



ISSN (E): 2277-7695  
ISSN (P): 2349-8242  
NAAS Rating: 5.23  
TPI 2022; 11(12): 5196-5199  
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[www.thepharmajournal.com](http://www.thepharmajournal.com)  
Received: 22-09-2022  
Accepted: 25-10-2022

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## To evaluate the therapeutic efficacy of *Achyranthes aspera* (Aghada) and *Allium sativum* (Garlic) extract on subclinical endometritis in crossbred cows

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### Abstract

The objective of the study was to evaluate the therapeutic efficacy of *Achyranthes aspera* (Aghada) and *Allium sativum* (Garlic) extract on subclinical endometritis (SCE) in postpartum crossbred cows. Total 83 cows, 30-60 days in milk (DIM) were screened from surrounding farms of Akola city with cytobrush technique with >5% PMN cells. Total 24 crossbred cows were selected and divided into three equal groups. First group (T<sub>1</sub>) was treated with 20 ml (10mg/ml) sterile hydroethanolic root extract of *Achyranthes aspera* (200 mg), second group (T<sub>2</sub>) was treated with 10 ml tincture of *Allium sativum* (Garlic) in 90 ml normal saline intrauterine for three consecutive days, respectively and third group (T<sub>3</sub>) cows showing PMN >5% in postpartum cows in uterine cytology were taken as positive control, without treatment. Blood was collected before treatment and at subsequent estrus for haematological and biochemical changes. Cows exhibiting spontaneous estrus from all groups were artificially inseminated as per AM and PM rule. The therapeutic efficacy and first service conception rate was higher in cows in T<sub>1</sub> followed by T<sub>2</sub> as compared to T<sub>3</sub>. The studied haematological and biochemical parameters in SCE cows were within normal physiological limit except TLC and neutrophils. There was significant decrease ( $p < 0.01$ ) in TLC and neutrophils and significant increase ( $p < 0.01$ ) in lymphocytes after treatment in T<sub>1</sub> and T<sub>2</sub>. It was concluded that the therapeutic efficacy and first service conception rate was higher in cows treated with *Achyranthes aspera* followed by *Allium sativum*.

**Keywords:** Cows, SCE, PMN cell, conception rate, therapeutic efficacy

### 1. Introduction

Subclinical endometritis disease is defined by the proportion of polymorphonuclear neutrophils (PMNs) exceeding operator-defined thresholds, usually about 5% of cells in samples collected by flushing the uterine lumen or by endometrial cytobrush, in the absence of clinical endometritis, about 35 to 40 days post-partum (Sheldon *et al.*, 2006) [1]. The treatment of endometritis with antibacterial agents, antibiotics and hormones has met with varying degree of success but due to overexploitation use of these agents lead to development of inconsistent recovery rate, high cost of treatment, residual effect of antibiotics & hormones in the milk & meat causing human health hazard. (Kala, 2005) [4]. About 60-85% of the population of developing world relies either on herbal or on indigenous forms of Complementary and Alternative Medicine (CAM) for their various general health related issues and countering several diseases (Kochnar, 1981) [5].

*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 antimicrobial, oestrogenic, immune stimulant, anti-inflammatory, antioxidant, diuretic, cardiac stimulant, antispasmodic and hepato protective (Dey, 2011) [3]. *Allium sativum* (Garlic) has been widely recognized as a valuable spice and after crushing the garlic bulb activate the enzyme allicin which has antimicrobial effects against many viruses, bacteria, fungi and parasites (Kemper, 2000) [6]. The rising incidence of postpartum subclinical endometritis in cows generated an interest to study the effect of *Achyranthes aspera* and *Allium sativum* on therapeutic efficacy and conception rate in subclinical endometrial cows.

### 2. Materials and Methods

The study was conducted on total 24 postpartum crossbred cows positive for subclinical endometritis belonging to ILFC, PDKV, Akola and surrounding Gorakshans of Akola city.

Total 83 cows between 30 to 60 DIM were screened for subclinical endometritis by collecting the mucous from uterus with cytobrush technique. Giemsa stain was used for detection of >5% PMN cells, Total 24 cows were included in study and divided in to three equal groups. The cows from Group I (T1, n=8) were treated with a 20 ml (10mg/ml) sterile hydroethanolic root extract of *Achyranthes aspera* (200 mg) intrauterine for three consecutive days. The cows from Group II (T2, n=8) were treated with a 10 ml tincture of Garlic in 90 ml normal saline intra uterine once daily for three consecutive days and the cows from Group III (T3, n=8) showing PMN  $\geq 5\%$  in postpartum cows in uterine cytology were kept as a control group and given no treatment up to the first estrus after selection (i.e. up to research period), then after uncured cows were treated hydroethanolic root extract of *Achyranthes aspera*.

The therapeutic efficacy was studied by undertaking uterine endometrial cytology on day seven after initiation of treatment, whereas the uterine endometrial cytology of untreated cows were undertaken on subsequent spontaneous estrus. All the animals were closely observed for spontaneous estrus exhibition by visual observation and inseminated with frozen semen and pregnancy was diagnosed on day 60 post insemination.

The blood biochemical parameters viz. Serum total protein, BUN, Creatinine and Haematological parameters viz. Haemoglobin, Packed Cell Volume, Total Erythrocyte Count, Total Leucocyte Count and Differential Leucocyte Count parameters were examined at the time of selection of animals before treatment and at subsequent spontaneous estrus after treatment.

The roots of *Achyranthes aspera* were collected in the month of January-February from the campus of Post Graduate Institute of Veterinary and Animal Sciences, Akola and authenticated by expert taxonomist Dr. S.P. Rothe, Botanist, Department of Botany, Shri Shivaji Science College, Akola (M.S.). Roots were cut into small pieces and shade dried in the department of Pharmacology and Toxicology, PGIVAS,

Akola. The shade dried roots of *Achyranthes aspera* were processed to get fine powder with the help of pulverizing machine. The freshly prepared powder of roots (50g) was immersed in hydro-ethanolic solution (30% distilled water + 70% ethanol) in a flask stoppered tightly with cotton plug and was kept at room temperature for 48 hours at 150 rpm in an orbital shaker. The contents of the flask were filtered through muslin cloth. The residue left in the flask was rinsed with little quantity of hydroethanolic 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 extract was stored in air-tight screw cap vials and kept in the desiccators until further used.

Claves of the plant *Allium sativum* (Garlic) were used for the preparation of tincture of *Allium sativum*, garlic cloves were dried and grind into powder out of which 30% powder was mixed with 70% alcohol. The mixture was placed in a water bath for 4-6 hrs at 65-70 °C and allowed overnight incubation at room temperature (25-30 °C). It was filtered and stored in air-tight screw cap bottles and refrigerated until further use. The garlic tincture (filtrate) 10 ml was mixed with 90 ml of normal saline and used (Alagar *et al.*, 2018) <sup>[1]</sup>.

## 2.1 Statistical Analysis

The data was analyzed by paired T test Design using online software of Web Agri Stat Package 2.0. developed by ICAR Research Complex Goa, Ela, Old Goa, Goa. 403 402. India.

## 3. Results and Discussion

The therapeutic efficacy and conception rate observed in treatment and untreated groups were depicted in Table 1 and the haematological and biochemical observation were depicted in Table 2 and 3, respectively.

**Table 1:** Therapeutic efficacy and first service conception rate in T1, T2 and T3 groups.

Sr.no	Parameters	Groups		
		T1	T2	T3
1.	Therapeutic efficacy (%)	(7/8) 87.50%	(6/8) 75.00%	(2/8) 25.00%
2.	Conception rate (%)	(4/8) 50.00%	(3/8) 37.50%	(2/8) 25.00%

The therapeutic efficacy was higher in *Achyranthes aspera* treated cows followed by *Allium sativum* as compared to untreated cows. The higher curative percentage of *Achyranthes aspera* might be attributed to its immunomodulatory, anti-inflammatory, antibacterial and estrogenic properties. (Dey 2011) <sup>[3]</sup>. The therapeutic efficacy of *Achyranthes aspera* observed in the study is in close agreement with Nikhade *et al.* (2019) <sup>[10]</sup> reported 80.00 percent therapeutic efficacy of *Achyranthes aspera* hydromethanolic extract in post-partum cows, while 20 percent of cows reported negative for subclinical endometritis in untreated (T3) control cows are in accordance with Bhardwaz *et al.* (2018) <sup>[2]</sup> reported 20% cows negative for SCE at subsequent estrus without any treatment. Similarly Kumar *et al.* (2013) <sup>[15]</sup> revealed 25% cows to be negative for subclinical endometritis. The therapeutic efficacy of *Allium sativum* observed in the present study are in close agreement with Alagar *et al.* (2018) <sup>[1]</sup> reported 66.67 percent therapeutic

efficacy with intrauterine administration of garlic extract and Bhardwaz *et al.* (2018) <sup>[2]</sup> reported 80 percent therapeutic efficacy in cows treated with garlic extract.

It was observed that cows treated with *Achyranthes aspera* showed higher first service conception rate followed by *Allium sativum* 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 *Achyranthes aspera* and *Allium sativum* in subclinical endometrities which had a detrimental effect on fertility causing an increase in calving to conception and a decrease in the rate of cows who become pregnant (Ricci *et al.*, 2015) <sup>[11]</sup>.

In the present research, the first service conception rate observed in the *Achyranthes aspera* treated cows are in close agreement with Syed (2016) <sup>[14]</sup> and Nikhade *et al.* (2019) <sup>[10]</sup>, reported 50 and 40% first service conception rate, respectively in subclinical endometritis cows treated with

*Achyranthes aspera* hydromethanolic extract. The first service conception rate with *Allium sativum* are in accordance with Bhardwaz *et al.* (2018) [2] reported 40 percent first service conception rate in endometrial affected cows treated with garlic extract. In contrast, Kumar *et al.* (2018) [8] reported 83.33 percent conception rate in repeat breeder cows with endometrial symptoms treated with 30 ml garlic extract.

Results of untreated cow of control group are in accordance with Moges and Jebar (2012) [9] and Nikhade *et al.* (2019) [10] observed first service conception rate of 21.4%, and 20% in subclinical endometrial cows, respectively. In contrast, Ricci *et al.* (2015) [11] and Syed (2016) [14] observed lower first service conception rate 13% and 16.67% in Subclinical endometritic cows, respectively.

**Table 2:** Haematological observations in T1, T2 and T3 groups.

Groups	Parameters	
	Haemoglobin (Hb) (g/dl)	
	Before	After
T1	9.96±0.54 <sup>a</sup>	10.12±0.62 <sup>a</sup>
T2	9.15±0.38 <sup>a</sup>	9.33±0.37 <sup>a</sup>
T3	9.28±0.34 <sup>a</sup>	9.33±0.35 <sup>a</sup>
Packed Cell Volume (PCV) (%)		
T1	30.37±1.85 <sup>a</sup>	29.97±1.87 <sup>a</sup>
T2	26.96±1.08 <sup>a</sup>	26.91±1.12 <sup>a</sup>
T3	25.25±0.47 <sup>a</sup>	25.02±0.44 <sup>a</sup>
Total Leukocyte Count (TLC)		
T1	12.93±0.19 <sup>a</sup>	10.00±0.35 <sup>b</sup>
T2	12.73±0.27 <sup>a</sup>	10.35±0.21 <sup>b</sup>
T3	12.87±0.21 <sup>a</sup>	12.6±0.22 <sup>a</sup>
Total Erythrocyte Count (TEC) (×10 <sup>6</sup> /cumm)		
T1	5.25±0.19 <sup>a</sup>	5.53±0.17 <sup>a</sup>
T2	4.58±5.11 <sup>a</sup>	5.11±0.25 <sup>a</sup>
T3	5.43±0.20 <sup>a</sup>	5.98±0.23 <sup>a</sup>
Neutrophil Count (%)		
T1	41.5±0.82 <sup>a</sup>	36.62±0.73 <sup>b</sup>
T2	42.12±0.83 <sup>a</sup>	37.62±0.90 <sup>b</sup>
T3	41.87±1.02 <sup>a</sup>	42.25±0.77 <sup>a</sup>
Lymphocyte Count (%)		
T1	53.62±1.05 <sup>a</sup>	58.75±0.78 <sup>b</sup>
T2	53.25±0.95 <sup>a</sup>	58.25±1.04 <sup>b</sup>
T3	53.62±1.17 <sup>a</sup>	53.5±0.92 <sup>a</sup>

Mean bearing different superscript differ significantly

The studied haematological parameters in subclinical endometrial cows were within normal physiological limit except TLC and increased levels of neutrophils within normal physiological limit. This substantial increase in mean total leukocyte count might be due to the release of neutrophils from the bone marrow via the leukocytosis-inducing-factor (LIF) of the plasma is promoted by leukocytosis induced as a result of infection. Bacterial products increase the concentration of LIF in bacterial diseases, so leukocytosis (neutrophilia) occurs in such diseases (Sastry, 2001) [12]. Increased levels of neutrophils within normal physiological limit might be due to leukocytosis caused as a result of infection, which facilitates the release of plasma leukocytosis-inducing-factor (LIF) neutrophils from the bone marrow. Bacterial products increase the concentration of LIF in bacterial diseases, so leukocytosis (neutrophilia) occurs in such diseases (Sastry, 2001) [12].

The significant decrease ( $p < 0.01$ ) in TLC and neutrophils and significant increase ( $p < 0.01$ ) in lymphocytes after treatment was observed in T1 and T2 groups. The non significant

difference observed in Hb, TEC, PCV and significant decrease in TLC and neutrophils count and significant increase in lymphocytes count after treatment in both the treatment group are in accordance with Syed (2016) [14] and Nikhate *et al.* (2019) [10] reported in cows treated with *Achyranthes aspera* and Kumar (2016) [16] in cows treated with garlic extract.

**Table 3:** Biochemical observations in T1, T2 and T3 groups.

Groups	Parameters	
	Total protein (g/dl)	
	Before	After
T1	6.69±0.03 <sup>a</sup>	6.56±0.02 <sup>a</sup>
T2	6.65±0.00 <sup>a</sup>	6.64±0.00 <sup>a</sup>
T3	6.70±0.04 <sup>a</sup>	6.78±0.10 <sup>a</sup>
Blood Urea Nitrogen (BUN) (mg/dl)		
T1	17.53±0.24 <sup>a</sup>	17.54±0.24 <sup>a</sup>
T2	17.96±0.12 <sup>a</sup>	17.99±0.12 <sup>a</sup>
T3	16.59±0.25 <sup>a</sup>	16.61±0.25 <sup>a</sup>
Creatinine (mg/dl)		
T1	1.36±0.01 <sup>a</sup>	1.39±0.02 <sup>a</sup>
T2	1.51±0.01 <sup>a</sup>	1.53±0.01 <sup>a</sup>
T3	1.49±0.03 <sup>a</sup>	1.43±0.04 <sup>a</sup>

Mean bearing different superscript differ significantly

All the studied serum biochemical parameters like total protein, blood urea nitrogen 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, BUN and creatinine after the treatment with *Achyranthes aspera* and *Allium sativum*.

The non-significant variation in mean values of serum total protein, BUN and creatinine after the treatment with *Achyranthes aspera* and *Allium sativum* are in close agreement with Syed (2016) [14] and Nikhade *et al.* (2019) [10], reported a mean total protein, BUN and creatinine level in treated with different herbal extract.

#### 4. Conclusion

The therapeutic efficacy and first service conception rate was higher in cows treated with *Achyranthes aspera* followed by *Allium sativum*.

#### 5. Acknowledgement

Authors are thankful to Associate Dean, PGIVAS, Akola and Gorakshan Sansthas for providing facilities to undertake this study.

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