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Formulation and characterization of a novel hydrogel loaded with honey silver nanoparticle with its wound healing activity

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ABSTRACT
To combat the increased chronicity of microbial infection with impaired wound healing and having superior antimicrobial activity, a new hydrogel formulation loaded with honey silver nanoparticles has been developed. The silver honey-loaded nanoparticles were synthesized using silver nitrate solution and characterized for particle size, zeta potential, surface morphology, and XRD analysis. Hydrogel is fully characterized by UV spectroscopy, pH determination, viscosity measurement, spread-ability, swelling index, in vitro drug release studies, and in vivo testing. The average particle size of honey silver nanoparticles was found to be 395.4 nm with a zeta potential of -3.6 mV. The surface morphology of silver honey showed a larger size and uneven shape of silver nanoparticles. The XRD patterns specify the crystalline structure of the nanoparticle. The zone of inhibition with maximum values against Streptococcus aureus was found to be 12 mm with 102 ug/ml concentration. The topical hydrogel exhibited a transparent appearance with a jelly-like consistency. The average viscosity ranged from 6020 to 2192 cP. Borax guar gum hydrogel demonstrated remarkable self-healing within 6 minutes. The hydrogel with 102 ug/ml had a higher swelling index i.e., 33%. The hydrogel was found to be free of skin irritancy. The progressive decrease in wound size underscores the hydrogel's potential to accelerate the healing process and support tissue remodeling. Complete healing was observed in the skin epithelial tissue, resulting in an intact skin layer. The guar gum hydrogel loaded with honey silver nanoparticles (102 ug/ml) shows improved antimicrobial and wound healing activity.
Keywords*: antibacterial wound healing, guar gum, honey, hydrogel, silver nanoparticle.*

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INTRODUCTION

Use of silver as antimicrobial agent is common since long time. Silver nanoparticles (AgNPs) have wide ranging antimicrobial activity better than frequently used antibiotics. Various types of mechanisms are involved in AgNPs antimicrobial activity, by penetrating the bacteria with damage of DNA or proteins, by disrupting bacterial cell walls, by making reactive oxygen species (ROS), and by penetrating and disrupting intracellular metabolism. Cheaper way of synthesis is the additional advantage of AgNPs over chemical and physical methods. Use of sunlight as reducing agent has advantages like non toxicity, non-polluting and traceless method (1).

Honey, natural sweet syrup created by bees from flower nectar or by other plant secretions with antimicrobial action against multiple microorganisms. Presence of polyphenols bee peptides, hydrogen peroxide, dicarbonyl methylglyoxal (MGO) and polyphenols plays major role in its antimicrobial activity (2). Physicochemical and antibacterial properties of honey vary according to environmental and botanical origins, composition of soil, type of bee and harvest season. Honey is used as a stabilizing and reducing agent in nanoparticle synthesis methods, due to presence of oxygen peroxide and glucose which reduces metallic ions, whereas gluconic acid and proteins stabilizes the formation of particles (3-4). Different nanomaterials and natural based formulations of honey were explored previously. Natural bionanomaterials have several advantages, like low cost, better drug delivery, and efficient against many bacteria. These are sterile, non-scarring, non-toxic, biodegradable, biocompatible with excellent mechanical and physicochemical properties (5)

Guar gums is a multipurpose plant-based biopolymer. Water absorbing property of guar gum is high with increased swelling strength (6). Guar gum is more used in food and biological industry as it is biodegradable, safe, and economic, it easily forms gel with water at normal temperature. Due to use of borax as a cross-linker with guargum, the boronate ester linkages provides self-healing properties to the gel without any external stimuli (7-9).

Now a day's hydrogels are preferred in wound treatment. Hydrogels provides moist environment and better fluid absorbance during wound healing. Hydrogel formulations preferred as the high-water content helps in reducing the pain during application. Hydrogels provide more spread ability and easy removal, doesn't stain, well suited with numerous excipients and water-soluble or miscible. Therefore, a hydrogel with biocompatible matrice and biologically active substances is the need in wound treatment (10).
In a guar gum hydrogel matrix, this work is an attempt to study and examine the synergistic benefits of combining the wound-healing abilities of honey with the antibacterial capabilities of silver nanoparticles. Use of guar gum with honey gives additional hydration effect to this formulation. Understanding the potential of this novel formulation may help in the creation of robust antimicrobial medicines for the treatment of wounds. This research intends to develop a biocompatible and efficient wound-healing material with enhanced antimicrobial capabilities by utilizing natural sources and minimizing the use of hazardous chemicals

MATERIAL AND METHODS

Materials

Honey (Maharashtra State Khadi and Village Industries Board, Satara), Silver Nitrate (Merck Life Science Private Limited, Mumbai), Sodium Hydroxide (Sisco Research Laboratories Pvt. Limited, Taloja), Borax (Vishal Chem, Mumbai), Glycerol (V.A. B Enterprises, Mumbai), Guar gum (Research Lab Fine Chem industries). All other reagents used were of pharmaceutical and analytical grade.

Methods

Formulation of antibacterial guar gum hydrogel loaded with silver and honey nanoparticles (Preparation of Silver NP):

To prepare the silver honey nanoparticles, approximately 0.085 g of silver nitrate was weighed and added to 50 ml of distilled water. Later, a 0.2% honey solution was prepared separately. A beaker is used to combine 20 ml of 0.01 M silver nitrate solution with 15 ml of the 0.2% honey solution. The mixture is thoroughly mixed and allowed to react under sunlight. Finally, after a reaction time of 10 to 15 minutes, the appearance of a distinct color indicates the successful preparation of the silver honey nanoparticles (solution A) (11).

Preparation of Hydrogel:

A volume of 5 ml of thus prepared Silver NP (solution A) was pipette out and diluted to 100 ml water. This resulting solution serves as the stock solution for subsequent dilutions. From the stock solution, 1 ml, 3 ml, and 6 ml were pipetted out individually and made up to 100 ml each using distilled water, resulting in concentrations of 17, 51, and 102 micrograms/ml, respectively. Taking 20 ml from each of the resulting concentrations (17, 51, and 102 ug/ml), the addition of 0.1 g of guar gum to each solution was followed by thorough stirring to achieve a homogeneous system. The resulting mixture was alkalized using200 µl of 0.1 M NaOH then 0.2% glycerol was introduced followed by, 500 µl of 4% borax with continuous stirring of the solution until a homogeneous hydrogel was obtained, ensuring that all components were well mixed and dispersed uniformly throughout the hydrogel matrix (8). **Table 1** describes the composition of the hydrogel

Characterization of silver nanoparticles

Determination of particle size and silver honey nanoparticles:

The particle size of silver honey nanoparticles was determined using the HORIBA Scientific SZ-100 nanoparticle analyzer. The sample cell was filled with around 5 ml of the nanoparticle solution for examination.

Determination of zeta potential of silver honey nanoparticles:

Using a HORIBA SCIENTIFIC AND SZ-100 nanoparticle analyzer, the zeta potential of the produced nanoparticles was assessed.

Determination of λmax by UV Spectroscopy

A computerized double-beam UV-visible spectrophotometer was used to measure the absorbance maximum of Silver-Honey nanoparticles and Honey. The scanning range of 200-400 nm was selected for honey and 300-600 nm for silver nanoparticles. To determine the wavelength at which maximum absorption occurred for each sample, the absorbance spectra were recorded.[12] With the use of double distilled water, a solution of nanoparticles and honey was created. The spectrum and absorbance measurements were captured using a Shimadzu UV-visible spectrophotometer UV-1800 having spectral bandwidth of 1 nm, precision of wavelength of 0.3 nm, and a pair of 10 mm quartz cells.

SEM of Silver Nanoparticles:

The surface morphology of the silver nanoparticles was observed through scanning electron microscopy.

XRD of Silver Nanoparticles:

The crystalline or amorphous nature of the silver nanoparticles was determined using X-ray diffractometry.

Characterization of Hydrogel

Physical Characteristics

The resulting hydrogel formulations' pH, colour, consistency, homogeneity, texture, grittiness and phase separation were all visually assessed.

Determination of pH:

The final pH of hydrogel formulations was assessed using a digital pH meter. The electrode was immersed in the hydrogel for 30 minutes until a consistent reading was obtained. One gram quantity of gel was dissolved in 25 ml of distilled water for the measurement. Multiple readings were taken at regular intervals. The pH of each formulation was measured in triplicate, and the mean of readings were calculated.[14]

Viscosity determination

The viscosity of the produced hydrogel was measured using a Brookfield digital viscometer. Spindle number 6 was utilized to measure the viscosity at 10 rpm and 25 °C. An appropriate wide-mouth container was filled with a sufficient amount of gel. The hydrogel was poured into the wide-mouth container, ensuring that the viscometer's spindle could submerge comfortably. Before the measurements, the hydrogel samples were allowed to settle for 30 minutes at the same temperature (25 ± 1°C).[15]

Self-healing activity

The self-healing capabilities of the hydrogels were assessed visually by dividing sample sections into two and colouring one piece with methylene orange for visualization. After separating the blocks, they were brought back together at room temperature and kept without any light, pressure, or chemical stimuli.

Swelling Index:

Degree of swelling was investigated by immersing the hydrogels in DI water at room temperature. Each hydrogel film weighing 0.1 g was soaked in 50 mL of water for a specified duration. Excess water was then removed by blotting with filter paper. Weight of the gels was constantly monitored until a constant value achieved. The swelling percentage was determined using the following formula:

$$\textbf{Swelling degree} = [(Ws - Wo)/Wo] \times 100$$

Where Ws is the swollen weight of the soaked hydrogel and Wo is the original weight of the dry hydrogel. All experiments were performed in triplicate.

In *vitro* release studies:

Release of Ag Nps from hydrogel formulation was studied using a Franz diffusion cell apparatus. Wistar rat skin was used as membrane. A specific weight of honey hydrogel equivalent to 10 mg was placed at top chamber in a precise volume of 5.5 pH phosphate buffer at room temperature and the amount of released honey was recorded at specific time intervals by measuring the absorbance of test sample with UV-Vis spectrophotometer (Shimadzu, 1800). The absorbance of the released honey was noted at λ max 480 nm. All experiments were performed in triplicates, the mean of the results were considered. Blank experiments were performed with plain bases.

***In vitro* antimicrobial studies:**

Antimicrobial studies were performed using the spread plate technique. Nutrient agar media was prepared and poured into sterile petri plates. A volume of 0.1 ml of freshly received Streptococcus aureus was spread onto each petri plate. Wells were created in the agar plate using a sterile borer, and 0.1 ml of each formulation (17 µg, 51 µg, and 102 µg) was added to the respective wells. The plates were then incubated at 25^0C for 24 hours. The zone of inhibition was subsequently measured and recorded for each formulation.[13]

***In vivo* wound healing studies:**

The excision wound model was employed in this study. Six animals were divided into three groups, receiving different concentrations (17 µg/ml, 51 µg/ml, and 102 µg/ml) of the treatment. Under Diethyl ether anesthesia, a wound was induced by removing the skin on the dorsum of the abdomen in all five groups. Evaluations were conducted at intervals of seven days (days 1, 7, 14, and 21), and photographs were taken from the time of wound creation until complete healing. Clinical parameters included observations of the wound's appearance, and the wound size was recorded**.**

RESULTS

Characterization of silver nanoparticles

Particle size and zeta potential of silver honey nanoparticles:

The instrument was operated at cumulative operations and the value was found to be Z average: 849.1nm PI: 0.464. The mean particle size of honey silver nanoparticles was found to be 395.4nm. The results are summarized in **Table 2**. The particle size distribution of silver nanoparticles is presented in **Figure 1.**

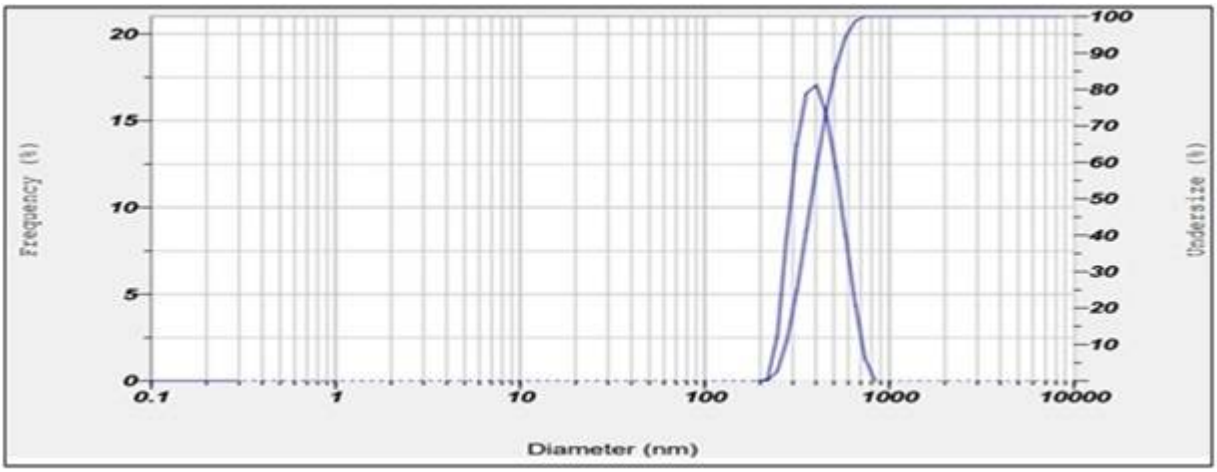

Figure 1. Particle size of silver honey nanoparticles

After analysis, the mean zeta potential of nanoparticles was found to be -3.6 mV **(Figure 2)** with electrophoretic mobility of 0.000028 cm^2/Vs indicating that the nanoparticles are within the nano range and exhibit stability.

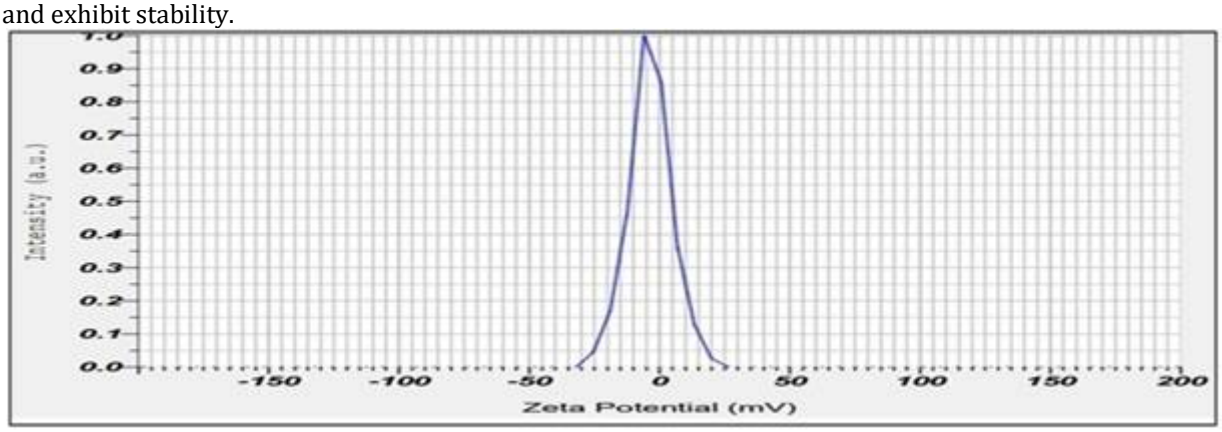

Figure 2. Zeta potential of silver honey nanoparticles

Determination of λmax by UV Spectroscopy:

A computerized double-beam UV-visible spectrophotometer was used to measure the absorbance maximum of Silver-Honey nanoparticles and Honey. The λmax of 0.2% pure honey and honey silver nanoparticles was found to be 273 nm and 448 nm respectively. **(Figure 3A and 3B).**

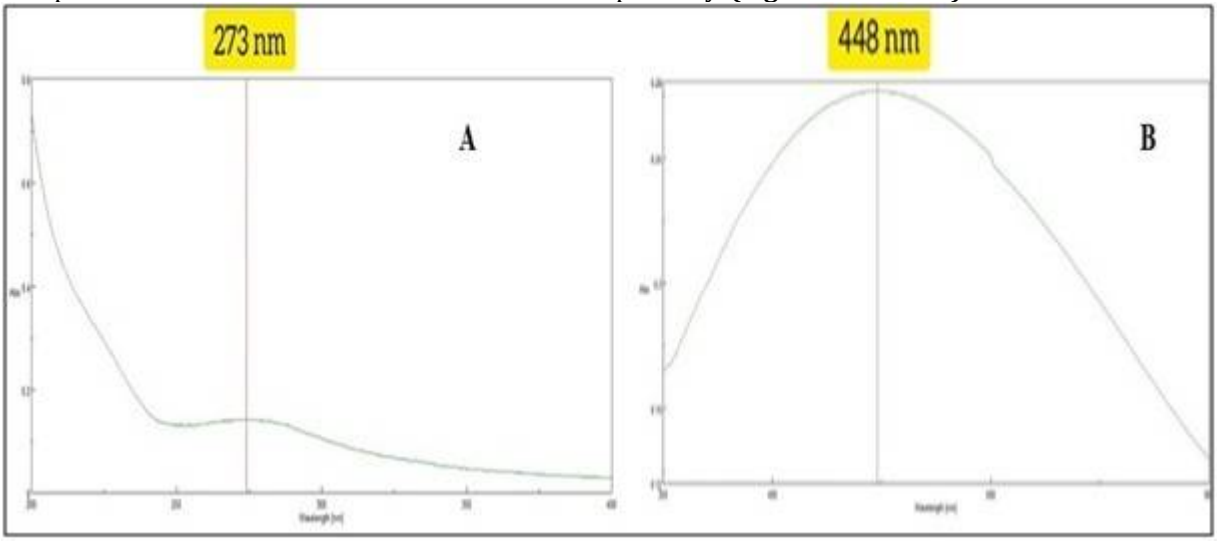

Figure 3. UV-visible spectroscopy of A: 0.2% pure honey; B: silver nanoparticles

SEM of Silver nanoparticles:

The surface morphology of silver honey is shown in **Figure 4**, with the larger sized and uneven shape of silver nanoparticles. Previous studies also show similar phyto-synthesized silver nanoparticles. Amounts of spherical sized silver nanoparticles were very few. The accumulation of particles might be because of the existence of cell components on the outer edge of silver nanoparticles which act as capping agent.

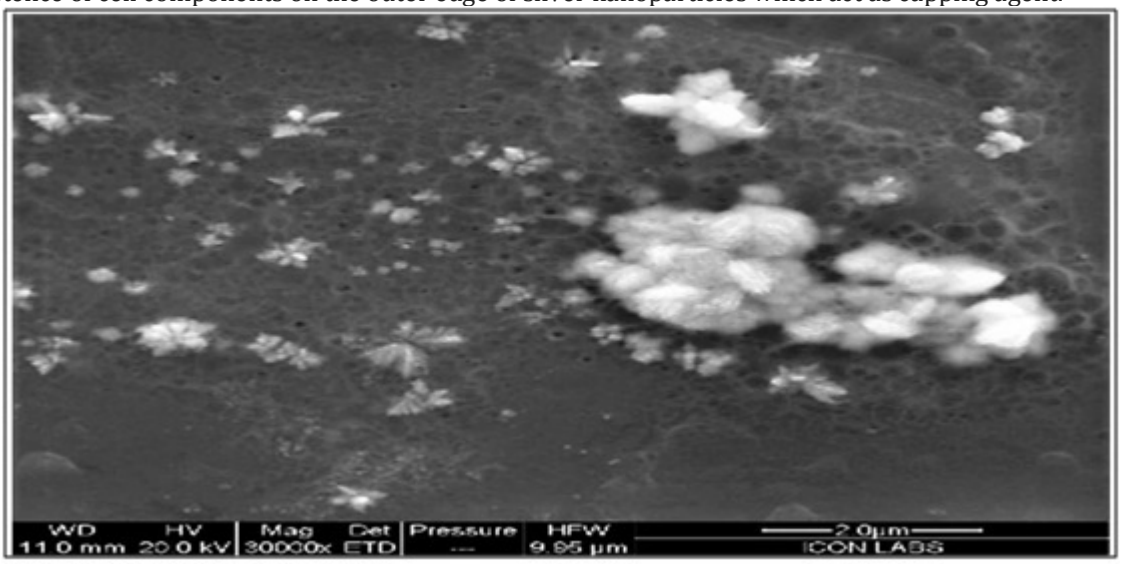

Figure 4. SEM of Silver Nanoparticles

XRD of Silver Nanoparticles:

The honey silver nanoparticles were additionally defined by the typical peaks observed in the XRD results. The XRD image of Ag showed the 4 sharp peaks that indicate the crystalline structure of the nanoparticle **(Figure 5)**. The regular crystalline size of silver nanoparticles was found to be around 20-90 nm. The XRD image has sharp peaks in the entire spectrum of 2Θ from 0–70 nm.

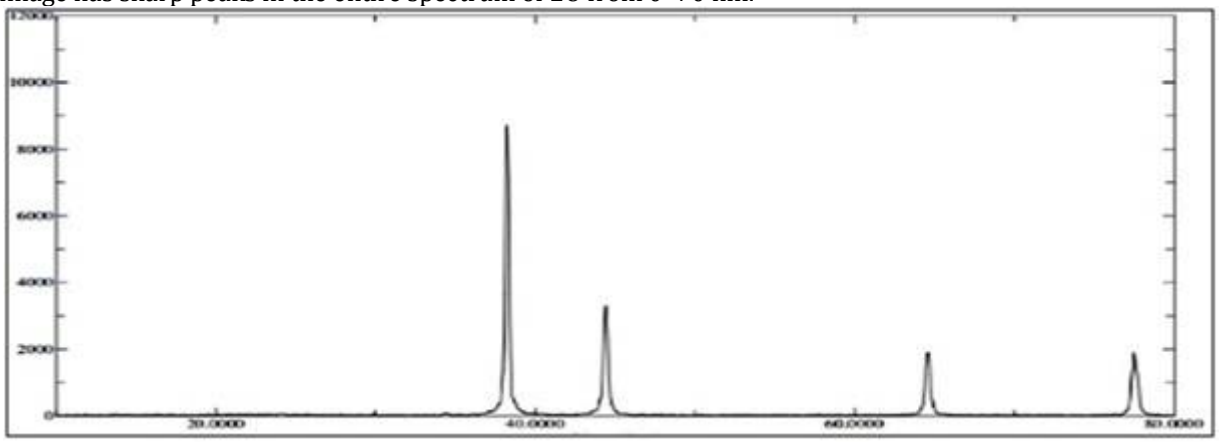

Figure 5. XRD of Silver Nanoparticles

Characterization of hydrogel:

Physical Characteristics

The topical hydrogel prepared using green synthesis of honey silver nanoparticles exhibited a transparent appearance with a jelly-like consistency. All the 3 concentrations 17ug/ml, 51ug/ml, and 102ug/ml are jelly-like and transparent. The pH of the hydrogel was observed to be in the range of 5.2 to 6.8. The results are summarized in **Table 4.**

Viscosity of the hydrogel:

The viscosity of the hydrogel decreased with increasing shear rate. The average viscosity of 17 ug/ml, 51 ug/ml, and 102 ug/ml was found to be 6020cP, 8687cP, and 2192cP. The comparative viscosity of different concentrations of the hydrogel is presented in **Table 5.**

Self-healing activity:

Each weight percent of borax guar gum hydrogel demonstrated remarkable self-healing within 6 minutes. Two blocks of the original hydrogel were immediately reconnected after being cut into two parts. The self-healed gel fully restored its rheological characteristics in 6 minutes, showing same strain-at-breakage and strain-stiffening as the hydrogel without cut. The observations demonstrate the exceptional self-healing

and gelling capabilities of borax cross-linked hydrogels.[16] The results can be seen in **Figure 6**. The time required for self-healing was found to be 6 minutes.

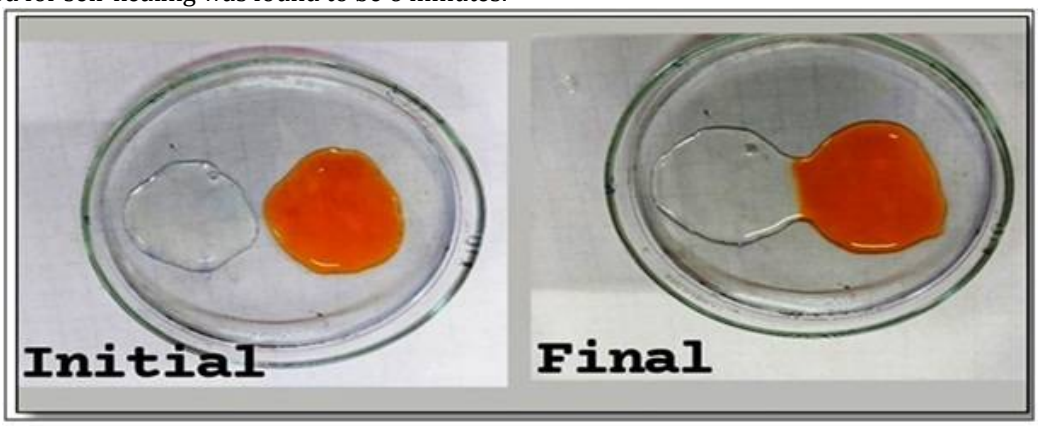

Figure 6. Self-healing activity of hydrogel

Swelling Index:

The hydrogels' swelling ratio was determined to assess their water absorption capacity. Equilibrium size was reached by the hydrogels after approximately two hours of soaking. The swelling ratio was observed to increase with an increasing borax composition from 17 µg to 102 µg wt.%, indicating a positive correlation with the degree of crosslinking within the hydrogel. This aligns with previous studies on guar gum/borax hydrogels. Additionally, the swelling ratio was found to increase under alkaline pH conditions, attributed to the formation of wider networks facilitated by the attraction of negatively charged borate and hydroxide ions. In our research, an alkaline environment was utilized for hydrogel formation. NaOH was utilized to adjust the initial pH of the solution of guar gum to 8 before adding varying amounts of borax and drying the film. Consequently, we anticipate that the observed rise in swelling ratio with composition of borax in our study will be significantly influenced by the repulsion between borate and hydroxide ions, similar to previous findings.[17] The results of the swelling index are presented in **Table 6.** Among the three concentrations, the hydrogel with 102ug/ml was found to have with higher swelling index i.e. 33%.

Skin irritancy test:

All the concentrations 17 ug/ml, 51 ug/ml, and 102 ug/ml are free of skin irritancy.

In *vitro* release studies:

In vitro release study observations showed that the 100 % release of Ag Nps from the hydrogel takes approximately 3.5 hrs and the concentration of Ag Nps doesn't show any effect on the release pattern **(Figure 7)**.

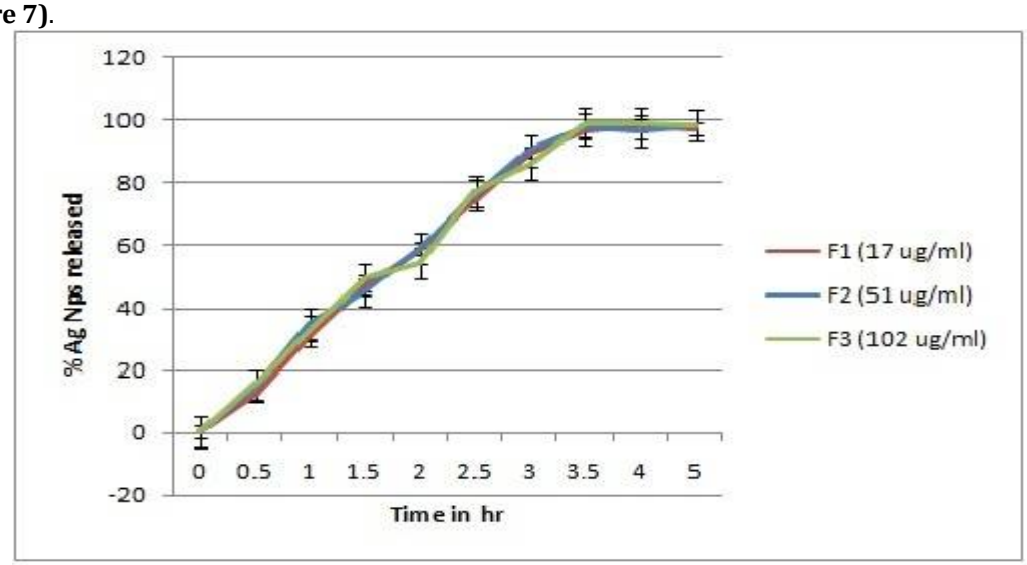

Figure 7. *In vitro* release studies

***In vitro* antimicrobial studies:**

The maximum zone of inhibition against Streptococcus aureus was found to be 12 mm of formulation having a concentration of 102 ug/ml. In the antimicrobial study, the nanoparticulate formulation

demonstrated effective inhibition of the growth of Streptococcus aureus in the zone of inhibition test, particularly at higher concentrations. The results are brifed in **Table 3** and **Figure 8**.

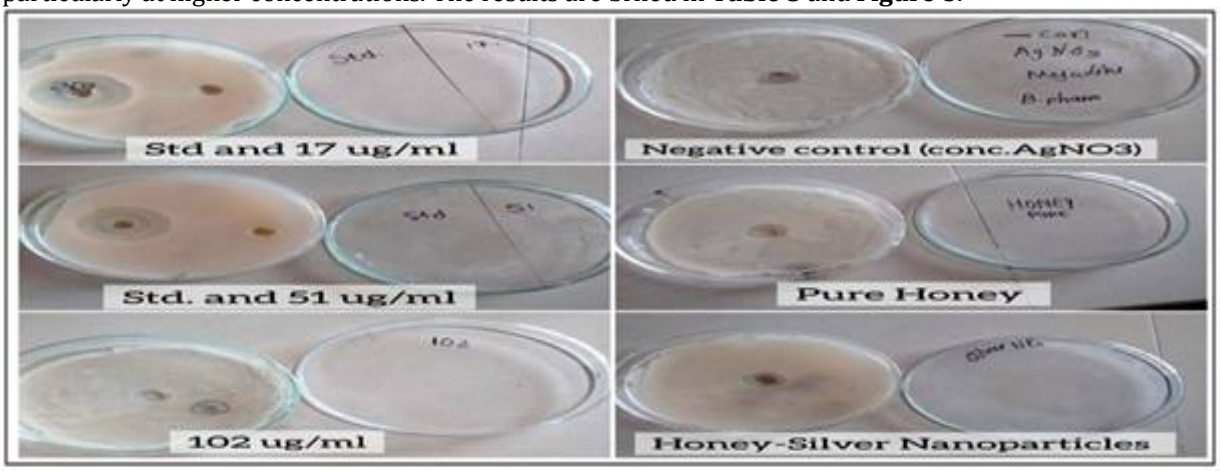

Figure 8. *In vitro* antimicrobial studies

***In vivo* wound healing studies:**

Over 21 days, a significant reduction in wound size was observed, indicating the hydrogel's effectiveness in promoting wound closure and tissue regeneration. The progressive decrease in wound size from day 1 to day 21 underscores the hydrogel's potential to accelerate the healing process and support tissue remodeling. These findings suggest that the formulated hydrogel with 102 µg/ml concentration of Ag Nps have promising implications for clinical applications, providing a valuable therapeutic approach for wound management and tissue repair. The results are presented in **Table 7** and **figure 9.**

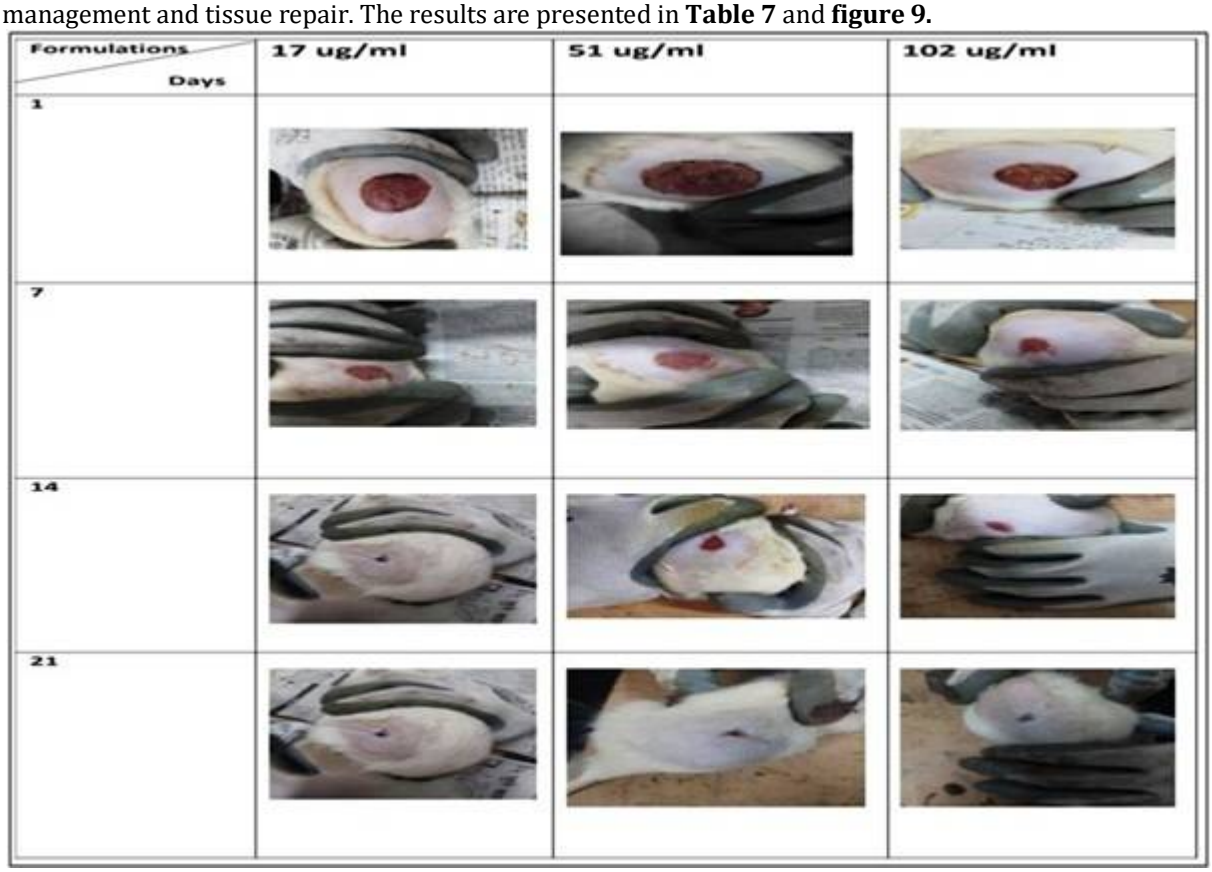

Figure 9. *In vivo* wound healing animal studies

Histopathology study:

In histopathological study, standard shows the formation of granulation tissue with neovascularization and deposition of collagenous stroma in the subcutis layer, accompanied by the re-epithelization of the skin epidermis. Minimal and focal hyperplasia of squamous epithelium was observed in the epithelial layer. In

the group treated with hydrogel formulation with 102 ug/ml Ag Np concentration, the formation of granulation tissue with neovascularization and deposition of collagenous stroma was observed in the subcutis layer, accompanied by re-epithelization of the skin epidermis. Minimal and focal hyperplasia of squamous epithelium was present in the epithelial layer. Complete healing was observed in the skin epithelial tissue, resulting in an intact skin layer (**figure 10)**.

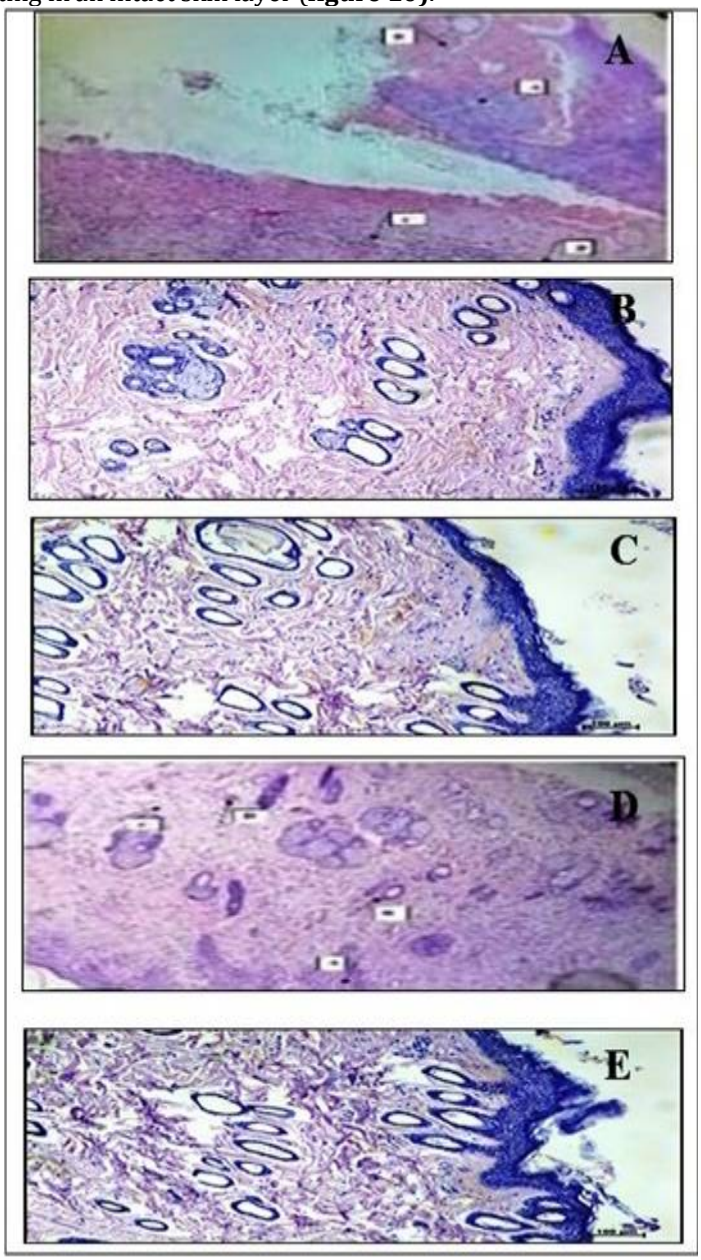

Figure 10. Histopathology of A: Standard group; B: Control group; C: animal tissue for 17 ug/ml formulation; D: animal tissue for 51ug/ml formulation and E: animal tissue for 102ug/ml formulation

Table 1. Composition of Antibacterial Hydrogel

Sr. No	Ingredients	Role of ingredient	F1	F2	F3
1.	Guar gum	Natural Polymer	0.1g	0.1g	0.1g
2.	Honey	Base	0.2%	0.2%	0.2%
3.	Borax	Crosslinker	4%	4%	4%
4.	Glycerol	Plasticizer	0.2%	0.2%	0.2%
5.	Silver Nitrate	Anti-microbial	17µg	51 µg	102 µg
6.	Water	Vehicle	q. s	q. s	q. s

Table 2. Particle size of silver honey nanoparticles.

Peak	S.P. Area Ratio	Mean	S.D.	Mode
1	1.00	395.4nm	102.2nm	377.1nm

Table 3. Zone of inhibition of formulations

Sr no	Group	Zone of Inhibition
1	Negative control	13mm
2	Standard	30mm
3	Pure Honey	11mm
4	Pure silver honey nanoparticles	15mm
5	17ug/ml nanoparticles	11mm
6	51ug/ml nanoparticles	11mm
7	102ug/ml nanoparticles	12mm

Table 4. Physical properties of the hydrogel

Sr. no.	Formulation	Consistency	Appearance	pH
1	17 ug/ml	Jelly like	Transparent	5.2
2	51 ug/ml	Jelly like	Transparent	6
3	102 ug/ml	Jelly like	Transparent	6.8

Table 5. Viscosity of hydrogel

Sr no	RPM	17ug/ml	51ug/ml	102ug/ml
1	10	10840	19000	4800
2	20	10800	9500	2400
3	30	3600	6367	1600
4	40	2700	4760	1200
5	50	2160	3808	960

Table 6. Comparative swelling index at different concentrations

Concentration	Day1	Day7	Day14	Day21
17ug/ml	2.5cm	2.1cm	1cm	0.6cm
51ug/ml	2cm	1.4cm	1.4cm	1cm
102ug/ml	2.2cm	1.7cm	1.4cm	0.8cm

Table 7. *In vivo* wound healing activity of hydrogel

Sr no	Time (in min)	17 ug/ml	51 ug/ml	102 ug/ml
1	0	1 gm	1 gm	1gm
2	15	1.10 gm	1.11 gm	1.12gm
3	30	1.20 gm	1.18 gm	1.20gm
4	45	1.25 gm	1.20gm	1.29gm
5	60	1.30gm	1.27gm	1.33gm
Swelling index		**30**	**27**	**33**

DISCUSSION AND CONCLUSION

This article thoroughly investigates the formulation, characterization, and wound-healing activity of a novel guar gum hydrogel loaded with honey silver nanoparticles. The study aims to explore the potential of this innovative composite material for wound healing applications, specifically evaluating the synergistic effect of honey and silver nanoparticles. The hydrogel demonstrates excellent film-forming properties, enabling the formation of a protective barrier on the wound site. Additionally, it exhibits remarkable hydration effects, promoting wound environment with more moisture that facilitates the process of healing. The formulation is synthesized using a green and eco-friendly approach, utilizing natural sources and reducing agents. This green synthesis method ensures minimal environmental impact and promotes sustainable wound healing solutions. Characterization studies reveal favourable physicochemical properties of the hydrogel, including physical characterization, swelling capacity, pH, viscosity, zeta potential, and particle size. In vitro release study support the effective nanoparticles release. Furthermore, in vitro and in vivo wound healing evaluations demonstrate the remarkable effectiveness of the hydrogel in promoting tissue regeneration and accelerating the healing process. The synergistic combination of guar gum and honey silver nanoparticles at 102 ug/ml concentration in hydrogel exhibits enhanced antimicrobial activity, wound closure, and reduced inflammation. These findings strongly support the

potential of the guar gum hydrogel loaded with honey silver nanoparticles as a promising therapeutic option for wound healing.

COMPETING INTERESTS

The authors declared no conflict of interest in this study

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