ORIGINAL ARTICLE

Potential effects of broccoli extract (*Brassica oleracea var. italica*) on collagen fiber density in the wound healing process

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ABSTRACT

Introduction: Wound healing is a protective function of the body that focuses on quick recovery, where the regeneration process in a hostile environment takes more time. In particular, the oral cavity is a remarkable environment in which wound healing occurs in warm oral fluid that contains millions of microorganisms. Collagen plays an important role in all phases of wound healing, which provides integrity and strength to tissues, and it is very important especially in the proliferation and remodeling phases. One of the materials that have potential related to collagen is broccoli (*Brassica oleracea var. italica*). Broccoli has the potential to regulate collagen; therefore, it could be an alternative to answer the problem related to anti-inflammatory, antioxidant, and antimicrobial effects. The aim of this research is to analyze the potential effects of broccoli extract on collagen image in wounds. **Methods:** This research used 18 male Wistar rats with wounds created by incisions on each of the rats' backs. These rats are divided into two groups, the control group, which received no treatment, and the treatment group, which was treated by applying the broccoli extract topically twice a day. Wound tissues on each group were observed microscopically on the 3rd, 7th, and 14th day. The density of collagen fibers was measured based on the calculation of semiquantitative score. **Results:** The result showed that wound healing in the treatment group looked better compared to that in the control group, with an increase in collagen score in the treatment group. The result of the statistical test Man-Whitney with confidence level 95% ($p < 0,05$) with observation on 3rd, 7th, and 14th days sequentially was $p = 0.100$, $p = 0,100$, $p = 0,700$. **Conclusion:** Broccoli extract which is applied topically, tends to have more benefits in terms of wound healing process, even though there is no significant difference found on the statistical tests.

KEYWORDS

broccoli, *brassica oleracea var.italica*, collagen, wound healing

INTRODUCTION

Wound healing requires multiple processes that occur in a specific sequence. Intact hemostatic and inflammatory mechanisms are needed to maintain the equilibrium and promote new tissue formation, triggering angiogenesis and epithelialization, together with collagen synthesis.[1] To facilitate healing with minimal scar formation, all tissues follow an essentially same pattern. Wound healing is a defensive function of the body that focuses on speedy recovery in situations when regeneration takes longer. The oral cavity, in particular, is a fascinating habitat in which wound healing occurs in warm oral fluid containing millions of bacteria. Oral mucosa wound healing consists of a set of consecutive responses that allow harm in this tissue to be closed. This process is critical to preventing the invasion of microorganisms and other agents into tissues and preventing chronic inflammation since the oral mucosa is continually exposed to traumatic and infectious challenges .[2, 3]

Collagen plays an important role in all phases of wound healing, which provides integrity and strength to tissues, and it is very important especially in the proliferation and remodeling phases. Collagen also functions as a basis for intracellular matrix formation in the wound area.[4,5] Secondary healing begins if the wound healing process is disturbed by infection, dehiscence, hypoxia, or immunological dysfunction. Granulation tissue development and epithelialization over this new tissue formation occur during this stage. This sort of wound is more prone to infection and can cause delayed wound healing. The changed shape of the collagen triple helix causes them to denature (unwind) much more quickly at lower temperatures.[6]

Currently, the use of herbal ingredients for chemical drugs substitution has been carried out. It is termed herbal medicine, particularly to solve various forms of wound healing disorders related to collagen. The use of herbal medicines for treatment has advantages, including more affordable price and less adverse effects.[7, 8] One of the most potential plants for this case is broccoli (*Brassica oleracea var. italica*).

It contains so many compounds, which can improve health, vitamins, minerals, and fibers, that broccoli is claimed as a vegetable with undeniable health benefits.[9,10] Broccoli flower extract contains several antioxidants and vitamins such as sulforaphane, indole, vitamins A, C, and E, beta carotene, quercetin, kaempferol, glutathione, and selenium.[11]

Vitamin C is an essential substance for collagen formation. It has the potential to increase type I collagen synthesis, and reduce oxidative stress parameters.[8] Broccoli also contains glucosinolate[12], which is reported to have antimicrobial effects.[13] Glucosinolate on broccoli plays a role in optimizing the formation of collagen by preventing tissue damage due to bacteria and its products.[14] Broccoli contains bioactive non-enzymatic compounds, is rich in nutritional antioxidants, such as vitamins C and E, and has non-nutritional antioxidants, such as carotenoids, phenolic compounds, especially flavonoids. Broccoli is also rich in polyphenols, chemicals that constitute a large part of phytochemicals and most antioxidants found in foods.

Polyphenols interfere with lipid oxidation and other molecules by releasing hydrogen atoms as free radicals. Flavonoids and their derivatives are the most common group of polyphenols. They are ideal scavengers against peroxyl radicals because of their specific reduction ability to alkyl peroxyl radicals, so they become effective inhibitors of lipoperoxidation. Broccoli is also said to contain flavonols and hydroxycinnamoyl derivatives. Some studies also said that anthocyanins in broccoli, the group of pigments in plants, are the most numerous coloured flavonoids and have high antioxidant activity.[15] Antioxidants in broccoli such as flavonoid and polyphenols have a potential to increase procollagen type-1 protein synthesis.[16] The aim of this research is to analyze the potential effects of broccoli extract on collagen image in wounds.

METHODS

This research is an experimental laboratory study using a post-test-only control group design. This research was conducted at the Chemistry Laboratory of the Faculty of Mathematics and Science for extracting broccoli. The treatment of experimental animals was carried out in the Faculty of Veterinary Medicine at Syiah Kuala University (Unsyiah) and continued with histology preparation at the Histology Laboratory of the Faculty of Veterinary Medicine at Unsyiah. Before initiating the study, all procedures were approved by the Research Ethics Board of the Faculty of Dentistry, Unsyiah for Animal Care and Use.

Eighteen male white Wistar rats (*Rattus norvegicus*) were obtained from the Faculty of Veterinary Medicine at Unsyiah. The inclusion criteria included: Male white Wistar (*Rattus norvegicus*) strain, average weight 200-250 grams, age two to three months, clinically healthy, characterized by agile movements, neat fur, not tangled or dull, and had never been used in another research.[13] Exclusion criteria included white Wistar rats (*Rattus norvegicus*) that did not have any appetite to eat, were infected during the treatment period or died during the intervention.[14] Rats used in the study were divided into two groups: the control and the treatment groups, each consisting of nine rats. The sample calculation was based on a completely random design. Each group was divided into three subgroups with three repetitions. The division of this group was based on the time of histological observation of collagen fibers : the 3rd, 7th, and 14th days.

Broccoli Extraction (*Brassica oleracea var. italica*) with Maceration Technique. Initially, broccoli was washed clean, cut into pieces, and dried in a cupboard at 45ºC for 48 hours. The dried broccoli was then made into powder by blending and stored in an airtight container.[15] Broccoli powder was placed in a flat-bottomed glass container and soaked with 96% ethanol. It was closed and left for 3 days, stirring occasionally. The resulting mixture was then filtered with white cotton material and filtered again using Whatman filter paper. The filtrate was then concentrated with an RC 600 rotary evaporator at 120 rpm until a thick extract was obtained.[1]

Wound making was done on the 0th day in all groups. The wounds were made in the back area of the rat skin that had been shaved. Before making a wound on the back of the rats, the rats were first anesthetized using a single intramuscular injection of 1-2 mg/kg xylazine hydrochloride and 10 mg/kg of ketamine hydrochloride; then, the shaved back area was cleaned with 70% alcohol. A 15 mm linear incision was made along the os. Vertebrae with 2 mm depth using a scalpel.[17] Broccoli extract (*Brassica oleracea var. italica*) was given topically to the treatment group twice a day, at 9 am. and 3 pm, with a six-hour interval using a syringe to administer up to 0.1 ml. The control group was not given any substances or treatment. After the completion of the treatment, all the rats were euthanized with ether; 10 ml of cotton soaked with ether placed in a suitably large container. They were then put into the container until they died. After collecting the rats' tissue, the micewere buried.[18]

Sample tissues were fixed in 10% buffered formalin, processed and blocked with paraffin at 40 to 60°C, and then sectioned into 5 µm thick, stained with hematoxylin-eosin, and observed under a microscope to see the change in collagen density. The observation and administration of collagen fiber density scores on preparations were carried out using microscope Meiji Techno Co.Ltd. Japan, DP-12 digital camera, and Top View software with 400 times magnification. This observation was equipped with a DP-12 digital camera. The density of collagen fibers was observed on day 3, 7, and 14 with a light microscope at 400 times magnification. The collagen fibers density was measured based on the calculation of 1 field of view and interpreted by veterinarian pathologists using a semiquantitative score. These histological preparations were observed under a microscope Meiji Techno Co.Ltd. Japan, DP-12 digital camera, and Top View software, and they were scored under these following criteria: (-) or 0: no visible collagen fibers; (+) or 1: collagen fibers were very thin or very little spread on the tissue, low collagen density; (++) or 2: collagen fibers are moderately spread and appear fused, moderate collagen density; (+++) or 3: collagen fibers spread a lot, high collagen density; and (++++) or 4: collagen are perfectly bound,

very high collagen density. Data collected were analyzed using SPSS and hypothesis testing was performed using the Mann-Whitney test analysis method.

Figure 1. Application of broccoli extracts

RESULTS

The results of collagen fiber density scores on each observation day (3rd, 7th, and 14th day) can be seen in Table 1, Table 2, and Table 3. There was no statistical significance of the result. The results of the statistical test Man-Whitney with a confidence level of 95% ($p < 0,05$) with observation on the 3rd, 7th, and 14th day sequentially is $p = 0.100$, $p = 0,100$, $p = 0,700$.

Figure 1 demonstrates the results of the histological presentation of rat skin tissue wounds that were sectioned and stained with hematoxylin-eosin (H&E) viewed at 400 X magnification. No collagen fibers were detected (Score 0) on the 3rd day of observation in the control group. The inflammatory cells in the wound area were still abundant. In the treatment group, the density of collagen fibers was observed on the 3rd day, reaching 10-50%; moderate collagen fibers were detected (Score +2), indicating a decrease in the number of inflammatory cells in the wounded area. In the control group, the density of collagen fibers observed on the 7th day reached less than 10%, with low collagen fiber density (Score +1) and amorphous substances still filling the wound area.

In the treatment group, the density of collagen fibers observed on the 7th day was 50-90%, densely collagen fibrous density was detected (Score +2) where very few inflammatory cells were found in the collagen. In the treatment group, the density of collagen fibers observed on the 14th day was 90-100%, with very tight collagen fibrous density (+4 Score) where the collagen fibers were heavily dense. The increase in collagen fiber density scores in the treatment groups on the 3rd, 7th, and 14th day observations was also seen in Figure 2, showing that the line graph of the average collagen density of the treatment group was higher than the line graph of the average collagen density of the control group:

Table 1. Collagen fiber density score on 3rd day

Variable	Repetition	Group control ± Ds	Treatment group ± Ds
Collagen fiber	1	0	+1
Density	2	0	+2
	3	+1	+2
Mean		0.33 ± 0.57	1.67± 0.57

Table 2. Collagen fiber density score on 7th day

Variable	Repetition	Group control ±Ds	Treatment group ± Ds
Collagen Fiber	1	+1	+2
Density	2	+1	+3
	3	+2	+3
Mean		1.33± 0.57	2.67 ± 0.57

Table 3. Collagen fiber density score on 14th day

Variable	Repetition	Group control ± Ds	Treatment group ± Ds
Collagen Fiber	1	+3	+3
Density	2	+3	+3
	3	+3	+4
Mean		3± 0	3.33± 0.57

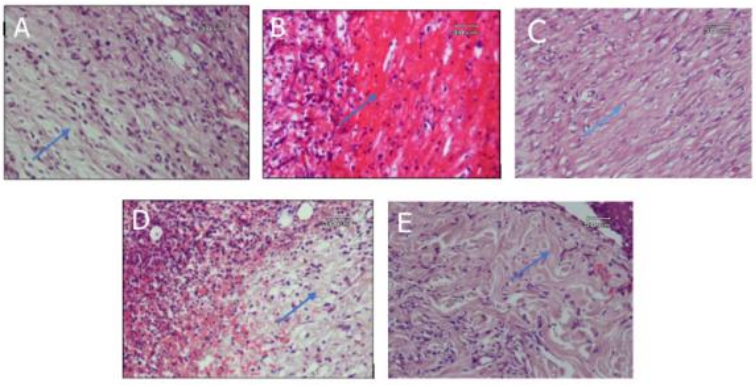

Figure 2. The results of histological presentation of rat skin tissue wounds that were sectioned and stained with hematoxylin-eosin (H&E) being viewed at 400X magnification. Figure A: no collagen fibers were found (Score 0) in the control group on the 3rd day; Figure B: densely collagen fibrous density was detected (Score +2) in the treatment group on day 3; Figure C. %, low collagen fiber density (Score +1) in the control group on 7th day; Figure D: Collagen fibers spread a lot (Score +3) in the treatment group on the 7th day; and Figure E: very tight collagen fibrous density (+4 Score) in the treatment group on the 14th day.

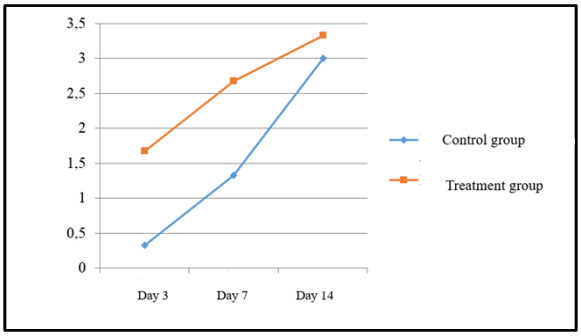

Figure 3. The highest graph of the average score of collagen fibers density obtained in the treatment group at the observation of the 14th day, while the lowest average score of collagen fibers density was found in the control group on the 3rd day observation. At the observation of the 7th day, the average score of collagen fiber density in the treatment group was higher compared to that in the control group.

DISCUSSION

On the 3rd day observation (Table 1), the density of collagen fibers in the treatment group showed a higher score than that in the control group, with an average score of collagen fibers density of 1.67. Figure B showed that the density of collagen fibers in the wounded area was medium (score +2). In Figure A, the collagen density in the control group was more tenuous. The results of this study suggest that the application of Broccoli extracts shorten the inflammation period. Inflammation is the first response during the healing period as a defense mechanism of the tissue, although a short duration in the inflammatory phase can shorten the healing process.[19]

On the 7th day observation (Table 2), the density of collagen fibers in the treatment group also showed a higher score than in the control group, with an average collagen fiber density score of 2.67. Figure 3 shows that the density of collagen fibers in the wound area is densely packed (score +3). Figure C shows less tight collagen density in the control group. This is because on the 7th day after injury, the proliferation phase has begun and the fibroblasts proliferation occurs. Fibroblasts have the function for synthesizing collagen. Collagen is secreted into the extracellular space as procollagen.

Furthermore, procollagen splits into terminal segments called tropocollagen. Tropocollagen then joins other tropocollagen molecules to form collagen filaments. These filaments join to form fibrils, which in the end form collagen fibers. The content of vitamin C contained in broccoli extracts helps the process of collagen synthesis. The hydroxylase enzyme needs vitamin C as a cofactor. This hydroxylase enzyme will form hydroxyproline and hydroxylysine, which are required for collagen chain.[12]

On the 14th day of observation (Table 3), the scores of the collagen fibers density in the treatment group increased in comparison to the control group, with the average collagen fiber density score of 3.33. Figure E shows the density of collagen fibers in the wound area being very dense (score +4). This condition is due to the antibacterial effect of glucosinolate, a substance in broccoli which plays a role in optimizing the formation of collagen by preventing tissue damage caused by bacteria and its products.[16]

The increase in collagen fiber density scores in the treatment group at all day observations was also seen in Figure 2, which showed that the line graph of the average collagen density of the treatment group was higher than the line graph of the average collagen density of the control group. In the control group, a wound healing process was observed on all observation days because the wound healing process occurred physiologically, such as in the sequence phase of hemostasis, inflammation, proliferation, and remodeling.[18] In the proliferation phase, fibroblasts migrate and proliferate to form new connective tissue and synthesize collagen, which gives integrity and strength to the tissue. In the remodeling phase, collagen type 3 will gradually replace type 1.[20]

The average collagen fibers density scores on each observation day, the 3rd, 7th and 14th day, showed statistically insignificant differences ($p > 0.05$). This insignificance could be due to the fact that the wounds treated with broccoli extract are wound in healthy experimental animals, so there is no difference in the control group because of the physiological healing that occurs in these healthy animals. Thus, it is necessary to design further studies to evaluate the effect of broccoli on burns or diabetic animals in order to determine whether the extract significantly affects the wound healing process. The other reason is that most wound herbal dressings cannot absorb thick and viscous exudates from the wound. True critical dose assessment of plant extracts and pure compounds is necessary.[17] According to Hakim et al[1], research, red algae from Pulo Aceh had a positive clinical and histological effect on wound healing, with an increase in the amount of collagen in normal rats' oral mucosal lesions following topical application of gel prepared from red algal extract. Study limitations include just observing one parameter, collagen, and only measuring it semiquantitatively.

CONCLUSION

Broccoli extract, which is applied topically, tends to have more benefits related to the process of wound healing, even though there is no significant difference found on statistical tests.

Author Contributions: research articles with several authors, a short paragraph specifying their individual contributions must be provided. The following statements should be used "Conceptualization, HFR and FF.; methodology, ARY.; software, ARY.; validation HFR, and. FF; formal analysis FF.; investigation, ARY.; resources, HFR.; data curation, ARY.; writing original draft preparation, FF.; writing review and editing, HFR; visualization, ARY.; supervision, HFR; project administration, FF.

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Informed Consent Statement: Not applicable for studies not involving humans.

Data Availability Statement: We encourage all authors of articles published in PJD journals to share their research data. In this section, please provide details regarding where data supporting reported results can be found, including links to publicly archived datasets analyzed or generated during the study. Where no new data were created, or where data is unavailable due to privacy or ethical restrictions, a statement is still required.

Conflicts of Interest: The authors declare no conflict of interest.

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