The effects of silkworm cocoon (*Bombyx mori*) wound dressing applications to the COX-2 expression and the number of neutrophils after skin excision wounds (*in vivo* research on Wistar rats) [version 1; peer review: awaiting peer review]

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**Abstract**

**Background:** Silkworm cocoons are materials that have fine regenerating abilities for the human body. Fibroin and sericin present in silkworm cocoons (*Bombyx mori*) are thought to have anti-inflammatory effects. This study aimed to know the effects of the use of wound dressing from silkworm cocoons toward COX-2 expression and neutrophils number in the inflammatory phase after skin excision.

**Methods:** Twelve male Wistar rats according to inclusion criteria were randomly divided into 4 groups, each group of 6, based on the decapitation time (the 3rd day and the 6th day) and based on the dressing material (moist gauze dressing as the control group and silkworm cocoons as the treatment group). Each group was performed an excision on the dorsal skin with subcutaneous depth using a 4 mm-round punch biopsy. Neutrophil cell observations were performed by Hematoxylin eosin staining (HE). COX-2 expression was found in preparations for immunohistochemical staining using rabbit monoclonal COX-2 antibody at sacrificed period on the 3rd and the 6th day after wound dressing application.

**Results:** The number of neutrophils and expression of COX-2 were analyzed using Two-way ANOVA and Independent t-test. The results showed a significant decrease in the number of COX-2 expression on inflammatory cells as well as the number of neutrophils (p<0.005) in the groups treated with wound dressing from silkworm cocoons on both the 3rd and 6th day.

**Conclusions:** It was concluded that the use of wound dressing from silkworm cocoons can inhibit COX-2 expression (p=0.000) and decrease the number of neutrophils in the inflammatory phase after skin excision (p=0.001).

**Keywords**

COX-2; fibroin; neutrophil; sericin; silkworm cocoons; skin excision
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Introduction
Wound healing is a complex dynamic process that results in restoration of anatomic continuity and tissue function after injury. Wound healing is divided into three stages that are interconnected and overlapped in time of occurrence, namely: 1) inflammation; 2) tissue formation (proliferation); and 3) tissue remodelling. Inflammation causes many substances to be released endogenously, known as inflammatory mediators. Arachidonic acid is one of the important inflammatory mediators. Arachidonic acid plays a role in the biosynthesis of prostaglandins through the cyclooxygenase pathway. Cyclooxygenase-1 (COX-1) plays a role in normal physiological functions such as mucus secretion to protect the digestive mucosa and to maintain kidney function. Cyclooxygenase-2 (COX-2) is an enzyme whose presence is influenced by tissue stimulation. These stimuli can be in the form of cytokines, bacterial lipopolysaccharides, inflammation, or other pathological conditions.

Inflammation also results in the accumulation of white blood cells, especially neutrophils and monocytes, at the site of injury to eliminate or limit the causative agent of injury. Neutrophils will perform margination, emigration, chemotaxis and phagocytosis. The short lifespan of neutrophils and the process of phagocytosis by neutrophils will affect the timing of the acute inflammatory phase and continue with the next phase of the healing process.

The wound care method that is currently developing is using the principle of moisture balance known as modern wound dressing, which is stated to be more effective in wound healing. Modern wound dressing is one of the wound care methods in a closed and moist way that is focused on keeping the wound from dehydration and improving wound healing process. Wounds with a moist condition can accelerate fibrinolysis, angiogenesis, reduce the risk of infection, form growth factors, and form active cells. Modern wound dressings that have been developed are made of synthetic polymers and are classified as passive, interactive and bioactive products, in the form of hydrocolloids, alginates, hydrogels, films, and foams.

Silkworms (Bombyx mori) produce cocoons that have many benefits, especially as a basic material for silk products. Silkworm cocoons have a structure, function and protection mechanism that is almost similar to human skin. Silkworm cocoons have been used for a long time as a medical basis and mainly as a suturing material, namely silk thread. Many studies have shown that the main proteins that make up the cocoon structure of silkworms, namely fibroin and sericin, are highly biocompatible materials and have fine regenerating abilities for human tissues. In vitro studies have also shown that fibroin helps cell migration and proliferation by acting as cellular and molecular modulators.

In vitro and in vivo studies on the use of silkworm cocoons as the base material for wound dressings have been carried out, but their use in skin excision wounds has never been proven and no studies have been conducted to determine whether or not there is an effect of using silkworm cocoons wound dressing on the inflammatory phase after skin excision. Based on these reasons, the idea emerged about the research on the effects of using silkworm cocoon wound dressing on wound healing after skin excision on Wistar rats in terms of the COX-2 expression and the number of neutrophils.

Methods
Wound dressing preparation
The making of wound dressings from silkworm cocoons was carried out by following the guidelines made by Yu, et al. (Figure 1). The cocoons used were oval in shape, then they were cut at both ends so that they became tubular and cut back on one side of the tube to form a rectangular sheet measuring 4 \times 1.5 cm. The silkworm cocoons that had been cut were then immersed in a beaker containing a solution of CaCl2-ethanol- H2O with a molar mass ratio of 1:2:8. The beaker containing the cocoon sheets was then placed in a water bath at 58°C for 90 minutes. The silkworm cocoons were then rinsed with sterile distilled water at room temperature four times and dried with silica gel desiccant for 24 hours. Sheets of silkworm cocoons that had been processed were formed into pieces of transparent film measuring 4 \times 1.5 cm with a thickness of 0.7 mm and were ready to be used as wound dressings on wounds. The wound dressing was then sterilized using a sterilization pack and ethylene oxide gas (EOG) at 36°C for seven hours.

Animals and group preparation
This research was approved by the The Research Ethics Committee of the Faculty of Veterinary Medicine, Universitas Gadjah Mada (Approval No. 00108/EC-FKH/Eks./2021). This research is a quasi-laboratory experimental study with 12 male Wistar (Rattus norvegicus) rats based on calculations using the resource of equation method with the minimum sample calculation formula in research with ANOVA design. Sample calculation: Minimum n= \frac{10}{kr +1} =10/(2 \times 2) + 1 = 3.5. Maximum n= \frac{20}{kr +1} = 20/(2 \times 2) + 1 = 6 (k = number of treatments, r = number of repeated measurements). The conclusion is the use of 6 rats meets the sample size requirements. They were randomly divided into four groups of euthanasia day by simple randomisation. Random numbers were generated using the standard = RAND() function in Microsoft Excel. That is, on the 3rd and 6th day after being given treatment, 6 subjects each. Subjects were taken with...
inclusion criteria including: males, three months old, weight $\pm$ 250 grams, looked healthy, physically active, and no anatomical defects. Exclusion criteria included: postoperative pain and infection, death before the euthanasia day and unstable body weight (weight loss $<$20% in a week). The rats were first adapted to the laboratory environment with temperature and humidity that had been adjusted to laboratory standards for one week, given standard feeding and drinking, and placed in individual cages.

Animals treatment
Rats were anesthetized by injection of Ketamine 10% (80-100 mg/kg body weight) and Xylazine (10-12.5 mg/kg body weight) intra peritoneal, then marking the back area to be shaved with a size of $3\times3$ cm with guidelines covering the area to be shaved in the excision that was 0.5 cm right and left of the vertebral column and 2.5 cm from the ears. The hair in the area was shaved and then a re-marking of the area to be excised was made. The skin was excised with a round punch biopsy with a diameter of 4 mm. The biopsy punch was pressed against the skin and then rotated while continuing to be pressed until the tool touched the fascia. The punch biopsy was pulled up along with the excised tissue. The tissue that had been cut by a punch biopsy was removed using tweezers and cut with surgical scissors so that a circular wound was obtained with the base of the wound being fascia. The blood that came out was cleaned with a cotton swab that had been dipped in saline solution (0.9% NaCl).

The post-excision wound of the rat's back was then covered with a film dressing from the cocoons of the silkworm (*Bombbyx mori*) in the treatment group and covered with sterile gauze moistened with saline in the control group (Figure 2). The entire edge of the film dressing was then covered with a Tegaderm film to provide stability to the film dressing so that it could last for the desired length of treatment. All rats were given analgesics in the form of Paracetamol 50 mg/kg body weight ad libitum orally after excision and the wound dressing was changed every day.

Figure 1. Silkworm cocoon dressing process.
The wound tissue sampling from the rats’ back skin of was with a subcutaneous depth of 3×3 cm around the punch biopsy wound area, then attached to cardboard using staples to prevent the tissue from curling before being stored in 10% formalin phosphate buffer solution. Rats were sacrificed by injection of Ketamine 150 mg/kg body weight and Xylazine 30 mg/kg body weight intra peritoneal after tissue sampling, then incinerated according to the standards of the Experimental Animal Laboratory of PSPG UGM.

Samples that had been soaked in formalin were taken to the Anatomical Pathology Laboratory of the Faculty of Medicine, Universitas Gadjah Mada to make preparations. The following parameters were assessed: IHC staining was carried out to see COX-2 expression and HE staining to see the number of neutrophils. COX-2 expression was brown in the cytoplasm in the wound margin area per 6 fields of view with 400x magnification. The calculation results for each field of view on each slide were added up and then divided by 6 to obtain the mean number of COX-2 expression in inflammatory cells. Neutrophils were observed by counting the number of neutrophil cells in the wound margin area using a 400× magnification light microscope, observed in 6 fields of view. The calculation results of each field of view on each slide were added up and then divided by six to obtain the mean number of neutrophils.

Statistical analysis
The data obtained from the observations were tested for normality of the data with the Shapiro-Wilk test and homogeneity test with Levene’s test. The results of the normality test showed that the data was not normally distributed, so data transformation was carried out. The homogeneity test showed that the data was homogeneous, then parametric analysis was carried out with the Two-way ANOVA test to determine the significant difference between the treatment groups and the time of observation. Statistical calculations were performed using statistical package for the social sciences (IBM SPSS Statistics 25) software at a confidence level of 95% (α < 0.05). Independent t-test was used to compare the significance between the control and treatment groups in one observation time.

Results
COX-2 Expression
The calculation of the amount of COX-2 expression in each group was carried out by observing it under a microscope with 400× magnification in 6 fields of view after IHC staining on the 3rd day and the 6th day of observations. The expression of COX-2 was indicated by the presence of brown color expression in the cytoplasm of inflammatory cells in the wound margin area. Figure 3 shows a microscopic picture of COX-2 expression on inflammatory cells, the cytoplasm of cells that absorbs brown color (red arrow) with IHC staining at 400× magnification shows that on the 3rd day of observation time more cells express COX-2 in the group. The control group (Figure 3A) is compared to the treatment group (Figure 3B), as well as on the 6th day, at 400× magnification, the cytoplasm of the inflammatory cells painted brown in the control group (Figure 3C) is more than that of the treatment group (Figure 3D).

The observations results of the number of COX-2 expressions in each treatment group at each observation time were calculated on the average and presented in Table 1.

Table 1 shows that the average number of COX-2 expressions on the 3rd day (39.4133) and the 6th day (20.4417) after excision in the control group is more than the treatment group on the 3rd day (19.4050) and 6th day (4.8050).
An illustration of the number of COX-2 expressions is presented in Figure 4.

Data analysis using Two-way ANOVA showed a significant difference between the number of COX-2 expressions between the observation times on the 3<sup>rd</sup> and the 6<sup>th</sup> day (p=0.001), as well as the comparison of the number of COX-2 expressions between the control and treatment groups (p=0.000), while between the time of observation and the treatment group p=0.614, which meant there was no interaction between the time of observation and the treatment group. The data analysis results of the difference in the number of COX-2 expressions can be seen in Table 2.

Table 1. The mean and standard deviation (SD) of COX-2 expression in each treatment group based on the time of observation.

<table>
<thead>
<tr>
<th>Time of observation</th>
<th>Group</th>
<th>Mean</th>
<th>Std. deviation</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>3&lt;sup&gt;rd&lt;/sup&gt; day</td>
<td>Control</td>
<td>39.4133</td>
<td>15.43508</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Treatment</td>
<td>19.4050</td>
<td>9.96840</td>
<td>6</td>
</tr>
<tr>
<td>6&lt;sup&gt;th&lt;/sup&gt; day</td>
<td>Control</td>
<td>20.4417</td>
<td>8.65926</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Treatment</td>
<td>4.8050</td>
<td>4.88992</td>
<td>6</td>
</tr>
</tbody>
</table>

An illustration of the number of COX-2 expressions is presented in Figure 4.

Figure 3. Microscopic photo of the histology of COX-2 expression on the healing of excision wounds on the back of Wistar rats with a magnification of 400×.

Figure 4. Mean number of COX-2 expressions based on observation time.
Independent t-test was to compare the mean number of COX-2 expression between treatment groups at each observation time. The results of the t-test showed a significant difference (p<0.05) between the control and treatment groups on both the 3rd day (p=0.024) and 6th day (p=0.003).

**Neutrophil number**

The calculation of the number of neutrophils in each group was carried out by observing them under a microscope with 400× magnification in 6 fields of view after HE staining on the 3rd day and the 6th day of observations. Figure 5 shows a microscopic picture of the number of neutrophils, the nucleus of neutrophil cells that absorbs purple color and is oval in shape (black arrow) with HE staining at 400× magnification, that appears on the 3rd day of observation time have more neutrophils in the control group (Figure 5A) compared to the treatment group (Figure 5B), as well as on the 6th day, at 400× magnification, the number of neutrophils in the control group (Figure 5C) is more than that of the treatment group (Figure 5D).

The mean calculation of the observation results of the neutrophils number in each treatment group at each observation time is presented in Table 3.

<table>
<thead>
<tr>
<th>Time of observation</th>
<th>Group</th>
<th>Mean</th>
<th>Std. deviation</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>3rd day</td>
<td>Control</td>
<td>65.5517</td>
<td>33.03853</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Treatment</td>
<td>30.1917</td>
<td>13.94647</td>
<td>6</td>
</tr>
<tr>
<td>6th day</td>
<td>Control</td>
<td>39.1267</td>
<td>25.08374</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Treatment</td>
<td>8.4333</td>
<td>1.42080</td>
<td>6</td>
</tr>
</tbody>
</table>
The mean number of neutrophils on the 3rd day obtained in the control group (65.5517) was more than the treatment group (30.1917). The mean number of neutrophils on the 6th day post-excision in the control group (39.1267) was also higher than the treatment group (8.4333) (Figure 6).

Homogeneity test results obtained homogeneous data results (p=0.117). The results of the analysis using Two-way ANOVA showed that there was a significant difference in the number of neutrophils (p<0.05) both between the days of observation and the treatment group, while between the time of observation and the treatment group was p>0.05, which meant that there was no interaction between the time of observation and the treatment group.

Table 4 shows that the number of neutrophils between the time of observation (the 3rd day and the 6th day) differs significantly (p=0.014), similarly the comparison of the number of neutrophils between the control and treatment groups is significantly different (p=0.001), while between the time of observation and the treatment group is p=0.797, which means that there is no interaction between the time of observation and the treatment group.

Independent t-test was to compare the mean number of neutrophils between treatment groups at each observation time. The results of the t-test showed a significant difference (p<0.05) between the control and treatment groups on both the 3rd day (p= 0.036) and the 6th day (0.014).

**Discussion**

The excision wound was chosen because the excision wound represents a secondary healing condition, has a greater risk of impaired healing, and has a large area of wound that requires dressing application. The excision wound model can be used in research for the healing process of skin wounds.\(^1\)

The calculation of the number of rat samples and the actions taken in this study were in accordance with the principle of reduction in the 3Rs (replacement, reduction, refinement).\(^1\) During the study, all Wistar rats were in healthy condition, stable body weight (did not experience weight loss), no postoperative infection, actively moving with the back area still covered with dressing, so that no rats were excluded in this study. The age of the rats in this study ranged from 3-4 months old. Rats aged 3-6 months can be an ideal comparison of tissues in the human body of young adults. Rats that are too young or too old will have a reduced ability to cope with environmental stress, which can lead to increased comorbidities and reduced skin blood flow.\(^2\)
The excision wound in this study was made on the back of the rats using a round punch biopsy with a diameter of 4 mm. The wound size of 4 mm was chosen because this size is sufficient to represent a secondary wound that can be covered by dressings easily and is still within the wound size limits that can be tolerated by rats, both with 1 and 2 wounds. Wound size that is too large can increase physical and metabolic stress in rats. Physical and metabolic stress can occur in large wounds relative to the total body surface area (TBSA) so that it can affect the results of wound healing studies. In order to prevent the effects of physical and metabolic stress, a wound size of less than 15 mm × 15 mm should be used in rats weighing 200 to 350 grams. Skin excision in rats can be done on any parts of the body because almost all parts of the rat’s body are movable skin, but generally it is done on the back of rats so that observations can be done more easily.10

The wound healing optimization can be done by following several basic principles, including (1) wound moisture, (2) adequate blood supply, and (3) infection minimization. These principles can be facilitated by the use of an ideal wound dressing. The ideal wound dressing requirement is to use a basic material that can protect and cover the wound, maintain moisture, is permeable to air so that the tissue gets an adequate oxygen supply, and is able to inhibit the growth of pathogens without inhibiting tissue growth. The ability of wound dressings to trap moisture in the wound area has proven that epithelial growth is twice as fast as wounds that are not treated with wound dressings. This happens because moisture will prevent the superficial dermis drying. Drying of the superficial dermis causes impaired cell migration and proliferation.15 Moist gauze soaked in NaCl selected as a control group in this study was also based on the principles of ideal wound dressing, so that it could be used as a comparison equivalent to silkworm cocoon wound dressing which also has certain humidity.

The use of silkworm cocoons in its history has been recognized as a pioneer of biopolymer materials in medical applications. Silkworm cocoons, since their discovery until now, have been used as a biocompatible suture material. There have been many studies showing that the main proteins that make up the cocoon structure of silkworms, namely fibroin and sericin, are highly biocompatible materials and have fine regenerating abilities for human body tissues.6,9 This study used silkworm cocoons which were degummed for 90 minutes to be used as the base material for wound dressings used in excision wounds of Wistar rats’ skin.

The time of observation in this study was carried out on the 3rd day and the 6th day after skin excision. The timing of the observation was on the 3rd day because it was the peak of the inflammatory phase as neutrophils reached the highest number in the wound area, while the 6th day was the time when the inflammatory phase had entered its final period.14 Another study on the back skin of rats that were injured using a 6 mm diameter punch biopsy without using a wound dressing showed that the number of neutrophils after a skin wound increased within 12 hours and reached a maximum value on the 2nd day, tending to level off until the 3rd day, then it will decrease drastically by 50% starting on the 5th day. On the 2nd day post-injury, many neutrophils were found in the dermis of the rats’ skin and on the 6th day only a few neutrophils and monocytes or macrophages in the tissue indicating the inflammatory phase were almost complete.15

The stimulus given in this study was an excision wound made using a punch biopsy. In the injured tissue there would be a wound healing process that began with the formation of blood clots and was then followed by an inflammatory phase. The wound healing process will not occur if there is no inflammation and it will be a source of pain. The inflammatory process causes an increase in inflammatory mediators, one of which is prostaglandin which is the result of biosynthesis of arachidonic acid through the cyclooxygenase pathway. Increased prostaglandins will stimulate pain nerves and increase the inflammatory response. The inflammatory response must be controlled because a continuous and long-lasting inflammatory response will cause tissue damage to get worse. Inhibition of the cyclooxygenase pathway is useful to reduce or eliminate inflammatory symptoms.16 The microscopic image of COX-2 expression in the treatment group that was given the silkworm cocoon wound dressing application showed that the inflammatory cells that expressed COX-2 were significantly decreased compared to the control group. Likewise, the microscopic image of the number of neutrophils in the treatment group was seen to decrease significantly compared to the control group on both the 3rd day and the 6th day observations.

The decrease in the amount of COX-2 expression in the treatment group could mean that the silkworm cocoons had the effect of inhibiting COX-2 expression and could be said to have anti-inflammatory effect. Silkworm cocoon wound dressing can inhibit COX-2 expression by the following mechanism: fibroin and sericin contained in silkworm cocoons have the ability to inhibit the activation of NFκB thereby inhibiting the synthesis of IL-1 and TNFα. Inhibition of IL-1 and TNFα synthesis causes reduced stimulation of cell membrane phospholipids, so that arachidonic acid is not released from cell membrane phospholipids by phospholipase activation. This situation causes reduced COX-2 protein synthesis and reduced prostaglandin biosynthesis so that the inflammatory response is reduced.15 The fibroin content in silkworm cocoons has an anti-inflammatory effect by inhibiting the signaling pathway associated with nuclear transcription factor (NF)-κB. Nuclear transcription factor (NF)-κB plays a role in transcription of a number of genes involved in the inflammatory pathway, cellular stress response, and tissue remodeling, including COX-2.15
The results also showed that the mean number of neutrophils in the treatment group given the application of silkworm cocoon wound dressing showed a significant decrease compared to the control group which was only covered with gauze moistened with NaCl, both on the 3rd day and the 6th day observations. The number of neutrophils in the skin of normal rats without wounds was also observed and the result was no neutrophils were found. The significant difference between the number of neutrophils in the treatment and control groups at each observation time was due to the silkworm cocoons having anti-bacterial and anti-inflammatory effects. Silkworm cocoon wound dressings contain sericin which has an antibacterial effect, especially against Streptococcus aureus and Escherichia coli, so it can prevent infections that can interfere the wound healing process, while gauze dressings do not have anti-bacterial abilities. The sericin protein in silkworm cocoons can be degraded and carried into the wound through exudate, skin absorption, and water vapor molecules generated by moisture in the wound area and the sweating process. The mechanism of decreasing the number of neutrophils in the treatment group could also occur due to the influence of the active substance fibroin contained in the silkworm cocoons which has an anti-inflammatory effect that is able to inhibit the activation of NFkB thereby inhibiting the synthesis of IL-1 and TnFα. Inhibition of IL-1 and TnFα synthesis causes vasodilation of blood vessels and increases capillary permeability so that there is no activation of complement factor C5a, and inhibits neutrophil adhesion to blood vessel walls. The adhesion of neutrophils to the vessel wall is the beginning of the movement of neutrophils into the tissue. Inhibition of neutrophil adhesion to blood vessel walls causes the infiltration of neutrophils into the tissue to decrease so that inflammation will decrease.

Conclusion
The application of silkworm cocoon wound dressing can reduce COX-2 expression (p=0.000) and reduce the number of neutrophils significantly (p=0.001) after skin excision.

Data availability
Underlying data
Figshare: Figure 1. Silkworm cocoon dressing process.
https://doi.org/10.6084/m9.figshare.19365596.v4

This project contains the following underlying data:
- JPG file showing the making of wound dressings from silkworm cocoons was carried out by following the guidelines made by Yu, et al. The cocoons used were oval in shape, then they were cut at both ends so that they became tubular and cut back on one side of the tube to form a rectangular sheet measuring 4 x 1.5cm. The silkworm cocoons that had been cut were then immersed in a beaker containing a solution of CaCl2-ethanol-H2O with a molar mass ratio of 1:2:8. The beaker containing the cocoon sheets was then placed in a water bath at 58°C for 90 minutes. The silkworm cocoons were then rinsed with sterile distilled water at room temperature 4 times and dried with silica gel desiccant for 24 hours. Sheets of silkworm cocoons that had been processed were formed into pieces of transparent film measuring 4 x 1.5 cm with a thickness of 0.7 mm and were ready to be used as wound dressings on wounds. The wound dressing was then sterilized using a sterilization pack and ethylene oxide gas (EOG) at 36°C for 7 hours.

Figshare: Figure 2. Making an excision wound. https://doi.org/10.6084/m9.figshare.19373192.v1

This project contains the following data:
- JPG file showing the wound excision on the skin of the rat's back with a punch biopsy and closed with a silkworm cocoon wound dressing on the right back and sterile gauze moistened with NaCl on the left back.

Figshare: Figure 3. Histology of excision wounds COX-2 expression. https://doi.org/10.6084/m9.figshare.19373168.v1

- JPG file showing microscopic photo of the histology of COX-2 expression on the healing of excision wounds on the back of Wistar rats with a magnification of 400x.

Figshare. Figure 2A. wound excision design. https://doi.org/10.6084/m9.figshare.19374851.v1
Figshare. Figure 2B. excision wound on the skin of the rat's back with a punch biopsy. https://doi.org/10.6084/m9.figshare.19374863.v2

Figshare. Figure 2C. close the excision wound with a silkworm cocoon wound dressing on the right back and sterile gauze moistened with NaCl on the left back. https://doi.org/10.6084/m9.figshare.19374866.v1

Figshare. Figure 3A. Microscopic photo of the histology of COX-2 expression on the healing of excision wounds control group on 3rd day. https://doi.org/10.6084/m9.figshare.19374893.v1

Figshare. Figure 3B. Microscopic photo of the histology of COX-2 expression on the healing of excision wounds treatment group on 3rd day. https://doi.org/10.6084/m9.figshare.19374893.v1

Figshare. Figure 3C. Microscopic photo of the histology of COX-2 expression on the healing of excision wounds control group on 6th day. https://doi.org/10.6084/m9.figshare.19374896.v2

Figshare. Figure 3D. Microscopic photo of the histology of COX-2 expression on the healing of excision wounds treatment group on 6th day. https://doi.org/10.6084/m9.figshare.19374896.v2

Figshare. Figure 5A. Microscopic photo of histology of neutrophils on excision wounds control group on 3rd day. https://doi.org/10.6084/m9.figshare.19374909.v1

Figshare. Figure 5B. Microscopic photo of histology of neutrophils on excision wounds treatment group on 3rd day. https://doi.org/10.6084/m9.figshare.19374908.v1

Figshare. Figure 5C. Microscopic photo of histology of neutrophils on excision wounds control group on 6th day. https://doi.org/10.6084/m9.figshare.19374914.v1

Figshare. Figure 5D. Microscopic photo of histology of neutrophils on excision wounds treatment group on 6th day. https://doi.org/10.6084/m9.figshare.19374923.v1


Figshare. Raw data of Cox-2 expression on control group 3rd day. https://doi.org/10.6084/m9.figshare.19376063

Figshare. Raw data of Cox-2 expression on treatment group 6th day. https://doi.org/10.6084/m9.figshare.19376066

Figshare. Raw data of Cox-2 expression on control group 6th day. https://doi.org/10.6084/m9.figshare.19376069


Figshare. Raw data of Neutrophil counts on control group 3rd day. https://doi.org/10.6084/m9.figshare.19376078

Figshare. Raw data of Neutrophil counts on treatment group 6th day. https://doi.org/10.6084/m9.figshare.19376081

Figshare. Raw data of Neutrophil counts on control group 6th day. https://doi.org/10.6084/m9.figshare.19376084

Figshare. Recapitulation data of Cox-2 expression and Neutrophil counts on treatment and control group 3rd and 6th day. https://doi.org/10.6084/m9.figshare.19376093

Extended data
Figshare: Table 1. The mean and standard deviation (SD) of COX-2 expression in each treatment group based on the time of observation. https://doi.org/10.6084/m9.figshare.19373978.v1

- Table 1. Shows that the average number of COX-2 expressions on the 3rd day (39.4133) and the 6th day (20.4417) after excision in the control group is more than the treatment group on the 3rd day (19.4050) and 6th day (4.8050).
Figshare: Figure 4. Mean number of COX-2 expressions based on observation time. https://doi.org/10.6084/m9.figshare.19373894.v1

- JPG file showing diagram illustration of COX-2 as described in Figure 3 and Table 1.

Figshare: Table 2. Statistical analysis. https://doi.org/10.6084/m9.figshare.19373990.v1

- Table 2. Shows statistical analysis using Two-way ANOVA of differences in the amount of COX-2 expression.

Figshare: Figure 5. Microscopic photo of histology of neutrophils on wound healing. https://doi.org/10.6084/m9.figshare.19373933.v2

- JPG file shows microscopic photo of histology of neutrophils on wound healing of Wistar rat back skin excision with 400x magnification.

Figshare: Table 3. Mean number of neutrophils in each treatment group based on observation time. https://doi.org/10.6084/m9.figshare.19374122.v1

- Table 3 shows the mean number of neutrophils on the 3rd day obtained in the control group (65.5517) was more than the treatment group (30.1917). The mean number of neutrophils on the 6th day post-excision in the control group (39.1267) was also higher than the treatment group (8.4333).

Figshare: Figure 6. Mean number of neutrophils based on the observation time. https://doi.org/10.6084/m9.figshare.19373960.v1

- JPG file diagram illustration of number of neutrophils as described in Figure 5 and Table 3.

Figshare: Table 4. Two-way ANOVA statistical analysis of differences in neutrophil counts. https://doi.org/10.6084/m9.figshare.19374185.v1

- Table 4 shows that the number of neutrophils between the time of observation (the 3rd day and the 6th day) differs significantly (p=0.014), similarly the comparison of the number of neutrophils between the control and treatment groups is significantly different (p=0.001), while between the time of observation and the treatment group is p=0.797, which means that there is no interaction between the time of observation and the treatment group.

**Reporting guidelines**

Data are available under the terms of the Creative Commons Attribution 4.0 International license (CC-BY 4.0).

In vivo animal studies follow the ARRIVE reporting guideline.

https://doi.org/10.6084/m9.figshare.19396481

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**References**

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