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Research Article

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Comparative Study on the Therapeutic Effect of Camel, Buffalo and Cow Milk against Tuberculosis Disease

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ABSTRACT

Introduction: One third of the world population currently suffers from tuberculosis and Pakistan ranks 5th in global tuberculosis burden. These health issues are currently creating an alarming situation in Pakistan due to poor eating habits, unhealthy and unhygienic living conditions, immune-compromised status of the health.

Objective: This study was conducted to compare the therapeutic potential of camel, buffalo and cow milk on the human subjects suffering from tuberculosis.

Methodology: The camel, buffalo and cowmilk analysis were carried out in Dairy Technology Laboratory. Chemical analysis of milk was done by proximate analysis. The efficacy study was conducted at District TB Hospital Faisalabad. After chemical analysis milk was offered to the patients suffered with tuberculosis and several parameters (age, body weight, BMI, ESR, Chest X-Ray and Sputum Smear Test) were recorded. Twenty-four patients diagnosed with tuberculosis were selected and divided into four groups. Group one was control group. Three treatments were framed (camel milk, buffalo milk and cow milk) and provided with 250ml of milk twice a day for forty days. Several parameters (body weight, Body Mass Index, Erythrocyte sedimentation rate (ESR), chest X-Ray and sputum smear test) were recorded.

Results: A significant increase in BMI of three groups (camel, buffalo and cow milk group) was observed. The significant decrease in ESR from 47.00 to 25.50 mm/hour was seen in P1 group while in P2 and P3 it was decreased from 49.17 to 40.83 mm/hour and 55.00 to 47.83 mm/hour respectively. Five patients were recovered by camel milk while only half patient showed negative report in sputum smear test in cow and buffalo milk fed group.

Conclusion: Camel milk had beneficial effect on reduction of tuberculosis in human. However, due to short duration of study the effect of camel milk on sputum conversions and chest X-ray was not significant.

Key Words: Tuberculosis, Camel milk, Buffalo milk, Cow milk, Body Mass Index (BMI), Proximate analysis

INTRODUCTION

Tuberculosis is a disease of ancient era and still posturing a major health issue globally. This disease caused by *Mycobacterium tuberculosis* which came forth before 70000 years ago as human pathogen in Africa and propagated throughout the continent by human migration.[1]According to World Health Organization, Pakistan ranked 5th among highly tuberculosis effected countries. In Pakistan 0.5 million new tuberculosis cases and 15000 cases of multi drug-resistant tuberculosis estimated to occur annually. This burden accounts for 61% of the global disease burden in Eastern Mediterranean constituency.[2]

The epidemiology of the tuberculosis is related to the number of factors including exposure to the infection and duration of infection, host characteristics, immune status, malnutrition, poverty, poor ventilation, crowding and smoking.[3] The infection occurs through the inhalation of small droplet nuclei by healthy individual. These droplets entered into the terminal alveoli of the lungs where they are ingested by the alveolar macrophages and destroyed. *M. tuberculosis* can also disrupt the nonphagocytic cells including alveolar endothelial cells, M cells, and type 1 and type 2 cells which are also called pneumocytes. These entered tubercle bacilli increase in number intracellularly and disseminated through the lym-

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phatic system.[4] The immune cells create guard shell around this tubercle within 2 to 8 weeks which is called granuloma. If the immune cells are unable to destroy the granuloma, they start multiply and spread infection throughout the body and causes extrapulmonary tuberculosis (tuberculosis of brain, kidney, bones abdomen).[5]

Food is very important for the survival of every living organism, because the food contain number of imperative bioactive components that are accountable for the growth and development of the body. From major five food groups, the milk is an essential part of a balanced diet bursting with number of bioactive components i.e.,immunoglobulins, lactoperoxidase, lactoferrin, vitamins, minerals such as calcium, magnesium, phosphorus etc. Each of these components has definite and precise function within the body.Milk has been used from centuries and proved as the good nutritional source. In spite of its great nutritional importance, the milk has been neglected because of many food faddists (people havingexaggerated and incorrect beliefs about the effect of food and nutrition on health particularly from prevention point of view) and vegetarians.[6] The milk of buffalo and cow contain number of important nutrients, minerals, vitamins and other biologically important bioactive components.[7] The buffalo milk has high percentage of saturated fatty acids and lower percentage of unsaturated fatty acids specifically oleic acid. Omega-3 polyunsaturated i.e., linolenic acid (C-18:3) and conjugated linoleic acid (fumaricacid) present in milk provide protective effect and decreases the incidence of mammary tumor.[8]

Camel milk is highly nutritious milk than other ruminants and also known as the desert's white gold as the nutritional profile of camel milk is very virtuous. It contains high amount of iron and vitamin-C which acts as potential antioxidant.[9] The nutritionally important unsaturated fatty acid and linoleic acid are present in high amount along with excellent source of fat-soluble vitamins such as vitamin A, E, D and K.[10]By keeping in view, the biological and physiological significance of the milk, the study was planned with the aim to appraise the effect of different types of milk (camel, buffalo and cow) against tuberculosis disease.

METHODOLOGY

Experimental model

To analyze the therapeutic effect of different types of milk against tuberculosis, the efficacy study was conducted at District TB Hospital Faisalabad Pakistan. The total numbers of patients were 24 which were divided into four groups (P_0, P_1, P_2, P_3) at the age of 16 to 35 years. Dietary patterns and disease history were also evaluated.

Study design:

The milk of camel, buffalo and cow was collected from the dairy farm of Faisalabad. The milk was pasteurized at 63ºC for 30 minutes and packed into sterilized bottles for transportation and made available to the patient for consumption. The patients were subjected to take 250 ml of milk twice a day (morning and evening) for 40 days.

Table 1: Treatment plan:

Treatment	Description	Quantity
To	-	-
T1	Camel milk	250ml
T2	Buffalo milk	250ml
T3	Cow milk	250ml

Proximate Analysis of Milk

The milk analysis was performed in the Dairy Technology Laboratory, National Institute of Food Science and Technology, University of Agriculture, Faisalabad. The proximate analysis of the milk including fat content, protein content, lactose content, pH, acidity, total solids and solids not fat was carried out by following the protocol of.[11] Whereas the lactoferrin content was determined) by using HPLC.[12]

Physiological markers

Anthropometric measurements including height, weight and Basal Metabolic Rate of the patients were studied. The weight of the patients was measured by weighing machine and expressed in kg. While the height of the patients was measured according to standard method and expressed in cm. the Body Mass index was measured by using Quetelet's index.[13]

$$\text{BMI: } \frac{\text{weight (kg)}}{\text{Height (m)}^2}$$

Biochemical Markers:

All biochemical tests including sputum smear, erythrocyte sedimentation rate and Chest x-ray were conducted in the laboratory of District TB Hospital, Faisalabad.

Statistical analysis

The data obtained from all the parameters was analyzed by using statistics 10 (FL, 32317 USA) according to.[14]

RESULTS

Proximate analysis of milk

Proximate analysis of camel, buffalo and cow milk was revealed that total solid contents were 14.32%, 13.43% and 12.43% in camel, buffalo and cow milk, respectively. Total

solid content was higher in camel milk and lower in cow milk. Results were not in line with the outcomes of [15] who reported that total solid contents were from 9.85 to 11.45% in camel milk. Omer and Eltinay, (2009) showed that camel milk contains 9.78% total solid contents.[16]The total solid content in camel milk was 13.2%. Solid not fat content was 9.80%, 7.05% and 6.93% for camel, buffalo and cow milk, respectively.[17] Solid not fat content was higher in camel milk and lower in buffalo and cow milk. Singh et al.(2013) compared chemical composition of camel and buffalo milk and stated that solid not fat content s was lower (6.91%) in camel milk and higher in buffalo milk (8.44%).[18] Likewise, [10] showed that solid not fat content was lower in camel milk (6.3%) than in buffalo milk ((7.5%). The acidity values were 0.13%, 0.23% and 0.19% for camel, buffalo and cow milk, respectively. Highest values of acidity were noted in buffalo milk while lowest values of acidity were recorded in camel milk. Acidity value was estimated by Mehmood and Usman, (2010) that was higher in camel milk and lower in buffalo and cow milk 0.21% and 0.17% respectively.[19] Fat content was 4.52%, 6.38% and 5.50% for camel, buffalo and cow milk respectively. Fat content was higher in buffalo milk and lower in camel milk. The values of protein contents were 3.95%, 4.63% and 3.75% for camel, buffalo and cow milk, respectively shown in table 2. Kanhal and Hamad, (2010) compared the chemical composition of different types of milk and revealed that fat content in camel milk was ranged from 2.9 to 5.4%, in buffalo milk 7.0 to 11.5% and 3.7 to 4.4% in cow milk. Highest protein was observed in buffalo milk while in camel and cow milk values were approximately same. The protein values of camel and buffalo milk observed by Singh et al. (2013) were (2.23%) and (4.11%) respectively. Values for moisture content were 86.80%, 82.90% and 85.97% of camel, buffalo and cow milk, respectively. Highest moisture content was in camel and cow milk while lowest moisture content was recorded in buffalo milk. Korlepara et al. (2017) stated 88.55 to 90.15% of the moisture content in camel milk.[15] Further, Kanhal and Hamad, (2010) compared chemical composition of camel milk with cow and buffalo milk and revealed that moisture content in camel milk was higher and ranged from (86 to 88%) as compared to buffalo and cow milk from 82 to 84% and 85 to 87% respectively.[20] The values of pH were 6.6%, 6.5% and 6.5% for camel, buffalo and cow milk, respectively. Ash content was 0.79%, 0.74% and 0.78% of camel, buffalo and cow milk, respectively. Gul et al. (2015) analyzed the camel milk and reported pH was from 6.2 to 6.5%.[21] Kanwal et al. (2004) found that the pH value of buffalo milk (6.5%) was higher than cow milk (6.3%).[22] Ash content was higher in camel milk and lower in buffalo milk. The values of lactose contents were 3.27%, 4.73% and 4.75% of camel, buffalo and cow milk, respectively. Lactose content was lower in camel milk and higher in cow milk. In our study the average lactoferrin contents were 0.46mg/ml, 0.33mg/ml and 0.25 mg/ml in camel, buffalo and cow milk respectively (table 2).Singh et al. (2013) compared chemical composition of camel and buffalo milk and reported that lactose content was lower in camel milk (3.86%) than buffalo milk (4.46%). Raghvendar et al.(2004) reported that lactose content in camel milk was lower (2.8 to 4.2%) than cow milk (4.8%).[23] Average lactoferrin contents were 0.46mg/ml, 0.33mg/ml and 0.25 mg/ml for camel, buffalo and cow milk, respectively presented in table 1. Lactoferrin content was higher in camel milk and lower in cow milk. Camel milk has higher amount of iron chelating protein known as lactoferrin. This protein removes free iron from joints of arthritic patients thereby improves arthritic.[24] Lactoferrinis among the protective proteins in camel milk with higher concentration and thus prevents microbial overgrowth and invading pathogens.[25]

Table 2: Proximate analysis of camel, buffalo and cow milk was done by standard methods.

Type of milk	Total solids	Solid not fat	Acidity	Fat	Protein	Moisture	pH	Ash	Lactose	Lactoferrin content
Camel	14.32±0.08	9.80±0.10	0.13±0.01	4.52±0.08	3.95±0.05	86.80±0.26	6.60±0.1	0.79±0.04	3.27±0.12	0.47±0.03
Buffalo	13.43±0.21	7.05±0.28	0.23±0.01	6.38±0.13	4.63±0.06	82.90±0.85	6.50±0.1	0.74±0.04	4.73±0.09	0.33±0.02
Cow	12.43±0.38	6.93±0.29	0.19±0.01	5.50±0.10	3.75±0.05	85.97±0.84	6.50±0.1	0.78±0.03	4.75±0.03	0.25±0.05

Body weight:

The average value of body weights has been shown in (Fig 1). Body weight of different patients was recorded before and after experiment. Statistical analysis revealed significant differencein body weight of all groups, camel milk group(P_1), buffalo milk group(P_2) and cow milk group(P_3). Analysis of variance regarding weight of patients of different experimental groups revealed that the treatment had highly significant effect ($p<0.01$) on body weight while time had non-significant effect ($p>0.05$) on body weight. Interaction of treatment and time had non-significant effect ($p>0.05$) on body weight (Table 3). The increase in weight was observed in P_1 group and values ranged from 49.83 to 51.33 kg, P_2 group weight ranged from 46.17 to 46.92 kg, while, P_3 group increase in weight was from 44.50 to 45.17 kg observed at the end of study. The weight reduction was observed in P_0

group (control group) that was from 40.50 kg to 39.58 kg during study. Patients received camel milk showed increase in body weight as compared to others.

Body Mass Index:

Body mass index of different patients was recorded before and after experiment. Analysis of variance regarding BMI of patients of different experimental groups revealed that patients received different milk had highly significant effect ($p<0.01$) on BMI while time had no effect ($p>0.05$) on BMI (Table 3). Further, interaction of treatment and time had non-significant effect ($p>0.05$) on BMI. Statistical analysis revealed significant difference in BMI among P_0, P_1, P_2 and P_3 group. Higher values of BMI were observed in P_1 group that was from 18.62 to 19.19 kg/m^2 while in group P_2 and P_3 the observed values of BMI were from 17.65 to 17.93 kg/m^2 and 17.57 to 17.83 kg/m^2 respectively (Figure 2). While in P_0 group the reduction in BMI was noted from 15.53 to 15.18 kg/m^2. Similar results were reported by [26] that BMI was lower in patient suffered with tuberculosis. Similarly,[27] reported that BMI was lower in patient suffered with tuberculosis than normal.

Erythrocytes Sedimentation Rate

Erythrocytes Sedimentation Rate of different patients was recorded before and after experiment. Analysis of variance regarding ESR of patients of different experimental groups revealed that patients received different types of milk had highly significant effect ($p<0.01$) on ESR. Time also had highly significant effect ($p<0.01$) on ESR reduction (Table 3). The results revealed significant difference in erythrocytes sedimentation rate among P_0, P_1, P_2 and P_3 group. In P_0 group the ESR reduced from 55.67 to 52.33 mm/hour (Figure 3). Further, ESR rate was lower at 40 days than 0 day of experiment. The reduced rate of ESR in P_1 group was 47.00 to 25.33 mm/hour. In P_2 and P_3 group, the reduction rate of ESR was 49.17 to 40.83 mm/hour and 55.00 to 47.83 mm/hour respectively. This might be possible that camel milk increases the antibodies status of patients which helps in reducing ESR. Mal et al.(2006) reported that camel milk reduces the ESR rate in patients suffering with tuberculosis while less reduction in ESR was noted in those received camel milk.[28] Erythrocyte sedimentation rate (ESR) referred the values of infection and inflammation within the body by counting the rate of red blood cells sedimentation at the bottom of the Westergren tube. The normal values for ESR in male ranging from 0-10 mm/hour and in females its value is 0-15mm/hour. Higher the value, higher will be the indication for inflammation. The value greater then 100mm/hour stated as the high infection and inflammation rate.[29]

Table 3: Analysis of variance regarding body weight, body mass index, Erythrocytes Sedimentation Rate of patients of different experimental groups

Source	DF	BW	BMI	ESR
Trt	3	228.65**	26.72**	750.5**
Time	1	3.00^{NS}	0.43^{NS}	1220.1^{NS}
Trt*Time	3	3.097^{NS}	0.45^{NS}	187.5^{NS}
Error	40	38.24^{NS}	3.11^{NS}	142.9^{NS}
Total	47			

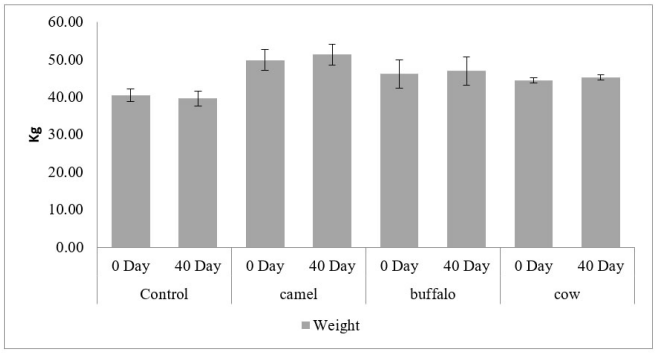

Figure 1: Effect of different milk intake on the weight of the individuals.

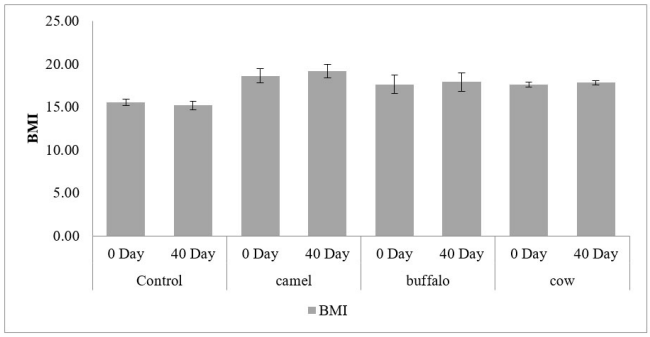

Figure 2: Effect of intake of different type of milk on BMI (Body Mass Index) of different experimental groups.

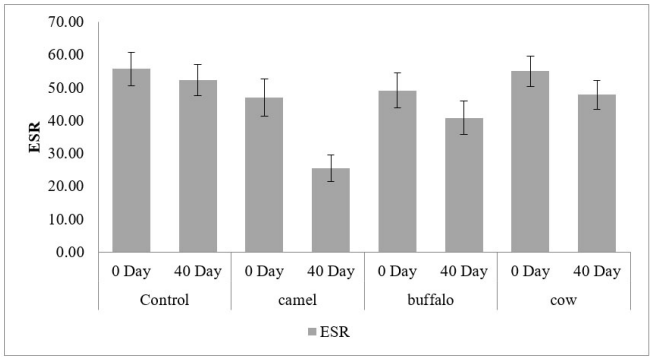

Figure 3: Effect of different treatments on ESR of different experimental groups.

Chest X-Ray:

Results of chest X-rays were similar (not pathophysiology was detected) in all patients suffering from tuberculosis. This might be because tuberculosis is detected early stages and treatment is just started. Because at an experimental study was conducted by [28] who reported that radiological reflection (as seen by x ray) was more pronounced in patients received camel milk than those received cow milk. This conclusion was made because of the presence of active tuberculosis (when the overt symptoms have appeared and disease has set its destination). In this study, all the patients were inducted at the early stage of diagnosis whose smear test was positive (indicating the presence of *M. tuberculosis* bacteria in their sputum), and they all are at early stage of diagnosis and treatment.The second reason is that, the study was consist of short period (40 days) and food has to take extended period of time to heal a body organ.

Sputum Smear Test:

Sputum examination was done according to Ziehl-Neeleson method.[30] Results of all patients were positive in P_0 group indicating the presence of *M. tuberculosis* in all patient's sputum. Five patients were recovered by camel milk while only half patient showed negative reports in sputum smear test in P_2 and P_3group (Figure 4). Mal et al.(2006) suggested that camel milk contains protective proteins which may have a possible role for enhancing the immune defenses mechanism. Antibacterial properties of these camel milk proteins destroyed *Mycobacterium tuberculosis*. Camel milk has been identified as assisting in the recovery processes of autoimmune diseases. It becomes apparent, that the powerful bactericide properties of camel milk have positive effect on the healing process.[31] Furthermore, camel milk contains abundant of beneficial protective proteins such as lysozyme, lactoferrin, lactoperoxidase, IgG, and IgA.[32] All mentioned protective proteins give antiviral, antibacterial, antifungal, antiparasitic effects. Lysozyme targets primarily to the invading pathogens, lactoferrin prevent microbial growth within the gut. This is iron rich protein. Immunoglobulin gives protection to the immune system against different kinds of infections.[33] Therefore, it is being used for number of diseases throughout the world i.e., dropsy, jaundice, tuberculosis, asthma etc.[32]

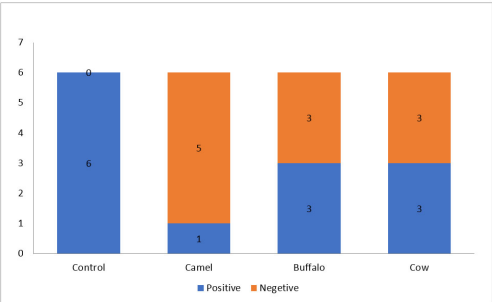

Figure 4: Overall disease recovery comparison

DISCUSSION

This research work was conducted to evaluate the effect of different types of milk against tuberculosis disease. Chemical analysis of cow, buffalo and camel milk was done by proximate analysis. Highest moisture contents 86.80% were noted in camel while low moisture content was observed in buffalo milk and in cow milk 82.90% and 85.97% respectively. Results were not in line with the outcomes of [15] who reported that total solid contents were from 9.85 to 11.45% in camel milk. Omer and Eltinay, (2009) showed that camel milk contains 9. 78% total solid contents [16]. Singh et al.(2013) compared chemical composition of camel and buffalo milk and stated that solid not fat content s was lower (6.91%) in camel milk and higher in buffalo milk (8.44%).[18] Likewise,[10] showed that solid fat content was lower in camel milk (6.3%) than in buffalo milk ((7.5%). Highest values of acidity were noted in buffalo milk while lowest values of acidity were recorded in camel milk. Acidity value was estimated by Mehmood and Usman, (2010) that was higher in camel milk and lower in buffalo and cow milk 0.21% and 0.17% respectively.[19] Kanhal and Hamad, (2010) compared the chemical composition of different types of milk and revealed that fat content in camel milk was ranged from 2.9 to 5.4%, in buffalo milk 7.0 to 11.5% and 3.7 to 4.4% in cow milk. The protein values of camel and buffalo milk observed by Singh et al. (2013) were (2.23%) and (4.11%) respectively. Korlepara et al. (2017) stated 88.55 to 90.15% of the moisture content in camel milk.[15] Further, Kanhal and Hamad, (2010) compared chemical composition of camel milk with cow and buffalo milk and revealed that moisture content in camel milk was higher and ranged from (86 to 88%) as compared to buffalo and cow milk from 82 to 84% and 85 to 87% respectively.[20] Gul et al. (2015) analyzed the camel milk and reported pH was from 6.2 to 6.5%.[21] Kanwal et al. (2004) found that the pH value of buffalo milk (6.5%) was higher than cow milk (6.3%).[22] In our study the average lactoferrin contents were 0.46mg/ml, 0.33mg/ml and 0.25 mg/ml in camel, buffalo and cow milk respectively (table 2). Singh et al. (2013) compared chemical composition of camel and buffalo milk and reported that lactose content was lower in camel milk (3.86%) than buffalo milk (4.46%).[18] Raghvendar et al. (2004) reported that lactose content in camel milk was lower (2.8 to 4.2%) than cow milk (4.8%).[23] Camel milk has higher amount of iron chelating protein known as lactoferrin. This protein removes free iron from joints of arthritic patients thereby improves arthritic.[24] Lactoferrinis among the protective proteins in camel milk with higher concentration and thus prevents microbial overgrowth and invading pathogens.[25]

The treatment had highly significant effect ($p<0.01$) on body weight. Patients of p_2 group had higher final weight and p_0 group had lower final weight. The treatment also had signifi-

cant effect ($p<0.01$) on BMI. Patients received camel milk had higher change in BMI ranged from 18.62-19.19 kg/m^2 and P_0 group had reduced BMI ranged from 15.53-15.18 kg/m_2. Similar results were reported by[26] that BMI was lower in patient suffered with tuberculosis. Similarly, [27] reported that BMI was lower in patient suffered with tuberculosis than normal.

The ESR value was significantly ($p<0.01$) decreased by the treatments. P_0 and P_3 group showed higher values of final ESR from 55.67 to 52.33mm/hour and 55.00-47.83 mm/hour respectively. While lower values of ESR were noted in P_1 group ranged from 47.00 to 25.50 mm/hour. Highest reduction in ESR was noted in P_1 group while lowest reduction was seen in P_0 group. Mal et al.(2006) reported that camel milk reduces the ESR rate in patients suffering with tuberculosis while less reduction in ESR was noted in those received camel milk.[28] Erythrocyte sedimentation rate (ESR) referred the values of infection and inflammation within the body by counting the rate of red blood cells sedimentation at the bottom of the Westergren tube. The normal values for ESR in male ranging from 0-10 mm/hour and in females its value is 0-15mm/hour. Higher the value, higher will be the indication for inflammation. The value greater then 100mm/hour stated as the high infection and inflammation rate.[29] Mal et al. (2006) suggested that camel milk contains protective proteins which may have a possible role for enhancing the immune defenses mechanism.[28] Antibacterial properties of these camel milk proteins destroyed Mycobacterium tuberculosis. Camel milk has been identified as assisting in the recovery processes of autoimmune diseases. It becomes apparent, that the powerful bactericide properties of camel milk have positive effect on the healing process.[31] Furthermore, camel milk contains abundant of beneficial protective proteins such as lysozyme, lactoferrin, lactoperoxidase, IgG, and IgA.[32] All mentioned protective proteins give antiviral, antibacterial, antifungal, antiparasitic effects. Lysozyme targets primarily to the invading pathogens, lactoferrin prevent microbial growth within the gut. This is iron rich protein. Immunoglobulin gives protection to the immune system against different kinds of infections.[33] Therefore, it is being used for number of diseases throughout the world i.e., dropsy, jaundice, tuberculosis, asthma etc.[32] Results of chest X-rays were similar in all patients suffering from tuberculosis. Five patients were recovered by camel milk while only half patient shows positive report in sputum smear test in cow and buffalo group.

CONCLUSION

Tuberculosis greatly affects the health status of the individual severely. The instant study signifies the effects of different milk on tuberculosis disease. By comparing the three types of milk, it was evident that the camel milk significantly improved the health status and BMI along with negative status of smear positive test and decreased value of Erythrocyte Sedimentation Rate. The long-term use of camel milk could improve the malnutrition as well as overall health status of the patients & as a result of which the patient can fight against disease efficiently. However further investigations are necessitated by conducting research on larger population, with greater population size, other hematological analysis such as Hb, WBC (Total and differential count), leukocytosis, neutrophilia with toxic granulation, thrombocytosis etc., developing and endorsing lactoferrin supplementation, evaluating its metabolic and safety aspects along with other multi-drug therapy used for treating tuberculosis in diseased individuals.

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Conflicts of Interest

The authors declare no competing financial interests. All authors have read and agreed to the published version of the manuscript.

Author Contributions

Funding acquisition, ST. and PAR.; Sources of material, ST, PAR, MZZ and MI; writing–original draft, ST, PAR and MZZ; data curation, SI, AI, AF and FA; methodology, AK, HH and ST; writing – review & editing, ST, PAR and MZZ

Supplementary data

No supplementary data available

Ethical clearance: None

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