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BIOTECHNOLOGY

Nanomaterial based therapeutic approach with bovine colostrums for the skin wound management in experimental model of rats

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ABSTRACT. Effective management of burn wounds is crucial for optimal healing, infection prevention, and pain relief. This study blends Bovine colostrum (BC) with Silver Nanoparticles (AgNPs) to create a specialized gel for burn wound care. The primary objective was to evaluate the efficacy of a gel containing 10% BC-loaded AgNPs in treating burn wounds, including wound healing, pain alleviation, infection prevention, and inflammation reduction. UV spectroscopy and zeta sizer were employed to analyze the AgNPs. An absorption peak at 300–400 nm indicated AgNPs formation, with an average size of 243.9 nm confirmed as nanoparticles. Infrared spectroscopy analysis showed distinct peaks representing carbohydrates, lipids, and proteins in the gel. The 10% AgNPs loaded BC gel accelerated wound healing, outperforming NanoColl by day 20. It also reduced oxidative stress, as evidenced by decreased thiobarbituric acid reactive substances (TBARS) and white blood cell counts (WBC). 10% BC gel demonstrates significant promise for burn wound care, as it effectively accelerates the healing process, provides relief from pain, prevents infections, and reduces inflammation.

Keyword: cow milk; silver nanoparticles; gel; oxidative stress; burn management.

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Introduction

'Wound' serves as a broad categorization encompassing injuries not only confined to the skin's external surface but also extending to deeper bodily tissues, organs, and internal structures. The gravity and management of a wound are influenced by diverse factors, including the specific wound type, its anatomical location, depth, and the individual's overall health status (Leaper &Gottrup, 1998). Multiple categories of wounds exist, encompassing an array of types such as incised wounds, lacerations, abrasions, contusions, ulcers, and burn wounds (Singer & Clark, 1999). A burn is described as skin injury brought on by intense heat or caustic substances. The frequent burn injuries are caused by heat and chemicals (Trabelsi et al., 2017). The most significant elements affecting the therapeutic care and any remaining morbidity or scarring are a burn wound's depth and/or its ability for healing. The process of a wound healing is active and dynamic, and it starts as soon as an injury occurs. The healing process might be prolonged by any delay in getting started of the body's response to injury (Okur et al., 2020). A variety of mechanisms, including coagulation, inflammation, matrix synthesis and deposition, angiogenesis, fibroplasia, epithelization, contraction, and remodelling, are involved in the complex process of wound healing (Fu et al., 1998).

Growth factors are polypeptides that coordinate the metabolism, growth, and differentiation processes of cells to control tissue repair. Fluids from wound sites are significant in this context because they represent a potential growth factor reservoir that is essential for promoting wound healing (Bennett & Schultz, 1993). Growth factors start the stimulation of cell growth by attaching to particular high-affinity receptors on cell surfaces. Despite being in very small numbers, they have a significant impact on the complex environment of wound repair. Growth factors have been studied in relation to the healing of burn wounds, and there are signs that they may be crucial in this process (Fu et al., 1998; Alemdaroğlu et al., 2006).

The majority of the time, treatment plans focus on applying the medication topically with the goal of promoting wound healing, reducing inflammatory response, and most importantly preventing opportunistic infections, which are frequently linked to serious wound damage (Brul & Coote, 1999).In majority of cases, antimicrobial ointment containing mafenide, povidone-iodine, silver nitrate, silver sulphadiazine and bacitracin are applied in minor cuts and burn to reduce the risk of infection. However, there are certain negative side effects and these topical antimicrobials only partially help the wound heal (World Health Organization [WHO], 2005). The necessity to find novel medications to treat wounds arises from this.

In the past few years, there has been a growing interest in exploring 'natural solutions' for healing wounds and discovering new sources of antioxidants. This field of research has expanded considerably, generating a substantial volume of literature. Applying treatments directly to the skin is crucial for addressing various skin ailments. While synthetic products for managing skin conditions are accessible in the market, there's also a surge in the pursuit of naturally derived remedies.

This study aims at to prepare the gel formulation containing of the BC with AgNPs for the topical application for wound management. BC is the first milk produced by cows after giving birth and is an abundant natural supply of macro- and micronutrients, immunoglobulins, and peptides that have antimicrobial and growth-promoting properties (Playford & Weiser, 2021).

The numerous immunological components found in colostrum make them suited for topical application to wounds. It can be applied topically or orally because of its antiviral, antibacterial, and anti-inflammatory effects. Colostrum contains seven separate growth promoters that have been linked to the development and repair of bodily cells. Three of the seven identified parameters are crucial to the healing of wounds. Epidermal Expansion Factor, Transforming Growth Factors, Fibroblast Growth Factors, and Insulin-like Growth Factor are nucleotides that directly act on Deoxyribonucleic acid (DNA) and Ribonucleic acid (RNA) to induce skin expansion, cellular growth, and repair. The tissues damaged by ulcers, trauma, burns, surgery, or inflammatory diseases can heal more quickly to these growth factors (Kshirsagar et al., 2015).

AgNPs mostly employed in therapeutic application due to its having broad-spectrum antimicrobial activity. Studies have been shown that, silver ion binds to electron carrying compound and neutralize thereby dimished the actions of DNA and cellular enzymes of the microbes. In the literature, no published report has been found on the use of BC with AgNPs in combination. Therefore, considering the application of BC and silver nanoparticle, this study proposes to formulate the gel of these two components for the wound healing potential in burn condition.

Material and methods

Collection of BC

BC (collected during the first 24 hours *postpartum*) was obtained from a local dairy farmer. Immediately after collection, BC was stored (−20°C) for 72 hours. After that following lyophilization, BC was kept at room temperature in plastic zipper bags and in sealed polystyrene boxes until it was used for oral supplementation.

Production of AgNPs

The 100 mL of 10^{-2}M silver nitrate solution (Aqueous) was mixed and heated till the solution reached at 90°C, in the solution dropwise 6 ml of trisodium citrate (1%) was added. After this, the solution was briefly stirred and left at room temperature to cool (Yakar & Dede, 2022).

Characterization of AgNPs

Characterization of synthesized AgNPs was done by UV–Visible spectroscopy, Zeta average size and Fourier Transform Infrared Spectroscopy (FTIR). For UV–Vis analysis absorbance in the range of 300–600 nm was observed. Zeta Average size revealed the average size distribution of AgNPs. The FTIR spectrophotometer was used to record spectra. For recording FTIR spectra for AgNPs wave number range was 3600- 800 cm^{-1} (Bhalekar et al., 2009).

Formulation of 10% AgNPs BC Gel

Gel was prepared using dispersion of the 1 gm of Carbopol 934 in 50 mL of above AgNPs solution and left it to swell for 30 minutes to form gel. In this mixture, methyl paraben and propylene glycol 400. pH of

AgNPs gel (6.8) at required skin pH was adjusted by using triethanolamine. Finally BC 10% was mixed with AgNPs gel at continuous stirring to obtain the gel at required consistency.

Experimental design

Twenty-four Wister rats weighing 150-200gm were used for the study. For one week animals were acclimatized to the standard laboratory conditions at 25±2^{0}C, relative humidity 44–56%, and 12:12 hours light and dark cycle. During the study the experimental animals were fed with standard Nutrivet pellet diets and water ad-libitum. The study protocol for the animal experimentation approved by Institutional Animal Ethics Committee (IAEC)and the study was performed according to the Committee for Control and Supervision of Experiments on Animals (CPCSEA) guidelines for the use and care of animals (Approval no. CPCSEA/IAEC/ PT-06/02-2K22).

Method of second-degree burn wound creation

The back hairs of rats were shaved and disinfected with betadine solution. Then, the animals were anesthetized by combination of ketamine hydrochloride (85mg kg^{-1} ip^{-1}) and xylazine hydrochloride (6mg kg^{-1} ip^{-1}) and a second-degree burn was created by the round metal coin (1.7 cm diameter) which was placed in boiling water at a temperature of 100°C and later on immediately placed on the shaved area behind the rats for 20 seconds. All the experimental animals were immediately resuscitated by injecting of lactated Ringer's solution (2 mL $100g^{-1}$ body weight, i.p.). After complete recovery of anaesthesia, the animals were then housed individually in separate cages. After wound creation; the animals were divided into four groups of each contained six animals as disease control, placebo control, standard NanoColl-Nanocrystalline Silver in Collagen base Gel and test 10% AgNPsBC gel. The respective group of animals were treated topically with 0.5g of test and standard gel once a day. To avoid the licking of wound by rats, the wound was covered with dressing (Guo et al., 2017).

Percent of wound healing

Daily the animals were monitored and observed for the percentage wound closure and photographs were taken. In this experiment, the wound area was measured with the help of transparent tracing paper on the days 0, 5, 10, 15, and 20. The percent wound contraction was calculated by considering the initial size of the wound on day 0 as 100% using the following formula (More et al., 2015).

$$\%\ Wound\ Contraction = \frac{(Initial\ wound\ area - Specific\ day\ wound\ area)}{(Initial\ wound\ area)} \times 100$$

Lipid peroxidation assay

The inhibition of lipid peroxidation was estimated from the isolated burn wound tissue in the form of TBARS which is an indicator of oxidative stress at 20-day trial period. In this, 0.3g of sample from the wound tissue was cut and homogenized in KCl solution. TBARS activity was estimated as per the method of Ohkawa and Farhoudi (Ohkawa et al., 1979).

Haematological parameter testing

Wound samples were collected in non-heparinized collection tubes for analysis of WBC.

Histopathological examination

At the end of the study, the animals were sacrificed under light anaesthesia and the full thickness of skin sample collected from site of burn area for histopathological examination. The excised skin samples fixed in formalin (10%) and dehydrated using ethanol. The skin sample was then clean using xylene and fixed in paraffin wax. The 5 µm sections were cut from the paraffin fixed tissue sample and stained with eosin and haematoxylin. Under the light microscope the section was observed and photographs were taken.

Statistical analysis

The data was statistically analysed using Graph Pad In-Stat version 3.10. All parameters were analysed by one-way analysis of variance (ANOVA), which was followed by the Tukey-Kramer multiple comparison test. The data are reported as the mean ± SEM. It was also considered statistically significant at $p<0.05$.

Results

UV-Visible Spectroscopy analyses of AgNPs

Both the UV range and the visible range are present in UV-Vis spectroscopy. The appearance of an absorption peak at a wavelength of 300–400 nm was an indicated the AgNPs have been formed (Figure 1).

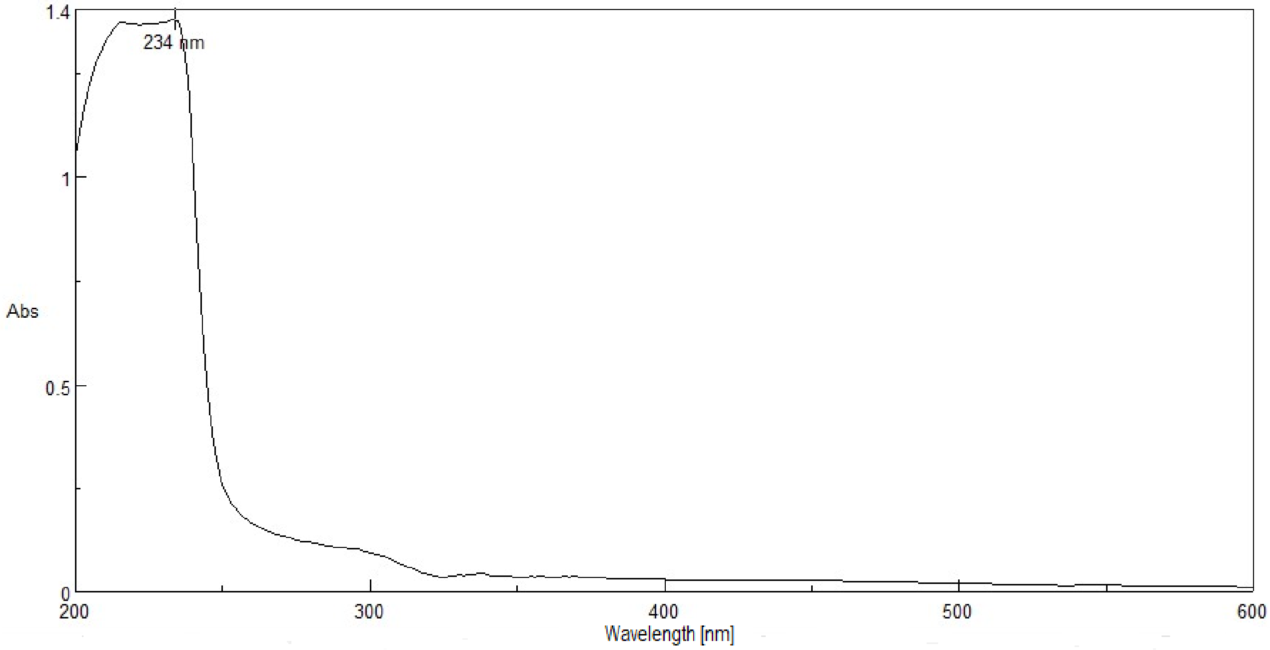

Figure 1. UV-Visible spectrum of AgNPs.

Zeta sizer analysis of AgNPs

The results of zeta sizer illustrate the particle size measurement, which was 243.9 d nm. This outcome supports the particles' characterization in the study and verifies the designation of them as Nanoparticles (Figure 2).

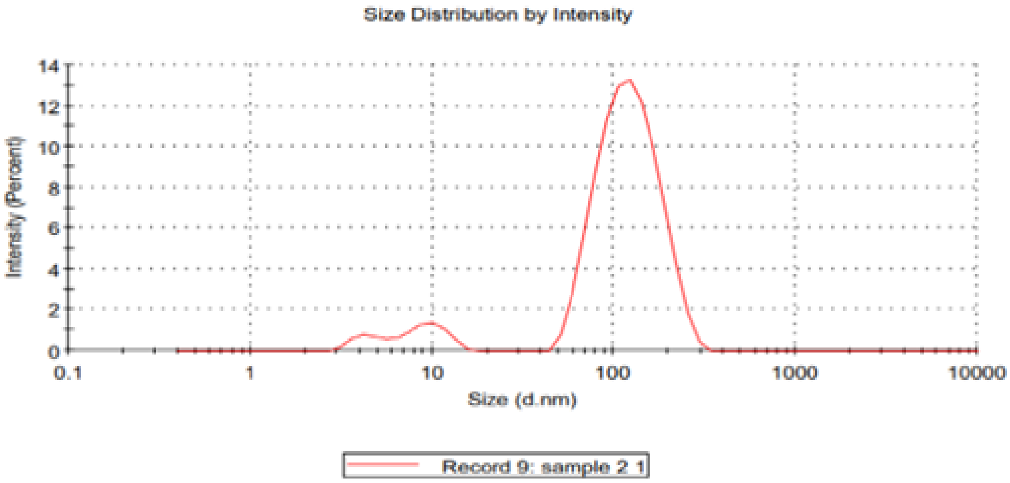

Figure 2. Zeta Average size of AgNPs.

FTIR Spectroscopy

The FTIR spectra of the 1% AgNPs BC gel showed the sharp peaks. The peak at 3289 cm^{-1} recognized for–OH, at 1628 cm^{-1} recognized for -NH bending vibration. There were recognition of N-H bending vibration of the primary amine, OH, CH in the ring and C-O-C and C-O stretching vibrations in the wavelength range of 1600–1000 nm. The peaks observed at 810 cm^{-1} recognized for Ag-O stretching vibration band (Figure 3).

Figure 3. Fourier Transform Infrared Spectroscopy of 10% AgNPs BC gel.

Effects on percent wound contraction

The result of the study showed the significant differences between the treatment groups, particularly on the 10, 15, and 20th days, with these differences being highly significant ($p<0.01$). 10% AgNPs BC gel on the dorsal surface of burn wound demonstrated 80% wound contraction ($p<0.01$) on 15th day while on 20th day there was complete wound closure compared to disease control wound(Table 1 and Figure 4).

Table 1. Effect of 10% AgNPsBC gel on percent wound contraction.

Groups	Day 5	Day 10	Day 15	Day 20
Disease Control	4.16±1.3	14.58 ±1.31	22.91±4.16	41.66±3.09
Placebo Control	13.54±1.9	41. 66±5.01a**	51.04±4.39a**	67.70±3.39a*** b***
10% AgNPs BC	14.58±3.09a*	44.79±7.29a***	77.08±5.74a*** b**	97.83±1.31a*** b***
NanoColl	16.66±3.48a*	37.5 ± 2.28a*	72.91±3.84a*** b*	95.83±2.08a*** b***

All values are represented in percentage as mean ± SEM, n=6 animals in each group. Data were analysed by one-way ANOVA, followed by Tukey-Kramer Multiple Comparisons Test. a: significant difference as compared to disease group; b: significant difference as compared to placebo group, and *p < 0.05, **p < 0.01, ***p < 0.001.

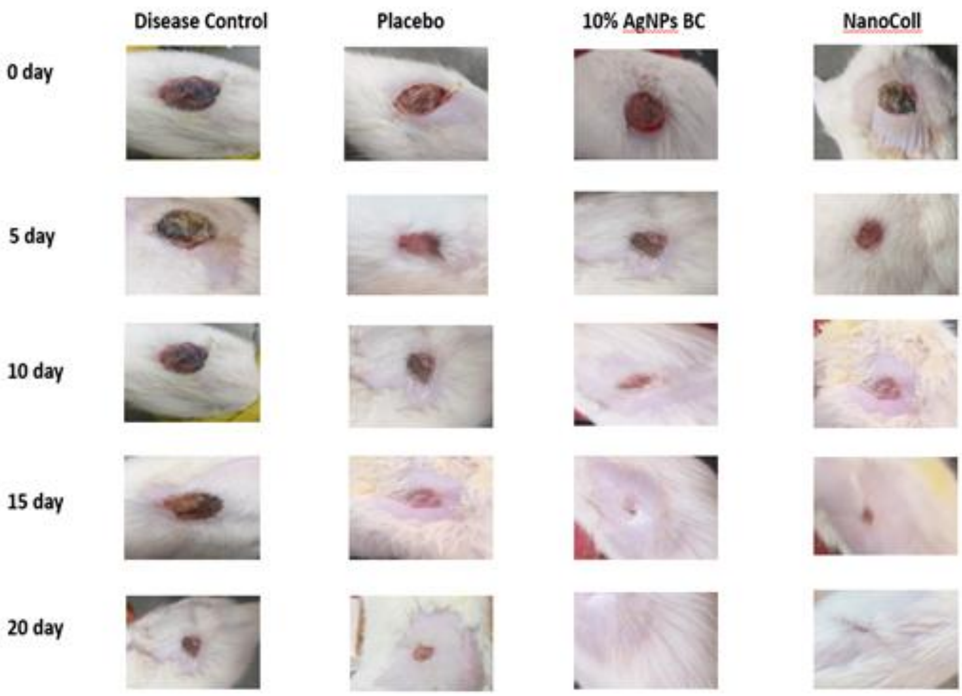

Figure 4. Effect of 10% AgNPsBC gel on wound contraction.

Effects on TBARS and WBC

The result showed the significant (p<0.05) increased of TBARS in the disease control indicates the high level of oxidative stress while topical application of 10% AgNPs BC gel showed significant (p<0.05) declined in the level indicates the antioxidant potential in 10% AgNPs BC gel. While the result of WBC count as an indicator of wound infection showed to increase the count in the burn wound. The WBC count was higher in wound of disease control group while in 10% AgNPs BC gel treated group, the count was found to significant low (p<0.05) (Figure 5).

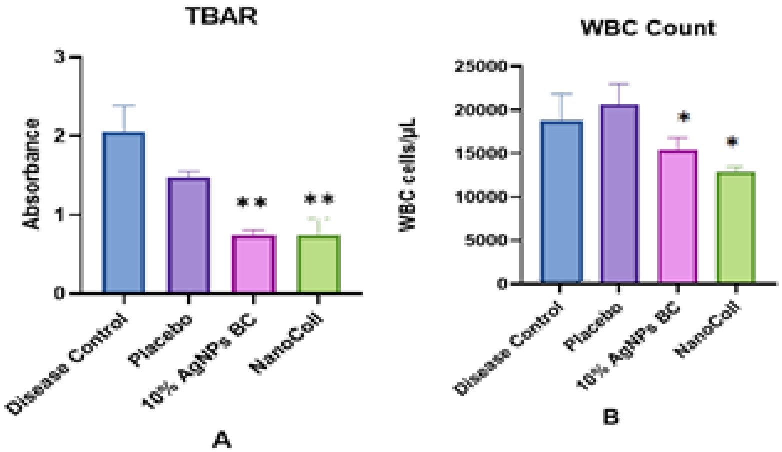

Figure 5. A. Effect on TBAR. B: Effect on WBC Count. All values are represented as mean ± SEM, Data were analysed by one-way ANOVA followed by Tukey-Kramer Multiple Comparisons Test, *p < 0.05, **p < 0.01 compared to disease control group.

Histopathological examinations of the burn wound tissue

Histopathological examinations of the burn wound tissue were conducted on the 20th day, and the histopathological characteristics of the tissue in all animal groups are depicted in Figures 6. In section A, disease control animals exhibited the presence of inflammatory cells, diminished collagen fibres, fibroblast cells, and blood vessels, along with visible scar tissue. In section B, the placebo control group animals showed necrotic cells and a lower presence of collagen fibres and blood vessels. Section C, representing the 10% AgNPs BC gel-treated group, demonstrated complete tissue regeneration with evident increases in fibroblast cells, collagen fibres, and blood vessels, along with a reduction in inflammatory cells. Section D, corresponding to the standard NanoColl group, indicates the minimum necrosis and marked collagen fibres and blood vessels (Figure 6).

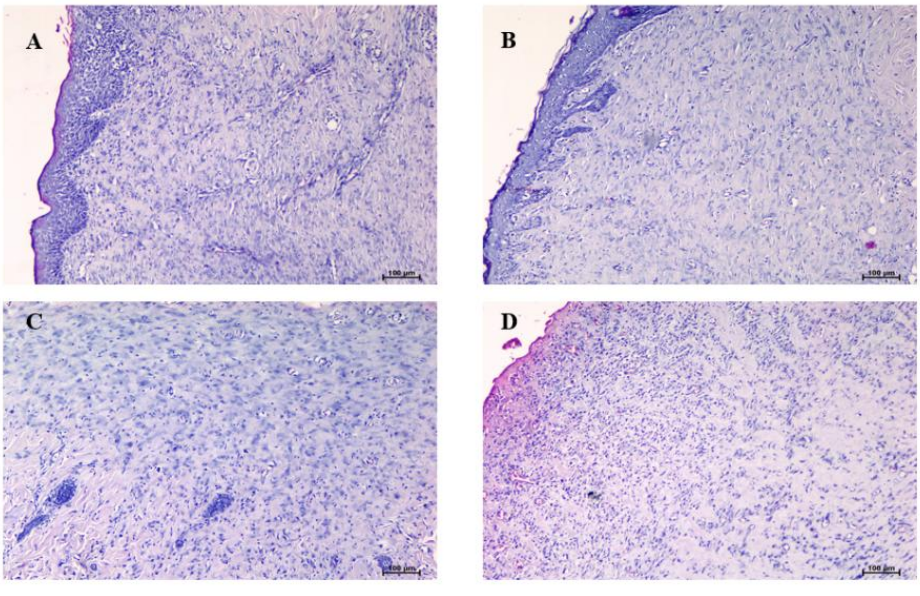

Figure 6. Effect on skin histopathology study in rats of A: Disease control, B: Placebo, C: 10% AgNPs BC gel, D: NanoColl.

Discussion

Burn injuries can lead to a range of complications, their severity depending on the degree and extent of the burn. Complications can exacerbate the healing process. In this study, burn wound infection is the most likely complication to occur. Therefore, effective topical treatments for burn wounds should incorporate an affordable, safe, and potent antibacterial component effective against both Gram-positive and Gram-negative bacteria, as well as Candida species (Trabelsi et al., 2017).

Burn wounds, characterized by significant inflammation, require treatment with anti-inflammatory and antioxidant agents to prevent delayed wound healing. In this study, we utilized BC embedded with AgNPs for managing burn wounds. BC contains various active substances that individually possess antibacterial, anti-inflammatory, and robust antioxidant properties (Yadav et al., 2016).

In the medical practices, nanoparticles of silver are widely used due to their non-toxic and wide ranging antimicrobial and other properties. The reported studies have also been revealed that silver alters and inhibits cellular enzymes and DNA by neutralizing the electron-carrying compounds (Zahoor et al., 2021). Previously several works has been reported on the AgNPs in alone as well as with the combination of other compounds (Yadav et al., 2016; Zahoor et al., 2021). While no published report on the combination of BC and AgNPs. Hence, this study aimed to develop a topical gel formulation containing AgNPs loaded with BC. The study's findings indicate that the formulated gel containing 10% BC contains AgNPs, as demonstrated by UV-visible spectrum and zeta particle sizer results.

UV-Vis spectroscopy is used to characterize and confirm the presence of reduced silver ions as AgNPs in the solution. The appearance of an absorption peak at a wavelength of 300–400nm was an indicated the AgNPs have been formed. The Zeta Sizer was used to determine the AgNPs average size distribution which was found to be at 243.9 d nm. This outcome supports the particles' characterization in the study and verifies the designation of them as nanoparticles. FTIR analysis was conducted to elucidate the structural characteristics of the 10% AgNPs BC gel, and upon closer examination it becomes evident that 10% AgNPs BC gel compositions exhibit distinct and prominent peaks in similar regions. One noteworthy peak appears at 3,289 cm^{-1}, indicating the presence of -OH, peak at 1,628 cm^{-1} recognized for the bending vibration of -NH. In the wavelength range spanning from 1,600 to 1,000 nm, several vibrations are observed, including -NH bending vibrations of primary amines, vibrations associated with -OH groups, cyclical CH groups, and stretching vibrations of C-O-C and C-O bonds. Notably, peaks at 810 cm^{-1} correspond to the stretching vibrations of Ag-O bonds, while a distinct peak at 2,985 cm^{-1} indicates the presence of lipids in BC, specifically representing antisymmetric CH_2 stretching. Furthermore, the IR bands at 1,032, 810, and 785 cm^{-1} primarily suggest the presence of carbohydrates in the sample. Additionally, a characteristic IR band in the range of 3600 to 3200 cm^{-1} primarily arises from the stretching vibration of -NH groups and is referred to as the Amide A band. It is worth noting that the Amide I band, which falls within the range of 1700 to 1600 cm^{-1}, is highly sensitive and is frequently utilized to assess the secondary structure of proteins (Carbonaro &Nucara, 2010; Mehra et al., 2022).

The primary objective of this study was to evaluate the effect of the 10% AgNPs BC gel in burn wound care management. Table 1 and Figure 4 represent the results of wound healing activity in burn model of rats. The results show that topical application of 10% AgNPsBC gel for 20 days results in complete wound healing. These findings are comparable with standard NanoColl (Nanocrystalline Silver in Collagen base Gel).This indicates that both 10% AgNPsBC gel and standard NanoColl are more effective in promoting wound contraction compared to the disease control group, highlighting their therapeutic potential.

Oxidative stress in burn wound tissue was also evaluated in this study. The findings indicate a reduction in oxidative stress, measured in terms of TBARS. These findings suggest that the 10% AGNPs BC gel has the potential to mitigate the damaging effects of free radicals during the wound healing process. These findings align with reported data on the antioxidant potential of AgNPs and BC (Zahoor et al., 2021; Santos et al., 2024).

Wound infection is a primary clinical concern in wound care. In this study, the impact of 10% AGNPs BC gel on WBC count in the wound area was assessed. The results showed a significantly higher WBC count in the disease control group compared to the treatment and standard NanoColl groups, indicating the presence of wound infection. Conversely, topical application of 10% AGNPs BC gel resulted in a significant ($p<0.05$) decreased WBC count.

Microscopic analysis yields valuable information regarding the gel's impact on cellular responses and overall wound healing progression (Bhatia et al., 2014). Histopathological examinations of the burn wound tissue were conducted on the 20th day, and the histopathological characteristics of the tissue in all animal groups are depicted in Figures 6. The finding revealed varying tissue responses (on 20th day) among the different groups, with the 10% AgNPs BC gel-treated group exhibiting reduced inflammation and enhanced tissue regeneration characteristics.

In the present study, the major limitations to use of BC are non-uniformity in chemical composition due to variation in source, stability and storage condition (Schogor et al., 2020; Mehra et al., 2022). Considering the stability issue of BC, there is need of details studies on the characterization of developed formulation of 10% AgNPs BC at accelerated temperature.

Conclusion

The overall results of the present investigation provide insights into contribution of AgNPs loaded with bovine colostrum for burn wound healing potential. The results conclude that, the developed formulation has strong wound healing potential which could be mediated by antioxidant property. Additionally, the AgNPs has reported to have strong antibacterial action that can correlate with our finding of WBC in the present study. The bovine colostrum contains the collagen and growth factors that may contributes in the tissue regeneration as well as for antioxidant and anti-inflammatory properties. Based on these reported literatures and our experimental findings it concludes that the 10% AGNPs BC gel would be beneficial in the management of burn wounds by managing the wound infection, inflammation, and promoting the regeneration process in wound care process.

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