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Effect of cow urine distillate fortified with *Curcuma amada* extract therapy on subclinical mastitis in cows

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Abstract
The present study was carried out to evaluate the effect of cow urine distillate fortified *Curcuma amada* extract therapy on subclinical mastitis (SCM) in cows. The study was conducted on cows at dairy farms. Thirty cows in the mid-lactation stage were randomly selected and divided into five equal-treatment groups *viz* T_0 was control, T_1 group was treated with Cow urine distillate @0.15 ml/kg body weight diluted in water by oral administration (post-milking), T_2 was given *Curcuma amada* aquous extract, T_3 was treated with *Curcuma amada*aquous extract @20 mg/kg b.w.t diluted in water by oral administration (post-milking) for 28 days. Group T_4 treated with morbofloxacin @8 mg/kg BW IM single dose (post-milking). The California mastitis test (CMT) was conducted on total 52 cows out of which 24 animals were found positive for SCM. The SCM prevalence was 46 percent. The study revealed significant alterations in the physical properties of milk samples from subclinical mastitis cows, including increased pH, total protein, albumin, and lactate dehydrogenase enzyme levels, alongside decreased milk fat, density, and viscosity. Hematological parameters also showed significant changes, with increased total white blood cell count, monocytes, and granulocytes, and decreased hemoglobin, packed cell volume, total red blood cell count, and lymphocytes. The anti-mastitic activity of CUD fortified *Curcuma amada* extract was found better than CUD or *C. amada* alone treated groups. However, the group which received standard drug morbifloxacin showed better anti-mastitic activity in SCM affected cows. The study demonstrates the efficacy of cow urine distillate and *Curcuma amada* extract in treating subclinical mastitis, offering a promising solution to this prevalent issue in the dairy industry.

Keywords: Cow urine, *Curcuma amada* extract, therapy, subclinical mastitis, cows

Introduction
Mastitis is one of the most severe and deadly illnesses that affect lactating animals. It is a parenchymal mammary gland inflammation, characterized by pathological abnormalities in glandular tissues as well as physical, chemical, and usually microbial changes in milk. It causes substantial economic losses to farmers and changes in the glandular tissues quantity. It adversely affects animal health, milk quality, quantity and the economics of milk production (Sharma *et al*., 2007) [20]. When there are no apparent external changes to the udder despite the infection, mastitis may appear in a subclinical form (Sharma *et al*., 2012) [19].
Currently, mastitis is mostly treated with allopathic medicines, but allopathic treatments are accompanied with residual effect, which poses a great health risk to the human population. Panchagavya therapy, or cowpathy, has been proposed as an alternative prophylactic and therapeutic approach to the health of livestock, which protects human health too (Dhama *et al*., 2013) [4]. Cow urine, in general, has antibacterial properties (Raad *et al*., 2013) [13]. In addition, traditional medicines *viz* plant extracts can be utilized in therapy. Cow urine possesses both antimicrobial and disinfectant properties. (Chauhan and Dhama, 2010) [2].
Curcuma amada roxb has wide conventional applications in folks medicine. Its antimicrobial, insecticidal, antifungal, hypotriglyceridemic, hypoglycemic or anti-hyperglycemic, anthelmintic, antiallergic, anti-inflammatory activity and antioxidant properties have been proved by several investigations (Jatoi *et al*., 2007; Samant, 2012) [7, 16]. Cow urine distillate can be fortified with *Curcuma amada* to enhance its medicinal properties, particularly antibacterial activity, which could be beneficial in treating diseases like mastitis. Therefore, the present study was planned to evaluate the therapeutic activity of *C.amada*-fortified cow

urine distillate in subclinical mastitis, with the objective to study effect of cow urine distillate fortified with *Curcuma amada* extract therapy on subclinical mastitis in cows.

Materials and Methods

The present work carried out at the Department of Veterinary Biochemistry, PGIVAS Akola and Adarsh Goseva Evam Anusandhan Prakalp Mhaispur, Dist. Akola and Dr Panjabrao Deshmukh Krishi Vidyapeeth Dairy Farm, Akola during 2023-24. Akola's geographic coordinates are latitude 20.7 °N and longitude 77.09 °E, with elevations ranging from 925 feet (287 meters) to 1036 feet (316 meters) above sea level. The climate of Akola is characterized as tropical savanna, with a humid subtropical climate in its vicinity. The city's local weather station serves as the national weather station. Annual temperatures in Akola fluctuate between a high of 47.6 °C (117.68 °F) and a low of 2.2 °C (35.96 °F). Due to its proximity to the Tropic of Cancer,

Before the start of the experiment, the experimental protocol was prior approved by the Institutional Ethics Committee for Veterinary Clinical Research (IEC-VCR) of Post Graduate Institute of Veterinary & Animal Sciences, Akola-vide IEC-VC Resolution number IEC-VCR (2) 2024 (08).

Procurement of Materials

Curcuma amadawasprocured from the local market and was identified by botanists. Prof. Dr. S.P. Rothe, Ex-principal, MB Science and Commerce College Akola.

Cow urine Distillate

Cow urine was procured from Adarsh Goseva Evam Anusandhan Prakalp, Mhaispur, Dist. Akola. The cow urine distillate was prepared in the Department of Veterinary Biochemistry, PGIVAS Akola. Procurement of biochemical reagents, CMT reagent, and CMT Paddle were procured from Akansha chemicals, Akola.

Selection of Cows

After screening for CMT and having SCC between 2.5 to 5.0×10^5 cells/ml from cattle farm, total 30 cattle in the mid-lactation period were selected randomly for experiment. The details of experimental treatment was as follows

Table 1: Details of experimental treatment

Sr. No.	Group	Treatment provided to Cows	Number of Cows
1.	T_0	Control	6
2.	T_1	treated with Cow urine distillate @0.15 ml/kg b.w.t diluted in water by oral administration (post-milking)	6
3.	T_2	treated with *Curcuma amada*aquous extract @20 mg/kg b.w.t diluted in water by oral administration (post-milking)	6
4.	T_3	treated with fortified or combined with *Curcuma amada* aquous extract (@20 mg/kg b.w.t), cow urine distillate (@0.15 ml/kg b.w.t) diluted in water by oral administration (post-milking)	6
5.	T_4	treated with morbofloxacin @8 mg/kg BW IM single dose (post-milking).	6
Total number of cows			30

Califonia Mastitis Test (CMT) CMT Reagent and CMT Paddle were used for diagnosis of subclinical mastitis. The milk pH of each milk sample was recorded with a digital pH meter on days 0^{th} 3^{rd},7^{th}, 14^{th} and 28^{th} days.

Somatic Cell Count (SCC)

The SCC was determined as described by Duttschaever and Leggatt, (1965) [6] and Dhakal, (2006) [3]. A square of 1 cm^2 was prepared on a glass slide with the diamond marker. Milk was mixed thoroughly before testing.

The SCC/ml = Total no. of cells counted in 25 fields × WF

Collection of milk samples

Milk samples were collected by hand milking method in the morning from each animal of the control and treated group on days 0^{th}, 3^{rd},7^{th}, 14^{th} and 28^{th} respectively at the time of milking. The first 3-4 streams of milk were discarded. Milk samples were collected in sterilized, dry plastic bottles with a volume of 50 ml to study somatic cell count, total viable count, and milk composition.

Physico-chemical parameters

Milk samples collected on days 0^{th}, 3^{rd}, 7^{th}, 14^{th} and 28^{th} were subjected to analysis of milk physico-chemical parameters such as colour, consistency, viscosity, fat and density.

Colour of milk recorded by Suresh *et al.* (2010) [25]. Consistency of milk recorded by Suresh *et al.* (2010) [25] method. The consistency of the milk was recorded as normal, watery, thick, ropy, or slimy. Viscosity of milk is determined by method (Mann *et al.*, 2012) [12]. Milk fat was determined by Gerber's method described by (Kleyn *et al.*, 2001) [9]. Milk density was determined by Lactometer method by (Mann *et al.*, 2012) [12]. Using a lactometer, the density or specific gravity of milk could also be determined. The lactometer was a specialized hydrometer calibrated within the range of 1.020 to 1.035 (or 20 to 35 as lactometer reading).

Milk Biochemical Parameters

On days 0^{th}, 3^{rd},7^{th}, 14^{th} and 28^{th}, the total protein in milk whey estimated by Biuret method described by Johnson and Swanson (1952), albumin content in milk whey was estimated by the BCG method described by Lieske *et al.*, (2005) [11] and the determination of lactate dehydrogenase (LDH) activity in milk whey as described by Larsen (2005) [10].

Complete blood count (CBC)

The CBC was evaluated or described by Jones and Allison (2007) [8] on the 0^{th},3^{rd},7^{th}, 14^{th} and 28^{th}days. Blood samples were collected from the jugular vein after taking all aseptic precautions, using a 16-gauge hypodermic sterile needle from subclinical mastitis positive cows as well as from healthy cows in a sterile ethylene diamine tetra-acetic acid (EDTA) tube. Haematological tests were also performed on the 0^{th},3^{rd},7^{th}, 14^{th} and 28^{th} days after treatment from 30 randomly divided cows in different treatment groups with subclinical mastitis. All collected samples were subjected to haematological investigation using an auto haematoanalyzer for the estimation of the following parameters.

Statistical analysis

The statistical analysis of the data obtained for different parameters like SCC, CBC, milk physiochemical parameters such as (colour, consistency, viscosity, fat and density) and Milk biochemical parameters (Milk pH, Total Protein,

Albumin, and LDH activity of milk) was done using one-way ANOVA with interaction described by Snedecor and Cochran (1980) [23]. The means were tested for significant differences using Completely randomized design experiment. The data were analysed statistically by Wasp 2.0 software.

Results and Discussion

The CMT was conducted on total 52 cows out of which 24 animals were found positive for SCM. The SCM prevalence was 46 percent of the total cows screened with CMT.

The study revealed that the overall pH value of treatment groups T_1, T_2, T_3 and T_4 increased significantly ($p<0.05$) as compared to control. Similar trend of pH values was observed at different intervals but non significant changes were observed between control and different treatment groups at 28th day. In treatment groups at different time intervals, the pH values found increased initially on day 0 and it was decreased progressively from day 3rd to 28th i.e. towards the end of experiment upon treatment with CUD, *C. amada* or CUD fortified urine. The results of the present study are in agreement with the results of earlier studies conducted by Swami *et al.*, (2017) [26] in which milk pH found to be increased initially and later found to decrease gradually toward normal in most of the cases in SCM. No earlier reports are available about milk pH post oral administration of Cow urine distillate, *Curcuma amada* or fortified cow urine distillate.

The milk total protein (TP) values of control and various treatments groups measured at different intervals increased significantly ($p<0.05$) as compared to control. Non significant improvement in milk TP was observed in T_3 group received cow urine distillate fortified with *C. amada*. The significant improvement in milk TP was observed in T_4 group which received standard drug morbifloxacin. The mean albumin (ALB) recorded (pooled mean) at different intervals in T_1, T_2, T_3 and T_4 groups was increased significantly ($p<0.05$) as compared to control. Significant ($p<0.05$) improvement in milk ALB was observed in T2 and T3 group received *C. amada* extract and cow urine distillate fortified with *C. amada*, respectively. The increase in milk protein during subclinical mastitis may be attributed to pathological changes resulting from mammary epithelium infection, leading to increased capillary permeability during mastitic infection (Rao, 1990) [14].

Average values of milk lactate dehydrogenase in control and different treatment groups of cows at different time intervals increased significantly ($p<0.05$) as compared to control, however non significant increase in milk LDH was observed in T3 group received cow urine distillate fortified with *C. amada*. The significant improvement in milk LDH was observed in T_4 group which received standard drug morbifloxacin. Similar trend of LDH values was observed at different intervals in which significant changes were observed among control and different treatment groups.

The average somatic cell count in different groups of cows at various intervals increased significantly ($p<0.05$) as compared to control, however non significant improvement in milk somatic cell count was observed in T_3 group received cow urine distillate fortified with *C. amada* as compared to CUD or *C. amada* treated groups. The significant increase in milk somatic cell count was observed in T_4 group which received standard drug morbifloxacin when compared to other treatment groups. The results of the present study also found similar with the results of earlier studies by Sumon *et al.* (2020) [24] stated that the increase in SCC suggested the inflammatory reaction that may be caused by a transfer of leukocytes to the udder after infection entry in the mammary gland. The rise in somatic cells in milk represents the animal's defense against the pathogen in terms of fighting sickness (Schalm, 1968) [18]. The findings of Berning and Shook (1992) [1] stated that the somatic cell count was proved to be a more effective means of distinguish between affected and unaffected individuals and support our observation that the SCC was able to discriminate the latent infections in the absence of udder inflammation indications.

The mean milk fat recorded (pooled mean) at different intervals in T_1, T_2, T_3 and T_4 groups decreased significantly ($p<0.05$) as compared to control, however non significant improvement in milk fat was observed in T_3 group received cow urine distillate fortified with *C. amada*. The significant improvement in milk fat was observed in T_4 group which received standard drug morbifloxacin when compared to other treatment groups. Like pooled mean values similar trend of fat values was observed at different intervals wherein milk fat values differ significantly among control and treatment groups. The results of the present study are in align with the results of earlier studies conducted by Singh *et al.* (1998) [22] observed a reduction in fat percentage in milk affected by subclinical mastitis (SCM).

The mean viscosity and density recorded (pooled mean) at different intervals in T_1, T_2, T_3 and T_4 groups were decreased significantly ($p<0.05$) as compared to control. The non significant improvement in milk viscosity and density were observed in T_3 group received cow urine distillate fortified with *C. amada*. The significant improvement in milk viscosity and density were observed in T4 group which received standard drug morbifloxacin when compared to T1 group. The milk viscosity and density were values decreased significantly in SCM cows in different groups on 0, 3rd,7th 14th and 28th day. Yesil *et al.* (2020) [28] conducted a study involving the analysis of 110 milk samples obtained from healthy black and white cows and determined the density of the milk to be 1.032±0.0003 g/ml. The results of the present study coincides with the results of earlier studies conducted by Rathaur *et al.* (2020) [15] observed a decrease in the specific gravity of milk from mastitis-affected cows, which they attributed to an increase in chloride levels and a decrease in lactose content.

The mean Hb recorded (pooled mean) at different intervals in T_1, T_2, T_3 and T_4 groups was decreased non significantly as compared to control. Non significant decrease in Hb was observed at different time intervals in treatment groups. The Hb values were found to improve nonsignificantly in cow urine and in CUD fortified group on 14th and 28th day and were found in the normal range. The overall PCV value of control and different treatment groups at different time intervals differ non significantly. In all treatment groups the PCV values found decreased initially upon entry of infection which was improved progressively from day 3rd to 28th i.e. towards the end of experiment upon treatment with CUD, *C. amada* or CUD fortified urine. This observation on Hb values aligns with the findings reported by Siddiqe *et al.* (2015) [21] in their studies on mastitis where decreased Hb values were observed.

The TLC values T_1, T_2, T_3 and T_4 groups increased significantly ($p<0.05$) as compared to control, however non significant improvement in TLC was observed in T_3 group received cow urine distillate fortified with *C. amada* as compared to group T1 and T2. The TLC values observed at different intervals in all treatment groups found non

significant when compared among the groups. In the initial stage upon entry of infection TLC values found increased which were decreased progressively from day 3rd to 28th i.e. towards the end of experiment.

The mean TEC recorded (pooled mean) at different intervals in T_1, T_2, T_3 and T_4 groups was decreased significantly ($p<0.05$) as compared to control, however non significant improvement in TEC was observed in T_3 group received cow urine distillate fortified with *C. amada* as compared to other treatment groups. The significant improvement in TEC was observed in T_4 group which received standard drug morbifloxacin when compared to other treatment groups. The results of the present study are in agreement with the results of earlier studies conducted by Yadav *et al.* (2022) [27] stated that the mean TEC values in both clinical and subclinical mastitis groups were significantly lower compared to the control group. This observation is also consistent with the findings reported by Siddiqe *et al.* (2015) [21] in their research on mastitis.

Average values of lymphocytes in T_1, T_2, T_3 and T_4 groups was decreased significantly ($p<0.05$) as compared to control, however non significant improvement in lymphocytes was observed in different treatment groups. In all treatment groups the lymphocytes values found decreased initially upon entry of infection which were improved progressively from day 3rd to 28th i.e. at different time intervals upon treatment with CUD, *C. amada* or CUD fortified urine. The monocytes values for various treatments T_1, T_2, T_3 and T_4 groups was differ non significantly as compared to control. In all treatment groups the monocytes values found increased initially upon entry of infection which were improved progressively from day 3rd to 28th i.e. at different time intervals upon treatment with CUD, *C. amada* or CUD fortified urine. The study revealed that the overall granulocytes value of treatment groups found to be increased. Granulocytes of T_2 group increased significantly ($p<0.05$) as compared to control, however granulocytes of in groups T_1, T_3 and T_4 found comparable to the control group (T_0) which indicate protective effect of CUD and CUD fortified extract. Improvement in granulocytes was observed in T3 group received cow urine distillate fortified with *C. amada*. The results of the present study are in coincides with the results of previous study by conducted Sarvesha *et al.* (2017) [17] discovered that the total milk leukocyte and granulocyte counts were notably higher, whereas lymphocyte and macrophage populations were significantly lower in animals infected with mastitis, indicating an infection in the mammary gland. Milk leukocyte count stands as one of the most reliable biomarkers for mastitis. The elevated level of milk leukocytes observed in this study suggests an immune response triggered by microorganisms in the mastitis-affected mammary gland (Djabri *et al.*, 2002) [5].

Conclusion

In conclusion based on various biochemical parameters of present study demonstrates that CUD fortified *Curcuma amada* extract showed positive activity in the control of SCM in cows. The anti-mastitic activity of CUD fortified *Curcuma amada* extract was found better than CUD or *C. amada* alone treated groups. However, the group which received standard drug morbifloxacin showed better anti-mastitic activity in SCM affected cows.

Conflict of Interest

Not available

Financial Support

Not available

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