

Formulation, Physical Characterization and Wound Healing Activity Evaluation of Carboxymethyl Chitosan-Curcumin Carbomer-Based Hydrogel

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ABSTRACT

The aim of this study was to determine the effect of different concentration of carboxymethyl chitosan and curcumin on physical characteristics and wound healing activity of carboxymethyl chitosan-curcumin hydrogel. Hydrogels were prepared using carbomer as a gelling agent and TEA was added to neutralize the carbomer hydrogel in order to make it swell. The obtained hydrogels were evaluated for its physical characterization such as organoleptic, viscosity, spreadability, drying time, pH, and wound healing activity on the burned wound in Wistar rats. The results showed that higher concentration of carboxymethyl chitosan significantly reduces viscosity and pH while its spreadability and drying time are significantly increased. Curcumin only affected two out of five physical characteristics: organoleptic and pH. Higher concentration of curcumin reduces its pH but statistical analysis showed no interaction between carboxymethyl chitosan and curcumin. The wound healing activity in Wistar rats with 2nd degree burn wound model indicates that carboxymethyl chitosan-curcumin hydrogel can significantly improve wound healing activity in rats compared to control group. Higher concentration of carboxymethyl chitosan can affect the physical characteristics of carboxymethyl chitosan-curcumin hydrogel i.e. a decrease in viscosity and pH, as well as an increase in spreadability and drying time. On the other hand, higher concentration of curcumin only affected the pH of the preparation. In the wound healing activity test, macroscopic observation showed that the combination of carboxymethyl chitosan-curcumin significantly increased the wound healing activity of 2nd degree burn in Wistar rats. However, the use of this hydrogel preparation statistically did not give significant improvement in wound healing process when compared with G1, G2, G3, G4, and positive control. Based on histopathology test results, it can be concluded that after 14 days of treatment the value of collagen deposition and PMN between groups 1, 2, 3, 4, and positive control and intergroup replication (1, 2, 3, 4, and positive controls) are in uniformity which indicates that the wound has undergone healing process. In addition, the four groups possess better results than negative controls which didn't receive any treatment.

Keywords: Hydrogel, Carboxymethyl chitosan, Curcumin, Wound healing.

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INTRODUCTION

Wound healing is a dynamic process consisting of four steps, namely hemostasis, inflammation, proliferation, and tissue remodeling phase.¹ Each stage of wound healing process must go on precisely. Prolongation or extension that occurs in the process will delay healing process or can inevitably become chronic, incurable wounds.² Wound healing is a result of cytokine, growth factor, and extracellular matrix interaction. Treatment of wounds needs to be done for both severe and minor injuries. Part of the wound treatment process is called wound dressing.³

The dressing can be used to promote and accelerate re-epithelization, collagen synthesis, and also to support of angiogenesis by making hypoxic environmental condition at the base of the wound and reduce pH level at the wound site, therefore decrease the possibility of wound to get infected by bacteria.³ Wound dressing can be classified into two categories: traditional and modern wound dressing, where the traditional wound dressing works as primary/secondary dressing to prevent wound from getting contaminated. When excessive wound drainage occurs, this type of dressing can be more humid and tends to be stickier at the wound site, so it may

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cause pain when it is removed. The modern wound dressing is developed to make the wounds stay hydrated and does not need removal after use.

A hydrogel is developed in modern wound dressing that has a high content of water (70–90%) which provides a humid environmental condition at the wound site and give cool and comfort effect when it is applied. Humid condition promotes wound healing process because cell regeneration mostly occurs in a humid condition. Bioactive addition in the dressing can increase the effect and accelerate the wound healing process.³

Carboxymethyl chitosan is a water-soluble chitin derivative and functional biomaterial which possesses many favorable biological properties such as biocompatibility, biodegradability, and bioactivity.⁴ Studies have suggested that carboxymethyl chitosan can effectively accelerate wound healing and reduce scar formation. Carboxymethyl chitosan can significantly accelerate wound healing process of 2nd-degree burn.⁵ Carboxymethyl chitosan is a bacteriostatic agent which is more effective compared to chitosan, and it increases fibroblast proliferation of skin and stimulates extracellular lysozyme activity on the skin.⁶ To upgrade the effectivity of healing process, carboxymethyl chitosan can be combined with another pharmacology material such as growth factor or antibacterial like curcumin.

Curcumin is a compound that has an effect on the wound healing process.⁷ The potential effect of curcumin in the wound healing process is associated with its anti-inflammatory,⁸ antibacterial, anti-infection,⁹ and anti-oxidant properties.¹⁰ Research conducted by Emiroglu *et al.* (2017) demonstrated that 0.01% curcumin for topical dosage form significantly reduces edema, cell hyperplasia, and leukocyte infiltration in the wound healing process.¹¹ Hydrogel physical characterization is important to ensure effectiveness and acceptability. The previous research showed that the formulation of hydrogel preparations with carboxymethyl chitosan concentration of 0.5%, 1%, and 0.05% curcumin gave steady results in terms of includes pH, viscosity, spreadability, and drying time after 30 days.¹²

The aim of this study was to determine the effect of carboxymethyl chitosan-curcumin concentration on physical characteristics (pH, viscosity, spreadability, drying time) and wound healing activity. The histopathological test was done to evaluate the skin physiology from the healing process.

MATERIALS AND METHOD

Material

Carboxymethyl chitosan (81.9% degree of substitution, 96.5% degree of deacetylation, viscosity 1% 22 mPa.s, China Eastar Group Co., Ltd.); Curcumin 95% (RD Health Ingredients Co., Ltd, China); propylparaben, carbomer 940, propylene glycol, 96% ethanol pro analysis (Merck), Triethanolamine (CV. Tristar Chemical); Ketamine® (PT Guardian Pharmatama); Xylazine®; Burnazin® (PT Darya-Varia).

Experimental animal

A total of 30 healthy Wistar mice (*Rattus norvegicus*) aged 2–3 months, weighed 150–250 g were used in this research.

Ethical clearance

This research was approved by the research ethics committee of the Faculty of Veterinary Medicine, Airlangga University, with a certificate number of ethical clearance No: 2.KE.083.05.2018.

Preparation of carboxymethyl chitosan-curcumin hydrogel

500 mg carbomer powder was dispersed into CO₂-free distilled water and stirred. Carboxymethyl chitosan was dissolved in distilled water then mixed with carbomer-water dispersion. 20 mg of propylparaben was dissolved in propylene glycol, then put into the mixture and mixed until homogenous. TEA was added gradually into the mixture, stirred continuously until pH 6 was obtained. Curcumin was dissolved in ethanol 96% then added to the hydrogel.

Characterization of carboxymethyl chitosan-curcumin carbomer-based hydrogel

Organoleptic

The organoleptic examination was done by observing the physical appearance of the preparation such as consistency, color, and odor of the hydrogel.

pH

1 g of hydrogel was diluted in 10 mL CO₂-free distilled water, then stirred, and pH was examined using calibrated SI Analytics LAB865 pH meter. pH measurement was conducted 3 times replication.

Viscosity evaluation

Viscosity was examined using Brookefield cup and bob RION Viscometer VT-04E. 140 g of hydrogel was placed in the

Table 1: Formula Prepared in This Research

Material	Formula (% w/w)			
	F I	F II	F III	F IV
Carboxymethyl Chitosan	0.5	0.5	1.0	1.0
Curcumin	0.05	0.10	0.05	0.10
Propyl paraben	0.02	0.02	0.02	0.02
Propylene glycol	2.0	2.0	2.0	2.0
Carbomer 940	0.5	0.5	0.5	0.5
Triethanolamine	0.75	0.75	0.75	0.75

sample chamber, and the spindle was dipped into the chamber, and the viscometer was run in 3replication for each sample.

Spreadability

1 g of hydrogel was put in the center of the glass plate then covered with another glass plate. The load was added on top of an upper glass plate gradually, starting from 0 to 5 g. The spreading diameter was measured when the preparation stopped spreading at each additional loading (± 5 minutes). The latest diameter recorded becomes the spreadability data of the preparation (3 times replication).

Drying time

1 g of hydrogel was applied to the glass plate then put into the incubator (36 ± 0.5°C), and the sample was weighed every 10 minutes until the constant weight was obtained (3 times replication).

Wound healing activity evaluation

Wistar rats were intramuscularly anesthetized with ketamine and xylazine. The hair on the skin of the back surface of rats was removed using a razor blade. The wound was made using an electric burner (85 °C) with an exposure time of 5 seconds.

Treatment

Before the treatment was given, adaptation was carried out on experimental animals for 7 days in animal cages. Experimental animals were randomly divided into 6 groups (4 test groups and 2 control groups) further explained below:

G1: Rats with 2nd-degree burn wound are treated with carboxymethyl chitosan-curcumin hydrogel of the selected formula

G2: Rats with 2nd-degree burn wound treated with carboxymethyl chitosan hydrogel

G3: Rats with 2nd-degree burn wound treated with curcumin hydrogel

G4: Rats with 2nd-degree burn wound treated with hydrogel basis

G5: Rats with 2nd-degree burn wound treated with silver sulfadiazine cream as control positive

G6: Rats with 2nd-degree burn wound, no treatment given

Each group was given the treatment according to the test group by topically applying 100 mg of hydrogel on a wound site twice a day for 14 days.

Wound healing percentage

Macroscopic visual was observed every day during the study, and the area of wounds was measured on days 1, 7, and 14. Wound healing percentage was calculated using the formula:

$$\% \text{ Wound Healing} = 100 - \% \text{ wound area}$$

(% wound area = wound area on day- X / wound area on day 0)

Table 2: Organoleptic Result of Hydrogel Preparation with Different Concentration of Carboxymethyl Chitosan and Curcumin F I, F II, F III, and F IV

Formula	Color	Odor
I	Orange	Curcumin like odor
II	Darker orange	Stronger curcumin like odor
III	Orange	Curcumin like odor
IV	Darker orange	Stronger curcumin like odor

Histopathology evaluation

The wounded area of rat skin was excised on day 7 and day 14 of treatment, followed by making histological slide preparation. Parameters observed were polymorphonuclear neutrophils (PMN), collagen deposition, degree of fibrosis, and angiogenesis. Scoring criteria: collagen deposition (normal bundle = 2, unorganized/edema = 1, amorphous = 0), PMN infiltration (0-10 = 2, 11-40 = 1, >40 = 0), angiogenesis (mild, moderate, and severe), degree of fibrosis (mild, moderate, and severe).

RESULTS AND DISCUSSION

Organoleptic evaluation

As shown in Table 2, there were some differences in the color and the odor of each formula. The increase in carboxymethyl chitosan concentration affected the consistency of the preparations, which appeared to be more dilute and possess stronger odor and color.

pH evaluation

The result of pH evaluation from F1, F2, F3, and F4 were 6.89 ± 0.02, 6.96 ± 0.08, 6.77 ± 0.02, and 6.84 ± 0.03, respectively. F1 and F2 (0.5% carboxymethyl chitosan) had higher pH compared to F3 and F4; this indicated that the addition of carboxymethyl chitosan concentration increased the acidity of hydrogel preparation because 1% aqueous carboxymethyl chitosan solution pH ranges at 4-5.¹³ On the other hand, higher concentration of curcumin increased the pH of the hydrogel because of its alkaline properties.

Viscosity

Based on the replicated test, viscosity data for F1, F2, F3, and F4 were 30.0 ± 0.0, 30.0 ± 0.0, 17.5 ± 0.0, and 17.5 ± 0.0 dPa.s, respectively. Carbomer has a chemical structure where each end of the chain has a carboxylic group (R-COOH), which is acidic when reacted with H₂O. At acidic pH, the carboxyl groups of carbomer are not ionized. TEA addition serves to form gel systems from hydrogels.¹⁴ If the addition of a base neutralizes the pH of the carbomer dispersion, the carboxyl group of carbomer will be ionized. The repulsive force between ionized groups causes hydrogen bonds in the carboxyl group to stretch, which will result in viscosity increment.¹⁵

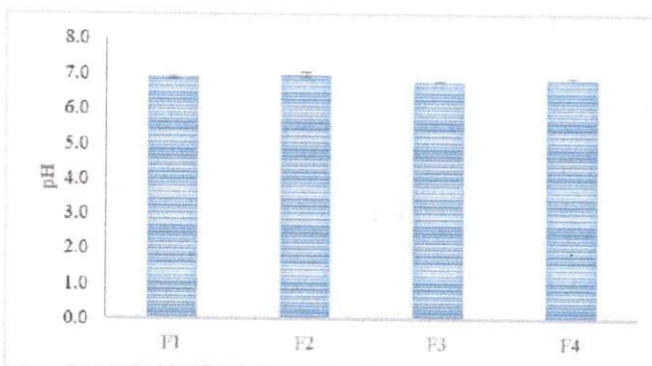


Figure 1: pH histogram of hydrogel preparation with different concentrations of carboxymethyl chitosan-curcumin F I, F II, F III and F IV.

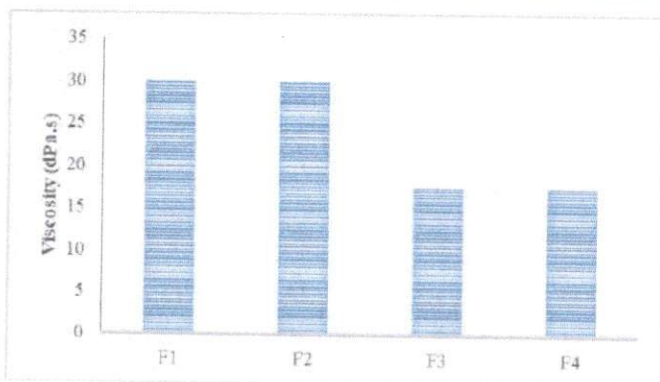


Figure 2: Viscosity evaluation results from carboxymethyl chitosan-curcumin hydrogel F I, F II, F III, and F IV.

On the other hand, carboxymethyl chitosan also has a carboxyl group in its structure, which can lead to competition between carboxymethyl chitosan and carbomer in the neutralization process by TEA. This can cause a decrease in repulsive force between carboxyl groups of carbomer and results in viscosity decrease. It can be concluded that different concentration in carboxymethyl chitosan (0.5% and 1.0%) affects the viscosity of carboxymethyl chitosan-curcumin hydrogel that curcumin does not.

Spreadability

Based on the replicated test, spreadability of hydrogel preparation was obtained 8.6 ± 0.17 , 8.9 ± 0.06 , 9.4 ± 0.10 , and 9.4 ± 0.10 cm for F1, F2, F3, and F4, respectively. The higher concentration of carboxymethyl chitosan is known to increase the spreadability of the hydrogel preparation. This is in accordance with the theory that the spreadability is influenced by the viscosity of the preparation: the thicker the preparation, the lower the spreadability of hydrogel preparation and vice versa.¹⁶

Drying time

Based on the replicated test, the drying time of F1, F2, F3, and F4 were 60 ± 0.00 , 60 ± 0.00 , 73.33 ± 5.77 , and 73.33 ± 5.77 min, respectively. Higher carboxymethyl chitosan concentration will increase the drying time of the preparation.

Wound healing activity

Visual

Visual wound observation in Figure 5 shows that the carboxymethyl chitosan-curcumin hydrogel has better wound healing activity by repairing the damaged tissue until the wound is well-closed. It is capable to significantly accelerate the

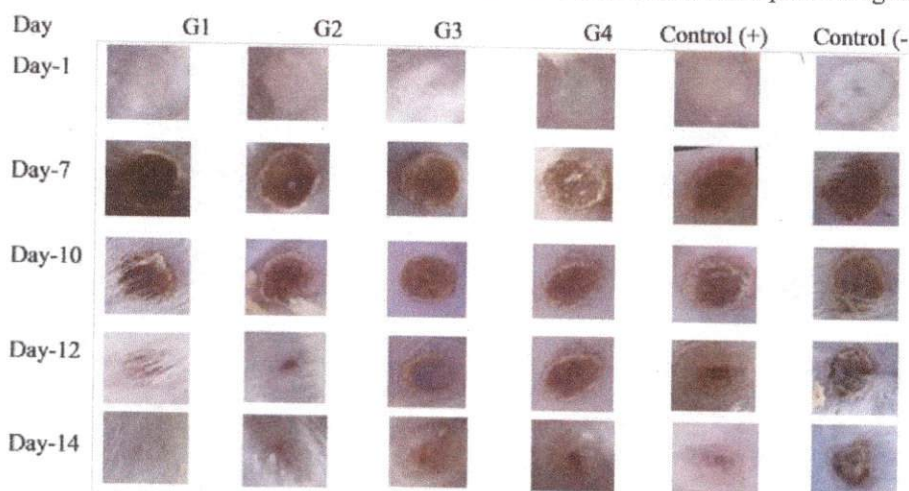


Figure 5: Wound visualization during treatment.

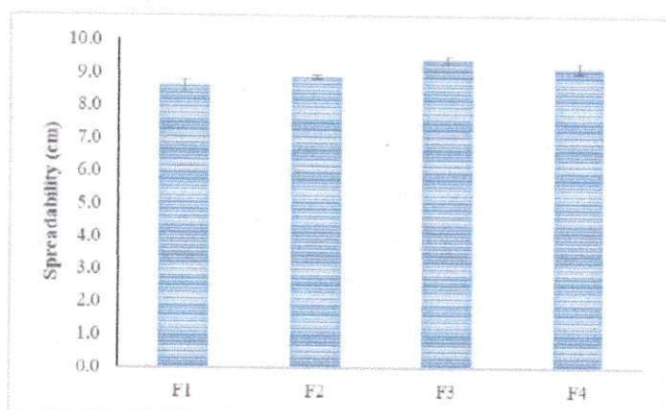


Figure 3: Spreadability histogram of hydrogel preparation with different concentration of carboxymethyl chitosan-curcumin F_I (0,5 % 0,05 %); F_{II} (0,5 % 0,10 %); F_{III} (1,0 % 0,05 %); F_{IV} (1,0 % 0,1%).

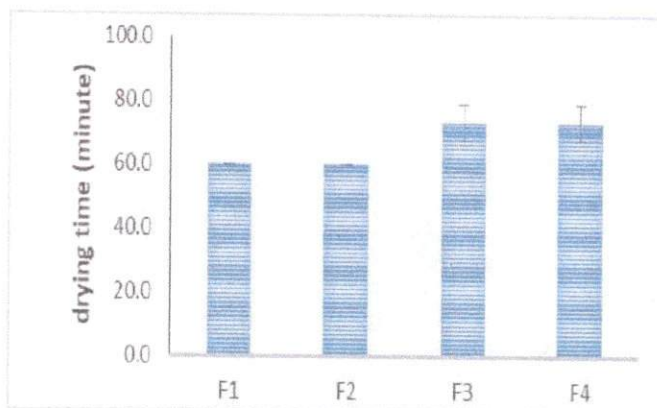


Figure 4: Drying time histogram of hydrogel preparation with different concentration of carboxymethyl chitosan-curcumin F_I (0,5 % 0,05 %); F_{II} (0,5 % 0,10 %); F_{III} (1,0 % 0,05 %); F_{IV} (1,0 % 0,1%).

wound healing process when compared to the G6 (untreated, natural healing process).

Based on wound healing percentage histogram (Figure 6), it can be seen that G6 (negative control) has the lowest healing percentage among other treatment groups while G1 (carboxymethyl chitosan-curcumin hydrogel) has the highest percentage of wound healing, even higher than the G5 (positive control). This indicated that the carboxymethyl chitosan-curcumin hydrogel improved the effectiveness of wound healing.

But the statistical analysis using one way ANOVA showed that there is a significant difference between G1, G2, G3, G4, and G5 with G6 but there no significant difference between G1, and G2, G1 with G3, G1 with G4, G1 with G5, G2 with G3, G2 with G4, G4 with G5, G3 with G4, and G3 with G5. So, it can be concluded that the combination of carboxymethyl chitosan-

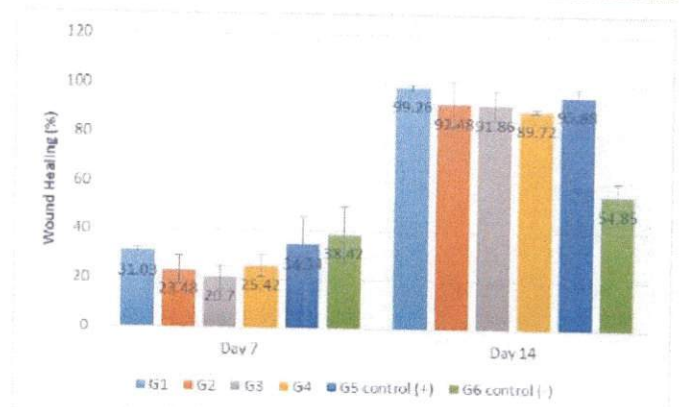


Figure 6: Wound healing percentage of different treatments: G1 (carboxymethyl chitosan-curcumin hydrogel), G2 (carboxymethyl chitosan hydrogel), G3 (curcumin hydrogel), G4 (hydrogel basis), G5 (positive control), and G6 (negative control).

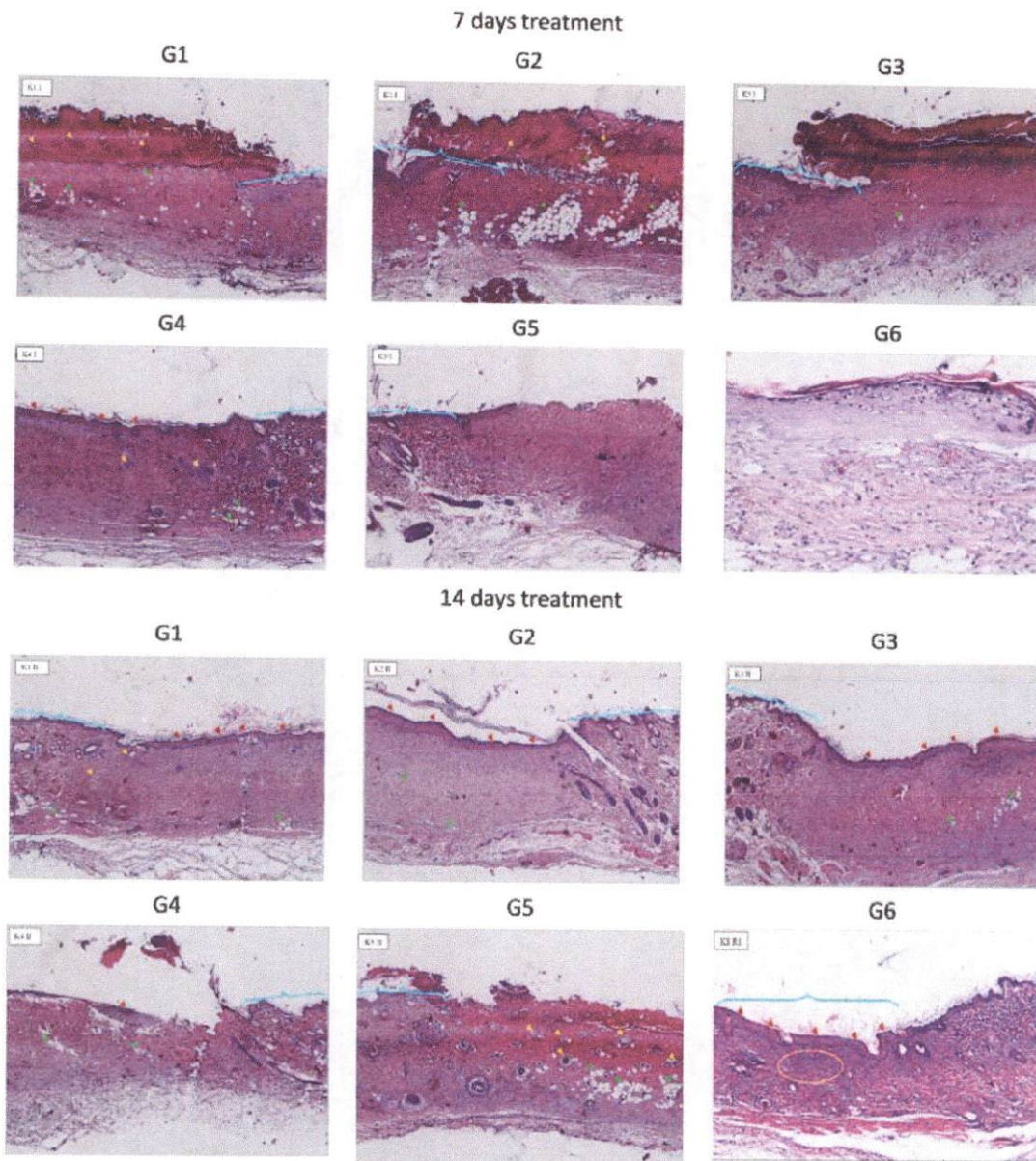


Figure 7: Histopathological images of albino rat's skin on treatment day 7 and 14.

Table 3: Histopathological Result

Group	Duration of treatment	Collagen deposition score	PMN score	Degree of angiogenesis	Degree of fibrosis
G1.2 I	7 days	2	1	medium	severe
G1.4 I	7 days	2	1	medium	severe
G1.6 I	7 days	1	1	mild	medium
G2.1 I	7 days	2	0	severe	severe
G2.4 I	7 days	2	0	severe	severe
G3.1 I	7 days	2	0	medium	severe
G3.4 I	7 days	2	0	medium	severe
G3.5 I	7 days	2	0	severe	severe
G4.3 I	7 days	2	0	medium	severe
G4.5 I	7 days	1	1	medium	medium
G5.2 I	7 days	1	1	mild	medium
G5.5 I	7 days	1	0	medium	medium
G6.1 I	7 days	1	0	mild	medium
G6.3 I	7 days	1	0	mild	medium
G1.1 II	14 days	2	2	mild	mild
G1.3 II	14 days	2	2	mild	mild
G1.5 II	14 days	2	2	mild	severe
G2.3 II	14 days	2	2	mild	medium
G2.5 II	14 days	2	2	mild	medium
G2.6 II	14 days	2	2	mild	Medium
G3.2 II	14 days	2	2	severe	Medium
G3.3 II	14 days	2	2	mild	Medium
G3.6 II	14 days	2	2	mild	Medium
G4.1 II	14 days	2	2	medium	Severe
G4.6 II	14 days	2	2	mild	Medium
G5.1 II	14 days	2	2	mild	Medium
G5.3 II	14 days	2	2	mild	Medium
G5.6 II	14 days	2	2	mild	Severe
G6.2 II	14 days	1	1	mild	medium
G6.4II	14 days	1	1	mild	medium

curcumin had similar activity compared to carboxymethyl chitosan or curcumin alone.

Figure 7 shows the histopathological features of the healing process of skin burns in various treatments (HE, 40x staining). The burn healing area was observed to form collagen coir (all groups), epithelialization (red arrow), fat tissue (green arrow), and hair follicles (yellow arrow) begin to form. Description: normal skin area.

The result of this study showed wound healing indicators, consisting of collagen deposition, PMN infiltration, angiogenesis, and fibrosis. Collagen deposition score, PMN score, degree of angiogenesis, and fibrosis were listed in Table 3 within a treatment duration of 7 and 14 days. Collagen deposition and PMN values for 7 days of treatment showed varied results between groups. However, it can be seen that K1 has the highest score on the PMN score. The histopathology result after 14 days showed collagen deposition and PMN scores between groups 1, 2, 3, and 4 as well as positive control and inter-group replication are all in uniformity, which showed that the wound had undergone healing after 14 days. However, the four test groups had better results than the negative control, which can be seen from the value of collagen deposition and PMN from negative control, which had a lower score after 14 days of treatment than the other groups.

CONCLUSION

The higher concentration of carboxymethyl chitosan could affect the physical characteristics of carboxymethyl chitosan-curcumin hydrogel, i.e., a decrease in viscosity and pH, as well as an increase in spreadability and drying time. On the other hand, a higher concentration of curcumin only affected the pH of the preparation. Macroscopic observation from the wound healing activity test showed that the combination of carboxymethyl chitosan-curcumin increased the wound healing activity of 2nd-degree burns in Wistar rats. From the histopathology test results, it can be concluded after 14 days of treatment the value of collagen deposition and PMN between groups 1, 2, 3, 4, and positive control and intergroup replication (1, 2, 3, 4, and positive controls) are in uniformity which indicates that the wound has undergone wound healing process.

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