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Evaluation of comparative efficacy of herbal gel and spray in sub clinical mastitis in bovines

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Abstract
Subclinical mastitis represents a major proportion of the burden of mastitis. Determining somatic cell count of milk are useful approaches to detect sub clinical mastitis. Four hundred and seventy-two milk samples from 259 lactating dairy cattle were screened in this study for subclinical mastitis using california mastitis test, mastrip and subsequent bacterial isolation. Comparison of indirect test was assessed by somatic cell count. Incidence of mastitis was evaluated in terms milk yield, month of lactation, involvement of quarter and parity. Comparative evaluation of efficacy of herbal spray and gel were done in two treatment groups having 10 animals in each groups. Mastitis was evaluated in these groups by somatic cell count. Overall prevalence of subclinical mastitis was 14.29%. Incidence of udder infection in cattle appeared to increase with the increase in average daily milk yield. Maximum incidence was observed during fourth lactation and 3rd parity. Main causative agent was staphylococcus followed by streptococcus, micrococcus 6 and *E. coli.* Application of herbal spray showed the more efficacies over herbal gel on subclinical mastitis.

Keywords: Mastitis, herbal spray, herbal gel, somatic cell count

1. Introduction
Mastitis is recognized as the most important and costly disease of dairy animals in terms of production loss, milk loss due to disposal after treatment, treatment loss, man power loss as well as premature culling [1, 2]. It is a global problem, characterized by physical, chemical and microbiological changes in the milk and pathological changes in the glandular tissue of the udder. Mastitis is recognized as the most important and costly disease of dairy animals [3, 4].
At least, 137 infectious causes of bovine mastitis are known to date, and in large animals the commonest pathogens are *Staphylococcus aureus*, *Streptococcus agalactiae*, other *Streptococcus* species and Coliforms [5]. Mastitis is mainly categorized into clinical mastitis and subclinical mastitis [6]. About 75-80% mastitis is subclinical, characterized by a significantly increased leukocyte count in milk [7]. In subclinical mastitis, there are no obvious clinical signs such as abnormal milk, udder swelling or tenderness, or systemic signs such as fever, depression. Subclinical mastitis causes two third losses of the total milk production due to affected quarters of animal [1]. In India, the incidence of subclinical mastitis was higher (10-50% in cows and 5-20% in buffaloes) as compared to clinical mastitis (1-10%) [8]. The subclinical mastitis is responsible for loss, approximately three times more as compared to clinical mastitis [9].
The subclinical mastitis (SCM) is defined as a quarter infected with a pathogen, having an increase in the cell content of the milk and absence of clinical signs. It is important to diagnose this disease in the subclinical stage. Subclinical mastitis can be diagnosed by somatic cell

Subclinical mastitis can be diagnosed by somatic cell counts, California Mastitis Test, White side test (WST) [10, 11]. Owing to its high sensitivity and specificity Somatic cell count is an important tool for diagnosis of mastitis. The high incidence of mastitis along with raised SCC is observed during rainy season due to unhygienic environment, more calving and animals in the stage of peak lactation. The present study therefore aims to diagnose sub clinical mastitis in crossbred cows by standard diagnostic tests and evaluate therapeutic efficacy of Herbal gel and spray against SCM.

2. Material and method

Milk samples were collected aseptically from apparently healthy quarters of 259 lactating cattle from organized and unorganized farm in and around Ranchi. The California Mastitis Test and mastrip test (Mastitis detection strip) was prepared and used for detection of subclinical mastitis on the survey field. The procedure of CMT was followed in this study as per manufacturer's instruction on quarter fore-milk samples. The CMT was conducted in milking shed at the start of milking of each cow. A plastic paddle with four shallow cups marked as left-fore (LF), left-hind (LH), right-fore (RF) and right-hind (RH) were used to detect the individual quarter incidence of subclinical mastitis. Approximately 2-3 ml of first striping of milk (fore milk) was taken from individual quarter in the respective cup of paddle. Then equal amount of CMT reagent was added to each cup of paddle. The content was mixed by gentle circular motion of paddle in the horizontal plane. Then the sample was observed for precipitation or gel formation and the result was recorded within 30 sec. as 0 (negative), T (trace), 1+, 2++, or 3+++ [12].

For analysis, 100 ml of positive milk samples freshly drawn milk from each quarter of the cows was collected separately in clean, well sterilized and previously dried sample bottle and immediately transported to the laboratory in cold chain and screened for SCM by SCC. Milk Somatic Cell Count (SCC) was determined as per the standard method described by Schalm *et al*. [13]. The milk smears were prepared from the test samples on a clean, grease-free glass slide and were dried in air. Thereafter, the slides were stained by Newman-Lampart stain. Cells under 25 random fields were counted under the oil immersion objective lens. Total number of cells/ml of milk was estimated by multiplying total number of cells in 25 fields with the working factor of the microscope used.

The milk samples found positive by one or many indirect tests were subjected to bacterial isolation by plating on brain heart infusion agar (BHI), macConkeys lactose agar (MLA), sabrouard dextrose agar (SDA). Plates were incubated 48 h at 37 ◦C and bacterial growth recorded at both 24 and 48 h of incubation. The typical colonies were sub-cultured in a selective broth and subjected to various tests *viz.*, Gram reaction, oxidase, catalase, IMViC, motility and growth on TSI slant for biochemical characteristics as per the standard bacteriological methods [14]. Prevalence in relation to age, no & stage of lactation was calculated. The relevant data including lactation number, milk yield, age and date of calving were recorded.

Animals exhibiting signs of subclinical mastitis as per direct test were selected for the study. Six healthy and twenty animals exhibiting signs of subclinical mastitis were selected for the current experimental study divided into three groups as mentioned in table 1.

Table 1: Experimental Design

Sr. No	Group Name	Animals/Group	Type of animals	Treatment
1	A	6	Cattle without SCM (Healthy Animals)	No Treatment
2	B	10	Cattle with SCM	Mastilep gel *(applied gently by massaging the udder; BID for 5 days)*
3	C	10	Cattle with SCM	Herbal spray to be applied on udder twice after milking, *BID for 5 days*

Record of milk yield, biochemical composition parameters of the milk i.e. fat, protein was analyzed by Milk analyzer on day 0, 5, 14 and 21. From all these three groups, milk samples were collected for Mastrip and Somatic cell count on 0, 5, 14 and 21 days. Efficacy of both the treatment groups against SCM was assessed individually on the basis of improvement in SCC. Comparative analysis of Mastilep gel and Herbal spray were performed.

3. Results and Discussion

Since direct microbiological investigation is not feasible, indirect tests are necessary to identify intra-mammary infections (IMI). The gold standard is to measure inflammation through cytological investigation, i.e., counting somatic cells. In addition, ''cow side'' tests such as the California Mastitis Test can also be used. In order to test for correlations between SCC and SCM of specific aetiology, or for the ability of the results to predict the incidence of SCM in organized or unorganized sectors, clinical diagnostic i.e. California mastitis test and microbiological analyses in this study.

Out of total number of 259 milch animals, 472 milk samples were examined for mastitis on the basis of history and examination. These cows were maintained in organized & unorganized farm and private owners in and around Ranchi.

A total of 37 cows were found positive for different types of mastitis. Out of 37, 14 were old and 23 were new cases of mastitis. Overall incidence and prevalence rate of mastitis were 8.88% and 14.29%, respectively (Table 2). Various authors have reported prevalence rate of 21.1% [15] 8.08% [16] and 7.69 *per* cent [17] in their respective studies. The difference observed in various studies might be due to difference in the managemental practices, hygienic conditions, care of the teat injuries, prompt treatment of clinical cases, culling of carriers, selective breeding and adaptation of mastitis control programme Saifudeen *et al.*, [18]

Table 2: Incidence and prevalence of mastitis

Total Milch Animals	Mastitis		Incidence rate	Prevalence Rate
	Old	New		
259	14	23	8.88%	14.29%

The relationship between milk yield and incidence of mastitis is presented in Table 3. As could be seen from the table, the incidence of udder infection in cattle appeared to increase with the increase in average daily milk yield. Incidence rate of

mastitis in relation to daily milk yield were maximum in animals yielding daily milk from 10 – 16 Kg daily (Table 3). Incidence of udder infection in cattle appeared to increase with the increase in average daily milk yield Saifudeen *et al.* [18] Similar results were observed by [19, 20, 21].

Table 3: Rate of incidence of mastitis based on cows examined in relation to daily milk yield

Daily milk yield in Kg	No. of Animal
4 – 5 (6)	--
5 – 6 (7)	--
6 – 7 (9)	--
7 – 8 (11)	--
8 – 9 (23)	2 (8.69%)
9 – 10 (34)	2 (5.88%)
10 – 11 (32)	4 (12.5%)
11 – 12 (33)	8 (24.24%)
12 – 13 (36)	7 (19.44%)
13 – 14 (27)	9 (33.33%)
14 – 15 (23)	3 (13.04%)
15 – 16 (18)	2 (11.11%)
Total 259	37 (14.29%)

Association between different stage of lactation and incidence of mastitis is displayed in Table 4. A browse of the table indicates that incidence decreased with the advancement in the stage of lactation in cows. The rate of incidence was higher in third and fourth month of lactation. Similar finding was also reported by Sudhan *et al.* [22]. Saini *et al.* [23] also observed increase in the incidence of subclinical mastitis with the increase in lactation number. This may be ascribed to gradual loss in immune system in the body of the animal with the increase in lactation number, which makes it susceptible to infection and it may also be ascribed due to inefficient sphincters.

Table 4: Rate of incidence of mastitis based on cows examined in relation to stage of lactation

Stage of lactation	No. of Animal affected
First (32)	2 (6.25%)
Second (41)	3 (7.31%)
Third (59)	14 (23.73.64%)
Fourth (54)	12 (22.22%)
Fifth (47)	4 (8.51%)
Sixth (26)	2 (7.69%)

As seen from the Table 5, increase in the number of lactations has caused a general increase in mastitis incidence in milch cows. The incidence rate of mastitis in relation to parity was maximum in third parity. Similar finding was also reported by Swami *et al.*, [24]

Table 5: Rate of incidence of mastitis based on cows examined in relation to parity

Parity	No. of Animal
1 (53)	4 (7.54%)
2 (82)	11 (12.19%)
3 (74)	17 (22.97%)
4 (27)	3 (11.11%)
5 and above (23)	2 (8.69%)

Quarter wise testing revealed that out of 53 quarters, 39 (73.58%) were found subclinical and 14 (26.42%) clinical. Involvement of right quarter with different types of mastitis was maximum (52.86%) and minimum in left fore quarter (7.55%) (Table 6). The higher incidence of SCM in right side quarters could be ascribed to the fact that cows mostly sit on right-side with the result right side quarters are frequently exposed to dung and soil and moreover due to pressure of the body of animal the milk dribbles out through the teats of high yielders and thus increasing their susceptibility to infections Swami *et al.*, [24].

Table 6: Quarter wise distribution of different types of mastitis

Type of mastitis	Quarter wise				Pooled
	LF	RF	LH	RH	
Subclinical	4 (7.55%)	6 (11.32%)	9 (16.98%)	20 (37.74%)	39 (73.58%)
Clinical	0 (0.00%)	2 (3.77%)	4 (7.55%)	8 (15.09%)	14 (26.42%)

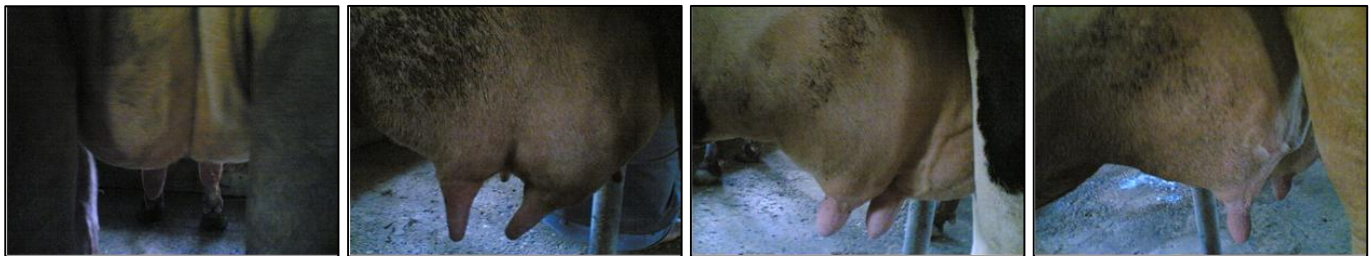

Fig 1: Inflamed udder due to mastitis

Out of 259 cows screened for mastitis only 37 were positive. The incidence of subclinical mastitis was 75.66% and clinical mastitis was 24.34% (Table 7).

Table 7: Distribution of different types of mastitis among positive cases

Types of Mastitis	Number of cows examined	Percent distribution
Subclinical	28 (37)	75.66
Clinical	9 (37)	24.34

Milk samples were subjected to indirect test *viz*. CMT and Mastrip test. CMT detected maximum 56 positive samples (11.86%) as compared to Mastrip which detected only 49 samples (10.38%). All the 56 samples positive on CMT test were subjected to cultural examination and only 53 samples were found positive. The results revealed that discrepancy in

positivity between cultural examination and CMT test was comparatively lower (0.63%) as compared to Mastrip test (0.85%) (Table 8). Sharma *et al.* [25] compared the sensitivity of CMT, SLST and SCC for the detection of subclinical mastitis in dairy cow and stated that CMT is most accurate, reliable diagnostic method after cultural isolation and SCC under field condition.

Table 8: Efficacy of indirect test of mastitis

Tests	No. of samples tested	No. of positive sample to indirect test	% positivity as per indirect test	No. of samples confirmed by cultural examination	% positivity by cultural examination	Absolute difference in % positivity
CMT	472	56	11.86	53	11.23	0.63
Mastrip	472	49	10.38	53	11.23	0.85

Table 9 showed that CMT detected 5.38% of false positive cases as compared to cultural examination. Simultaneously Mastrip test gave false negative result in 12.5%. CMT test detected 3 such cases which were not positive by cultural examination. This may occur due to trauma or injury.

Table 9: Comparison of indirect tests

Tests	True positive	True negative	False positive	False negative
CMT	53	0	3 (5.38%)	0
Mastrip	49	3	0	7 (12.50%)

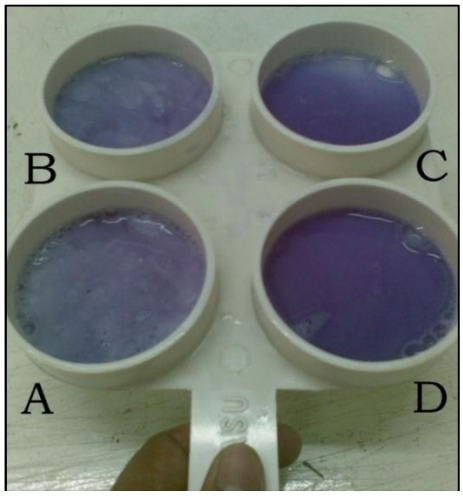

Fig 2: CMT test

To isolate bacteria, bacteriological examinations were done as per standard procedure on positive milk samples from different test. The resulting growth was identified by using primary and secondary tests. Out of 56 milk samples which were positive on indirect tests, bacteria were isolated from 53 samples. The result of identification of microorganism revealed that out of 53 samples, maximum no. of them were infected with Staphylococcus 33 (62.26%) followed by streptococcus 11 (20.75%), micrococcus 6 (11.32%) and *E. coli* 3 (5.66%) (Table 10).

Table 10: Etiological agent wise distribution of mastitis

Number of sample found positive	Types of bacteria	No. of positive samples
53	Staphylococcus	33 (62.26%)
	Streptococcus	11 (20.75%)
	Micrococcus	6 (11.32%)
	E. Coli	3 (5.66%)

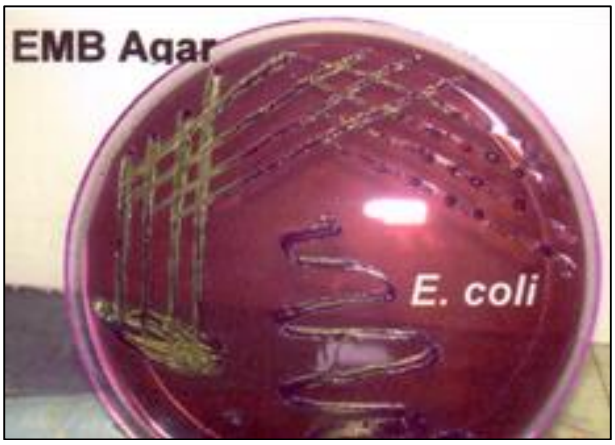

Fig 3: *E. coli* spp at EBM agar

Fig 4: Streptococcus spp at Blood agar

Fig 5: Staphylococcus spp at Blood agar

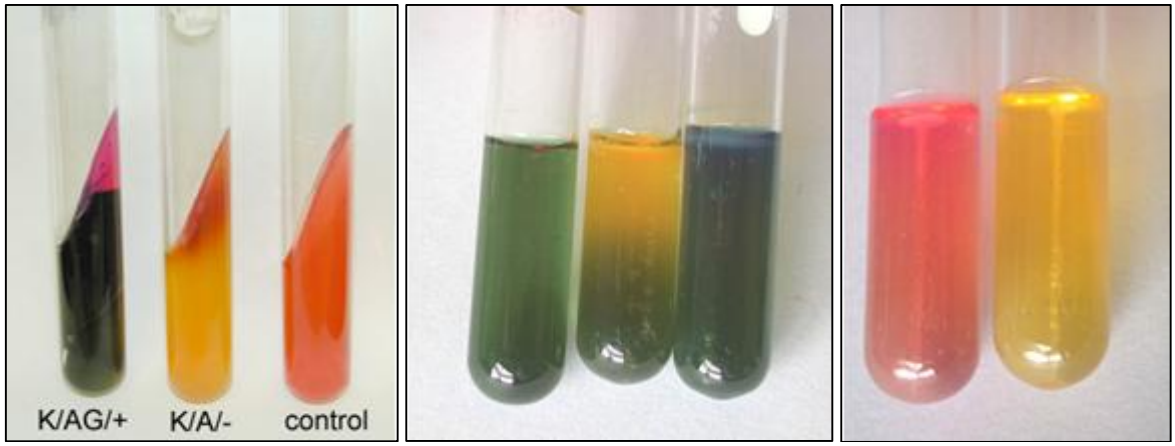

Fig 6: Chemical test for identification of spps

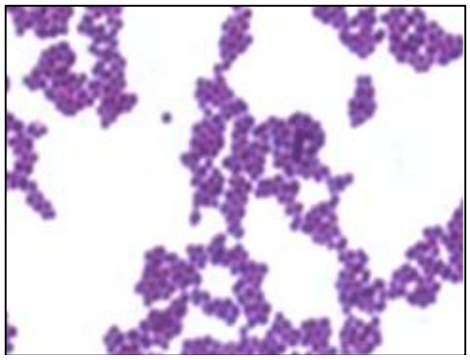

Fig 7: Staphylococcus spp at X1000

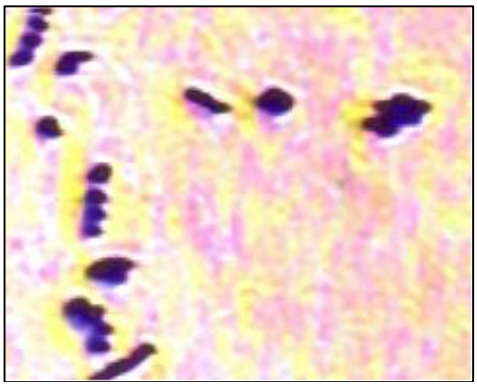

Fig 8: Streptococcus spp atX1000

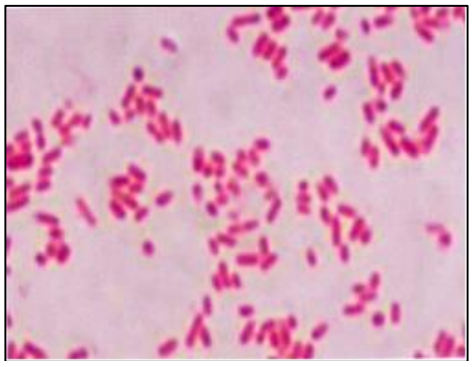

Fig 9: *E. coli* spp at X1000

The high prevalence of staphylococci has been reported by several workers Verma *et al.* [26] and Iqbal *et al.* [27] Distribution of pathogens in mastitis changes over time, therefore, bacteriological examination at herd level must be taken regularly to monitor udder health. The higher incidence of *Staphylococci* indicates unhygienic milking practices as this pathogen is mainly spread during milking via milker's hands. The bovine mammary gland can be a significant reservoir of enterotoxigenic strains of *Staphylococcus aureus* whereas prevalence of *E. coli* is an indication of poor hygienic practices in dairy as these organisms originate from the cow's environment and infect the udder through the teat canal. Contamination of end of the teat is a major predisposing factor in development of environmental mastitis Bradley [7].

Cows were randomly divided in three groups T1, T2 & T3 of 6 healthy cows on T1 and 10 cows having subclinical mastitis in T2 and T3 each. Treatment was given in group T2 and T3. SCC (×105 cells/ml) of milk from normal cows and the cows affected with mastitis are presented in Table-11. The SCC ($3.21\pm0.179 \times 10^5$ cells/ml) was lowest in normal milk and higher in mastitic milk ($6.34\pm0.183\times 10^5$ cells/ml). The present findings are in agreement with Tawheed *et al.*[28] who reported a significant reduction in CMT, SCC, TBC, EC and pH post treatment in subclinical mastitis affected animals. Milk protein percent decreased significantly with an increase in the severity of inflammation. Milk protein decreased significantly in mastitic milk (Table 11). Decrease in protein content in milk from infected animal milk due to high increase in the activity a proteolyticine enzyme (plasma) that cause extensive destroyed for milk protein in udder before milk removal. Shreekumar *et al.* [29] also noticed that the total protein in subclinical mastitis milk was decreased. Milk fat percent did not differ between milk samples from inflamed and non-inflamed quarters (Table 11). Group T3 recovered rapidly with the decrease in SCC (4.01±0.192), increase in protein content of milk (4.29±0.06) and negativity on mastrip test at 5^{th} day of treatment. Milk fat percent did not differ between milk samples from inflamed and non-inflamed quarters.

Table 11: Result of treatment of mastitis in different trial group in relation to SCC, Milk fat & protein and Mastrip test

Group	Days of treatment	Somatic cell Count (10^5 cells/ml)	Milk Fat	Milk Protein	Mastrip
T1	0	3.21±0.179	3.64±0.13	4.51±0.07	-ve
	5	3.19±0.189	3.62±0.11	4.59±0.06	-ve
	14	3.31±0.166	3.59±0.21	4.50±0.06	-ve
	21	3.22±0.173	3.71±0.19	4.54±0.08	-ve
T2	0	6.34±0.183	3.69±0.16	4.02±0.09	+ve
	5	4.99±0.188	3.73±0.23	4.17±0.11	+ve
	14	4.16±0.201	3.66±0.14	4.34±0.08	+ve
	21	4.08±0.221	3.72±0.09	4.43±0.07	-ve
T3	0	6.09±0.167	3.68±0.11	4.06±0.12	+ve
	5	4.01±0.192	3.77±0.14	4.29±0.06	-ve
	14	3.98±0.209	3.74±0.08	4.41±0.09	-ve
	21	3.41±0.119	3.78±0.12	4.48±0.05	-ve

Eighty percent of the animals recovered at 5 days after treatment in group T3 as compared with 50% in group T2 and 90% recovered on 14th day in T3 as compared to 60% in group T2 at 14 days of treatment which remain the same at the end of experiment (Table 12).

Table 12: Efficacy of treatment in different trial groups

Groups	Days of treatment	Recovered cows	Efficacy %
T1			
T2	0	0	0
	5	2	20
	14	5	50
	21	6	60
T3	0	0	0
	5	8	80
	14	9	90
	21	9	90

4. Conclusion

Out of 472 milk samples, 37 cows were found positive for different types of mastitis. Overall incidence and prevalence rate of mastitis were 8.24% and 13.26%, respectively. Incidence rate of mastitis in relation to daily milk yield were maximum in animals yielding daily milk from 10 – 14 Kg daily. The rate of incidence was higher in second and fourth month of lactation and rate of prevalence were maximum in 8th to 10th month of lactation. The incidence and prevalence rate of mastitis in relation to parity was maximum in third parity. Quarter wise testing revealed that involvement of right quarter with different types of mastitis was maximum and minimum in left fore quarter. Identification of microorganism revealed that maximum no. was infected with Staphylococcus followed by streptococcus, micrococcus and *E. coli.* Eighty percent of the animals recovered at 5 days after treatment in group T3 as compared with 50% in group T2 whereas 90% recovery was seen on 14th day of treatment in T3 as compared to 60% in group T2 which remain the same at the end of experiment.

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