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Research article

Comparison of gel ethanol green tea (*Camellia sinensis*) extracts and gel green tea water extracts for burns grade II on Marmut (*Cavia cobaya*) see from histopathology

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Abstract

Burns are a condition that can be experienced by every human being. Burns are often difficult to heal without the right treatment regimen. Bioplacenton is a gel that is often used to treat burns. Green tea has unique characteristics where the polyphenols can cause gradual growth and protect normal epithelial cells from carcinogenic substances. Among the content of polyphenols found in green tea, EGCG (*epigalokatekin galat*) and ECG (*epikatekin galat*) are the most abundant and very beneficial for the healing process of burns.This study aims to determine the difference in the speed of healingsecond degree burns between the administration of bioplacenton gel, CMC-Na gel base, green tea ethanol extract gel and green tea water extract with concentrations of 3%, 5%, and 7%, respectively, which included healing time and shrinkage of the size of burn size. This study was a laboratory experimental study using 27 guinea pigs that were induced by second degree burns and divided into groups of groups; control, control (-), bioplacenton (P1) gel, 3% green tea water extract, 5% green tea water extract, 7% green tea water extract, 3% green tea ethanol extract, 5% green tea ethanol extract, extract green tea ethanol 7%. Observation of healing time and shrinkage of wound diameter was carried out for 14 days. Data were analyzed using One way ANOVA statistical test and Kruskal wallis. The conclusions of this study are (1) Green tea ethanol extract and green tea water extract can be made in gel preparations which are proven by the results of testing of preparations that remain stable in 12 weeks, and do not irritate. (2) The preparation of green tea extract gel in water and ethanol has effectiveness in healing burns and can improve collagen in burns tissue. (3) The concentration of green tea ethanol extract and green tea water extract at the highest concentration is 7% which is most effective in healing burns and repairing collagen tissue and fibroblasts.

Introduction

In everyday life, people use heat sources such as fire, chemicals, and also electricity in their activities, so it is not uncommon to find burn cases [1]. According to WHO, in 2004, there have been accidental fires of 7.1 million in the world. In the same year, WHO recorded 310,000 people died from burns, according to the 2013 Basic Health Research, the prevalence of burns that occurred in Indonesia was 0.7%. This highest prevalence occurs at the age of 1-4 years [2].

Burns will cause damage to various organs, including the skin. In response to damaged tissue, the body can replace damaged tissue, improve its structure, strength, and function through the process of wound healing. The wound healing process is a complex and dynamic process that involves interactions between different cells and mediators [3].

Plant extracts have known to be used topically as therapy for healing burns. Some of these plants have similarities in several characters, such as producing flavonoid compounds with phenol structure. These phytochemical components are very reactive with other compounds, such as oxygen and other biological molecules, to neutralize free radicals. Some data states that plants with phenolic compounds that are beneficial to human health can found in green tea plants [4].

Green tea comes from drying and steaming from fresh tea leaves so there is no oxidation and causes the catechins contained in green tea is very high. In contrast, black tea undergoes a full fermentation stage before drying and steaming. During the fermentation process the catechins in black tea become oxidized and condensed and transformed into polyphenols with larger molecules such as teaflavin and tearubinin [5].

The polyphenols in green tea have unique characteristics where they can cause gradual growth and protect normal epithelial cells from carcinogenic substances. Among the polyphenol content found in green tea, EGCG (epigalokatekin galat) and ECG (epikatekin galat) are the most abundant and very beneficial ingredients for the process of healing burns [6].

Green tea leaves are very many benefits, but not many people know about the benefits of green tea leaves, especially in healing against burns. Therefore, the author tries to conduct a study entitled "Comparison of the Effectiveness Test of Green Tea Extract (Camellia sinensis) on Water and Ethanol for Grade II Burns on Marmots (Cavia cobaya) viewed from histopathology".

Material and method

Materials

razors, scissors, metal plates 2 cm in diameter, porcelain plates, mortar and stamper, spatula, dropper, syringe, plastic pot, analytical balance, rotary evaporator, freeze dryer, tweezers, pins, cotton, napkins, glass objects, and glass objects cover glass brand Sail Brand, scalpel brand surgical blades, Tweezers brand Chirurgie, Tissue Processor brand Sakura VIP 5, Paraffin Embedding Center brand Microme, Rotary Microtome brand Microm-Microtome HM 315, Water bath brand Microm-SB 80, Slide warmer brand Sakura Model PS-D, Toshiba brand Refrigerator, ESCO brand Fumehood, Olympus Model Microscope, and Xiaomi brand camera for documentation, green tea water, green tea ethanol extract, bioplacenton, CMC-Na, glycerin, propylene glycol, distilled water, and lidocaine HCl injection, ethanol 96%, alpha-naphthol, plaster, sterile gauze, 10% formalin solution for fixation of histopathological preparations, alcohol, ethanol, xylol, NaCl, hematoxylin and eosin dyes, soften.

Animals

The animals used are healthy adult guinea pigs, which are 27 males weighing 250-300 grams. Garut is in a healthy condition. Before the treatment, guinea pigs adapted for one week, and ethical clearance was approved.

Extraction method

As much as 1000 g of green tea leaves macerated green tea into a vessel, pour 6 L liquid and cover. Spread for 3 x 24 hours protected from light while stirring, filter, squeeze the lees with aqua dest until a filtrate of 1.5 L (Immersion I) obtained. Green tea pulp is obtained and re-soaked by maceration; the vessel is closed and left in a cool place 15-25^{o}C and not exposed to light, for 3x24 hours. Filtered again and obtained as much as 6.5 L. Filtrate amount of twice the soaking of green tea leaves with water is 8 liters. To separate the water from the extract, it is collected and concentrated by evaporating the freeze dryer until the remaining one-third portion obtained until a concentrated extract of 60g obtained.

Phytochemical screening

Phytochemical screening of tea extract refers to the procedure performed by others researcher [7]. Phytochemical contains flavonoid, alkaloid, glycoside, terpenoid and steroid, tanin, saponin test.

Formulation of gel

Formulation of gel base

In this study, Green tea extract gel preparations made in ethanol and water, which will then tested for their healing effect on burns on marmot skins. The preparations made based on the standard formula of CMC-Na base gel according to others researcher [8] are as follows CMC-Na 5g, Gliserin 10g, Propylene glycol 5g, Distilled water 100ml.

Formulation of extract gel

The gel formula made with a variety of concentrations of green tea ethanol extract and water extract : 3%; 5%; 7%; and gel base as blanks of 50g each.

Testing of gel preparations for burn healing

This study used 27 marmots, which divided into nine groups, each group consisting of 3 marmots. Determination of sample size done by using the Federer formula.

Group 1 : Control group
Group 2 : Gel without extract of tea
Group 3 : Bioplacenton (Positive control)
Group 4 : Gel ethanolic extract of green tea 3%
Group 5 : Gel ethanolic extract of green tea 5%
Group 6 : Gel ethanolic extract of green tea 7%
Group 7 : Gel water extract of green tea 3%
Group 8 : Gel water extract of green tea 5%

Group 9 : Gel water extract of green tea 7%

Execution of burns

In this study, burns on guinea pigs carried out using a 2cm diameter metal plate which was preheated using free fire for 5 minutes then placed on the back of the guinea pig which was first shaved on its back and was anesthetized with Lidocain HCL® subcutaneously injection with dose of 1mL for 10 seconds. Furthermore, leave for 24 hours and observed. The occurrence of infection in the wound characterized by the presence of red inflammation and the formation of bullae. Then the skin blisters that suffered burns were applied to the gel according to each group of guinea pigs thinly three times a day (the wound was not closed) and observed as seen from his pathology on the 7th day and the 14th day [9]. Skin sampling took on day seven and day 14. Guinea pigs in each diet with chloroform. In the part that given, a burn made excision with a degree II A depth and fixed with 10% formalin. Then the skin tissue is made histopathological preparations.

Parameter of research

The parameters observed in this study were the density of collagen formed in the healing of burns, fibroblasts, and inflammatory cells. Histopathological scoring parameters for the distribution density of collagen tissue, fibroblasts, and inflammatory cells were carried out based on the calculation of 5 visual fields on the 400 x magnification object.

Statistical analyze

Data on changes in the healing histopathology of guinea pig skin burns based on collagen density, fibroblast, and inflammation cell scores were analyzed using the One-Way Anovakarena test for more than two groups of variables. Data analysis performed with the Statistical Packed for Social Science (SPSS) computer program for windows.

Result and discussion

Determination of plant

The determination of plants carried out to ensure the correctness of tea plants to avoid mistakes in the selection of plant material used in research. The results of the identification of plants carried out at Medanarium Herbarium (MEDA), University of North Sumatra, showed that plants included in the tribe. Based on the results of determination, the plants used in this study are tea plants with a scientific name (*Camellia sinensis* (L). O.K)

Phytochemical screening result

The results of phytochemical screening extract obtained showed the presence of flavonoids, saponins, tannins, glycosides. The results of the phytochemical screening of ethanolic extract can be seen in table 1.

Table 1. Result of phytochemical screening of green tea of ethanol and water extract.

No.	Screening contents	Ethanol extract	Water extract
1.	Flavonoid	+	+
2.	Alkaloid	+	+
3.	Saponin	+	+
4.	Tanin	+	+
5.	Glikosida	-	+
6.	Steroid/triterpenoid	_	_

Information (+) = positive result
(-) = negative result

Alkaloids thought to have the ability to be antibacterial by the mechanism of disturbing the constituent components of peptidoglycan in bacterial cells so that the cell wall layers are not formed intact and cause the death of these cells [10].

Flavonoids can function to capture free radicals so that they can prevent damage to skin tissue. Flavonoids can enhance the process of mitogenesis of cell interactions and adhesion of molecules, which play a role in the phase of cell proliferation, which accelerates the process of healing of burnt tissue. Flavonoids also shorten the time of inflammation (inflammation), which can inhibit healing [11]. Based on the potential and mechanism possessed by green tea leaves, this study conducted with the hope that the administration of green tea leaf steeping could potentially improve grade II-a burn tissue in guinea pigs [12]. Tannin is a component that widely found in plant extracts which is efficacious as an astringent and able to shrink wounds, stop bleeding and reduce inflammation, tannins are useful as an antiseptic and also heal burns by presenting protein because there is an antibacterial power [13].

Saponins found in plants can stimulate collagen formation, which plays a role in the process of wound healing [14]. Saponin also has the ability as a cleanser and antiseptic that functions to kill or prevent the killing of microorganisms that can arise in the wound so that the wound does not experience a severe infection.

Steroids/triterpenoids are known to accelerate the wound healing process mainly because they have microbial and astringent activity, which have a role in wound shrinkage and increase the rate of epithelialization [13].

Evaluation Results of Tests for Healing Burns on Genuine pig (Gel Green tea ethanol extract)

This test intended to determine at what concentration of green tea ethanol extract can heal burns the fastest. This study uses two types of control, namely, positive control

(without treatment) and negative control (CMC-Na gel basis). The results of observing the healing time of a burn can be seen in table 2.

Based on table 2, it can observe that the control group experienced a longer healing process when viewed from the healing process of large areas of burns. This table showed that without the preparation of green tea ethanol extract gel does not affect the acceleration of healing of wounds, so it said the negative group, bioplacenton, and the green tea extract test group 3%, 5%, 7% experienced normal wound healing process.

Figure 1 shows that the longest average healing time of burns found in the control group with a mean of 1.78 ± 0.27 days and the way of the fastest time of healing of wounds seen in the bioplacenton group with an average of 1.30 ± 0, 81 days and green tea ethanol extract group 7% 1.36 ± 0.73 days. From the data obtained, it can see that the wound healing process for the untreated guinea pig group occurs longer wound healing process. Treatment for the guinea pig group based on the gel intended as a useful control proving whether ethanol extract and green tea water extract can use to accelerate the healing of burns.

Evaluation results of tests for healing burns on genuine pig (Gel Green tea water extract)

This test intended to determine at what concentration of green tea water extract can heal burns the fastest. This study uses two types of control, namely, positive control (without treatment) and negative control (CMC-Na gel basis). The results of observing the healing time of a burn can be seen in table 3.

In the study of green tea water extract activity on the healing of burns on the backs of guinea pigs, data obtained extract concentrations of 3%, 5%, 7%, and Bioplacenton began to notice changes on the third day, on the 14th day there was a change in the percentage of healing. This table showed that green tea water extracts have activity in healing burns.

Tabel 2. The average reduction in the burn area of green tea ethanol extract.

Group	The average reduction in the burn area of Green Tea Ethanol Extract			Average ± SD (cm)
	Heal (Day)			
	1	7	14	
Control	2	1.90	1.3	1.78 ± 0.27
F1 Control (-)	1.99	1.8	1.3	1.57 ± 0.49
F2 Bioplacenton	1.99	1.19	0.48	1.30 ± 0.81
F3 EEGT 3 %	1.98	1.2	0.5	1.58 ± 0.47
F4 EEGT 5 %	2	1.2	0.49	1.46 ± 0.62
F5 EEGT 7 %	2	1.51	0.56	1.36 ± 0.73

Information : EEGT (Extract Ethanol Green tea).

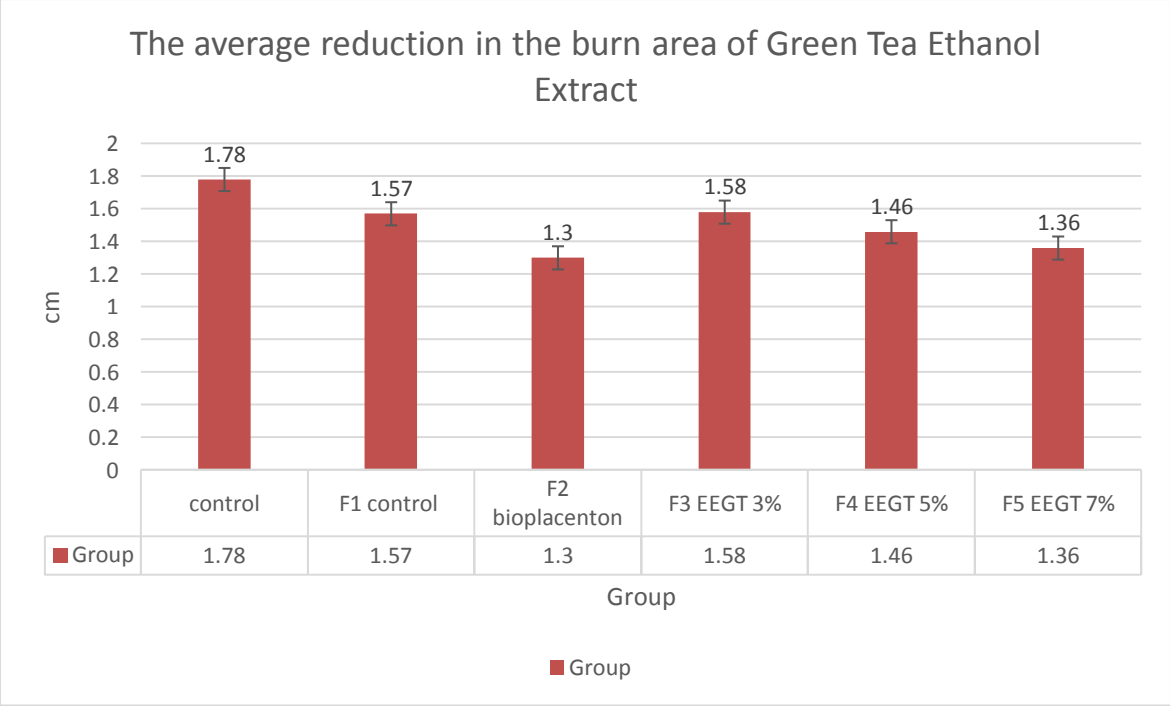

Figure 1. The average reduction in the burn area of green tea ethanol extract.

Tabel 3. The average reduction in the burn area of green tea water extract.

Group	The average reduction in the burn area of Green Tea Water Extract			Average ± SD (cm)
	Heal (Day)			
	1	7	14	
Control	2	1.89	1.51	1,80 ± 0,26
F1 Control (-)	2	1.79	1.40	1,73 ± 0,31
F2 Bioplacenton	2	1,43	0,30	1,24 ± 0,87
F3 EWGT 3 %	2	1,67	1,09	1,59 ± 0,46
F4 EWGT 5 %	2	1,61	0,79	1,47 ± 0,62
F5 EWGT 7 %	2	1,49	0,45	1,31± 0,79

Information : EEGT (Extract Water Green tea).

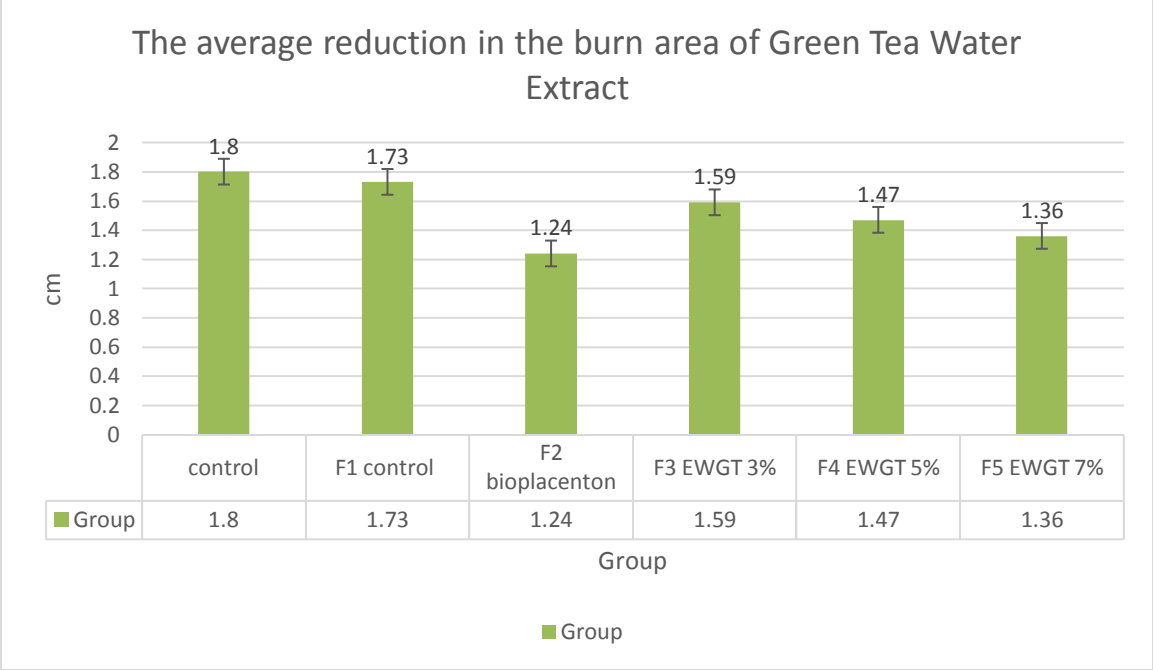

Figure 2. The average reduction in the burn area of green tea water extract.

Figure 2 shows that the longest average healing time of burns is in the control group with an average of 1.78 ± 0.27 days and the fastest average healing time of wounds is in the bioplacenton group with an average of 1.30 ± 0, 81 days and green tea ethanol extract group 7% 1.36 ± 0.73 days. The average duration of wound healing in each group needs to be tested for normality and homogeneity of the variants first as a condition for fulfilling the assumptions of the ANOVA test and the Post Hoc Duncan test. The normality test uses the Shapiro Wilk test, and the variant homogeneity test uses the Lavenne analysis. The results of the normality and homogeneity test of variance showed p-value> 0.05, which means the data is typically distributed and has a homogeneous variant. Anova and Post Hoc Duncan Test to find out the average comparison between groups and significant differences between groups.

From both figures 1 and 2, the green tea ethanol extract gel and green tea water extract can be seen that a better improvement found in green tea ethanol extract compared to green tea water extract because ethanol solvents forget the universal sweep solvent where ethanol can attract phytochemical compounds that are polar or non-polar so that more active compounds that play a role in the healing process of burns become faster.

Result of histopathology test

Histological observations on the healing of burns done by comparing the description of histology from the ethanol extract group of green tea 3%, 5%, 7%; Green Tea Water Extract 3%, 5%, 7%; Bioplacenton, and Control (-). This observation made on the 14th day after treatment. Results are shown in figure 3A to 3I.

In the control that is without treatment, visible inflammatory cells that spread very tightly, collagen fibrous tissue has not formed, many evident inflammatory cell infiltration. The number of inflammatory cells seen because of the inflammatory response in the injured tissue. Inflammatory cells stimulated to the wound, so there are still many inflammation cells. In the control group, the possibility of infection is substantial because no treatment can prevent disease (Figure 3A).

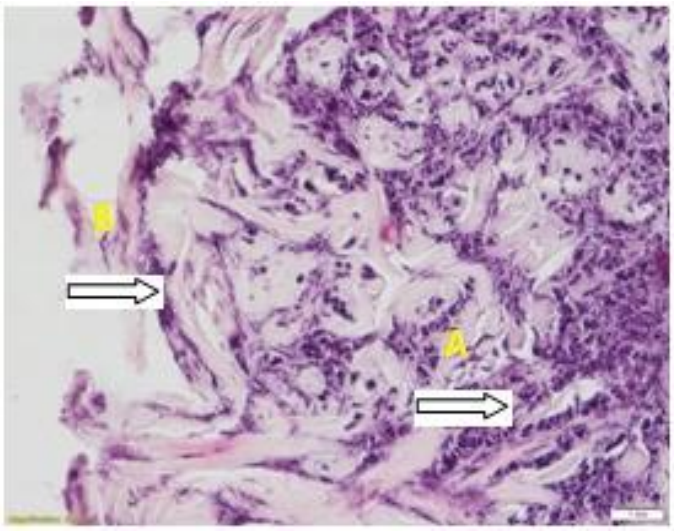

Figure 3A. Histology of control.
A: Inflammation cells, B: Collagen.

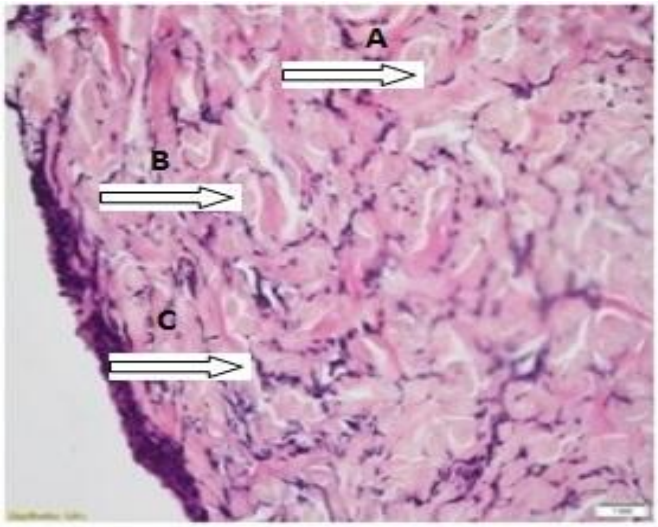

Figure 3C. Histology of water extract of green tea 3%.
A : Fibroblast, B : Collagen, C : Inflammation cells.

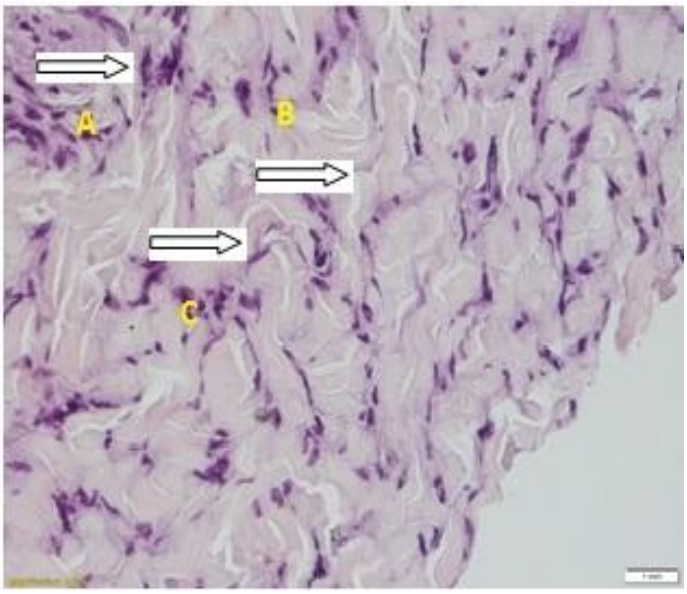

Figure 3B. Histology of negative control.
A : Inflammation cells, B : Collagen fibers,
C : Fibroblast.

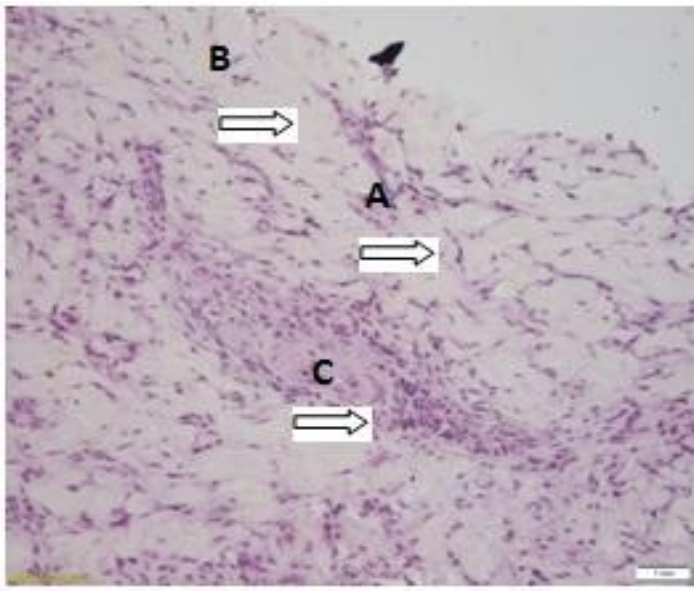

Figure 3D. Histology of water extract of green tea 5%.
A :Fibroblast, B :Collagen, C :Inflammation cells.

Treatment using CMC-Na on control (-) on day 14 caused fewer infiltration of inflammatory cells compared with the control group and began to found with fibroblasts and collagen fibers that spread with moderate density (Figure 3B).

In the picture of histology burns on the skin of guinea pigs, the group of green tea extracts on day 14th visible infiltration of tightly spread inflammatory cells, fibroblasts, and collagen fibers spread with moderate density. Inflammatory cells stimulated to the wound area histology. There is a lot of inflammatory cell infiltration (Figure 3C).

Inflammation of inflammatory cells spreads thin collagen fibers, the number of fibroblasts covered with moderate density. The involvement of inflammatory cells, which still dominates the injured area, indicates that the inflammation process is ongoing (Figure 3D).

The epidermis layer is not fully formed, inflammation cells spread with low density, and the collagen tissue begins to solid. Collagen fibers have reorganized to form webbing. Shows the fabric has undergone a process of healing burns and found fibroblasts and collagen (Figure 3E).

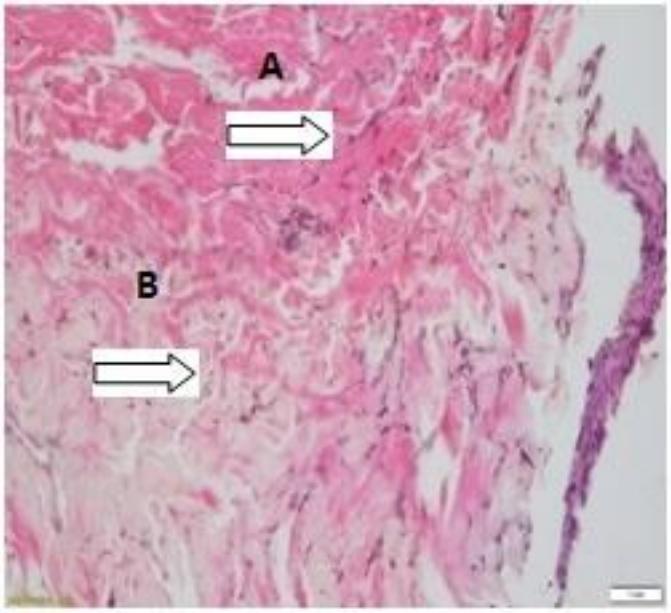

Figure 3E. Histology of water extract of green tea 7%. A :Fibroblast, B :Collagen.

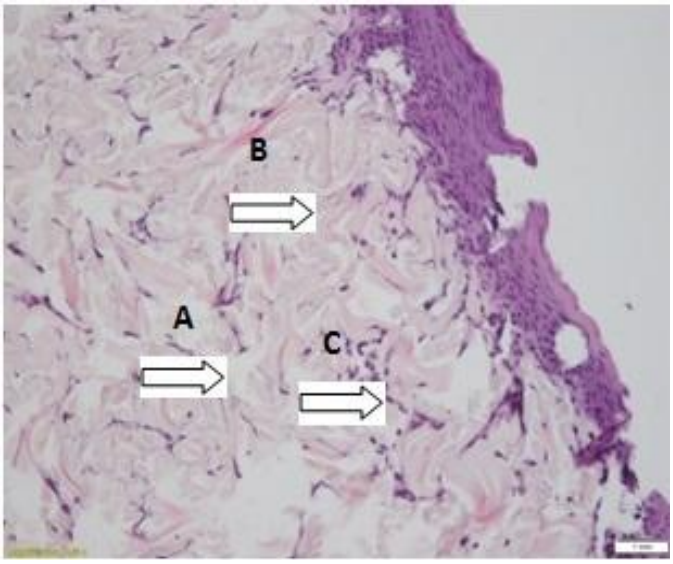

Figure 3F. Histology of ethanol extract of green tea 3%. A : Fibroblast, B : Collagen, C : Inflammation of cells.

Inflammation of inflammatory cells is still visible, the epidermis layer of the skin has not formed fully characterized by an unseen corneum layer and a thick layer of epithelium, collagen fibers spread with moderate density (Figure 3F).

Infiltration of inflammatory cells spread with low density, thickened collagen fibers. Inflammation of inflammatory cells is the least found in green tea ethanol extract because it contains flavonoids and tannins which have antiseptic power so that it can use as infection prevention. The involvement of inflammatory cells that still dominate the wound area indicates that the inflammatory process is ongoing (Figure 3G).

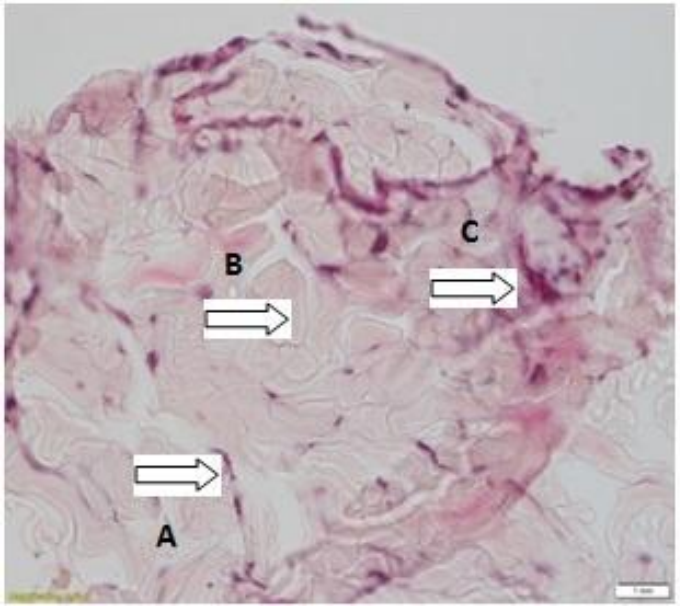

Figure 3G. Histology of ethanol extract of green tea 5%. A : Fibroblast, B : Collagen fibers, C : Inflammation of cells.

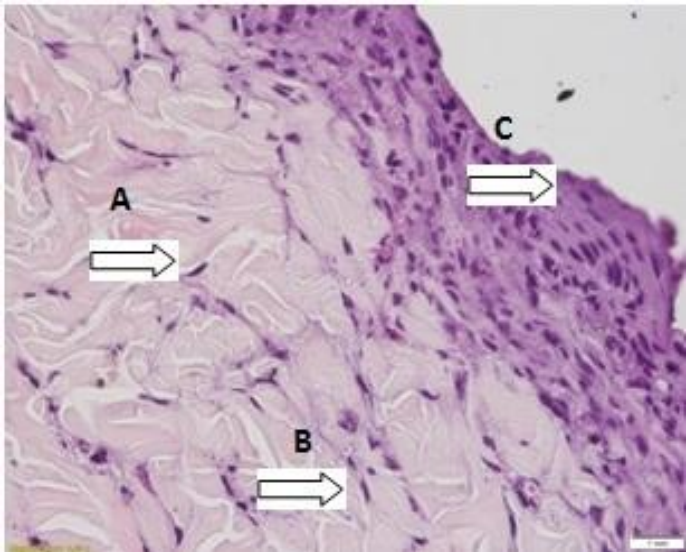

Figure 3H. Histology of ethanol extract of green tea 7%. A : Fibroblast, B : Collagen, C : Epithelial cells.

Treatment using ethanol extract of green tea gives epithelialization to form, and the dermis is fully formed. Many new blood vessels, very little visible infiltration of inflammatory cells and tight collagen fibers (Figure 3H). The density of the collagen bioplacenton on the 14th day was higher than the control group. These results can occur because of the treatment group that was given a burn with a bioplacenton. In the process of wound healing, several factors have contributed to the process, including fibroblast cells, which will stimulate the proliferation phase. In this phase, fibroblast cells can synthesize the collagen as an extracellular matrix that functions as new tissue (Figure 3I).

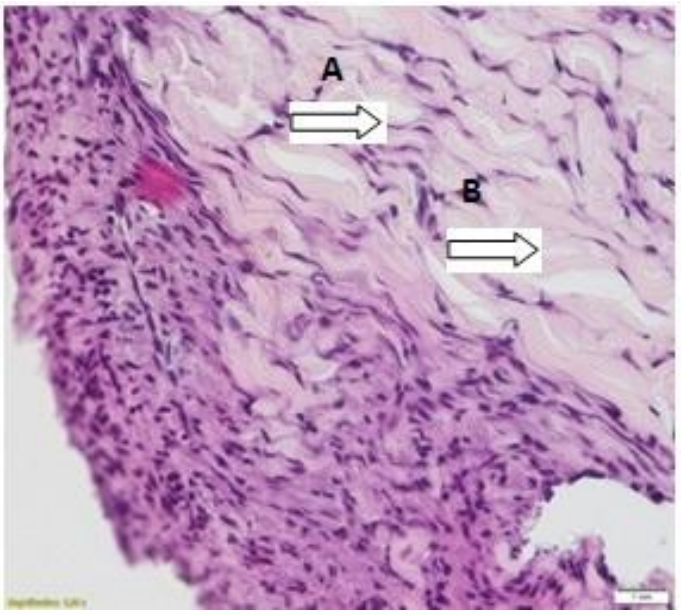

Figure 3I. Histology of Bioplacenton.
A : Fibroblast, B : Collagen fibers.

Based on the ANOVA test for a one-way pattern to see the histopathological picture of collagen density in the healing of guinea pig skin burns. Statistical test results using Variant Analysis (ANAVA) with a value ($P < 0.05$) showed no effect of each treatment group.

Conclusion

Green tea ethanol extract and green tea water extract can be made in gel preparations, which proven from the test results of preparations that remain stable in 12 weeks, and are not irritating. Green tea extract gel preparations in water and ethanol have effectiveness in healing burns and can improve collagen in burn tissue. The concentration of ethanol extract of green tea and green tea water extract at the highest concentration of 7% is the most effective in healing burns and repairing collagen tissue and fibroblasts.

Author contributions

All the authors have contributed equally in designing, drafting the manuscript as per the journal submission format. All authors read and approved the final manuscript.

Conflicts of interest

The authors declare that there are no competing conflicts of interest.

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