Moringa oleifera Extract-Loaded Hydrogel: Assessment of Wound Healing Potential in Animal Model

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Abstract

The quest for effective wound healing strategies has been an enduring challenge throughout history. Recent advancements have highlighted the potential of phytopharmaceuticals as exceptional candidates for wound dressings, owing to their economically viable, natural abundance, ease of accessibility, biocompatibility, biodegradability and inherited therapeutic attributes. This study introduces an innovative approach to wound care by formulating a carbopol-based hydrogel infused with *Moringa oleifera* extract. The incorporation of *Moringa oleifera* extract into the hydrogel leverages its remarkable antibacterial, antioxidant, and anti-inflammatory properties, aiming to create a superior wound dressing that not only accelerates wound healing but also maintains an optimal moisture environment at the wound site. The formulated hydrogel demonstrated an remarkable in vitro release of 87.01±0.74%, and substantial spreadability. Cell viability assessments using the MTT assay on NIH/3T3 cell lines confirmed the hydrogel's biocompatibility, while in vivo wound excision model studies revealed significantly enhanced wound closure in animal groups treated with the MH1 gel compared to other treatment groups. Histopathological evaluations further substantiated these findings, showing accelerated re-epithelialization and collagen deposition. This research underscores the potential of the *Moringa oleifera*-loaded hydrogel as a highly effective wound dressing, offering a promising solution for advanced wound management.

Keywords: Gallic acid, Hydrogel, *Moringa oleifera*, Rutin, Wound dressings.

1. Introduction

The protective barrier of our body is skin that has an important role in preventing microbial invasion. According to WHO (World Health Organization) the mortality due to skin injuries including severe burns is as high as 300,000 annually and thus is a major medical issue need to be taken into consideration. Wounds can be caused due to a variety of reasons such as burns, injuries, post operative and are of chronic type categorized as non-healing wounds (diabetic wounds) and are very confronting to heal (1). Wound healing is a complex process that progress in four steps viz. hemostasis phase followed by inflammatory phase, and proliferation phase, finally ceases with remodeling phase (2). An effective dressing is a prerequisite to get faster healing for both acute as well as chronic wounds. An ideal wound dressing must be bestowed with crucial properties comprising biocompatibility, anti-microbial potential, non-toxic, non-irritant, significantly permeable, high-grade absorbency, maintain moist wound environment, non-stick to skin *etc.* (3). Further the scar formation is one of the major problems associated with wound, and it can be only prevented during wound healing process. Wound healing also involves a well-orchestrated integration of biological and molecular events of cell migration, cell proliferation, and extracellular matrix (ECM) deposition (4). During wound healing process, growth factors released from fibroblasts, macrophages, neutrophils, keratinocytes, and endothelial cells influence all phases of wound healing and occur by providing signals for various cellular activities. Management of chronic wound involves the use of antibiotics, anti-inflammatory agents, or combination of both, but some of these drugs are associated with unwanted side effects hence the need for other alternatives without producing toxicity (5).

There are numerous kinds of novel wound dressing materials such as nanofibers, hydrogels, films, scaffold, foams *etc*. (6). Recently hydrogels having a 3D network structures hydrophilic in nature, have emerged as a substantial wound dressing of choice owing to laden with requires attributes that aids in swift wound healing (7). Hydrogels are bestowed with numerous properties that are required for an ideal wound dressing such as ease of applicability, good strength, biocompatibility, biodegradability, high air permeability and can maintain moist environment along with good adhesion. All improves the overall wound healing process by influencing the stage of wound repair (8). A variety of polymers have been utilized for the development of hydrogel such as carbopol, chitosan, alginates, collagen, gelatin and many more (9). Hydrogels made from carbopol are widely employed as biomaterials for wound therapy. Carbopol is a hydrophilic, biocompatible, and mucoadhesive polymer derived from polyacrylic acid (10). Carbopol polymers are made by crosslinking of acrylic acid with polyalkenyl ethers or divinyl glycol. They have potent flow and buffering capacity, high viscosity, biocompatibility, bioadhesive properties, thermal stability, and patient acceptability. Furthermore, carbopol-based hydrogel can be mixed with other active ingredients to create a matrix substance for diverse wound healing applications.

Recently phytopharmaceuticals have been utilized for various health issues owing to natural origin, less adverse effect, high antioxidant potential and low/no toxicity. *Moringa oleifera Lam*, more commonly known as Moringa pterygosperma Gaertn, and locally horseradish tree belongs to Moringaceae family. In India it is more popular as drumstick tree and has been reported to bestowed with numerous medicinal properties such as anti-diabetic, anti-

inflammatory, anti-hypertensive, antispasmodic, antiseptic, antimicrobial, anti-hyperlipidaemic, antioxidant *etc*.(11). Since ancient era it has been used to treat skin sores, sore throats, eye infections and in recent decades it has been explored for wound healing potential also (12). *Moringa oleifera Lam* leaves contains numerous beneficial phyto components such as vitamins, isothiocyanates, polyphenol, kaempferol, carotenoids, tannins, rutin, saponins, flavonoids, gallic acid, chlorogenic acid, glucosinolates, quercetin, oxalates, vicenin-2, and phytates, which are responsible for imparting pharmacological/therapeutical properties (12, 13). A high concentration of phenolics and flavonoids enhances wound healing, mostly through antioxidant and antibacterial activity, wound contraction, and an increase in epithelialization. An abundance of flavonoids lowers lipid peroxidation by delaying or reducing the development of cell necrosis, as well as enhancing vascularity and hence wound healing occurs (14).

In this research, an effort was made to fabricate an effective wound dressing by incorporating *Moringa oleifera* extract into a carbopol-based hydrogel. The presence of *Moringa oleifera* extract is proposed to accelerate wound healing due to antibacterial, antioxidant and anti-inflammatory properties, while the carbopol hydrogel will facilitate wound healing process through maintain moist environment and creating a physical barrier that limits microbial growth as well as providing a cooling and soothing effect on the skin. The optimal hydrogel wound dressing material was evaluated in terms of spreadability, swelling index, viscosity, cell biocompatibility, in vivo wound healing.

2. Material and methods

2.1 Procurement of plant materials

Moringa oleifera leaf extract was purchased from Vital Herbs**,** Delhi, India.

2.2 Procurement of chemicals

Carbopol-940 and triethanolamine were sourced from Hi media. Rutin was received as a complimentary sample from Herbo Nutra, India. Gallic acid, aluminium chloride, trichloroacetic acid, Diethyl ether, ethanol and butylated hydroxyl toluene were purchased from Sigma Aldrich Pvt Ltd, Singapore. Cell line (NIH/3T3fibroblast cells) was purchased from NCCS Complex, University of Pune campus, Pune. Dialysis membrane-50 (pore size 2.4 nm and MW 12000 to 14000 Dalton) was obtained from Hi-media. All other chemicals used were of analytical specifications.

2.3 Experimental

2.3.1 Estimation of flavonoid and phenolic concentrations

The main natural components of *Moringa oleifera* are various flavonoid and phenols. The concentration of two phytoconstituents components rutin and gallic acid was determined by the authors in their previous study and these two components are taken as biomarkers for further studies. Total flavonoids present in *Moringa oleifera* was determined employing aluminium chloride colorimetric method and calculated as rutin (mg/g) at wavelength of 420 nm, and the total phenolic content was determined through folin-ciocalteu method and was calculated as gallic acid equivalents (GAE) at wavelength 550 nm through ultraviolet spectrophotometer (Labtronics LT-2910) (4).

2.3.2 Formulation of Moringa oleifera extract hydrogel

The hydrogel was prepared employing direct dispersion method described elsewhere (15). Briefly, propyl paraben was dissolved in distilled water maintained at 80ºC, and in a separate beaker accurately weighed predetermined quantity of carbopol 940 in varying quantities (0.5, 0.75, and 1.0% w/v) was dispersed in distilled water at 40ºC under constant stirring over a period of 1 h. These two solutions were mixed to get a homogenous mixture. In a third beaker accurately weighed *Moringa oleifera* extract was dispersed in ethanol and added to the above mixture under continuous stirring over a period of 1 h. Finally, the pH of all the hydrogel was adjusted to pH 6, using triethanolamine. The obtained formulations were kept overnight to get a homogenous hydrogel. The various formulation of hydrogels is shown in **Table 1**. Additionally, a blank hydrogel MH4 (without extract) was also formulated which was only used for *in vivo* studies for comparison. The prepared hydrogels were further subjected to various *in vitro* and *in vivo* evaluations (16).

Table 1. Composition of *Moringa oleifera* -loaded carbopol hydrogels

Formulation code	**Carbopol-940**	***Moringa oleifera* Extract**
MH1	0.5%	5% w/w
MH2	0.75%	5% w/w
MH3	1.0%	5% w/w
MH4	0.5%	-

2.3.3 Morphological appearance and pH

The morphological appearance of the prepared hydrogels was examined visually with respect to color, homogeneity, consistency, and phase separation (if any). The pH of the hydrogel was measured with a digital pH meter (NIG333, India). The measurements were done in triplicate, and the average pH value was determined.

2.3.4 Viscosity

The mechanical properties of hydrogels with different ratios of carbopol were assessed by Brookfield DV-III Ultra rheometer (Brookfield Viscometers Ltd., UK with spindle number #C95). The cylindrical vessel was filled with the sample, and it was left to equilibrate at an ambient temperature. At varying angular velocity of 25, 50, 75, and 100 rpm, the viscosity was measured in mPas. The measurement was taken thrice, and average values were obtained (17).

2.3.5 Spreadability

This test is used to measure the ability of the gel to extend on the skin surface after application. 0.5 g of formulated herbal gel was placed in the center of a circle with 7.5 cm diameter, drawn on one of two glass slides, and the other slide was placed on top of it. Using a scale, the diameter of the pressed gel was measured after 100.0 g of mass was placed on the upper slide for 60 seconds (18). The procedure was carried out three times for every formulation and means were determined. Then spreadability was calculated using below equation (1)

$S = \frac{W \times L}{T}$……. Equation 1

where W= weight (g) or load placed on slides, L = length (cm) of pressed gel moved on glass slide and T = time in seconds for which weight was tied on slides.

2.3.6 Swelling index

Briefly, 1 g of the pre-dried hydrogel (W_o) formulation was immersed in distilled water for a period of 3h. At two-time intervals (1h and 3h), the soaked gels were taken out, and the excess surface water was removed and was weighed again (W_1). The measurements were conducted thrice, and mean values were calculated. The swelling index is calculated using equation (2).

$$\text{Swelling Index} = \frac{W1-W0}{W0} \times 100 \ldots\ldots\ldots \text{Equation 2}$$

2.3.7 Drug content

100 mg of the prepared hydrogel was mixed with distilled water, and the mixture was allowed to stand for 2 h. The resulting mixture was centrifuged, and the supernatant was collected and filtered. A sample of 100µL from the supernatant was taken and diluted with ethanol and examined using UV Spectrophotometer (Labtronics LT-2910) at 365 and 260 nm wavelengths. Results were calculated as mean ± SD (n=3).

2.3.8 In vitro release study of Moringa oleifera extracts loaded hydrogel

Franz diffusion cell apparatus was used to determine in vitro release of drugs at 37±0.5 °C. The effective diffusional area was 4.90 cm^2 and the volume of the receptor chamber was 50 mL to maintain sink condition which was filled with phosphate buffer (pH 7.4). The pretreated Cellophane® membrane-50 (pore size 2.4 nm, MW 12000 to 14000 Dalton) was assembled between the donors and receptors compartment, and 0.3 g hydrogel samples were placed in the donor chamber, respectively. The receptor compartment was filled with phosphate buffered (pH 7.4) and was stirred at 50rpm using magnetic stirrer. Samples of 1 ml were taken at predefined time over 24 h and analyzed for drug content using a UV spectrophotometer (Labtronics LT-2910) at 360 and 265 nm against a blank, respectively and the sink condition was maintained by replacing the sample by the same volume of fresh medium (19).

2.3.9 Cell viability and biocompatibility

The biocompatibility of prepared hydrogels and naïve *Moringa oleifera* extract was evaluated in NIH/3T3 fibroblast cell line. The NIH/3T3 cell line is a fibroblast cell line, which is the primary cell type in connective tissue, isolated from a mouse embryo and it was mostly used to check the biocompatibility of formulations. The MTT cytotoxicity evaluation is based on the principle of reduction of MTT reagent [3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazoline bromide] to azure formazan in living cells which facilitates the enzymatic activity measurement of the succinate dehydrogenase and subsequently cell vitality (20, 21).

Culture plates seeded with NIH/3T3 cells were incubated with *Moringa oleifera* extract and its hydrogel over a period of 72 h. The MTT assay was carried out as per the method described elsewhere. After the incubation period of 72 h, the supernatant was discarded and DMSO was added. The absorbance fluorescence was recorded at 620 nm wavelength with Biorad Microplate Reader Benchmark (Tokyo, Japan), and the percent cell viability was estimated (22).

On the basis of results recorded through *in vitro* evaluation formulation MH1 (0.5 % w/w carbopol-940) was selected as optimized formulation due to highest release and subjected to in vivo studies.

2.3.10 In vivo wound healing assessment

Selection of Animals

The *in vivo* studies were conducted on the approved animals (*Albino Wistar* rats) as per the approved protocol Registration number (1451/PO/Re/S/11/CCSEA) granted through institutional committee. The Wistar rats weighing in a range of 140-200 g of male/female sex were selected and kept separately in polypropylene cages under pathogen-free environment maintained at temperature 25±2°C and 55±5% RH, and 12 h of alternate light/dark cycle. The animals were fed with a standard commercial pellet diet with free access to drinking water. Subsequent to 1 week of acclimatization, the animals were randomly divided into four groups having 6 animals each group as recorded in Table **2.** The assessment of *in vivo* wound healing potential of hydrogels was done in a wound excision model, and treatments were evaluated for their wound healing potential was marked in terms of wound closure and histopathology. The Group 1 was taken as negative control i.e. receive no treatment, group 2 received plain hydrogel (Carbopol loaded hydrogel without *Moringa oleifera* extract), group 3 was received treatment with marketed formulation i.e. Povidone iodine ointment and group 4 was treated with *Moringa oleifera* extract- loaded carbopol hydrogel (MH1 hydrogel).

Table 2. Experiment groups and treatment.

	Treatments	**Number of animals**
ment Group 1	No treatment	6
II Treatment Group 2	Blank hydrogel (MH4)	6
III Treatment Group 3	Povidone ointment	6
IV Treatment Group 4	*Moringa* hydrogel (MH1)	6

Excision wound healing model

The animals' dorsal surfaces were shaved using electric razor, and on the dorsal shaved area of the rats a spherical, full-thickness excision wound (10 mm) was produced with a biopsy punch (Acumen Schimatle, India) under anaesthesia induce through Diethyl ether. The wounds were created right below the neck to prevent scratching and licking by animals in all the groups. The treatment was done via topical administration of respective hydrogels (once daily) over a period of 14 days and the animals were observed for wound closure i.e. wound healing. The area of wound was measured on the 1, 7, and 14 day. Wound contraction was measured as a % of reduction in wound area and calculated using below mentioned equation 3 (23, 24).

$$\textbf{Percentage Wound closure} = \frac{\text{Wound area on day 1- Wound area on day n}}{\text{Wound area on day 1}} \times 100 \qquad \text{Equation 3}$$

2.3.11 Histopathological studies

The histopathological assessment of the wounded area delivers insight into the wound healing process and its progression. On days 7^{th} and 14^{th}, a few animals from each group were sacrificed, and the skin wound region of the animals was excised and histologically examined. The removed tissues were immersed in formalin and processed using an automatic tissue processor (Leica TP 1020, Germany). Tissue was preserved in paraffin wax and sliced to a thickness of 3 μm with a microtome (Leica RM2125 RTS; Germany). The slices were stained with haemotoxylin and eosin and examined under a microscope (Model Eclipse Ci-L, Nikon Corporation, Tokyo, Japan) at 20X and 40X magnifications. The tissue samples were analyzed for scab/thrombus, inflammation, epithelialization, and granulation tissue.

2.3.12 Skin dermal irritation test

The dermal irritation test was performed on animals of each group to detect skin reaction following one time application of the carbopol-hydrogel. Following 24 h of shaving, rats were administered topically with 0.5 g plain carbopol hydrogel, of Moringa extract loaded hydrogel (MH1), and marketed formulation. Animals of group I were left untreated (normal control). Dermal irritation was observed for skin reactions like Moderate erythema (dark pink), moderate to severe erythema (light red) and severe erythema (extreme redness) after 1, 4, 24, 48, and 72 h, respectively (25, 26).

3. Results and Discussion

3.1 Estimation of flavonoid and phenolic concentrations

In the previous experiment conducted by authors the total flavonoids present in Moringa oleifera was determined employing aluminium chloride colorimetric method and calculated as rutin (mg/g) and the total phenolic content was determined through folin-ciocalteu method and was calculated as gallic acid equivalents (GAE). The total flavonoid content (TFC) was quantified as mg rutin equivalent per gram extract dry weight, and the values were found to be 79 mg/g of Moringa oleifera extract. Further, the total phenolic content (TPC) was quantified as mg gallic acid equivalent per gram extract dry weight, and the values were found to be to be 84.6 mg/g of Moringa oleifera extract. Based on these results the biomarkers gallic acid and rutin were selected for in. vitro studies.

3.2 Formulation of Moringa oleifera extract hydrogel

The hydrogel formulation MH1, MH2 and MH3 were successfully prepared utilizing 0.5%, 0.75% and 1% of carbopol-940 to obtain the hydrogels of variable viscosity and strength. The prepared hydrogel established that the fabrication process and the optimized conditions employed to develop hydrogels was facile and suitable. All the three-hydrogels demonstrated analogous organoleptic attributes such as transparent texture, clarity, no signs of phase separation and exists as 3D networks. The hydrogels exhibit white to glossy off-white color and showed no signs of grittiness or lumps visually subsequent to formulation over a period of 24 h.

3.3 Morphological appearance and pH

The developed hydrogel formulation MH1, MH2 and MH3 were glossy off-white in color, homogenous, free of lumps or grittiness, and phase separation. The carbopol-hydrogels were found to be uniform, with no evidence of separation of phases. All the three formulation exhibits pH values ranging from 6.5 to 7.0, which are considered acceptable and safe owing to extreme similarity to the typical pH of human skin. Topical hydrogels having a strongly acidic or basic pH might affect the skin's structure, producing irritation when topically applied on skin (27). The values of physicochemical evaluation is mentioned in Table 3.

3.4 Viscosity of hydrogels

Viscosity and homogeneity are the two most important characteristics of a gelling solution. Viscosity of an ideal hydrogel should be of consistency that can be easily applied over the skin surface. The wound dressing should hold an optimal viscosity to facilitate comfortable application on the skin surface. Viscosity of carbopol-hydrogels decreased with increasing shear rate but increased with increasing carbopol-940 concentration at various rpm. This is possibly due to the development of dense polymer network and a substantial increase in the hydrodynamic interactions between particles at greater levels of carbopol polymer, which raises the viscosity (28, 29).

3.5 Spreadability

Spreadability is an attribute of semisolid formulations and refers to the amount of work (mJ) necessary to spread the formulation over the skin surface. A hydrogel possessing suitable spreadability is considered reliable for easy application determines and evenness and swiftness of application on the applied skin surface. The spreadability of carbopol-hydrogels decreased with increasing carbopol concentrations. Formulation MH1, MH2 and MH3 exhibited a spreadability value of 57±0.1cm, 55±0.1cm and 49±0.03cm respectively. It was observed that as the concentration of carbopol polymer increases, it also increases the cohesiveness of hydrogels, and the spreading ability of a topical hydrogel is inversely related to its cohesiveness since strong cohesive forces limit spreadability. We selected MH1 hydrogel for its soft consistency and spreadability. It was depicted that a higher concentration of polymer causes an increased viscous and subsequently less spreadability is attained. Furthermore, hydrogels with low concentration of carbopol are necessary to healing of wound and prevent tissue loss at the site of wound during the removal of the hydrogel dressing (28).

3.6 Swelling index

The ideal wound dressing must hold an attribute of substantial swelling i.e. water holding capacity so as to absorb/accommodate the wound exudates during the process of wound healing. The hydrogels have good swelling index, and they can retain significantly high amount of fluid preventing development of dry bed at the wound vicinity, that facilitates healing process, that is why they are preferred wound dressing of choice. The swelling percentage of found to be significantly high in all the three hydrogels with a value of 98.14±0.1%, 91.67±0.01%, and 78.26±0.03% for hydrogel formulations MH1, MH2, and MH3 respectively (Fig. 1). The hydrogels attained equilibrium within 2 h of experimental conditions and no further increase in water uptake was observed after 2 h. The basic mechanism behind swelling of hydrogels or uptake of water can be attributed to the fact that water molecules initially absorbed into the hydrogel via hydrogen bonding with the

hydrophilic groups found in the carbopol polymers. Finally, additional water molecules absorbed into the carbopol-hydrogel network, causing it to swell. Further, the viscosity of a neutralized 0.5% w/v Carbopol 940 aqueous dispersion ranges from 40,000 to 60,000 cps. Carbopol 940 in an aqueous solution (neutral pH) act as an anionic polymer, as it loses protons from its side chains and acquiring a negative charge. This attribute of carbopol 940 is credited to impart substantial water retention ability enabling good swelling upto several folds of its original volume (30).This means that the hydrogels can absorb water and wound exudates both. Hydrogels provide a moist environment for the wound bed, promoting wound healing. Also, they help in rehydrating dead tissues, heal debridement and the cooling of the wound surface (31).

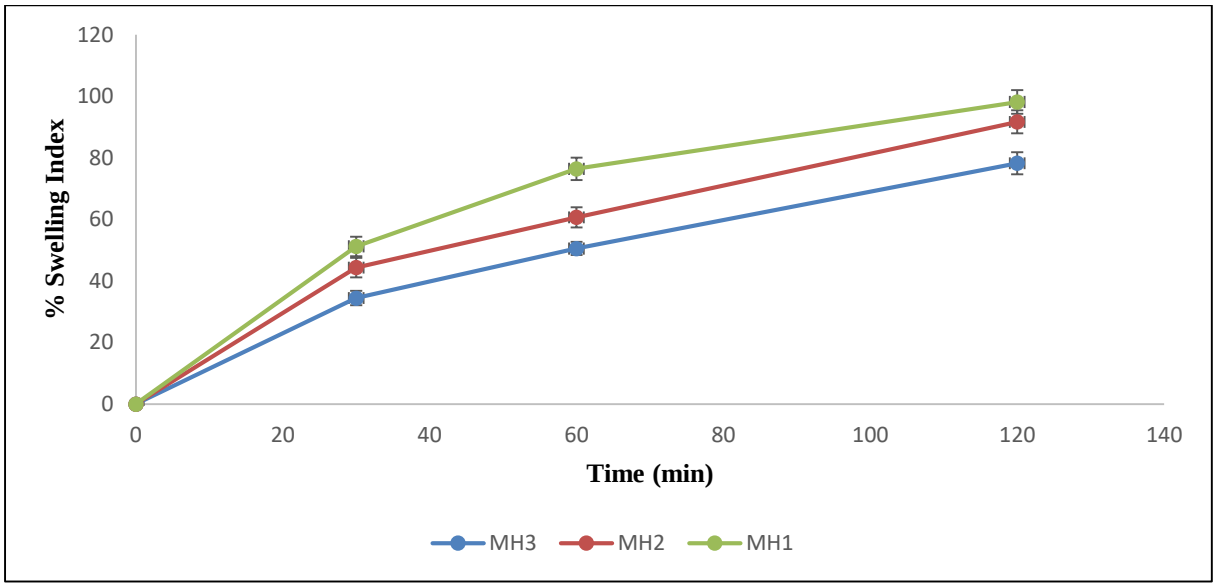

Fig. 1. Percentage swelling index versus time profile of developed hydrogels.

Table 3. Physiochemical evaluation of hydrogels.

Formulation code	pH*	Spreadability* (cm)	Viscosity *at 25 rpm shear rate (m.Pa.S)	Viscosity* at 100 rpm shear rate (m.Pa.S)	Swelling index* (%)
MH1	6.75±0.05	59 ± 0.1	2303±0.1	940±0.1	98.14±0.1
MH2	6.7±0.2	55 ± 0.3	3064±0.2	1037±0.1	91.67±0.1
MH3	6.7±0.1	4.9 ± 0.03	4480±0.5	1281±0.2	78.26±0.03
MH4	6.5± 0.03	5.8 ±0.5	2843±0.43	1687±0.1	88.23±0.3

*Data are given in triplicates (n=3)

3.7 Drug content uniformity

The drug content of MH1, MH2, and MH3 hydrogels was found to be 99.99±1.3, 98.99±2.4 and 99.98±2.0 at 260 nm (gallic acid) and 99.99±1.3, 99.99±0.24 and 99.99±2.1 at 365nm (rutin), respectively. Based on the result, it was concluded that each of the carbopol loaded hydrogels had approximately same amount of drug incorporated, indicating that the gels are capable of loading a significant amount of medication respectively.

3.8 In vitro release study

Diffusion studies were used to conduct *in vitro* release of gallic acid and rutin from *Moringa oleifera* extracts-loaded Hydrogel (MH1, MH2, and MH3) over a period of 24 h. The samples were examined with UV spectrophotometer (Labtronics-LT 2910) at 260nm and 365nm for gallic acid and rutin respectively. *In vitro* drug release study revealed that all three herbal hydrogels released their contents throughout 24 h period. The hydrogel exhibited a percent release of 87.01±0.74%, 60.69±0.82% and 51.08±0.64% from MH1, MH2 and MH3 respectively for rutin (when measured UV spectrophotometrically at 365nm). Further, the percent release for gallic acid was found to be 88.36±0.5%, 55.72 ±0.4% and 49.29±0.2% respectively from MH1, MH2 and MH3 when measured UV spectrophotometrically at 260 nm. The release profile suggested that the fractions are released from the polymeric matrix by a gradual diffusion was shown in Fig. 2. Over a period of 24 h, the hydrogel MH1 exhibited highest percentage drug release of 88.36±0.5% while MH3 exhibited lowest percent release of 49.29±0.2%. This could be attributed to the presence of various concentration of carbopol in respective formulation which affects the release of extract from carbopol loaded hydrogel formulation. The development of a tight, rigid gel causes a reduction in release of drug from carbopol polymer. As the drug mixed with the carbopol-hydrogel, the viscosity of the matrix increases resulting in the entrapment of drug so the percentage of drug release declines as the concentration of carbopol increases (32, 33). Furthermore, the in vitro release data was evaluated for various kinetic model where highest R^2 values of 0.9234, 0.9630 and 0.9393 for MH1, MH2, and MH3 respectively, indicating that release followed Higuchi diffusion kinetics, and the release followed diffusion-controlled mechanism. Also, the n values for Korsmeyer–Peppas model were found to be 0.991 to 1.287 and 0.925 to 1.14 for formulations MH1, MH2, and MH3 respectively, indicating that the hydrogel's diffusion was non-fickian, *i.e.* the release is dependent on the swelling and erosion of the polymer matrix (34).

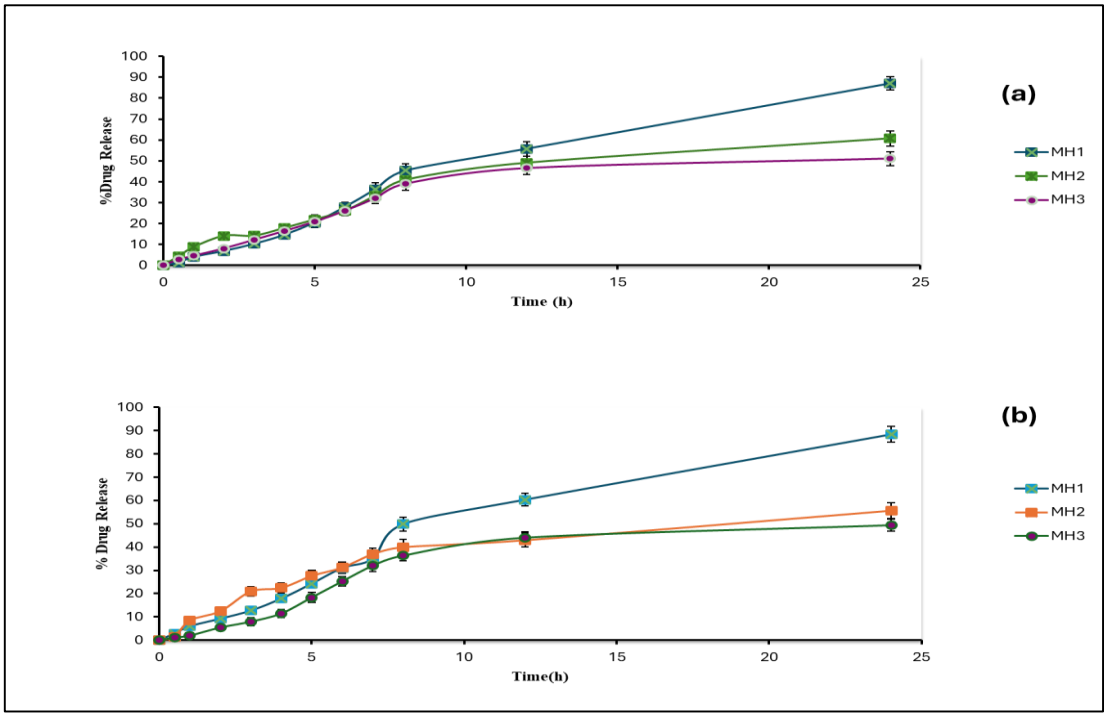

Fig. 2. *In vitro* percentage cumulative drug release profiles of MH1, MH2 and MH3 hydrogels at (a) 360 nm and (b) 265 nm.

3.9 Cell viability and biocompatibility of hydrogels

The NIH/3T3 cell lines were treated with hydrogels and pure *Moringa oleifera* extract over a period of 72 h (Fig. 3 a&b). The results of MTT assay demonstrated good cell proliferation and growth with good viability. This indicated that the hydrogels and the extract is biocompatible. The enhanced cell proliferation and sustained viability could be credited to antioxidant and nutritive content of *Moringa oleifera* which could have facilitated cell growth (22).

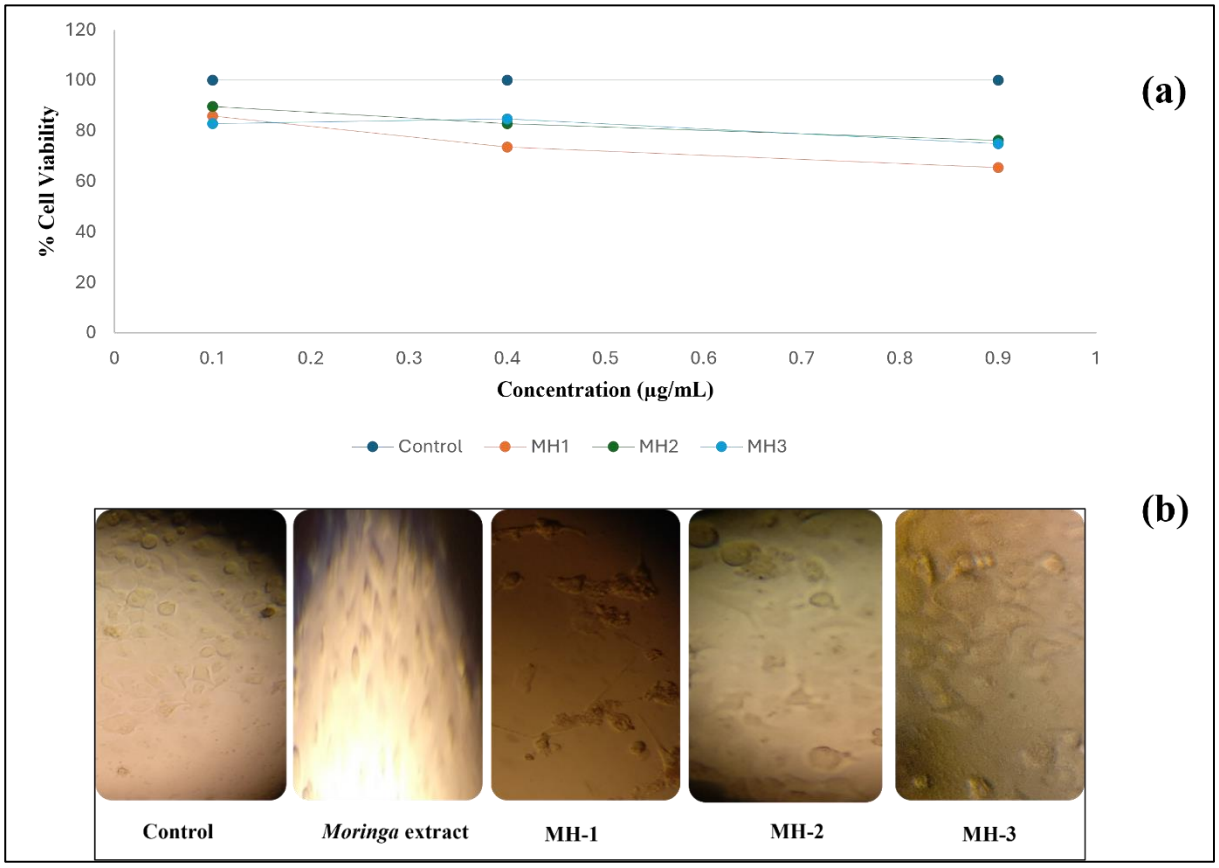

Fig. 3. (a) Cell viability of NIH/3T3 cells after treatment with different formulations **(b)** Microscopic images of NIH/3T3 cell lines treated with *Moringa oleifera* extract and hydrogels (after MTT Assay).

Based on the *in vitro* results, formulation MH1 was chosen as the optimal batch among all hydrogel formulations because it had good pH, viscosity, spreadability, morphology, swelling index, and highest *in vitro* drug release. MH1 hydrogel was then subjected to in vivo studies, wound healing studies, dermal skin irritation study, and histopathological studies.

3.10 In vivo studies

The in vivo studies were carried out for wound closure and histopathology subsequent to completion of experiment study period.

Wound-healing activity

The wound-healing process of hydrogels was evaluated utilizing an excision wound model. Fig. 4. clearly assessed between group 4 (treated with MH1 hydrogel) and group 1 (untreated).

Every wound area was examined for inflammation, dryness, and infection at 1, 7, and 14 days. On the first day, an inflammation and redness was observed at the wound area, which continued on day 3rd. On 7th day, group 4 developed scabs, and reduction in wound size was shown to be faster than in groups 1 and 2. On the 14th day, there was no sign of inflammation or infection in group 4 (MH1 treated) and group 3 (Marketed formulation Betadine®). The extract-loaded hydrogel (MH1) was able to sustain visible dryness in the treated wounds while preventing pathological fluid discharge. Fig. 5. depicts the rate of wound closure in each group over time. On day 1, there was no significant difference in percentage wound closure across the various groups. On 7th day, wounds in group 4 and group 3 have a significantly higher ($p<0.05$) percentage of closure (20±0.5%) than groups 1 (5±0.4%) and 2 (10± 0.5%). On day 14, group 4 had a higher percentage of wound closure (50±0.9%) compared to groups 1((10±0.2%) and 2 (20±0.6%). Group 3 exhibited highest wound closure rate of 60±0.7% on day 14. A comparison between percent wound closure of group 3 and group 4 showed insignificant difference, suggesting that group 4 has similar wound healing potential as of marketed formulation group 3. The significant wound healing potential could be due to high level of phytoconstituents such as flavonoids, bioflavonoids, tannins, saponins, triterpenes and steroids *etc.* that are present in *Moringa oleifera* extract. A high concentration of phenolics and flavonoids promotes wound healing, mostly through antioxidant and antibacterial properties, reduces wound size, and improves epithelial cell production. Flavonoids decrease the degradation of lipids by preventing or slowing the onset of cell necrosis and increasing vascularity, which facilitates wound healing. The results of in vivo study also demonstrated that combining carbopol polymer and *Moringa oleifera* extract speeds up wound contraction and epithelialization. The antibacterial qualities of carbopol, along with the ability of *Moringa oleifera* extract to induce collagen formation and epithelialization of open wounds, make it a feasible dressing for accelerated wound healing (35).

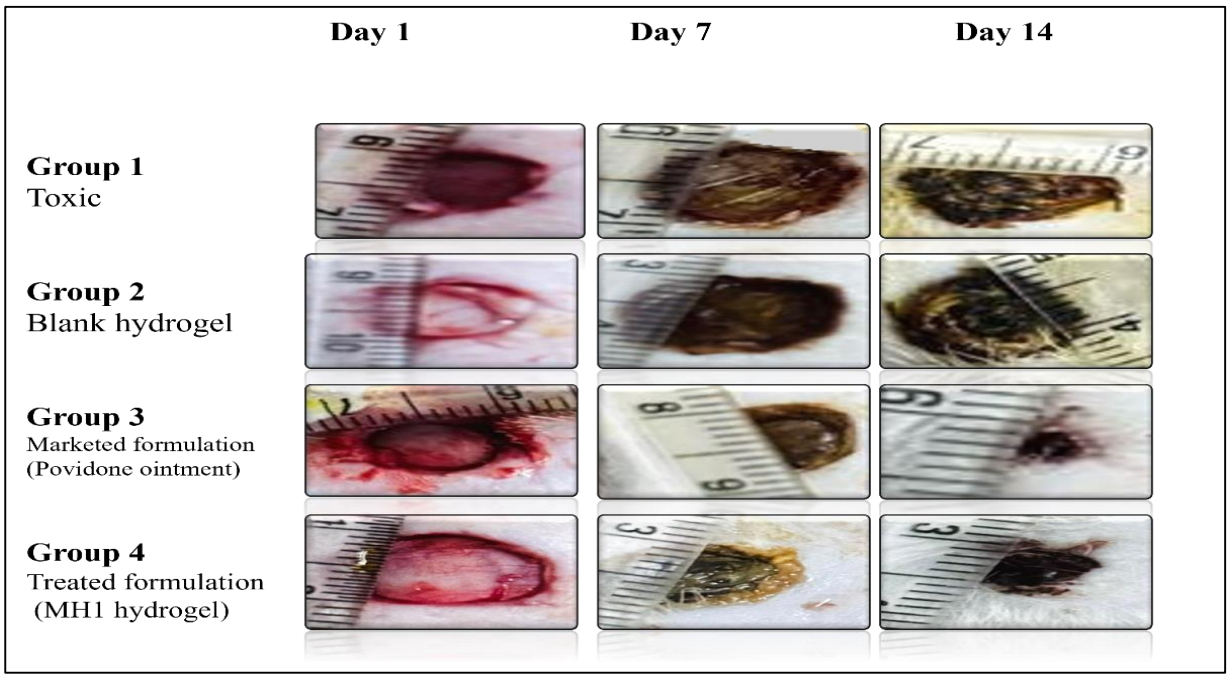

Fig. 4. Excision wound area (mm) of rats on day 1, 7 & 14 treated with different formulations.

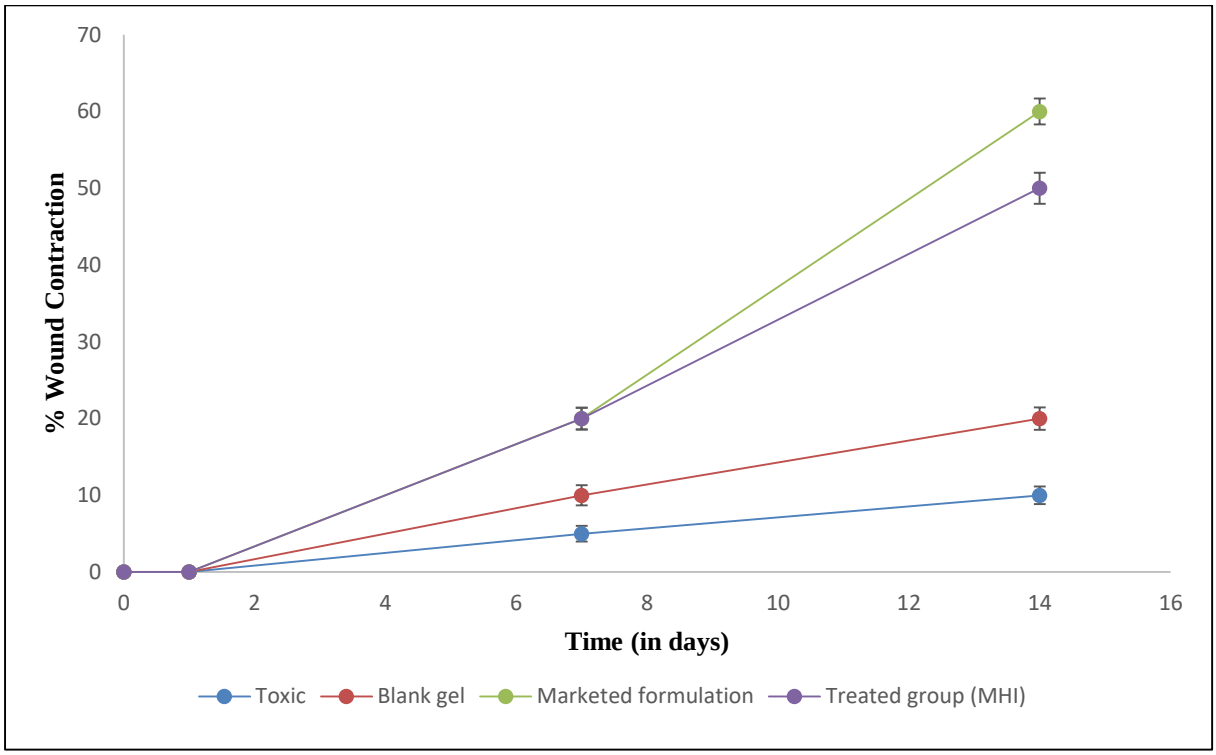

Fig. 5. Percent wound reduction in different formulations (on 1st, 7th& 14th day).

3.11 Histopathological studies

Fig. 6. depicts the histological modifications in wounds from groups 1 to 4 on days 7 and 14, respectively. On day 7, scabs formed on the epidermal layer in groups 1 and 2, while in the dermal and subcutaneous regions of the skin, granulation tissues replaced them. While group 4 displayed a considerable growth of epithelial cells & scabs on the epidermal layer fell off earlier than groups 1 and 2. Dermal and subcutaneous layers of the skin have an abundance of newly formed blood capillaries, fibroblast cells and connective tissue contributing to the faster restoration of injured skin. On 14th day, the low degree of epithelial growth and the high degree of keratinisation and development of blood vessels was found in group 3 and group 4. This suggested the rapid healing of wound. The presence of significant phenols, flavonoids, and other compounds in *Moringa oleifera* extract accelerates wound healing, as seen by the development of fibroblasts in group 4 wounds (36).This change was not found in groups 1 & 2, indicating a slower healing process. All of these results indicated that carbopol and *Moringa oleifera* extract had complementary effects on the wound healing process. Beneficial features of carbopol for designing potential wound dressing materials include its antimicrobial, hydrating action, and its porous structure increased air permeability (37).

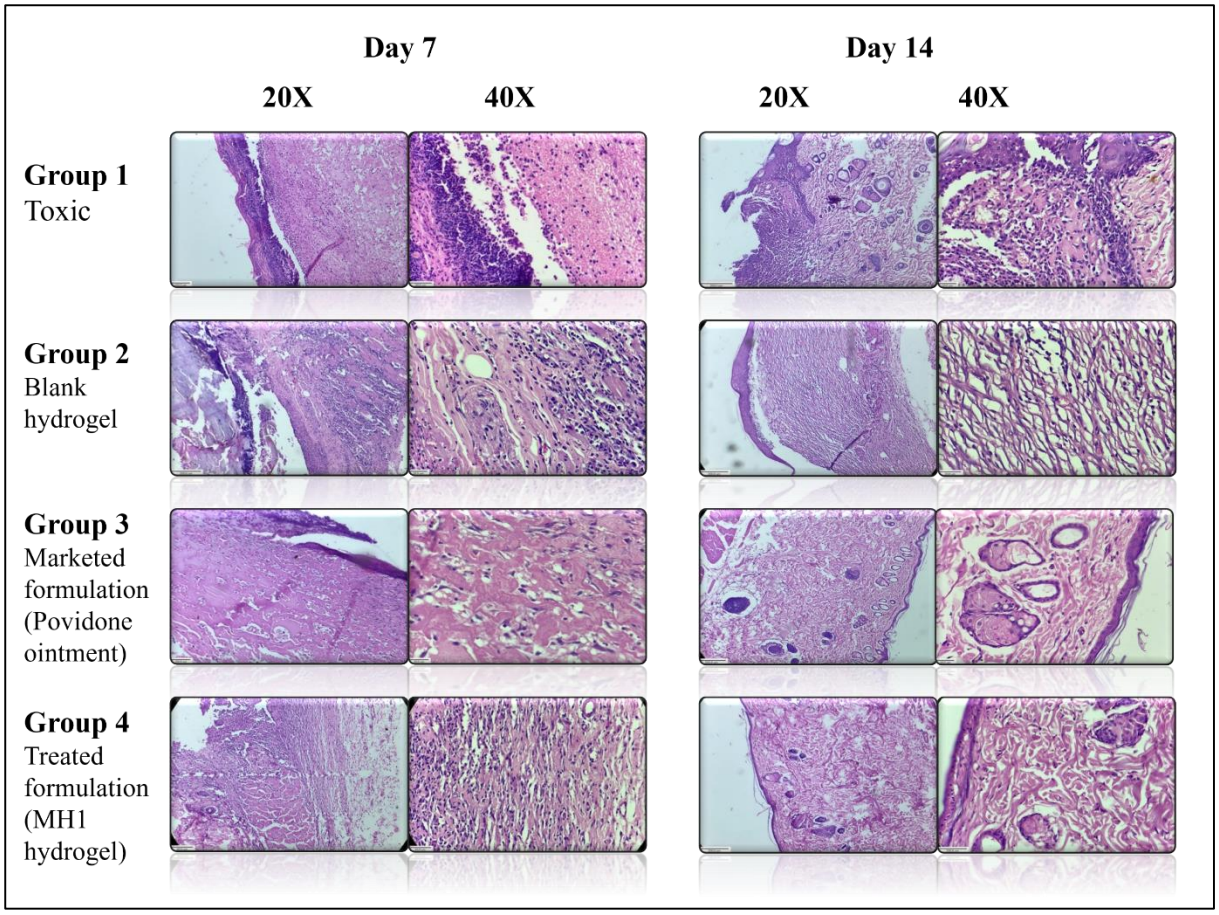

Fig. 6. Histopathological images show healing process in the different groups (7th & 14th day) at 20x and 40x.

3.12 Skin irritation study

Skin irritation investigations demonstrated no signs of erythema or edema on the skin for up to 72 h after application of blank hydrogel and *Moringa oleifera* hydrogel, with a Draize-scale score of 0. The skin irritation investigation found that the formulations are non-irritating and can be used to dress wounds. This could be attributed to the biocompatible and natural herbal components utilized in the composition, such as *Moringa oleifera* extract and carbopol-940. Blank and *Moringa oleifera*-loaded hydrogels were non-irritant for 72 h (mean score 0). The study was a successful attempt to improve the skin tolerability of a formulation, hence increasing patient compliance. The results of hydrogel are shown in supplementary data **(Supplementary Table A1).**

4. Conclusion

The hydrogels loaded with *Moringa oleifera* extract were successfully fabricated and the hydrogels showed a substantial wound healing potential. The improved wound healinga as well comparable wound healing to the marketed formulation (betadine®) has been credited to the antioxidant and anti-inflammatory propertied of *Moringa oleifera* extract which enhanced the properties of hydrogel to accelerate wound healing process. The hydrogel showed good biocompatibility, antimicrobial and antioxidant attributes, that are contributing factors for effective and accelerated tissue regeneration during wound healing process.

Additionally, the porous architecture of hydrogel's offer good absorption of wound extrudates while maintaining the moist environment at the wound bed, concomitantly giving modified release profile that facilitated creation of suitable environment for substantial cell proliferation and migration. The findings of the studies suggested that the developed hydrogel is a promising wound dressing, however further studies are required to establish these hopeful *in vitro* and *in vivo* results accompanying in for its clinical usage.

Data availability

Not applicable.

CRediT authorship contribution statement

Shaheen Ansari: Methodology, Investigation, Data curation, Writing – original draft. Pragya Yadav: Writing – review & editing, Validation. Sikha Srivastava: Methodology, Writing, Data curation. Poonam Parashar: Conceptualization, Writing – review & editing, Validation, Supervision, Project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

1. Qin W, Wu Y, Liu J, Yuan X, Gao J. A comprehensive review of the application of nanoparticles in diabetic wound healing: therapeutic potential and future perspectives. International Journal of Nanomedicine. 2022;17:6007.
2. Vijayakumar V, Samal SK, Mohanty S, Nayak SK. Recent advancements in biopolymer and metal nanoparticle-based materials in diabetic wound healing management. International Journal of Biological Macromolecules. 2019;122:137-48.
3. Singh MA, Wahi A, Teotia AK, Thota P, Varshney R, Gupta M. Novel Phytopharmaceuticals Used for Wound Healing. Novel Phytopharmaceutical for Management of Disorders: CRC Press; 2024. p. 250-61.
4. Singh RG, Negi PS, Radha C. Phenolic composition, antioxidant and antimicrobial activities of free and bound phenolic extracts of Moringa oleifera seed flour. Journal of functional foods. 2013;5(4):1883-91.
5. Qadir A, Jahan S, Aqil M, Warsi MH, Alhakamy NA, Alfaleh MA, et al. Phytochemical-based nano-pharmacotherapeutics for management of burn wound healing. Gels. 2021;7(4):209.
6. Yadav P, Gupta M, Singh SP, Parashar P. Biocompatible Electrospun Hydrogel Fibers for Advanced Wound Healing Therapies. Current Pharmaceutical Design. 2024.

7. Zhang X, Qin M, Xu M, Miao F, Merzougui C, Zhang X, et al. The fabrication of antibacterial hydrogels for wound healing. European Polymer Journal. 2021;146:110268.
8. Chen J, Zhao X, Qiao L, Huang Y, Yang Y, Chu D, et al. Multifunctional On-Demand Removability Hydrogel Dressing Based on in Situ Formed AgNPs, Silk Microfibers and Hydrazide Hyaluronic Acid for Burn Wound Healing. Advanced Healthcare Materials. 2024;13(8):2303157.
9. Silva JP, Dhall S, Garcia M, Chan A, Costa C, Gama M, et al. Improved burn wound healing by the antimicrobial peptide LLKKK18 released from conjugates with dextrin embedded in a carbopol gel. Acta Biomaterialia. 2015;26:249-62.
10. Al-Bazzaz FY, Ismail ST. Topical HPMC/Carbopol 934 Gel for Wound Healing: Formulation and in-Vivo Evaluation. Pharmakeftiki. 2024;36(2).
11. Padayachee B, Baijnath H. An updated comprehensive review of the medicinal, phytochemical and pharmacological properties of Moringa oleifera. South African Journal of Botany. 2020;129:304-16.
12. Mohammad Shafie N, Raja Shahriman Shah RNI, Krishnan P, Abdul Haleem N, Tan TYC. Scoping Review: Evaluation of Moringa oleifera (Lam.) for Potential Wound Healing in In Vivo Studies. Molecules. 2022;27(17):5541.
13. Akanji O, Sonibare M. Evaluation of wound healing activity of Erythrophleum suaveolens (Guill. & Perr.) Brenan and Moringa oleifera lam. on infected albino rats. 2015.
14. Paswan SK, Srivastava S, Rao CV. Wound healing activity of ethanolic extract of Selaginella bryopteris on rats. Pharmacognosy Journal. 2020;12(2).
15. Kaur D, Raina A, Singh N. Formulation and evaluation of carbopol 940 based glibenclamide transdermal gel. Int J Pharm Pharm Sci. 2014;6(8):434-40.
16. Verma J, Kanoujia J, Parashar P, Tripathi CB, Saraf SA. Wound healing applications of sericin/chitosan-capped silver nanoparticles incorporated hydrogel. Drug Delivery and Translational Research. 2017;7(1):77-88.
17. Lin YY, Lu SH, Gao R, Kuo CH, Chung WH, Lien WC, et al. A Novel Biocompatible Herbal Extract-Loaded Hydrogel for Acne Treatment and Repair. Oxid Med Cell Longev. 2021;2021:5598291.
18. Saher T, Manzoor R, Abbas K, Mudassir J, Wazir MA, Ali E, et al. Analgesic and Anti-Inflammatory Properties of Two Hydrogel Formulations Comprising Polyherbal Extract. J Pain Res. 2022;15:1203-19.
19. Wang Z, Hu Y, Xue Y, Zhu Z, Wu Y, Zeng Q, et al. Mechanism insight on licorice flavonoids release from Carbopol hydrogels: Role of “release steric hindrance” and drug solubility in the release medium. European Journal of Pharmaceutical Sciences. 2022;179:106307.
20. Tyliszczak B, Drabczyk A, Kudłacik-Kramarczyk S, Bialik-Wąs K, Sobczak-Kupiec A. In vitro cytotoxicity of hydrogels based on chitosan and modified with gold nanoparticles. Journal of Polymer Research. 2017;24(10):153.
21. Nwe N, Furuike T, Tamura H. Selection of a biopolymer based on attachment, morphology and proliferation of fibroblast NIH/3T3 cells for the development of a biodegradable tissue regeneration template: Alginate, bacterial cellulose and gelatin. Process Biochemistry. 2010;45(4):457-66.

22. Ningrum DR, Hanif W, Mardhian DF, Asri LATW. In Vitro Biocompatibility of Hydrogel Polyvinyl Alcohol/Moringa oleifera Leaf Extract/Graphene Oxide for Wound Dressing. Polymers. 2023;15(2):468.
23. Anand S, Pandey P, Begum MY, Chidambaram K, Arya DK, Gupta RK, et al. Electrospun Biomimetic Multifunctional Nanofibers Loaded with Ferulic Acid for Enhanced Antimicrobial and Wound-Healing Activities in STZ-Induced Diabetic Rats. Pharmaceuticals. 2022;15(3):302.
24. Pandey A, Yadav P, Mishra S, Srivastava S, Parashar P. Biomimetic resveratrol-loaded sericin hydrogel: Augmented antimicrobial and wound healing potential in animal model. Journal of Drug Delivery Science and Technology. 2024;101:106145.
25. Dwivedi D, Pandey S, Asif S, Awasthi V, Kaur G, Nimisha N. Investigating the potential of Quercetin enthused nano lipoidal system for the management of dermatitis. Research Journal of Pharmacy and Technology. 2021.
26. Mohamad N, Mohd Amin MCI, Pandey M, Ahmad N, Rajab NF. Bacterial cellulose/acrylic acid hydrogel synthesized via electron beam irradiation: accelerated burn wound healing in an animal model. Carbohydr Polym. 2014;114:312-20.
27. Huang X, Bao X, Liu Y, Wang Z, Hu Q. Catechol-Functional Chitosan/Silver Nanoparticle Composite as a Highly Effective Antibacterial Agent with Species-Specific Mechanisms. Scientific Reports. 2017;7(1):1860.
28. Al haushey L. Study of different factors affecting spreadability and release of ibuprofen from carbopol gels using screening design methodology. ACTA Pharmaceutica Sciencia. 2024.
29. Jain S, Patel N, Madan P, Lin S. Formulation and rheological evaluation of ethosome-loaded carbopol hydrogel for transdermal application. Drug Dev Ind Pharm. 2016;42(8):1315-24.
30. Ismail SH, Hamdy A, Ismail TA, Mahboub HH, Mahmoud WH, Daoush WM. Synthesis and Characterization of Antibacterial Carbopol/ZnO Hybrid Nanoparticles Gel. Crystals. 2021;11(9):1092.
31. Khan BA, Khan A, Khan MK, Braga VA. Preparation and properties of High sheared Poly(Vinyl Alcohol)/Chitosan blended Hydrogels films with Lawsonia inermis extract as wound dressing. Journal of Drug Delivery Science and Technology. 2021;61:102227.
32. Park S-H, Chun M-K, Choi H-K. Preparation of an extended-release matrix tablet using chitosan/Carbopol interpolymer complex. International journal of pharmaceutics. 2008;347(1-2):39-44.
33. Jain S, Patel N, Madan P, Lin S. Formulation and rheological evaluation of ethosome-loaded carbopol hydrogel for transdermal application. Drug Development and Industrial Pharmacy. 2016;42(8):1315-24.
34. Yadav P, Rastogi V, Porwal M, Upadhyay P, Verma A. 3(2) Designing and optimization of aceclofenac transdermal films using response surface methodology: investigating the effect of hydrophilic and hydrophobic matrix on ex vivo and in vivo permeation characteristics. Journal of Pharmaceutical Investigation. 2017;47(6):541-58.

35. Vergara-Jimenez M, Almatrafi MM, Fernandez ML. Bioactive Components in Moringa Oleifera Leaves Protect against Chronic Disease. Antioxidants (Basel). 2017;6(4).
36. Muhammad AA, Arulselvan P, Cheah PS, Abas F, Fakurazi S. Evaluation of wound healing properties of bioactive aqueous fraction from Moringa oleifera Lam on experimentally induced diabetic animal model. Drug Des Devel Ther. 2016;10:1715-30.
37. Das PE, Majdalawieh AF, Abu-Yousef IA, Narasimhan S, Poltronieri P. Use of A Hydroalcoholic Extract of Moringa oleifera Leaves for the Green Synthesis of Bismuth Nanoparticles and Evaluation of Their Anti-Microbial and Antioxidant Activities. Materials. 2020;13(4):876.

Supplementary Table A1 . The irritation scores of hydrogels to the rat skin

Formulation		Time (h)			
	1	4	24	48	72
Blank hydrogel	0	0	0	0	0
MH1	0	0	0	0	0
Control	0	0	0	0	0
Mean	0	0	0	0	0