kept for evaporation of solvent at room temperature. After complete evaporation, the petri dish was once again weighed to know the amount of extract. The per cent extractability was 9.00%. The extract was stored in airtight screw cap vials and kept in the desiccators until further use.

3.7 *Azadirachta indica*

3.7.1 Preparation of extract and determination of per cent extractability

For preparation of methanolic fraction the neem oil obtained from local market, it was subjected to fractionation by mixing equal volume of methanol. This mixture was vigorously shaken for 10 minutes and poured in a separating funnel and allowed to stand for another 10 minutes. The uppermost, methanol miscible fraction was collected and kept in a vacuum desiccator for complete evaporation of solvent and stored till use.

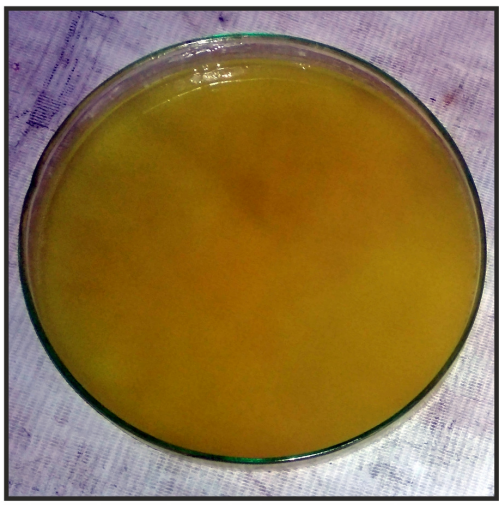
Leaves of *Achyranthes aspera*

Powder of *Achyranthes aspera*

Oil of *Azadirachta indica*

Plate 6. Details of plants used for present study

Hydromethamolic extract of *Achyramthes aspera*



Hydromethamolic extract of *Azadirachta indica*

Plate 7. Hydromethamolic extract used for present study

3.8 Prevalence of Subclinical Endometritis

The prevalence study was carried out by screening in 87 animals surrounding Akola city by endometrial cytology.

Prevalence of subclinical endometritis was determined. The percent prevalence of subclinical endometritis was determined by using the formula:-

$$\text{Percent prevalence} = \frac{\text{Number of positive animals}}{\text{Total number of animals screened}} \times 100$$

3.9 Treatment of Subclinical Endometritis

Total 30 cows showing PMN cells ≥ 5% threshold level in postpartum cows were treated into following groups. In each group ten subclinical endometritis affected cows were selected with average body weight range 250-300.

a) **Group I (n=10)** = Cows will treated with a hydromethanolic fraction of neem oil 25 ml intra uterine for three consecutive days.

b) **Group II (n=10)** = Cows will treated with a hydromethanolic extract of *Achyranthes aspera* 10 mg /kg body wt. intra uterine once daily for three consecutive days.

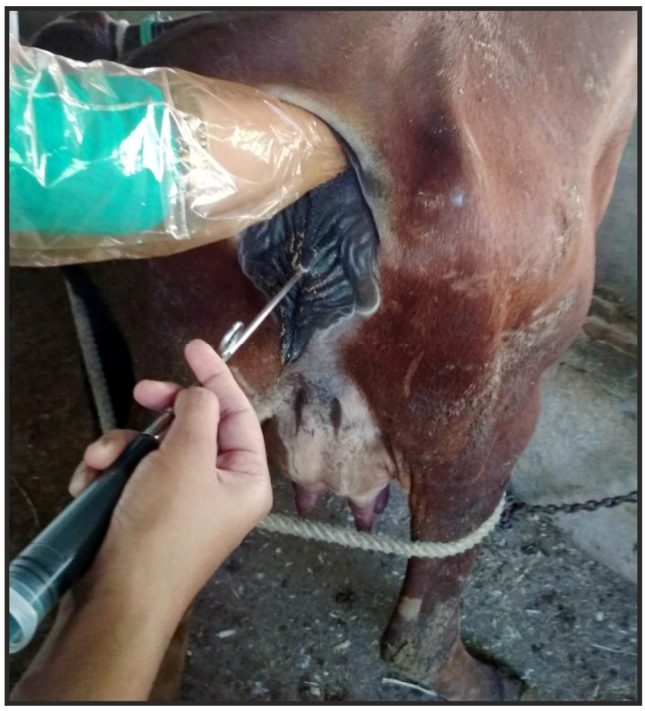
c) **Group III (n=10) =** The cows showing PMN ≥5% in postpartum cows in uterine cytology will be control group and given no treatment.

3.10 Post Treatment Procedure

All the selected animals under study were closely observed for spontaneous estrus exhibition by visual observation.

3.10.1 Artificial Insemination (A.I.)

Animal was restrained with rope or in crate as per the availability. Using lukewarm water dirt and dung was cleaned. Thawing of frozen semen straw was done in sperm processor at 37°C for 30 seconds.



I/U administration of *Achyranthes aspera*

I/U administration of *Azadirachta indica*

Plate 8. Drug used for subclinical endometritis

AI Gun was loaded with thawed straw and AI was done in esturs cows following AM-PM rule by depositing semen in the mid-cervix.

3.10.2 Conception rate (CR)

Conception rate - Conception rate is calculated by the formula

$$\text{Conception rate} = \frac{\text{Number of the animal conceived}}{\text{Number of the animal Inseminated}} \times 100$$

The conception rate in all the groups was determined by per-rectal (P/R) examination on 60 day post A.I.

3.11 Statistical Analysis

Data generated during the experiment was subjected to statistical analysis using computer statistical software Wasp ICAR Goa 2.0. Significance of paired values before and after treatment were tested using independent / paired't' test.

CHAPTER IV

RESULTS AND DISCUSSION

The present study entitled “Comparative Efficacy Of Different Herbal Extract On Subclinical Endometritis In Postpartum Cows” was carried out on thirty subclinical endometritic cows diagnosed with endometrial cytology by cytobrush technique and were divided into three equal groups for the present study. First group (T_1) was treated with 25 ml sterile hydromethanolic fraction of neem oil intra uterine for three consecutive days. Second group (T_2) was treated with 20 ml (10 mg/ml) sterile hydromethanolic leaf extract of *Achyranthes aspera* (200 mg) intrauterine for three consecutive days. Third group (T_3) was kept as untreated control. The results obtained in the present study are presented in tabular form and discussed under following objectives.

1. To study the prevalence of subclinical endometritis in post partum dairy cows in and around Akola city.
2. To evaluate the therapeutic efficacy of *Azadirachta Indica* and *Achyranthes aspera* in Subclinical endometritic cows.
3. To study the efficacy of *Azadirachta Indica* and *Achyranthes aspera* on conception rate in subclinical endometritic cows.
4. To study the hematological and biochemical parameters in subclinical endometritic cows.

4.1 Prevalence of Subclinical Endometritis in Postpartum Cows

During the present study, total 87 cows from 30-60 days in milk (DIM) without any vaginal discharge were enrolled from different farms, Gorakshan sanstha around Akola city.

The prevalence of subclinical endometritis in different herds is given in Table 1 and Figure 1.

The overall prevalence of subclinical endometritis with 5% threshold level from 30-60 days in milk was 35.63% (31/87). Herd level prevalence was 30.00% (6/20), 44% (11/25), 27.27% (3/11), 33.34% (4/12), 30% (3/10) and 44.45% (4/9) on farms ILFC, PDKV, Gorakshan akola, Rashtria shala, Nimbi shetki shala, Guldhi and Gow sanstha Dabki road, respectively.

Table 1. Prevalence of subclinical endometritis in different farms in and around Akola city

Name of Farm	No of animals screened	Positive for SCE	Herd level prevalence (%)
ILFC, PDKV	20	6	30.00%
Gorakshan, Akola	25	11	44.00%
Rashtria shala,	11	3	27.27%
Nimbi shetki shala	12	4	33.34%
Guldhi	10	3	30.00%
Gow sanstha Dabki road	09	4	44.45%
Total	**87**	**31**	**35.63%**

The overall prevalence of subclinical endometritis in the present study are in accordance with previously published data from confinement housing systems. Kasimanickam *et al.* (2004) diagnosed subclinical endometritis in clinically healthy dairy cows and described a prevalence of 35% and 34% for 20 to 33 days postpartum and 34 to 47 days postpartum, respectively. Green *et al.* (2009) reported a prevalence of 37% subclinical endometritic cows. Cheong *et al.* (2011) reported a herd level prevalence of SCE of 36.1%. Senosy and Osawa (2013) revealed the prevalence of SCE to be 33.3% and 30.4% in cold and temperate season, respectively at ≥5 % threshold level. Lima *et al.* (2013) observed prevalence of SCE of 29.5% with 5% threshold level. Melcher *et al*. (2014) revealed the prevalence of SCE of 33% by counting 100 cells. Carneiro *et al.* (2014) reported a prevalence of SCE of 26%. Singh *et al.* (2016) observed the prevalence of SCE was 29.4% at ≥4 % threshold level. Dini *et al*. (2015) reported the prevalence of SCE 30 day postpartum as 38.5% using the threshold of 18%. Syed Anwar (2016) reported prevalence of 36.14% in post partum cows in and around Akola city showing herd level

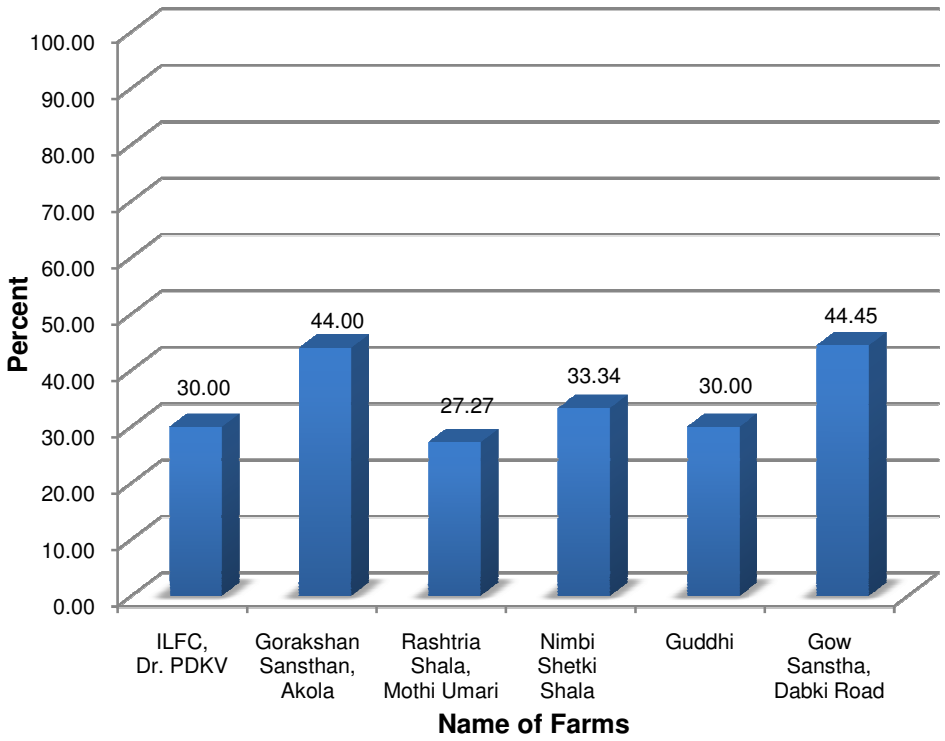


Fig. 1. Prevalence of subclinical endometritis in different farms in and around Akola city

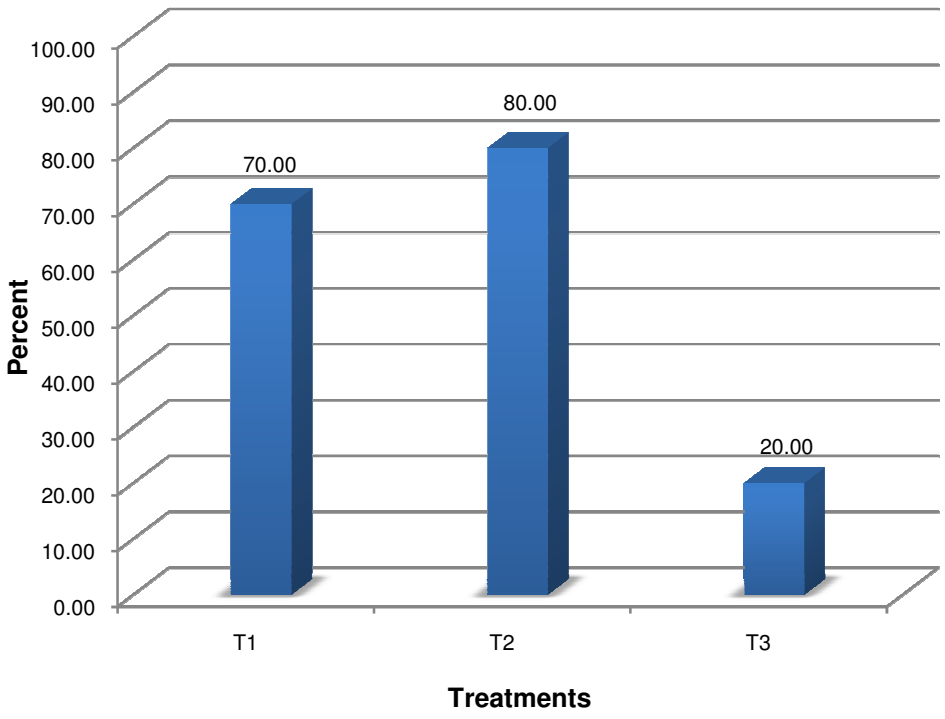


Fig. 2. Therapeutic efficacy of *Azadirachta Indica* (T_1), *Achyranthes aspera* (T_2) and untreated control cows (T_3) in subclinical endometritic cows

prevalence of 30% (6/20), 46.66% (7/15), 40% (8/20), 30% (3/10), 33.34% (4/12) and 33.34% (2/6) in (ILFP), Dr Punjabrao Deshmukh Krishi Vidyapeeth, Akola; Shri Gorakshan Sanstha Akola, Shri Gorakshan Sanstha Mhaispur, Hanuman Gosewa Gorakshan Sanstha, Kumbhari and Shri Gorakshan Sanstha (Umri).

In contrast to present findings, lower prevalence of SCE obtained by Dubuc *et al.* (2010) who reported 13.5% prevalence for cytological endometritis at ≥6% threshold level of PMN cells. Kaufmann *et al.* (2010) who observed the overall prevalence of SCE was 12.4% at 18 % threshold level of PMN cells. Ribeiro *et al.* (2013) reported 13.4% prevalence in subclinical endometritic cows at ≥5 % threshold level and Barrio *et al.* (2015) who observed 14.9% prevalence of SCE in 35-45 days in milk.

The difference in percentage of prevalence with earlier findings may be due to difference threshold level of PMN cells, difference in postpartum day of diagnosis, variation in season, variation in Body Condition Score and different counting methods for PMN cells (Senosy and Osawa 2013, Carneiro *et al*., 2014 and Melcher *et al*., 2014).

4.2 Therapeutic Efficacy of *Azadirachta Indica* and *Achyranthes aspera* in Subclinical Endometritic Cows

The therapeutic efficacy of *Azadirachta indica* and *Achyranthes aspera* in subclinical endometritic cows were shown in Table 2 and Fig. 2. The therapeutic efficacy was measured on the basis of curative percentage by endometrial cytology on the subsequent heat after the treatment.

The therapeutic efficacy on the basis of curative percentage in groups *Azadirachta Indica* and *Achyranthes aspera* were 70.00% (7/10) and 80.00% (8/10) after the treatment and in untreated group it was 20.00% (2/10), respectively. From the present study it was revealed that the curative efficacy was higher in *Achyranthes aspera* treated cows followed by *Azadirachta Indica* treated cows as compared to untreated control cows. The higher curative percentage of *Achyranthes aspera* and *Azadirachta indica* might be due to immunomodulatory, antiinflamatory and antibacterial as well as estrogenic property of *Achyranthes aspera.*

Table 2. Therapeutic efficacy of *Azadirachta Indica* (T_1) and *Achyranthes aspera* (T_2) in subclinical endometritic cows

Treatments Groups (n=6)	No of Animals Treated	No of Animals Negative in endometrial cytology	Percentage (%)
T_1	10	7	70.00%
T_2	10	8	80.00%
T_3	10	2	20.00%

The therapeutic efficacy of *Azadirachta Indica* observed in thepresent study is in accordance with Amit kumar *et al.* (2013) who reported 75% therapeutic efficacy with hydro acetoinic neem bark extract, where as higher therapeutic efficacy was observed by Amit kumar *et al.* (2013) and Harendra kumar *et al.* (2013) 96.02% with hydroalcholic neem bark and methanolic extract of neem oil, respectively which are in not accordance with present study.

The difference in therapeutic efficacy of *Azadirachta Indica* with earlier findings might be due to difference in breed ,post partum time period of treatment, season, body condition score and variation in PMN cells counting. (Senosy and Osawa 2013, Carneiro *et al*., 2014 and Melcher *et al*., 2014).

Therapeutic efficacy of *Achyranthes aspera* observed in present study is in close accordance with Syed anwar (2016) who reported 66.66%, therapeutic efficacy of hydromethanolic extract of *Achyranthes aspera* in post partum cows, whereas 20 % cows observed negative for subclinical endomerities in untreated cows which are in aggregment with Amit kumar *et al.* (2013) and Harendra kumar *et al.* (2013) both of whom reported 25 % cows to be negative for subclinical endometrities cows.

4.3 First Service Conception Rate in Experimental cows

All the cows exhibited spontaneous estrus in groups treated with *Azadirachta indica*, *Achyranthes aspera* including untreated groups they were artificially inseminated as per Am and Pm rule. In the present study, 5 out of ten cows were found to be pregnant with 50 percent

conception rate in *Azadirachta Indica* group, 4 out of ten cows was found to be pregnant with 40.00 percent conception rate in *Achyranthes aspera* group. Whereas, out of ten cows only two cow was found to be pregnant with 20.00 percent in untreated group, respectively

Table 3. First service conception rate in subclinical endometritic cows with different treatments.

Treatments Groups (n=10)	Number of Animals Treated	Number of Animals Inseminated	Number of Animals Pregnant	First Service Conception Rate (%)
T_1	10	10	5	50.00
T_2	10	10	4	40.00
T_3	10	10	2	20.00

From the present study it was observed that cows treated with *Azadirachta Indica* showed higher first service conception rate followed by *Achyranthes aspera* treated cows as compared to untreated cows.

The higher first service conception rate observed in cows from treatment groups as compared to untreated group might be due to better therapeutic efficacy of *Azadirachta indica* or *Achyranthes aspera* in subclinical endometrities which had a determintal effect on fertility causing an increase in calving to conception and a decrease in the rate of cows who become pregnant (Ricci *et al.* 2016).

The first service conception rate in *Achyranthes aspera* group observed in present study is in close aggrement with Syed Anwar (2016) who recorded 50 % first service conception rate in subclinical endometritis cows treated with methanolic extract of *Achyranthes aspera.*

The first service conception rate in *Azadirachta Indica* observed in present study is in accordance with Amit kumar *et al.* (2013) who reported 50% conception rate in endometritic cows treated with 30 ml hydroacetonic neem bark , whereas Amit kumar *et al.* (2013) and Harendra kumar *et al.* (2013) reported higher conception rate 62.50% and 71.42 % in endometric cows treated with hydroalcholic and methanolic fraction of neem oil, respectively which is not accordance with present study.

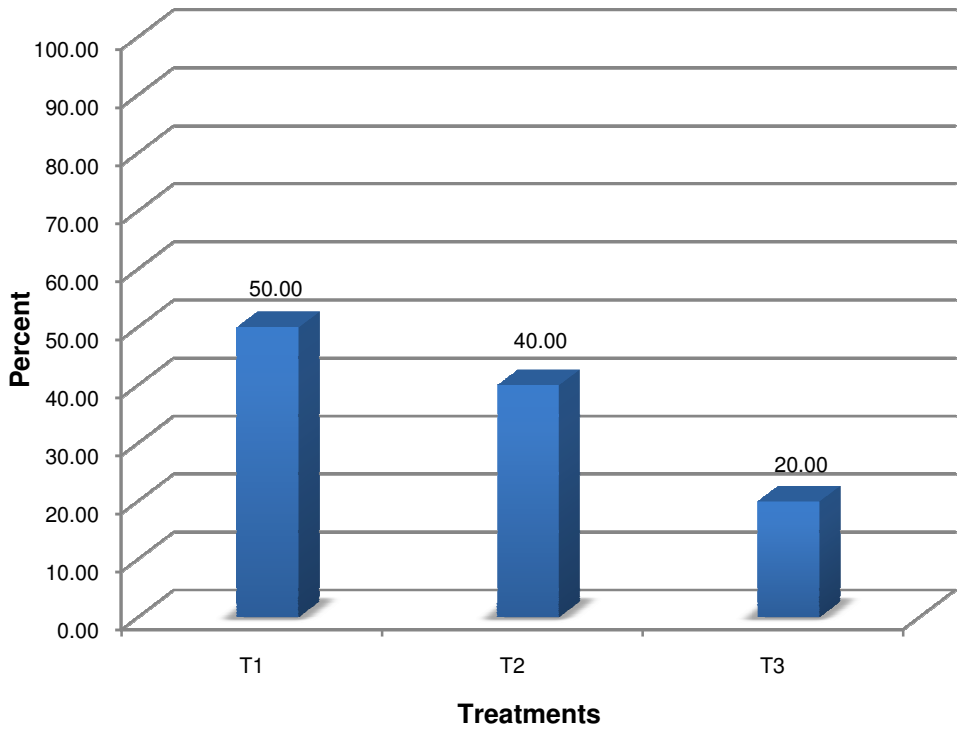


Fig. 3. First service conception rate in subclinical endometritic cows with different treatments

A lower first service conception rate than the present study for A*zadirachta indica* observed by Harendra kumar *et al.* (2013) 25% in endometritic cows treated with ethanolic fraction of neem seed powder.

Results of untreated cow control group are in agreement with Moges and Jebar (2012) and Ricci *et al.* (2015) who observed first service conception rate of 21.4 % and 13 % in Subclinical endometritic cows, respectively. Similarly Barlund *et al.* (2008) reported 14.2 % conception rate in endometritic positive cows.

In the present study the difference in conception rate in earlier findings may be due to the variation in curative efficacy of different drugs used. Lima *et al.*. (2013) stated that the cows with subclinical endometritis from the early postpartum period have greater depression in measures of fertility at first AI.

4.4 Hematological Parameters

4.4.1 Hemoglobin (Hb) (g/dl)

The estimated mean hemoglobin levels in different treatment groups and in control group are given in Table 4. and Fig 4. As per Brar *et al*. (2004) the normal physiological range of hemoglobin is 8 to 14 g/dl in cows.

Table 4. Mean values of blood hemoglobin level (g/dl) in subclinical endometritis affected cows treated with T_1 (*Azadirachta Indica extract*), T_2 (*Achyranthes aspera* extract) and T_3 (untreated control) before and after treatment.

Treatments/ Groups (n=10)	Hemoglobin (Hb)(g/dl)		t Statistics Value	P Value
	Before	After		
T_1	9.32 ± 0.19^a	10.54 ± 0.20^b	-9.045	0.000
T_2	8.56 ± 0.26^a	8.61 ± 0.26^a	-0.429	0.678
T_3	8.41 ± 0.12^a	8.44 ± 0.11^a	0.031	0.763

Mean bearing same superscript in a row do not differ significantly

The mean hemoglobin level (g/dl) in *Azadirachta indica*, *Achyranthes aspera* and untreated control groups were 9.32 ± 0.19, 8.56 ± 0.26 and 8.41 ± 0.12, respectively before treatment and 10.54 ± 0.20, 8.61 ± 0.26 and 8.44 ± 0.11, respectively after treatment on 7th day.

From the present study it was observed that the mean hemoglobin level in subclinical endometritic cows were within the normal physiological limit in all groups before and after treatment.

From the present study it was observed that there was significant increase ($p<0.001$) in mean hemoglobin level after treatment on 7th day in *Azadirachta indica* group whereas it was observed to be non significantly different in *Achyranthes aspera* group and untreated group before and after treatment.

Significant increase in haemoglobin level in *Azadirachta indica* treated cow might be due to that in repeat breeding cows erythropoietin is diminish presumbly because of inflammatory cytokinines leading to lowed erythropoesis. As neem is having anti-inflammatory property (Amit Kumar *et al.,* 2013) it reduces the inflammatory cytokines naturally to improve erythropoesis (Thrall, 2004).

In the present study the normal mean haemoglobin level observed in subclinical endometritic cows are in agreement with Heidarpur *et al.* (2014), Green *et al.* (2005) and Ahmed *et al.* (2016) who recorded normal level of mean haemoglobin values in post partum subclinical endometrities and endometritic cows. In contrast to the present study Nazifi *et al.* (2008) , Belice *et al.* (2012) and Syed Anwar (2016) observed lower level of mean haemoglobin value in post partum metritic and subclinical endometritic cows.

Nonsignificant increase in *Achyranthes aspera* treated cows after treatment and in untreated cows is in close accordance with Syed Anwar (2016) who reported no change in haemoglobin level after treatment.

4.4.2 Packed Cell Volume (PCV) (%)

The estimated mean Packed Cell Volume (PCV) percentage levels in different treatment groups and untreated control group are given in Table 5 and Fig 5. As per Brar *et al.* (2004) the normal physiological range of PCV is 24 to 44 % in cows.

The mean packed cell volume (%) in *Azadirachta indica*, *Achyranthes aspera* and untreated control groups were 29.1 ± 2.74, 24.6 ± 0.87 and 22.45 ± 0.87, respectively before treatment and 32.9 ± 0.67, 24.7 ± 0.78 and 22.52 ± 1.02, respectively after treatment on 7th day.

From the present study it was observed that the mean packed cell volume (%) in subclinical endometritic cows were in the normal physiological range**.**

Table 5. Mean values of Packed Cell Volume (%) in subclinical endometritis affected cows treated with T_1 (*Azadirachta indica extract*), T_2 (*Achyranthes aspera* extract) and T_3 (untreated control) before and after treatment

Treatments/ Groups (n=10)	Packed cell volume (PCV)(%)		T statistics value	P Value
	Before	After		
T_1	29.1 ± 2.74^a	32.9 ± 0.67^a	-1.275	0.234
T_2	24.6 ± 0.87^a	24.7 ± 0.78^a	-0.499	0.629
T_3	22.45 ± 0.87^a	22.52 ± 1.02^a	-0.133	0.897

Mean bearing same superscript in a row do not differ significantly

From the present study it was observed that the mean packed cell volume was non-significantly increased in *Azadirachta indica*, *Achyranthes aspera* and untreated control groups after the treatment and untreated control group on 7th day.

The packed cell volume level of subclinical endometritic cows are in agreement with earlier findings of Green *et al.* (2009), Heidarpour *et al.* (2014) and Syed Anwar (2016) who observed PCV level in subclinical endometritic cows within the normal physiological range. In contrast with present findings Nazifi *et al.* (2008) reported the low level of mean heamatocrit in SCE affected cows.

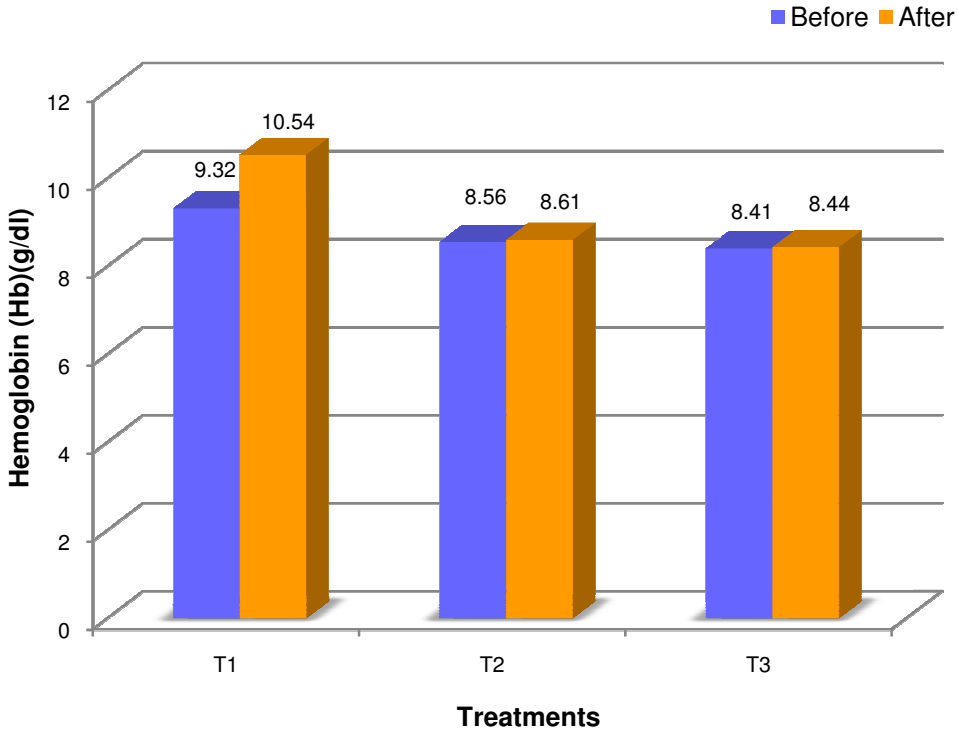


Fig. 4. Mean values of blood hemoglobin level (g/dl) before and after treatment in different groups

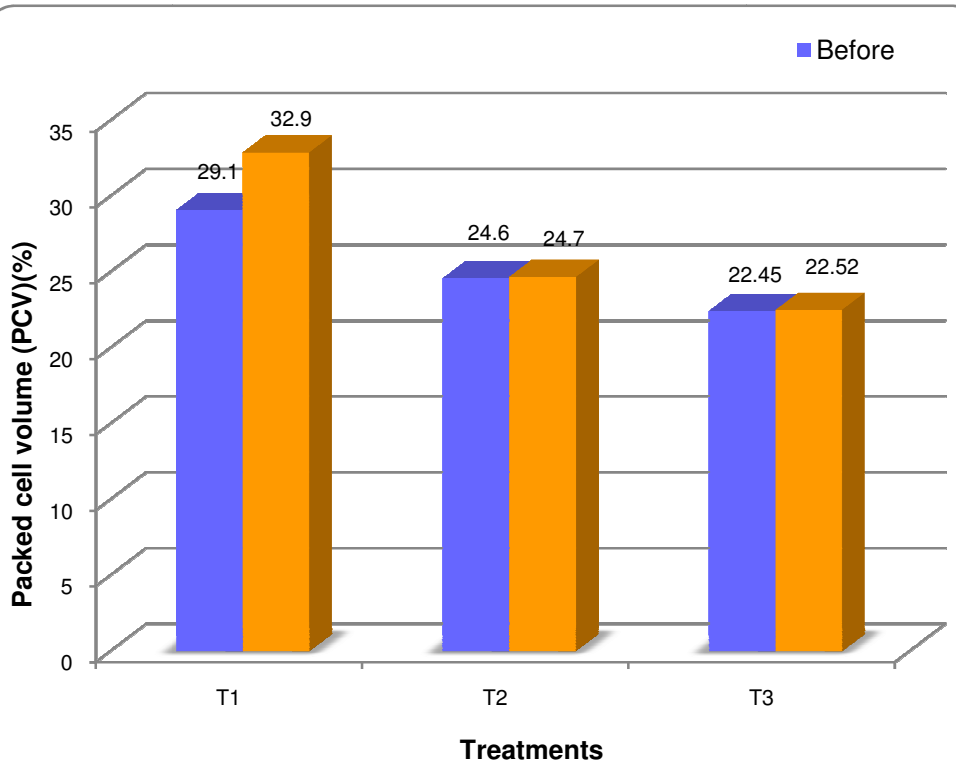


Fig. 5. Mean values of Packed Cell Volume (%) before and after treatment in different groups

Similarly in the present study the mean PCV in *Azadirachta Indica* cows after treatment show nonsignificant difference which is not in accordance in Amit Kumar *et al.* (2013) who reported significant increase in PCV values in repeat breeding cows after treatment by using hydroalcholic neem bark.

4.4.3 Total Leukocyte Count (TLC) (×10^3 /cumm

The mean values of total leukocyte count (×10^3 /cumm) in all different treatment and untreated control groups before and after treatment are shown in Table 6 and Fig. 6. As per Brar *et al.*. (2004) the normal physiological range of TLC is 4 to 11 ×10^3 /cumm in cows.

Table 6. Mean values of blood Total Leukocyte Count (×10^3 /cumm) in subclinical endometritis affected cows treated with T_1 (*Azadirachta Indica extract*), T_2 (*Achyranthes aspera* extract) and T_3 (untreated control) before and after treatment.

Treatments/ Groups (n=10)	Total leukocyte count (TLC) (×10^3 /cumm)		t statistics value	P Value
	Before	After		
T_1	12.32 ± 0.08^a	10.04 ± 0.11^b	15.159	0.000
T_2	12.44 ± 0.10^a	9.43 ± 0.12^b	51.370	0.000
T_3	12.53 ± 0.06^a	11.02 ± 0.14^a	0.000	1.0

Mean bearing same superscript in a row do not differ significantly.

The mean values of *total* leukocyte count (×10^3 /cumm) in *Azadirachta Indica, Achyranthes aspera* and untreated control groups were 12.32 ± 0.08, 12.44 ± 0.10 and 12.53 ± 0.06 respectively before treatment and 10.04 ± 0.11, 9.43 ± 0.12 and 11.02 ± 0.14, respectively after treatment on 7th day.

From the present study it was observed that the mean values of total leukocyte count in subclinical endometritic cows increases than the normal physiological limit. Leukocytosis induced as a result of infection promotes the release of neutrophills from the bone marrow through leukocytosis-inducing-factor (LIF) of the plasma. The concentration of LIF is increased in bacterial diseases by bacterial products; hence leukocytosis (neutrophilia) occurs in such disease (Sastry, 1989).

In the present study the mean values of total leukocyte count significantly decreased after the treatment in T_1 and T_2 groups on 7th day, however there was no significant difference observed in untreated control (T_3) group on 7th day.

These significant decreases in TLC level in subclinical endometritic treated cows suggest that the two treatments were effective for curing the uterine infection.

The total leukocyte count level observed in the present study before treatment is in accordance with Heidarpour *et al.* (2014) and Syed Anwar (2016) who reported the mean level of total leukocyte count to be above the normal physiological limit in SCE cows. In contrast to present observations Nazifi *et al.* (2008) and Green *et al.* (2009) reported the mean level of total leukocyte count within the normal physiological limit.

The total leukocytes count observed in the present study after the treatment significantly decreased which was in accordance with Heidarpour *et al.* (2014) and Patil *et al.* (2015) who revealed that TLC count significantly decreased after the treatment in postpartum subclinical endometritis and metritis, respectively.

4.4.4 Total Erythrocyte Count (TEC) (×10^6/cumm)

The mean total erythrocyte count (×10^6/cumm) in *Azadirachta Indica*, *Achyranthes aspera* and untreated control groups of subclinical endometritic cows before and after treatment are shown in Table 7 and Fig. 7. As per Brar *et al*.. (2004) the normal physiological range of TEC is 5 to 9.5 ×10^6 /cumm in cows.

Table 7. Mean values of blood Total Erythrocyte count (×10^6/cumm) in subclinical endometritis affected cows treated with T_1 (*Azadirachta Indica* extract), T_2 (*Achyranthes aspera* extract) and T_3 (untreated control) before and after treatment.

Treatments/ Groups (n=10)	Total Erythrocyte Count (TEC) (×10^6 /cumm)		t statistics value	P Value
	Before	After		
T_1	6.89 ± 0.49^a	7.04 ± 0.14^a	-0.316	0.759
T_2	5.03 ± 0.24^a	5.21 ± 0.22^a	-1.602	0.143
T_3	4.98 ± 0.21^a	5 ± 0.38^a	-0.042	0.967

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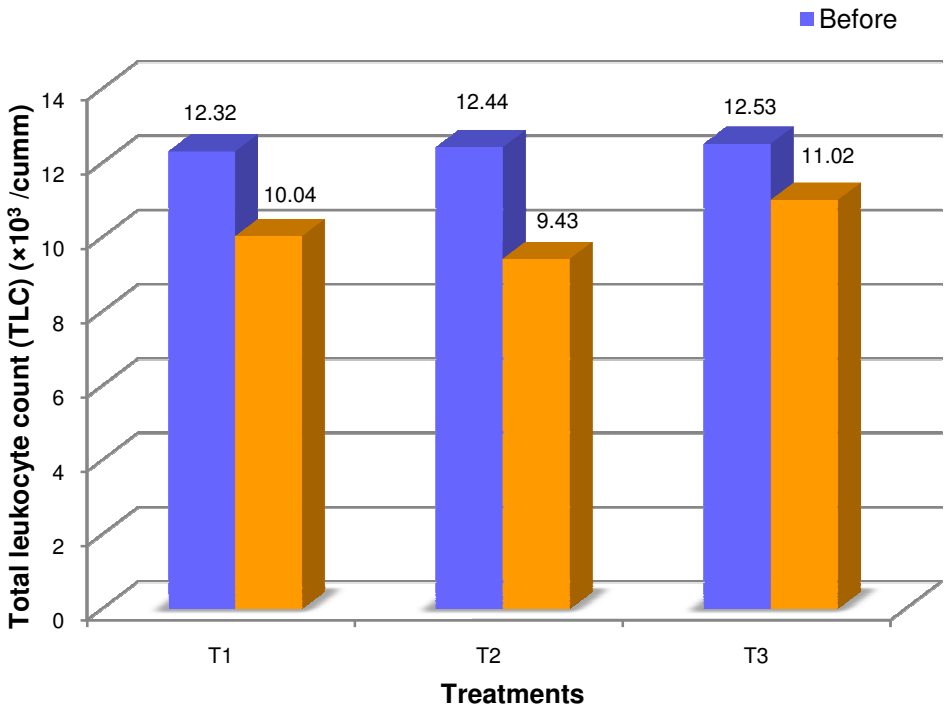

Fig. 6. Mean values of Total leukocyte count (TLC) (×10³ /cumm) before and after treatment in different groups

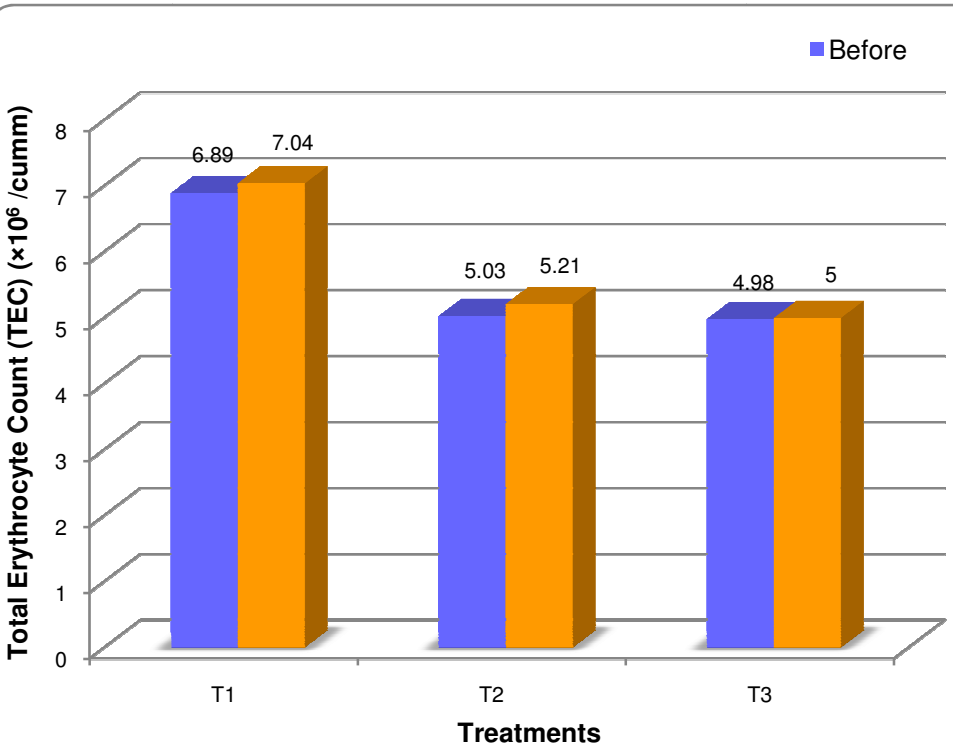

Fig. 7. Mean values of Total Erythrocyte Count (TEC) (×10⁶ /cumm) before and after treatment in different groups

The mean total erythrocyte count ($\times 10^6$/cumm) in *Azadirachta Indica*, *Achyranthes aspera* and untreated control groups were 6.89 ± 0.49, 5.03 ± 0.24 and 4.98 ± 0.21, respectively before treatments and 7.04 ± 0.14, 5.21 ± 0.22 and 5 ± 0.38, respectively after treatments on 7th day.

From the present study it was observed that the concentration of total erythrocyte count was within the normal physiological limits in subclinical endometritic cows.

From the present study it was observed that a moderate non-significant increase in the mean values of total erythrocyte count was observed after the treatment in all two treated groups and untreated control group on 7th day.

In the present study the total erythrocyte count of subclinical endometritic cows observed were in agreement with Nazifi *et al.* (2008), Green *et al.* (2009) Heidarpour *et al.* (2014) and Syed Anwar (2016) who reported normal level of total erythrocyte count in subclinical affected cows.

Observed values of total erythrocyte count after the treatment in the present study increased non-significantly which is in accordance with Heidarpour *et al..* (2014) who revealed non-significant slight increase in TEC level after treatment with cloprostenol sodium and benzathine cephapirin with cloprostenol sodium in subclinical endometritic cows. Similarly Syed Anwar (2016) reported a nonsignificant increase after treatment with *Achyranthes aspera* and Tinosopra cordifolia.

4.4.5 Differential Leucocytes Count (DLC)(%)

4.4.5.1 Neutrophils (%)

The mean neutrophil count (%) in *Azadirachta Indica, Achyranthes aspera* and untreated control group of subclinical endometritic cows before and after treatment are shown in Table 8 and Fig. 8. As per Brar *et al.* (2004) the normal physiological range of neutrophil 15-45%.

Table 8. Mean values of blood Neutrophil count (%) in subclinical endometritis affected cows treated with T_1 (*Azadirachta Indica extract*), T_2 (*Achyranthes aspera* extract) and T_3 (untreated control) before and after treatment

Treatments/ Groups (n=10)	Neutrophils (%)		t statistics value	P Value
	Before	After		
T_1	42.6 ± 0.58[a]	36.3 ± 1.08[b]	8.126	0.000
T_2	43.8 ± 0.46[a]	32.4 ± 1.96[b]	5.968	0.000
T_3	41.9 ± 1.00[a]	42.3 ± 1.00[a]	-1.809	0.103

Mean bearing same superscript in a row do not differ significantly.

The mean neutrophils (%) count in *Azadirachta indica*, *Achyranthes aspera* and untreated control groups were 42.6 ± 0.58, 43.8 ± 0.46 and 41.9 ± 1.00, respectively before treatment and 36.3 ± 1.08, 32.4 ± 1.96, and 42.3 ± 1.00, respectively after treatment on 7th day.

From the present study it was observed that the mean neutrophils (%) count was towards higher side of the normal physiological limit in subclinical endometrtic cows. In the present study the mean neutrophils (%) count in T_1 and T_2 group was decreased significantly ($p<0.01$) after the treatment on 7th day where as there was no significant change in observed in T_3 group on 7th day.

The present findings of mean neutrophils (%) count in SCE affected cows are in agreement with Nazifi *et al.* (2008), Green *et al.* (2009) and Syed Anwar (2016) who reported the mean neutrophils count (%) was towards higher side but within normal limit in subclinical endometritic cows.

Increased in neutrophil levels may be due to leukocytosis induced as result of Infection promotes the release of neutrophils from the bone marrow through leukocytosis-inducing-factor (LIF) of the plasma. The concentration of LIF is increased in bacterial diseases by bacterial products; hence leukocytosis (neutrophilia) occurs in such disease (Sastry, 1989).

Harendra Kumar *et al.* (2013) stated, significant decline in bacterial load was observed at post treatment estrus in both treatment groups (methanolic fraction of neem oil treated cows and neem seed

powder treated cows). Due to decline in bacterial count the mean neutrophils was also decreased.

In the present study significant decrease in neutrophil count after treatment with *Azadirachta Indica* is in accordance with Harenrda kumar *et al.* (2013) were report decrease in neutophil count after treatment due to reduce in the bacterial load with treatment of Azadiracta Indica.

In contrast to the present study Amit Kumar *et al.* (2013) reported increase in neutrophil count after treatment suggesting an effective immunomodulatory effect of *Azadiracta Indica*. Signifacant decrease in neutrophil count after treatment with *Achyranthes aspera* observed and non significant difference in untreated cows in presence is in close aggrement with Syed Anwar (2016) who reported decrease in neutrophil count after treatment with *Achranthes aspera*. Similarly he also observed no difference in neutrophil count in untreated cow.

From the present observation regarding significant decrease in neutrophil % after treatment indicates the antibacterial, immunomodulatory and anti-inflammatory effect of *Azadirachta Indica* (Harendra kumar, Amit kumar 2013) and *Achyranthes aspera* (Vasudeva *et al.*, 2002, Naidu *et al.*, 2006 and Kumar *et al.*, 2009).

4.4.5.2 Lymphocyte (%)

The mean lymphocyte count (%) in *Azadirachta Indica*, *Achyranthes aspera* and untreated groups of subclinical endometritic cows before and after treatment are shown in Table 9 and Fig. 9.As per Brar *et al.* (2004) the normal physiological range of lymphocytes is 40 to 75 % in cows.

The mean values of blood Lymphocyte count (%) in *Azadirachta Indica*, *Achyranthes aspera* and untreated groups were 53.1 ± 0.56, 52.4 ± 0.71 and 54.1 ± 1.05, respectively before treatment and 59.7 ± 1.21, 63.7 ± 2.05 and 54.1 ± 1.03, respectively after treatment on 7^{th} day.

From the present findings it was revealed that the mean values of blood Lymphocyte count (%) was within the normal physiological limit in SCE cows. Whereas the mean lymphocyte (%) count in T_1 and T_2

groups increased significantly ($p<0.01$) after the treatment on 7th day and there was no significant change observed in T_3 group on 7th day.

Table 9. Mean values of blood Lymphocyte count (%) in subclinical endometritis affected cows treated with T_1 (*Azadirachta indica* extract), T_2 (*Achyranthes aspera* extract) and T_3 (untreated control) before and after treatment.

Treatments/ Groups (n=10)	Lymphocytes (%)		t statistics value	P Value
	Before	After		
T_1	53.1 ± 0.56^a	59.7 ± 1.21^b	-5.361	0.000
T_2	52.4 ± 0.71^a	63.7 ± 2.05^b	-5.603	0.000
T_3	54.1 ± 1.05^a	54.1 ± 1.03^a	0.000	1.0

Mean bearing same superscript in a row do not differ significantly.

From the present findings it is evident that the post treatment increase in the mean lymphocytes % of SCE affected cows are in agreement with the earlier findings of Nazifi *et al.* (2008), Green *et al.* (2009) and Syed Anwar (2016) who reported mean lymphocytes percent was in normal physiological limit in subclinical endometrities cows.

Significant ($p<0.01$) increase in mean lymphocytes percent after the treatment with methanolic extract of neem is in agreement with earlier findings of Amit Kumar *et al.* (2013) who reported a significant ($p<0.05$) increase in the mean values of lymphocytes after treatment with hydro-alcoholic neem bark and hydro-acetonic neem bark (I/U) in endometritic cows.

In the present study significant ($p<0.01$) increase in lymphocyte % after treatment with *Achyranthes aspera* and non significant difference in untreated cows is in close aggrement with Syed Anwar (2016) who reported significant increase in lymphocyte % with *Achyranthes aspera* leaf extract in subclinical endometrities cows. Similary he observed no change in untreated cows.

From the present observation regarding significant increase ($p<0.01$) in lymphocyte % after treatment indicates the antibacterial, immunomodulatory and anti-inflammatory effect of *Azadirachta Indica* (Harendra kumar, Amit kumar 2013) and *Achyranthes aspera* (Vasudeva *et al.*, 2002, Naidu *et al.*, 2006 and Kumar *et al.*, 2009).

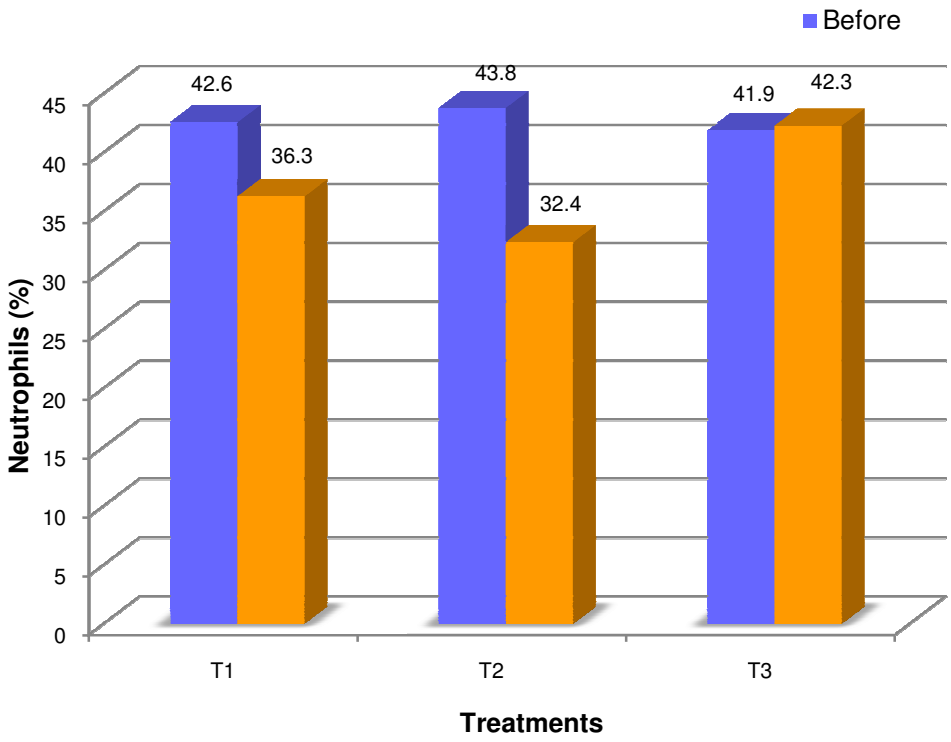

Fig. 8. Mean values of Neutrophils (%) before and after treatment in different groups

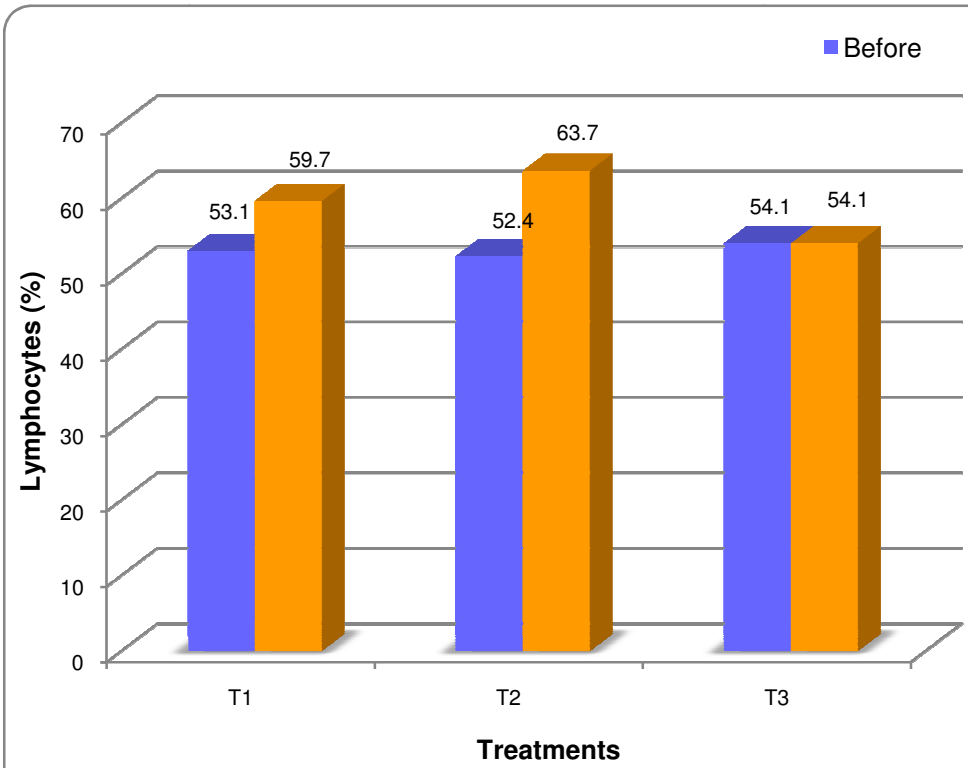

Fig. 9. Mean values of Lymphocytes (%) before and after treatment in different groups

4.4.5.3 Eosinophils (%)

The mean eosinophil count (%) in *Azadirachta indica*, *Achyranthes aspera* and untreated control groups of subclinical endometritic cows before and after treatment are shown in Table 10 and Fig. 10. As per Brar *et al.* (2004), the normal physiological range of eosinophils is 0 to 15 % in cows.

Table 10. Mean values of blood Eosinophil count (%) in subclinical endometritis affected cows treated with T_1 (*Azadirachta indica* extract), T_2 (*Achyranthes aspera* extract) and T_3 (untreated control) before and after treatment.

Treatments/ Groups (n=10)	Eosinophils (%)		t statistics value	P Value
	Before	After		
T_1	2.1 ± 0.23[a]	2.0 ± 0.25[a]	0.318	0.57
T_2	1.6 ± 0.22[a]	1.6 ± 0.16[a]	0.000	1.00
T_3	1.8 ± 0.38[a]	1.7 ± 0.21[a]	0.361	0.726

Mean bearing same superscript in a row do not differ significantly.

The mean values of blood eosinophil count (%) in *Azadirachta indica*, *Achyranthes aspera* and untreated control groups were 2.1 ± 0.23, 1.6 ± 0.22, and 1.8 ± 0.38, respectively before treatment and 2.0 ± 0.25, 1.6 ± 0.16 and 1.7 ± 0.21, respectively after treatment on 7th day.

From the present study it was observed that the mean eosinophil (%) count was within the normal physiological limit in subclinical endometritic cows. Whereas the mean eosinophil (%) count in T_1, T_2 and T_3 groups were observed nonsignificat variation after the treatment on 7th day.

The normal mean eosinophils (%) count in subclinical endometritic cows observed in present study are in accordance with Nazifi *et al.* (2008), Green *et al.* (2009) and Syed Anwar (2016).

The mean eosinophils (%) count after treatment are in agreement with Heidarpour *et al.* (2014) who reported a non-significant change in eosinophil level after treatment with Cloprostenol sodium and benzathine Cephapirin with Cloprostenol sodium in subclinical endometritic cows. Syed Anwar (2016) also reported a nonsignificant difference in

eosinophils count after treatment with *Achyranthes aspera* and untreated cows.

Similarly Patil *et al.* (2015) observed non-significant changes in the mean eosinophils count after the treatment with metronidazole (I/U), Ciprofloxacin and Tinidazole (I/U), Cflox-Tz (I/U) along with single (I/M) injection of $PGF_2\alpha$, Ceftiofer single S/C in postpartum metritic cows.

4.4.5.4 Monocytes (%)

The mean values of monocyte (%) in all groups of subclinical endometritic cows before and after treatment are shown in Table 11 and Fig. 11. As per Brar *et al.* (2004) the normal physiological range of monocytes is 2 to 8 % in cows.

Table 11. Mean values of blood Monocyte count (%) in subclinical endometritis affected cows treated with T_1 (*Azadirachta indica* extract), T_2 (*Achyranthes aspera* extract) and T_3 (untreated control) before and after treatment.

Treatments/ Groups (n=10)	Monocytes (%)		t statistics value	P Value
	Before	After		
T_1	2.2 ± 0.29[a]	2. ± 0.25[a]	0.514	0.619
T_2	2.2 ± 0.38[a]	2.3 ± 0.53[a]	0.000	1.000
T_3	2.2 ± 0.46[a]	1.9 ± 0.56[a]	1.000	0.343

Mean bearing same superscript in a row do not differ significantly.

The mean level of monocyte count (%) in *Azadirachta indica*, *Achyranthes aspera* and untreated control groups were 2.2 ± 0.29, 2.2 ± 0.38 and 2.2± 0.46, respectively before treatment and 2 ± 0.25, 2.3 ± 0.53 and 1.9 ± 0.56, respectively after treatment on 7th day.

From the present study it was revealed that the mean level of monocyte count (%) in all the groups were within normal physiological limit. There was no significant difference in the mean monocytes percent after the treatment in all three treatment groups including control group on 7th day.

The mean values of monocytes count observed in *Azadirachta indica, Achyranthes aspera* and untreated control group in

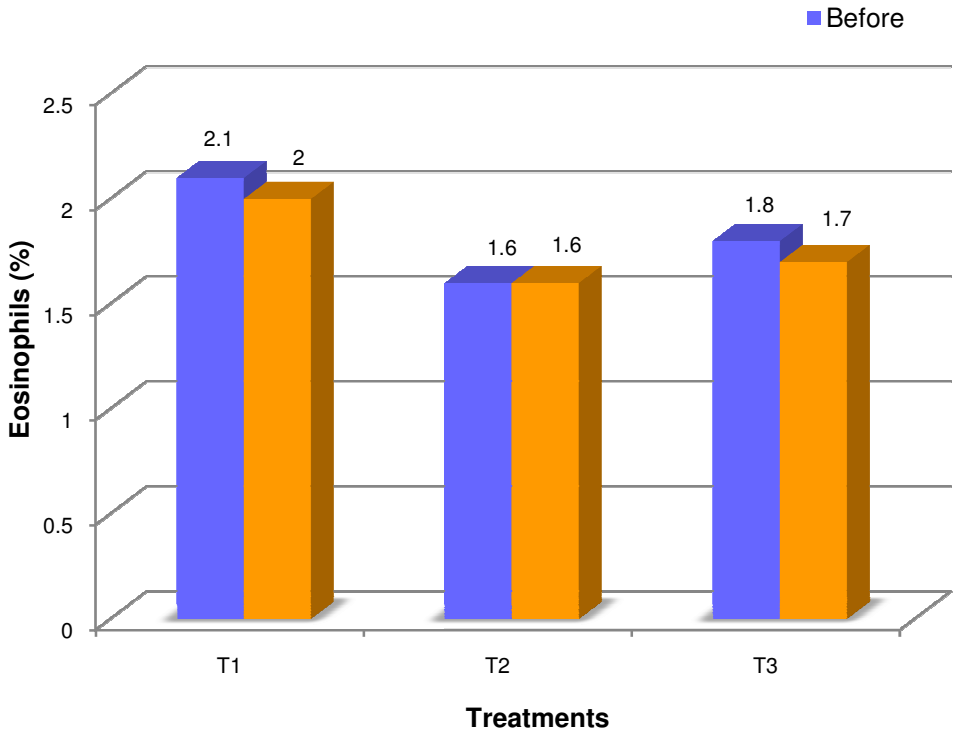

Fig. 10. Mean values of Eosinophils (%) before and after treatment in different groups

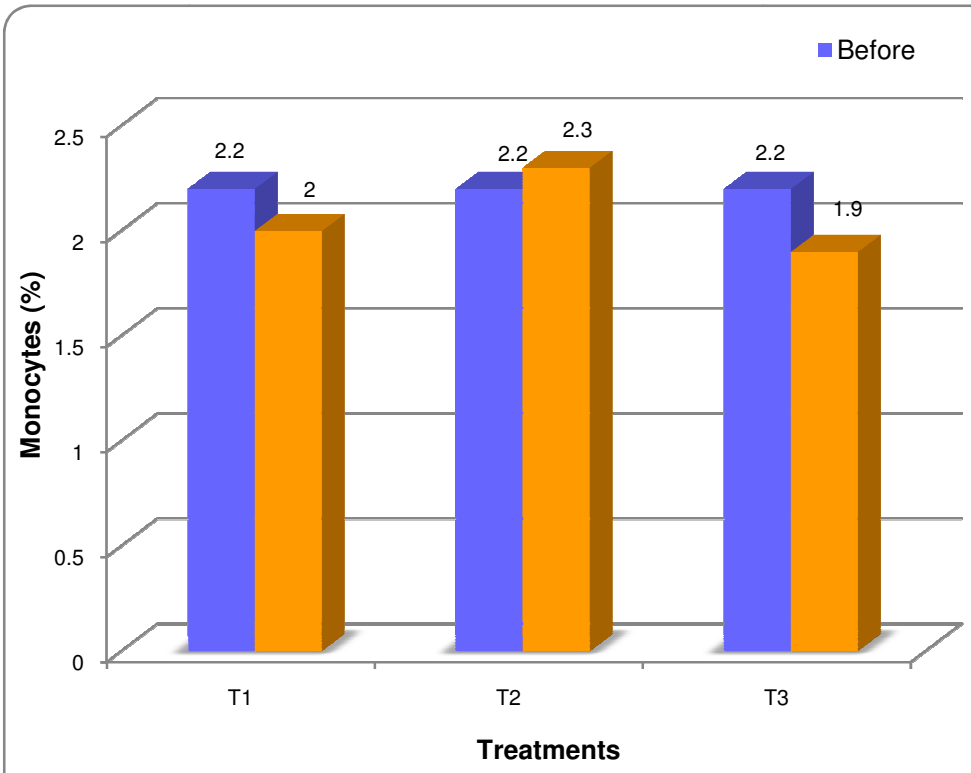

Fig. 11. Mean values of Monocytes (%) before and after treatment in different groups

present study are closely in accordance with Nazifi *et al*. (2008) and Syed Anwar (2016) who reported the mean values of monocytes in SCE cow was within the normal limit. Similarly Ahmad *et al.* (2016) reported mean values of monocyte in normal physiological range in endometritic cows.

The non significant difference in before and after treatment in treatment groups and in untreated group on day 7 observed in present study is in close aggrement with Syed Anwar (2016) who reported non significant difference after treatment in *Achyranthes aspera* group and untreated cows.

In contrast with present findings Heidarpour *et al.* (2014) reported a significant decrease in monocyte level after the treatment with cloprostenol sodium and benzathine cephapirin with cloprostenol sodium in subclinical endometritic cows.

4.5 Biochemical Parameters

4.5.1 Total Serum Protein (g/dl)

The mean total serum protein values (g/dl) in *Azadirachta indica*, *Achyranthes aspera* and untreated control groups of subclinical endometritic cows before and after treatment are shown in Table 12 and Fig. 12. As per Brar *et al*. (2004) the normal physiological range of total serum protein is 6.6 to 7.8 g/dl in cows.

Table 12. Mean values of total serum protein (g/dl) in subclinical endometritis affected cows treated with T_1 (*Azadirachta indica* extract), T_2 (*Achyranthes aspera* extract) and T_3 (untreated control) before and after treatment.

Treatments/ Groups (n=10)	Total protein (g/dl)		t statistics value	P Value
	Before	After		
T_1	6.78 ± 0.07[a]	6.70 ± 0.05[a]	0.829	0.42
T_2	6.84 ± 0.16[a]	6.7 ± 0.10[a]	1.083	0.30
T_3	6.91 ± 0.26[a]	6.94 ± 0.19[a]	-0.101	0.92

Mean bearing same superscript in a row do not differ significantly.

The mean level of total serum protein (g/dl) in *Azadirachta indica*, *Achyranthes aspera* and untreated control groups were 6.78 ± 0.07, 6.84 ± 0.16 and 6.91 ± 0.26, respectively before treatment and 5.5 ± 0.29, 6.7 ± 0.10 and 6.94 ± 0.19, respectively after treatment on 7th day.

The present study revealed that the mean level of total serum protein was within the normal physiological limit in subclinical endometritic cows and there was non-significant variation after the treatment in two treatment groups and also in untreated control group on 7th day which is in close accordance with Syed Anwar (2016) who reported mean total protein level in *Achyranthes aspera* treated and untreated group was within normal physiological levels.

Similarly the level of total serum protein observed in present study was correlated with earlier findings of Sanchez *et al.* (2014) who observed the similar findings of serum total protein value in higher PMN profile of subclinical endometritic cows. However Ruginosu *et al.* (2011) and Biswal *et al.* (2013) observed the similar results of mean serum total protein in puerperal genital infection and in endometritic cows, respectively.

In contrast to present study Green *et al.* (2008) and Akbar *et al*. (2014) observed the slight higher level of serum total protein in SCE affected cows and Reddy *et al.* (2012) found total protein level was significantly increased in endometritic cows as compared to cyclic and noncyclic cows.

From the present study the mean total protein after the treatment is in agreement with earlier findings of Biswal *et al.* (2013) who observed that there was non-significant difference in total protein level in pre and post treated cows with oyster glycogen.

4.5.2 Serum Albumin (g/dl)

The mean serum albumin level (g/dl) in *Azadirachta indica*, *Achyranthes aspera* and untreated control groups of subclinical endometritic cows before and after treatment are shown in Table 13 and Fig. 13. As per Brar *et al*. (2004) the normal physiological range of serum albumin is 2.0 to 3.5 g/dl in cows.

Table 13. Mean values of serum albumin (g/dl) in subclinical endometritis affected cows treated with T_1 (*Azadirachta indica* extract), T_2 (*Achyranthes aspera* extract) and T_3 (untreated control) before and after treatment.

Treatments/ Groups (n=10)	Albumin (g/dl)		t statistics value	P Value
	Before	After		
T_1	2.61 ± 0.15[a]	3.01 ± 0.14[a]	- 1.781	0.10
T_2	3.23 ± 0.08[a]	3.28 ± 0.05[a]	- 0.425	0.68
T_3	3.27 ± 0.13[a]	3.31 ± 0.09[a]	- 0.254	0.80

Mean bearing same superscript in a row do not differ significantly.

The mean serum albumin level in (g/dl) in *Azadirachta indica*, *Achyranthes aspera* and untreated control groups were 2.61 ± 0.15, 3.23 ± 0.08 and 3.27 ± 0.13, respectively before treatment and 3.01 ± 0.14, 3.28 ± 0.05 and 3.31 ± 0.09, respectively after treatment on 7th day.

From the present study it was observed that the mean level of serum albumin within the normal physiological limit in subclinical endometritic cows and there was no significant difference after the treatment in two treatment groups and also in untreated control group on 7th day.

The level of serum albumin in subclinical endometritic cows observed in present study are closely related to earlier findings of Green *et al.* (2009), Heidarpour *et al.* (2012), Akbar *et al.* (2014) Sanchez *et al.* (2014) and Syed Anwar (2016) who observed the serum albumin level in normal physiological limit in SCE affected cows.

The present findings of mean serum albumin level after the different treatments is in agreement with earlier findings of Heidarpour *et al.* (2012) who reported a non-significant rise in serum albumin level after the treatment with cloprostenol sodium and benzathine cephapirin with cloprostenol sodium in subclinical endometritic cows. Similarly, Syed Anwar (2016) found a non significant rise in subclinical endometritic cows after treatment with *Achyranthes aspera* as well as in untreated group.

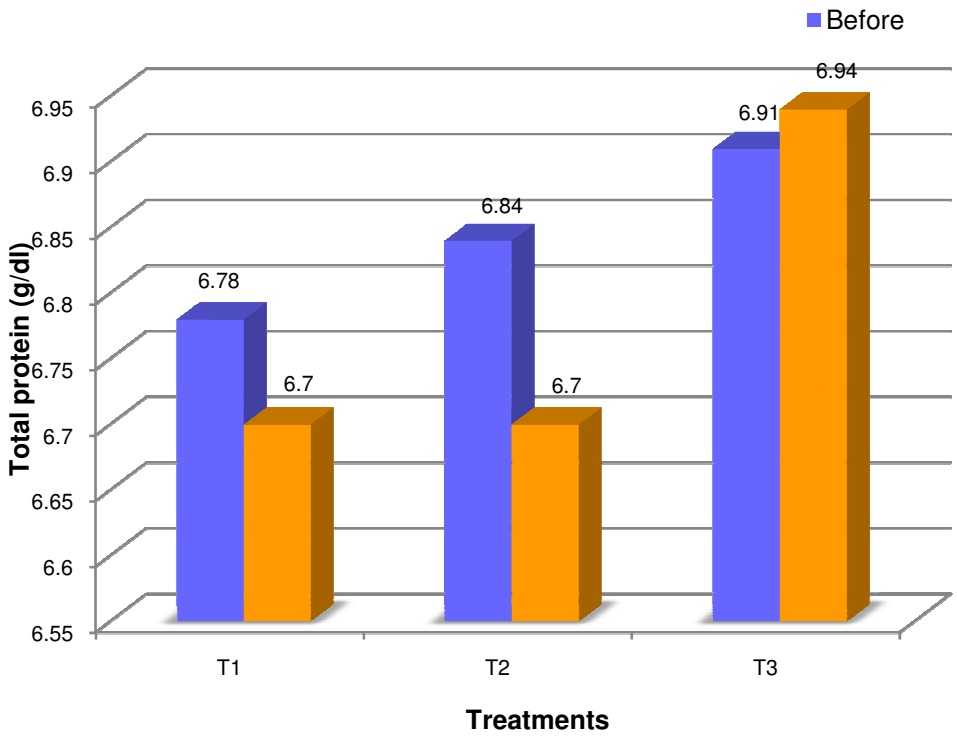

Fig. 12. Mean values of Total protein (g/dl) before and after treatment in different groups

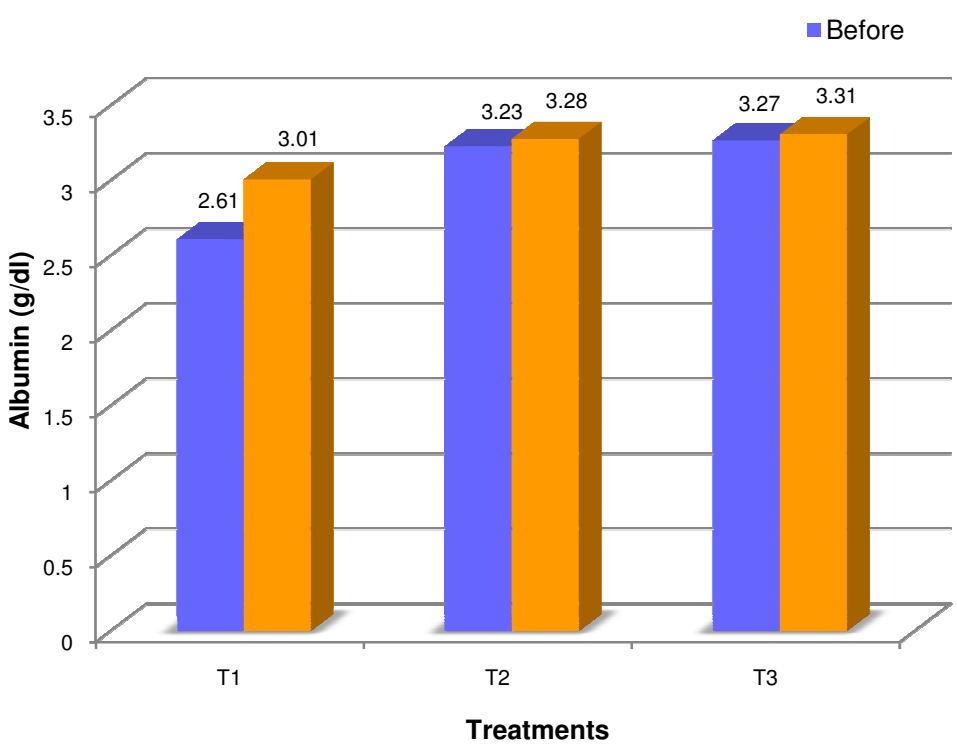

Fig. 13. Mean values of Albumin (g/dl) before and after treatment in different groups

4.5.4 Aspartate Amino Transferase (AST) (IU/L)

The mean values of AST in *Azadirachta indica*, *Achyranthes aspera*, and untreated control groups of subclinical endometritic cows before and after treatment are shown in Table 14 and Fig. 14. As per Brar *et al.* (2004) the normal physiological range of serum AST level is 60 to 118 IU/L in cows.

Table 14. Mean values of serum AST level (IU/L) in subclinical endometritis affected cows treated with T_1 (*Azadirachta indica* extract), T_2 (*Achyranthes aspera* extract) and T_3 (untreated control) before and after treatment.

Treatments Groups (n=6)	AST (IU/L)		t statistics value	P Value
	Before	After		
T_1	70.24 ± 3.70[a]	66.52 ± 3.57[b]	6.144	0.000
T_2	73.37 ± 1.81[a]	59.85 ± 1.27[b]	4.988	0.000
T_3	72.89 ± 2.65[a]	73.12 ± 2.90[a]	- 0.057	0.950

Mean bearing same superscript in a row do not differ significantly.

The mean serum AST level (IU/L) in *Azadirachta indica*, *Achyranthes aspera* and untreated control groups were 70.24 ± 3.70, 73.37 ± 1.81 and 72.89 ± 2.65, respectively before treatment and 66.52 ± 3.57, 59.85 ± 1.27, and 73.12 ± 2.90, respectively after treatment on 7th day.

From the present study it was observed that the mean serum AST level in subclinical endometritic cows was in the normal physiological level and from the results after the treatment it was revealed that there was highly significant ($p<0.01$) decrease in their post treatment values *Azadirachta indica* and *Achyranthes aspera* treated group. However, there was no change in untreated control group on 7th day.

The observed serum AST level in present study are in aggrement with Heidarpour *et al.* (2012), Biswal *et al.* (2013), Akbar *et al.* (2014) , Walker *et al.* (2015) and Syed Anwar (2016) who observed the mean AST level to be within the normal physiological limit.

The present findings for AST after the treatment are closely related to Heidarpour *et al.* (2012) who treated SCE cows with cloprostenol sodium alone and Benzathine cephapirin with cloprostenol sodium and observed the significant decrease after the treatment. Biswal *et al.* (2013) also treated endometritic cows with oyster glycogen as an immunomodulator and observed a significant decreased in AST level after the treatment. Similarly Syed Anwar (2016) observed significant reduction in serum level of AST following *Achyranthes aspera* treatment and there was no significant difference in untreated group at day 7.

Significant decrease in AST level in *Azadirachta indica* treated group which might be due to neem leaf minimize chemically induced liver damage by stablising levels of serum marker enzymes and boosting levels of antioxidants, like those found in vitamins C and E in natural carotenoids, which neutralize free radicals and prevent damage (Sharma *et al.* 2011) and Kale *et al.* (2005) who stated the protective effect of neem leaves on hepatotoxicity induced by antitubercular drugs in rats.

The significant reduction of AST enzyme in the serum after treatment might be attributed to their recovery following therapy (Biswal *et al.* 2013).

4.5.5 Alanine Amino Transferase (ALT) (IU/L)

The mean serum ALT level (IU/L) in *Azadirachta indica*, *Achyranthes aspera* and untreated control groups of subclinical endometritic cows before and after treatment are shown in Table No.15 and Fig. 15. As per Brar *et al.* (2004) the normal physiological range of serum ALT level is 6.9 to 35.3 IU/L in cows.

Table 15. Mean values of serum ALT level (IU/L) in subclinical endometritis affected cows treated with T_1 (*Azadirachta indica* extract), T_2 (*Achyranthes aspera* extract) and T_3 (untreated control) before and after treatment.

Treatments/ Groups (n=10)	ALT (IU/L)		t statistics value	P Value
	Before	After		
T_1	24.83 ± 1.59[a]	25.6 ± 1.57[a]	-1.427	0.187
T_2	24.90 ± 1.73[a]	25.01 ± 1.84[a]	-0.198	0.847
T_3	24.84 ± 1.44[a]	24.67 ± 1.88[a]	0.080	0.93

Mean bearing same superscript in a row do not differ significantly.

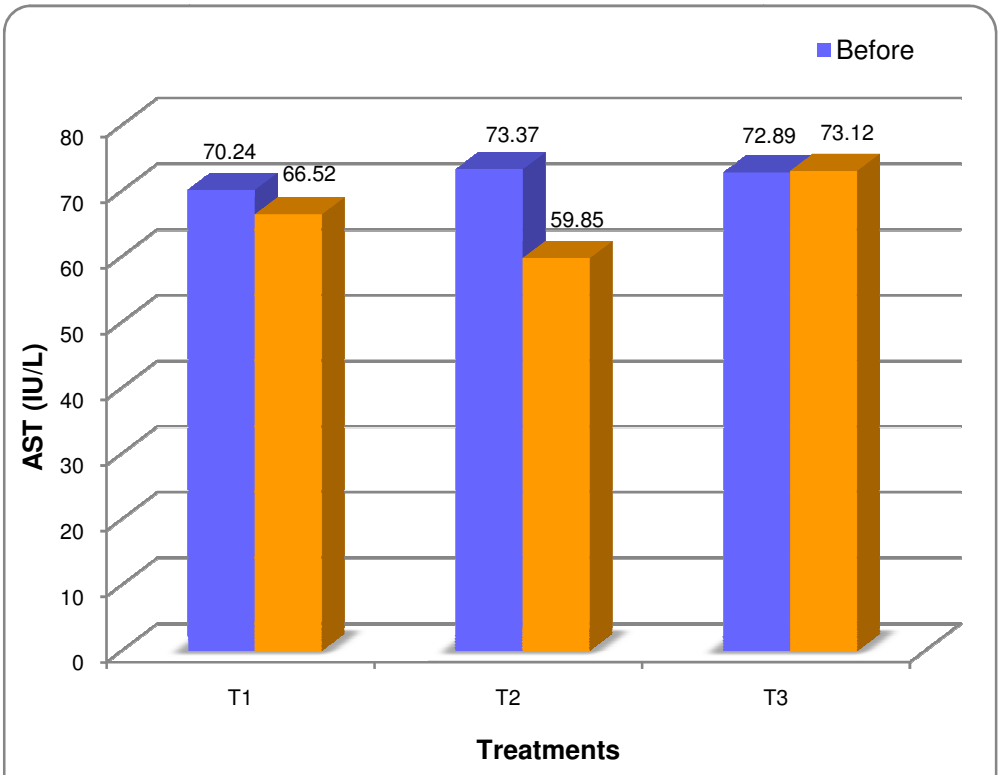

Fig. 14. Mean values of AST (IU/L) before and after treatment in different groups

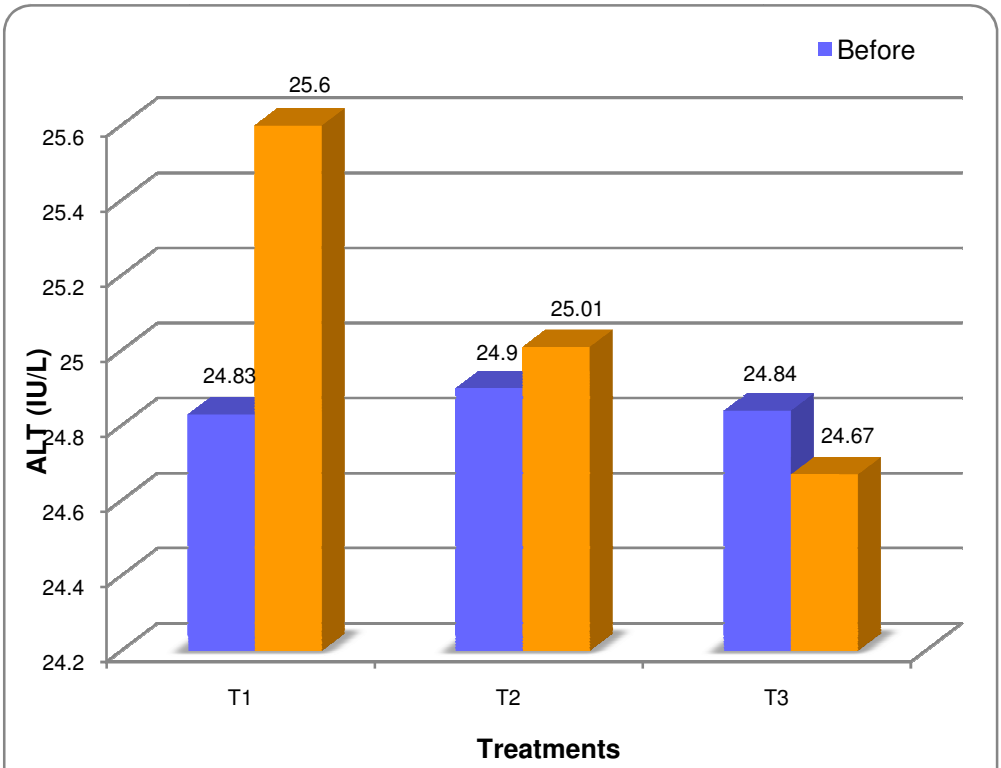

Fig. 15. Mean values of ALT (IU/L) before and after treatment in different groups

The mean serum ALT level (IU/L) in *Azadirachta indica*, *Achyranthes aspera* and untreated control groups were 24.83 ± 1.59, 24.9 ± 1.73 and 24.84 ± 1.44, respectively before treatment and 25.6 ± 1.57, 25.01 ± 1.84 and 24.67± 1.88, respectively after treatment on 7th day.

The observed values from present study of serum ALT in subclinical endometritic cows indicated that the serum ALT levels were within the normal physiological limit in all groups and there was no significant difference after the treatment in two treatment groups and also in untreated control group on 7th day.

The serum level of alanine aminotransferase (ALT) observed in present study after treatment are in accordance to Biswal *et al*. (2013) who treated the endometritic cows with oyster glycogen and reported that the serum level of ALT was non-significantly different before and after treatment and Syed Anwar (2016) who reported nonsignificant difference in *Achyranthes aspera* treated group in subclincal endometritic cows and also in untreated group.

4.5.6 Blood Urea Nitrogen (BUN) (mg/dl)

The mean serum BUN level (mg/dl) in *Azadirachta indica*, *Achyranthes aspera* and untreated control groups of subclinical endometritic cows before and after treatment are shown in Table 16 and Fig. 16. As per Brar *et al*. (2004) the normal physiological range of serum BUN level is 6 to 25 mg/dl in cows.

Table 16. Mean values of blood urea nitrogen (mg/dl) in subclinical endometritis affected cows treated with T_1 (*Azadirachta indica* extract), T_2 (*Achyranthes aspera* extract) and T_3 (untreated control) before and after treatment.

Treatments/ Groups (n=10)	BUN (mg/dl)		t statistics value	P Value
	Before	After		
T_1	15.82 ± 0.76^a	15.42 ± 0.96^a	1.047	0.32
T_2	15.96 ± 0.74^a	16.05 ± 0.78^a	-0.084	0.93
T_3	16.4 ± 0.79^a	16.61 ± 0.60^a	-0.205	0.84

Mean bearing same superscript in a row do not differ significantly.

The mean serum BUN level (mg/dl) in *Azadirachta indica, Achyranthes aspera* and untreated control groups were 15.82 ± 0.76, 15.96 ± 0.74 and 16.4 ± 0.79, respectively before treatment and 15.42 ± 0.96, 16.05 ± 0.78, and 16.61 ± 0.60, respectively after treatment on 7th day.

The BUN level observed in present study was within the normal physiological limit in all subclinical endometritic cows before treatment and the mean BUN level was not-significantly differ after the treatment in two treatment groups and also in untreated control group on 7th day.

Present findings of BUN level in subclinical endometritic cows are in agreement with Barrio *et al*. (2015) and Ruginosu *et al.* (2011) observed no variable difference of blood urea level in subclinical endometritic cows and in puerperal genital infection, respectively. Similarly Syed Anwar (2016) reported nonsignificant difference in *Achyranthes aspera* treated group and in untreated group. In contrast to present findings Ahmad *et al*. (2004) reported the higher values of mean serum BUN level (mg/dl) in endometritic cows.

4.5.7 Creatinine

The mean serum creatinine level (mg/dl) in *Azadirachta indica, Achyranthes aspera*, and untreated control groups of subclinical endometritic cows before and after treatment are shown in Table 17 and Fig. 17. As per Brar *et al.* (2004) the normal physiological range of serum creatiine level is 6 to 25 mg/dl in cows

Table 17. Mean values of serum creatinine (mg/dl) in subclinical endometritis affected cows treated with T_1 (*Azadirachta indica* extract), T_2 (*Achyranthes aspera* extract) and T_3 (untreated control) before and after treatment

Treatments/ Groups (n=10)	Creatinine (mg/dl)		t statistics value	P Value
	Before	After		
T_1	1.70 ± 0.09^a	1.75 ± 0.09^a	-0.609	0.55
T_2	1.33 ± 0.08^a	1.35 ± 0.06^a	-0.210	083
T_3	1.50 ± 0.11^a	1.41 ± 0.09^a	0.568	0.58

Mean bearing same superscript in a row do not differ significantly.

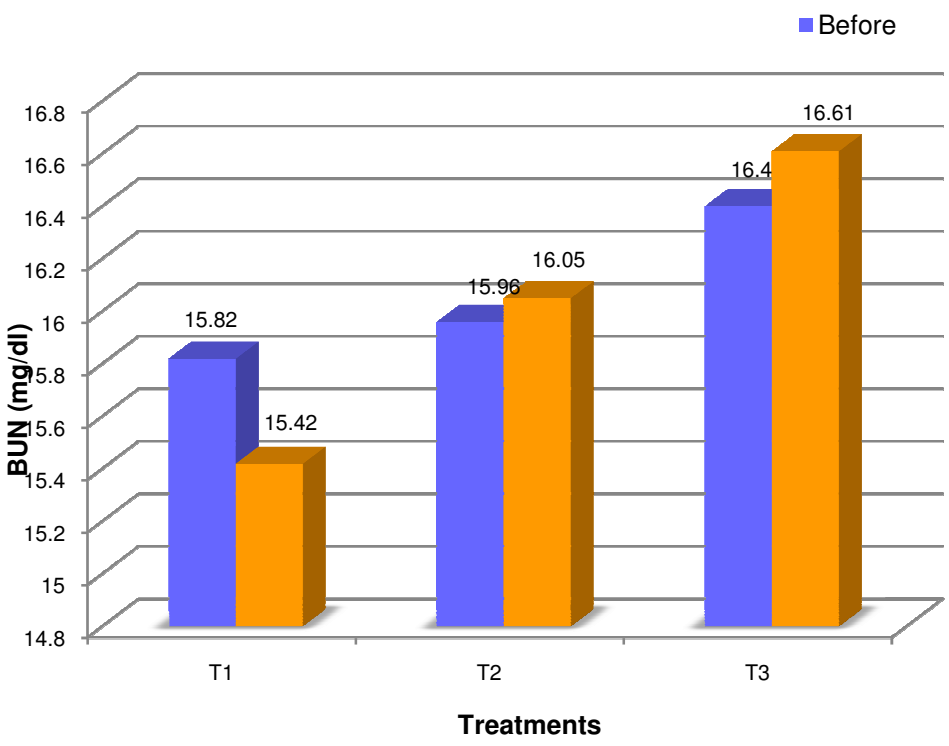

Fig. 16. Mean values of BUN (mg/dl) before and after treatment in different groups

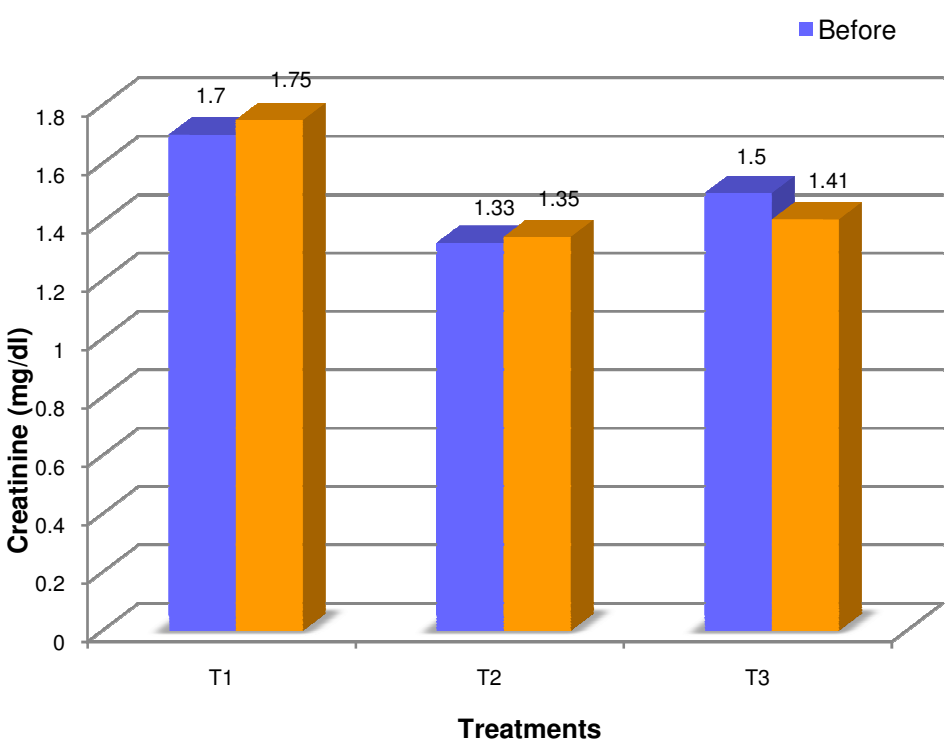

Fig. 17. Mean values of Creatinine (mg/dl) before and after treatment in different groups

The mean serum creatinine level in *Azadirachta indica, Achyranthes aspera* and untreated control groups were 1.70 ± 0.09, 1.33 ± 0.08 and 1.50 ± 0.11, respectively before treatment and 1.75 ± 0.09, 1.35 ± 0.06 and 1.41 ± 0.09, respectively after treatment on 7^{th} day.

The level of serum creatinine observed in present study was well within the normal physiological limit in all subclinical endometritic cows before treatment and the mean creatinine level was different with non significant effect after the treatment in all the treatment groups including untreated control group on 7^{th} day.

The normal level of creatinine observed during present study before and after treatment revealed that intrauterine infusion of *Azadirachta indica* and *Achyranthes aspera* extract did not have any adverse effect on kidney function test.

The literature regarding effect of *Azadirachta indica* and Achyranthes *aspera* on creatinine level was not available to compare.

CHAPTER V

SUMMARY AND CONCLUSIONS

The present study on “Comparative Efficacy Of Different Herbal Extract On Subclinical Endometrities In Postpartum Cows” was carried out with the objectives to study the prevalence of subclinical endometritis in postpartum dairy cows in surroundings of Akola city and to evaluate the therapeutic efficacy and first service conception rate of *Azadirachta indica* and *Achyranthes aspera* in subclinical endometritic cows.

A total of 87 postpartum dairy cows 30-60 days in milk were examined for signs of clinical endometritis. Cows without signs of clinical endometritis were examined for subclinical endometritis with the cytobrush technique. Cows with ≥ 5% polymorphonuclear cells (PMN) in the cytological sample were considered as affected by subclinical endometritis.

Total thirty postpartum cows showing PMN cells ≥ 5% threshold level were selected and divided into three groups comprising ten cows in each group. In T_1 group, cows treated with a 25 ml of hydromethanolic fraction of *Azadirachta indica* intrauterine for three consecuties days. In T_2 group, cows treated with a 20 ml (10mg/ml) sterile hydromethanolic leaf extract of *Achyranthes aspera* (200 mg) intrauterine for three consecutive days and T_3 group **c**ows showing PMN ≥5% in postpartum cows in uterine cytology were taken as untreated positive control group. Blood samples were collected before treatment (0 day) and seven days after the treatment for heamatological and biochemical changes. For curative efficacy endometrial cytology performed in all 30 cows from the three group on consecutive oestrus by using cytobrush technique. All cows exhibited spontaneous estrus were artificially inseminated as per AM and PM rule and pregnancy diagnosis was carried out on day 60 post insemination. Curative efficacy was studied by performing uterine cytology on spontaneous estrus after treatment.

5.1 Prevalence of Subclinical Endometritis in Postpartum Cows

The overall prevalence observed during the study of subclinical endometritis 30 to 60 days postpartum cows with ≥5% threshold level was 35.63 %.

5.2 Therapeutic Efficacy of *Azadirachta indica* and *Achyranthes aspera* in Subclinical Endometritic Cows

The therapeutic efficacy on the basis of curative percentage in *Azadirachta indica* and *Achyranthes aspera* treated cows were (7/10) 70.00% and (8/10) 80.00% after the treatment and in untreated cows it was (2/10) 20.00%, respectively. The curative efficacy was higher in *Achyranthes aspera* treated cows followed by *Azadirachta indica* as compared to untreated cows.

5.3 First Service Conception Rate

The first service conception rate in *azadirachta indica, Achyranthes aspera* and control cows were 50.00, 40.00 and 20.00, respectively. From the present study it was observed that cows treated with *Azadirachta indica* showed higher first service conception rate followed by *Achyranthes aspera* treated cows as compared to untreated cows.

5.4 Hematological Profile

Hematological parameters like Hb, PCV, TEC and DLC were within the normal physiological limit in all subclinical endometritic cows and TLC was above the normal physiological limit in all subclinical endometritic cows.

Hematological examination of subclinical cows showed significant difference in hemoglobin level after treatment with *azadirachta indica* treated cows whereas the PCV and TEC showed nonsignifinant

difference at different time interval. Nonsignifinant difference in mean hemoglobin, PCV and TEC after treatment with *Achyranthes aspera* and untreated cows on day 7. There was significant decrease observed in TLC count after the treatment in both *Achyranthes aspera* and *Azadirachta indica* treated cows, whereas there was no change in mean levels of Hb, PCV, TEC and TLC levels on 7^{th} day in untreated control cows.

In differential leukocyte count, mean values of neutrophils count significantly ($p<0.01$) decrease after the treatment in *Azadirachta indica* and *Achyranthes aspera* treated cows. Similarly lymphocyte count showed significant ($p<0.01$) increase after the treatment with *Azadirachta indica* and *Achyranthes aspera.* Whereas there was no significant difference in untreated control cows.

Biochemical parameters

Serum biochemical parameters like total protein, albumin, AST, ALT, BUN and creatinine were within the normal physiological limit in all subclinical endometritic cows. There was non-significant variation in mean values of serum total protein, albumin, ALT, BUN and creatinine after the treatment with *Azadirachta indica* and *Achyranthes aspera*. Whereas highly significant ($p<0.01$) decrease in AST in *Azadirachta indica* and *Achyranthes aspera* treated cows. There was no change in mean levels of serum total protein, serum albumin, AST, ALT serum BUN and creatinine levels on 7^{th} day in untreated control group.

The higher curative efficacy of *Achyranthes aspera* and *Azadirachta indica* observed in present study indicate that the hydromethanolic extract of *Achyranthes aspera* and *Azadirachta indica* were effective in the treatment of subclinical endometritis in postpartum cows.

5.6 Conclusions

The following conclusions were drawn from the findings of present study.

1) The prevalence of subclinical endometritis in postpartum cows 30-60 DIM in surrounding of Akola city was 35.63 % at ≥5 % threshold level of PMN cells.

2) The therapeutic efficacy was higher in *Achyranthes aspera* treated cows followed by Azadirachta *indica* treated cows as compared to untreated control cows.

3) The first service conception rate was higher in *Azadirachta indica* treated cows followed by *Achyranthes aspera* treated cows as compared to untreated control cows.

4) Heamatological parameters like Hb, PCV, TEC and DLC were observed in normal physiological limit except TLC in subclinical endometritic cows.

5) Biochemical parameters like serum total protein, albumin, AST, ALT BUN and creatinine levels were within the normal physiological limit in subclinical endometritic cows.

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APPENDIX - I

Days in milk, day of treatment, day of exhibition of estrus and pregnancy status in *Achyranthus aspara* treatment group.

Sr. No.	Animal name	Body weight	Days in milk	Day of treatment	Day of exhibition of estrus	Pregnancy status
1	109	250	30	31	71	non pregnant
2	313	269	55	56	69	non pregnant
3	747	285	45	46	72	non pregnant
4	BLACK	296	32	33	80	pregnant
5	593	270	49	50	74	non pregnant
6	UMBRI 1	300	58	57	66	non pregnant
7	UMBRI 2	289	60	61	65	pregnant
8	12	278	40	41	76	pregnant
9	14	299	60	61	75	non pregnant
10	18	261	43	44	72	non pregnant
	AVERAGE	279.7	47.2	48	72	pregnant
	SE	16.149613	10.5337553	10.90361816	4.335896678	-

APPENDIX II

Days in milk, day of treatment, day of exhibition of estrus and pregnancy status in Azadirachta indica treatment group

Sr . No.	Animal name or no.	Body weight	Days in milk	Day of treatment	Day of exhibition of estrus	Pregnancy status
1	NIMBI I	250	56	57	71	Non pregnant
2	NIMBI II	278	60	61	70	pregnant
3	GIR I	299	53	55	69	pregnant
4	GIR II	268	52	53	74	Non pregnant
5	DARK BLACK	289	55	56	76	pregnant
6	SIDE VILLAGE I	300	58	59	70	Non pregnant
7	SIDE VILLAGE II	288	59	60	69	Non pregnant
8	SIDE VILLAGE III	280	50	52	71	Non pregnant
9	ND I	260	51	53	70	pregnant
10	ND II	294	54	55	70	pregnant
	Average	280.6	54.8	56.1	71	-
	SE	16.82062226	3.425395354	3.107338983	2.60	-

APPENDIX III

Days in milk, day of exhibition of estrus and pregnancy status in Untreated control group

Sr. No.	Animal name or no	Body weight	Days in milk	Day in treatment	Day of exhibition of estrus	Pregnancy status
1	401	260	59	-	81	NON PREGNANT
2	402	272	47	-	82	PREGNANT
3	403	300	38	-	83	NON PREGNANT
4	404	250	30	-	82	NON PREGNANT
5	405	290	32	-	84	NON PREGNANT
6	RS I	270	42	-	83	NON PREGNANT
7	RS II	289	50	-	82	NON PREGNANT
8	GAURI	276	55	-	80	PREGNANT
9	KAVERI	270	60	-	80	NON PREGNANT
10	BHURI	310	48	-	83	NON PREGNANT
	Average	278.7	46.1	-	82	
	SE	18.39112105	10.53512643	-	1.333333333	

VITA

The author, **Miss. Nikhade Chaitali Tilakrao**, was born on 5th May, 1992 at Ambajogai, Dist. Beed, Maharashtra, India. She passed his Secondary School Certificate (S.S.C) Examination in the First Division in year 2008, from Saint Anthony English Medium School, Ambajogai. She passed her Higher Secondary School Certificate (H.S.S.C.) Examination in the second division, in the year 2010, from Yogeshwari Mahavidyalaya, Ambajogai, Dist. Beed.

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She believes that she will use her knowledge in a constructive manner for veterinary profession.

THESIS ABSTRACT

a)	**Title of the thesis (in Capital letters)**	:	**COMPARATIVE EFFICACY OF DIFFERENT HERBAL EXTRACT ON SUBCLINICAL ENDOMETRITIS IN POSTPARTUM COWS**
b)	**Full name of student**	:	**Nikhade Chaitali Tilakrao**
c)	**Name and address of Major advisor**	:	**Dr. S. G. Deshmukh** Assistant Professor, Department of Animal Reproduction, Gynaecology and Obstetrics, PGIVAS, Akola
d)	**Degree to be awarded**	:	M.V.Sc.
e)	**Year of award of degree**	:	2018
f)	**Major subject**	:	Animal Reproduction, Gynaecology and Obstetrics
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j)	**Signature, Name and address of forwarding authority**	:	

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ABSTRACT

The project entitled “Comparative efficacy of different herbal extract on subclinical endometritis in postpartum cows” was conducted on thirty subclinical endometritic cows.

Total 87 cows 30-60 days in milk were examined from surrounding farms of Akola city with cytobrush technique. Cows with ≥5% PMN cells in endometrial cytology were regarded as affected by subclinical endometritis.

Out of 87 cows, total 30 cows showing PMN cells ≥ 5% threshold level in postpartum cows were selected and divided into three groups comprising ten cows in each group. In T_1 group cows treated with a 25 ml sterile hydromethanolic extract of *Azadirachta indica* intra uterine for three consecutive days. In T_2 group cows treated with a 20 ml (10mg/ml) sterile hydromethanolic leaf extract of *Achyranthes aspera* (200 mg) intrauterine for three consecutive days and T_3 group **c**ows showing PMN ≥5% in postpartum cows in uterine cytology were taken as positive control group and given no treatment. Blood samples were collected before treatment and seven days after the treatment for heamatological and biochemical changes. For curative efficacy endometrial cytology was performed in all 30 cows from all the groups on consecutive oestrus by using cytobrush technique. Cows exhibited spontaneous estrus from all groups were artificially inseminated as per AM and PM rule.

In the present study the overall prevalence of subclinical endometritis at 30-60 days postpartum was 35.63 %. The overall prevalence of subclinical endometritis with 5% threshold level from 30-60 days in milk was 35.63% (31/87). Herd level prevalence was 30.00% (6/20), 44% (11/25), 27.27% (3/11), 33.34% (4/12), 30% (3/10) and 44.45% (4/9) on farms ILFC, PDKV, Gorakshan Akola, Rashtria Shala, Nimbi Shetki Shala, Gudadhi and Gow Sanstha Dabki Road, respectively.

The therapeutic efficacy on the basis of curative percentage in *Azadirachta indica*, *Achyranthes aspera* treated cows *and* untreated cows were 70.00%, 80.00% and 20.00%, respectively after the treatment. The curative efficacy was higher in *Achyranthes aspera* treated cows followed by *Azadirachta indica* treated cows as compare to untreated control cow.

The first service conception rate in *Azadirachta indica*, *Achyranthes aspera* treated cows and untreated control cows were 50.00%, 40.00% and 20.00%, respectively. Conception rate was higher in *Azadirachta indica* treated cows followed by *Achyranthes aspera* as compared to untreated cows.

All the studied heamatological and serum biochemical parameters in subclinical endometritic cows were within the normal physiological limit except TLC. The TLC count was above the normal physiological limit. There was

significant decrease in TLC, neutrophills and AST level after the treatment, whereas significant increase in lymphocyte counts after the treatments in *Azadirachta indica*, *Achyranthes aspera* treated cows. It was also observed that there was no significant difference in all studied heamatological and serum biochemical parameters on 7^{th} day in untreated control cows.

From present study it was concluded that there was higher prevalence of subclinical endometritis in postpartum cows in surrounding farms of Akola city. The therapeutic efficacy was higher in *Achyranthes aspera* treated cows followed by *Azadirachta indica* treated cows as compared to untreated control cows and first service conception rate was higher in *Azadirachta indica* treated cows followed by *Achyranthes aspera* as compared to untreated control cows.

प्रबंध सारांश

१.	**प्रबंधाचे शिर्षक**	: **''दुग्धोत्पादन कालावधीतील गाईच्या गर्भाशयाच्या सुत्पदहावरील विविध वनौषोधींच्या अर्काची तुलनात्मक कार्यक्षमता''**
२.	**विद्यार्थ्याचे पूर्ण नांव**	: **निखाडे चैताली तिलकराव**
३.	**मुख्य मार्गदर्शकाचे नांव व पत्ता**	: **डॉ. एस. जी. देशमुख** सहाय्यक प्राध्यापक, पशुप्रजनन, स्त्रीरोग व प्रसुतीशास्त्र विभाग, स्नातकोत्तर पशुवैद्यक व पशुविज्ञान संस्था, अकोला.
४.	**प्रदान केली जाणारी पदवी**	: एम.व्ही.एस्सी. (पशुप्रजनन, स्त्रीरोग व प्रसुतीशास्त्र)
५.	**पदवी प्रदान करण्याचे वर्ष**	: २०१८
६.	**मुख्य विषय**	: पशुप्रजनन, स्त्रीरोग व प्रसुतीशास्त्र
७.	**प्रबंधामधील एकुण पाने**	: ८२
८.	**प्रबंध सारांशामधील एकुण शब्द**	: ५२३
९.	**विद्यार्थ्याची सही**	:
१०.	**प्रबंधक कार्यवाहीस्तव पाठविणाऱ्या अधिकाऱ्याची सही, नाव व पत्ता**	:

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स्नातकोत्तर पशुवैद्यक व पशुविज्ञान संस्था,
अकोला.

सारांश

गाईमधिल गर्भाशयाच्या सुप्त दाहावर कडुनिंब आणि आघाडाची उपचारात्मक क्षमता बघणे याचा प्रायोगिकरित्या अभ्यास पशुजनन व स्त्रीरोग शास्त्र विभाग स्ना.प.प.वि. संस्था अकोला येथे करण्यात आला.

एकुण ३० गायीमध्ये कडुनिंब आणि आघाड्याच्या वनस्पतींचा गर्भाशयाच्या सुप्त दाहावरील उपचारात्मक क्षमतेचा अभ्यास सदर प्रयोगांतर्गत करण्यात आला. सदर अभ्यासात

अकोला सवभोतालच्या परीसरातील एकुण ८७ व्यालेल्या गायी मध्ये ज्या ३० ते ६० दिवसाच्या दुग्धोत्पादन कालावधीत होत्या अशा गायीच्या सायटोब्रश तंत्राच्या आधारे गर्भाशयाची उतीशास्त्रात्मक तपासणी करण्यात आली. ज्या गायीच्या गर्भाशयाच्या उतीशास्त्रात्मक अभ्यासात पॉलीमार्फोन्युक्लीयर (पी.एम.एन.) पेशींचे प्रमाण ५% किंवा अधिक होते. अशा गायींना गर्भाशयाचे सुप्त दाह झालेला असल्याचे निदान करण्यात आले.

एकुण ८७ व्यालेल्या गायी पैकी ३० गायी मध्ये पी.एम.एन. पेशींचे प्रमाण ५% किंवा अधिक आढळुण आले. त्यांना निवडुन **तीन** गटात, प्रत्येकी १० गायी प्रमाणे समान रित्या विभागण्यात आले. गट क्र. १ ($T_१$) च्या गायींना **कडुनिंब** वनस्पतीच्या **बिया** विरहीत वेलेचा निरर्जंतुक हायड्रोमिथॅनोलीक अर्क २५ मी.ली.या प्रमाणे गर्भाशयात सलग ३ दिवस गर्भाशयाच्या सुप्त दाहावर उपचार म्हणून देण्यात आला.

गट क्र. २ ($T_२$) च्या गायींना आघाडा या वनस्पतीच्या पानाच्या निरर्जंतुक हायड्रोमिथॅनोलीक अर्क २० मी.ली. (१० मी.ग्रा./मी.ली.) या प्रमाणे गर्भाशयात सलग ३ दिवस (प्रतीदिन २०० मि.ग्रा. या दराने) गर्भाशयाच्या सुप्त दाहावर उपचार म्हणून देण्यात आला.

तसेच गट क्र. ३ ($T_३$) मधिल गायी व्याल्यापश्चात पि.एम.एन. पेशींचे प्रमाण ५% किंवा अधिक होते त्यांना विनाउपचार (नियंत्राण गट) म्हणून संबोधण्यात आला.

रक्त घटक चाचणी आणि जैव रासायनिक बदलांचा अभ्यास करण्यासाठी सदर गायींचे उपचार देण्यापूर्वी आणि उपचारा पश्चात ७ व्या दिवशी रक्त नमुने घेण्यात आले.

सदर केलेल्या उपचारांच्या क्षमतेच्या शहाणीशेसाठी सर्व ३० गायींमध्ये उपचारा नंतर माज दाखविल्यावर सायटोब्रश तंत्राच्या आधारे गर्भाशयाच्या उती शास्त्रात्मक अभ्यास करण्यात आला. **तीनही** गटातील गायीमध्ये माजावर आल्यानंतर सकाळ व सायंकाळी या नियमाप्रमाणे कृत्रिम रेतन करण्यात आले.

सदर तपासणीत ज्या गायींमध्ये पि.एम.एन. पेशींचे प्रमाणे ५% पेक्षा कमी आढळुण आले अशा गायींना गर्भाशयाचा सुप्त दाह दुरूस्त झाल्याचे निदान करण्यात आले.

सदर अभ्यासात गायीमध्ये व्याल्यानंतर ३० ते ६० दिवसातील एकुण ३५.६३% गर्भाशयाचे सुप्त दाहाचे प्रमाण आढळुण आले. **गर्भाशयाच्या सुप्त दहाचा एकूण प्रसार ३५.६३ टक्के आढळला. कळपातील प्रसाराची पातळी ३०.०० टक्के (६/२०), ४४.४५ टक्के (११/२५), २६.२६ टक्के (३/११), ३३.३४ टक्के (४/१२), ३० टक्के (३/१०) आणि ४४.४५ टक्के (४/९), आय.एल.एफ.सी., पं.दे.कृ.वि., गोरक्षण अकोला, राष्ट्रीय शाळा,**

निंबी शेतकी शाळा, गुडदी आणि गोसंस्थान दाबकी रोड, अकोला मध्ये अनुक्रमे आढळली.

उपचारापश्चात T_1, व T_2 या गटांमध्ये उपचारानंतर दुरूस्ती प्रमाणाच्या आधारे सरासरी अनुक्रमे ७०.००% व ८०.००% उपचारात्मक क्षमता आढळली तसेच T_3 या नियंत्राण गटात उपचारात्मक क्षमता सरासरी २०.००% आढळुन आली. तुलनात्मक रित्या आघाडा गटाची उपचारात्मक क्षमता कडुनिंब व नियंत्रण गटा पेक्षा सरस आहे. प्रथम फळविल्या पश्चात गर्भधारणेचे प्रमाण T_1, T_2 व T_3 या गटात अनुक्रमे ५०.०० % ४०.००% व २०.००% इतके आढळुन आले.

तुलनात्मक रित्या कडुनिंब गटाचे गर्भधारणेचा दर आघाडा व नियंत्रण गटापेक्षा सरस आला.

सर्व गटामध्ये गर्भाशयाचा सुप्त दाह झालेल्या गायी मध्ये एकुण पांढऱ्या पेशी वगळता रक्त घटक चाचणी व जैवरसायनीक चाचणी पश्चात सर्व घटक त्यांच्या सर्व साधारण पातळीत आढळले. या पैकी पांढऱ्या पेशींचे प्रमाणे हे सर्वसाधारण पातळी पेक्षा अधिक आले.

उपचारा पश्चात सातव्या दिवशी कडुनिंब व आघाडा या गटांमध्ये एकुण पांढ-या पेशी, न्युट्रोफील्स व ए.एस.टी. ची पातळी यामध्ये लक्षणीय रीत्या घट तर लिमफोसाईट पेशींमध्ये लक्षणीय वाढ आढळुन आली.

सदर अभ्यासा अंती असे आढळुन आले की, अकोला लगतच्या परीसरातील गायीमध्ये व्याल्यापश्चात गर्भशयाच्या सुप्त दाहाचे प्रमाण अधिक आढळुन आले. तसेच आघाडा वनस्पतीच्या अर्काची उपचारात्मक क्षमता आणि प्रथम फळविल्या पश्चात गर्भधारणेचे प्रमाण कडुनिंब अर्कापेक्षा सरस आली.