

RESEARCH ARTICLE

Nigella Sativa Seed Extract Ointment Increasing the Amount of Healing Fibroblast and Collagen on Balb/C Mice

Salep Ekstrak Biji Nigella Sativa Meningkatkan Jumlah Fibroblas dan Kolagen pada Penyembuhan Luka Sayat Mencit Galur Balb/C

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ABSTRAK

Pendahuluan: Luka yang tidak sembuh dalam waktu lama oleh berbagai sebab merupakan masalah yang sering ditemukan di masyarakat. Alternative yang dapat dipakai untuk mempercepat penyembuhan luka adalah *Nigella Sativa* (NS). **Tujuan:** untuk mengetahui peningkatan jumlah fibroblas dan kolagen akibat pemberian salep ekstrak biji NS 40% dan 60% pada penyembuhan luka sayat.

Metode: Dalam *post test only control group design*, sebanyak 48 ekor mencit jantan galur balb/c, dibagi 2 kelompok besar masing-masing 24 ekor. Setiap kelompok kemudian dibagi lagi menjadi 4. Salep ekstrak biji *Nigella sativa* dengan konsentrasi 40% (NS-40) dan 60% (NS-60) dioleskan sebanyak 3x sehari selama 7 hari (fibroblas) dan 14 hari (kolagen). Evaluasi hasil diperoleh dari preparat yang masing-masing menggunakan pengecatan HE dan Masson's Trichrome.

Hasil: analisis statistik *Mann Whitney* menunjukkan bahwa jumlah fibroblast dan kolagen pada kelompok C-G, NS-40, dan NS-60 lebih tinggi bermakna dibanding C-N, $p < 0,05$. Jumlah fibroblast pada NS-40 dan NS-60 tidak berbeda bermakna dibanding C-G, demikian pula dengan CN-40 dengan CN-60, $p > 0,05$. Sedangkan jumlah kollagen pada CN-60 lebih tinggi bermakna dibanding CN-40, $p < 0,05$.

Kesimpulan: pemberian salep ekstrak biji *Nigella sativa* dengan konsentrasi 40% dan 60% pada luka sayat selama 7-14 hari mampu meningkatkan jumlah fibroblas dan kolagen, sehingga terjadi penyembuhan luka sayat lebih singkat.

Kata kunci: salep ekstrak biji jinten hitam (*Nigella sativa*), fibroblas, kolagen, luka sayat.

ABSTRACT

Introduction: Untreated cuts in some period of time, caused by various reasons, can be a problem. Alternative therapy used to speed up the healing of the cut is *Nigella Sativa* (NS). **Objective:** to investigate the number of fibroblast and collagen by giving 40% and 60% NS seed extract on the cut.

Methods: *post test only control group design*, 48 male balb/c strain rats were divided into 2 large groups, with each cage contained 24 rats. Each group then divided to 4. Each group is subdivided into 4. *Nigella sativa* seed extract ointment with a concentration of 40% (NS-40) and 60% (NS-60) smeared 3x daily for 7 days (fibroblasts) and 14 days (collagen). Evaluation derived from preparations using HE staining and Masson's Trichrome respectively.

Results: *Mann Whitney* analysis shows that the number of fibroblasts and collagen in the CG group, NS-40, and NS- 60 Higher than CN, $P < 0.05$. The number of fibroblasts in the NS-40 and NS-60 was not significantly different compared to the CG, as well as CN-40 with CN-60, $p > 0.05$. While the number of collagen in CN-60 were significantly higher than CN-40, $p < 0.05$.

Conclusion : administration of *Nigella sativa* seed extract ointment with a concentration of 40% and 60% in cuts for 7-14 days increase the number of fibroblasts and collagen, resulting in a shorter healing period.

Keywords: black cumin seed extract ointment (*Nigella sativa*), fibroblasts, collagen, cuts.

INTRODUCTION

Cuts, that take longer to recover accompanied by a decline in the patient's immune system makes them more vulnerable to exposed wound microorganisms, causing infections (Anthony, 2004). Incidence of wounds each year increases, both acute and chronic wounds. In the United States there are an estimated 3 million patients have impaired wound healing and estimated millions of dollars per year is used for wound

care to heal faster. Research in America, indicated patients with cuts were 3.5 per 1000 population. Injury incidence rate by etiology of the disease in the world is quite high, so it needs attention. The etiology of these wounds include surgical wounds as much as 110.3 million cases, 1.6 million cases of traumatic wounds, abrasions there are 20.4 million cases, burns 10 million cases, 8.5 million cases of decubitus ulcers, venous ulcers 12.5 million Cases, diabetic ulcers 13.5

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Mastuti, et al.

million cases, amputation 0.2 million per year, 0.6 million carcinoma per year, melanoma 0.1 million, skin cancer complications in 0.1 million cases (Med Market Diligence, 2009).

The wider community povidone iodine is usually used to treat cuts, but the use of povidone iodine can cause allergies and hinder the process of wound healing and scarring, resulting in the need of other treatment alternatives that better (Henry, 2007). *Nigella sativa* (NS) or have a popular name of black seed (black seed) or Seed (Habbat al baraka) turned merupak an plant that has many benefits. One of the benefit is anti-inflammatory role in the wound healing process (Parakh, 2010). *Nigella sativa* can stimulate the activity of cytokines Macrophag Activating Factor (MAF) thereby improving the function of the macrophages plays a role in cellular immune system. *Nigella sativa* also contains saponins that play a role in helping the process of wound healing (angiogenesis) via Vascular Endothelial Growth Factor (VEGF). In addition, the content of zinc in the NS can also help the cut healing in experimental animals. Zinc is known to have the ability to help accelerate the healing of wounds, both as an important activator of enzymes in the formation of protein as well as the bodily defense. Zinc function in replication of fibroblasts, the synthesis of collagen, and also collagen crosslinking in tissue injuries (Niluh, 2012). Zinc deficiency can result in decreased Tensile Strength and delays in wound closure, therefore lubrication NS seed extract is expected to prevent the possibility (Dharma *et al.*, 2010).

This study was conducted to prove the increased number of fibroblasts and collagen in mice balb/c with cuts after administration seed extract ointment NS in dosage by 40% and 60%.

METHODS

In experimental research with draft "*post test only control group design*", as much 48 strain male mice balb/c, are divided into two major groups, each consisting of 24 male for checking the number of fibroblasts and collagen. A total of 24 individuals in each group were then divided into 4 groups randomly, each consisting of 6 rats. Negative control group (CN), only distilled water smeared. Garamycin group (GG) smeared with ointment garamicyn. Group *Nigella sativa* (NS-40) ointment smeared NS 40%. Group *Nigella sativa* (NS-60) smeared with ointment NS 60%. Before getting ointment, The backs s ach Mice Made cuts Along 2 cm?. Ointment smeared 3x daily for 7 days (fibroblasts) and for 14 days (collagen). Day 8 and 14 skin tissue sampling was conducted to make preparations, then

on each of the preparations stainings with HE and T richrome were carried out. A statistical analysis made to count the number of fibroblasts and collagen.

The Preparation of *Nigella Sativa* Extract

Dried *Nigella sativa* (black cumin) seeds of 448 grams, and pulverized in a blender and then added with 96% ethanol of 800 ml, in order to get thick extract with a concentration of 100% weighing 52.2 grams.

Ointment Preparation

Ointment of *nigella sativa* seeds was made using petroleum jelly base material obtained from pharmacies. NS seed extract of 8 grams, added with 12 grams of vaseline resulted in seed extract ointment NS with 40% concentration. Furthermore, to make an ointment with a concentration of 60% by adding 12 grams of seed extract NS on 8 grams of vaseline.

Creating the Wound

Before the cuts were made, first fleece mice will be slashed in the back and shaved clean. Scalpel affixed with a pressed on the backs of mice with a depth of 0.3 cm, then shifted in the direction along the 2 cm. Before making cuts, the mice were anestezized using ether. Then, NS seed extract ointment were applied to the cut according to the treatment group evenly, with a sterile cotton bud as much as 3 times a day.

PREPARATIONS MAKING

Skin tissue is cut on the wound with a thickness of + 2 mm, included in the cassette to the process. Tapes containing skin tissue were put on Tissue Processor with the following sequence: fixation, dehydration, clearing, impregnation, to the the paraffin block. Paraffin blocks were cut using a tool mikrotome with a thickness of 2-3 microns, stretched on the water with a temperature of 45 °C, and was captured with a glass object. Tissues on each glass object were conducted HE staining and Masson's trichrome land then the readings were conducted.

Statistical Analysis

The analysis used in this study is the Kruskal - Wallis followed by Mann Whitney, considering the data obtained is not normal and homogeneous. Statistical analysis was considered significant when p-value <0.05. This research was conducted in the laboratory of Chemistry and Biology, Faculty of Medicine, Sultan Agung Islamic University and the Laboratory

Table 1. The number of Fibroblasts with HE staining (Hematoksili eosin)

Variables	Group				P Kruskall wallis
	C-N	G-G	NS-40	NS-60	
	n = 6 Mean (SD)				
Fibroblast (Σ)	6.83	30.83	155	63.3	0.046
Collagen (Σ)	2.83	14,7	55.43	70.2	0.000

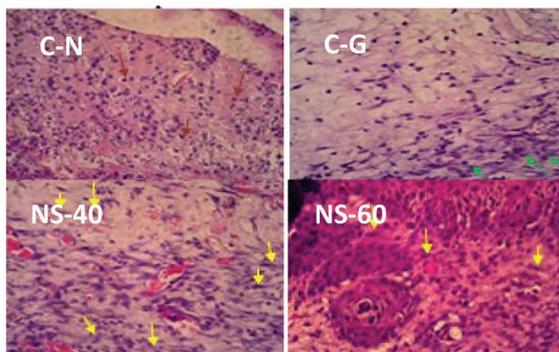


Figure 2. Gambaran mikroskopis dengan pembesaran 400 x dengan pengecatan masson's tricrome. C-N: fibrosit masih sangat sedikit; C-G: fibroblast lebih banyak; NS-40: fibroblast lebih banyak; NS-60: fibroblast mulai berkurang dan luka mulai sembuh terjadi reepitelisasi

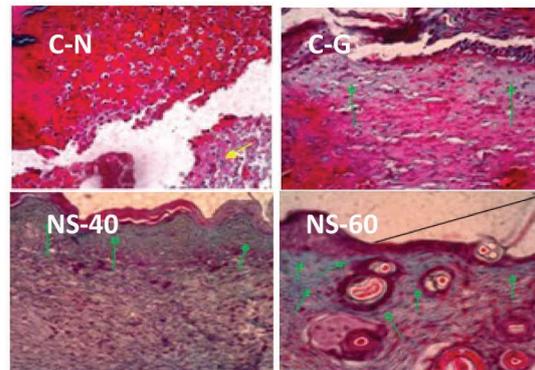


Figure 3. Gambaran mikroskopis dengan pembesaran 400 x dengan pengecatan masson's tricrome. C-N: tampak fibrin; C-G: kolagen semakin tampak berwarna kehijauan; NS-40: jumlah kolagen semakin banyak; NS-60: kolagen tambah banyak dan tampak berwarna kehijauan.

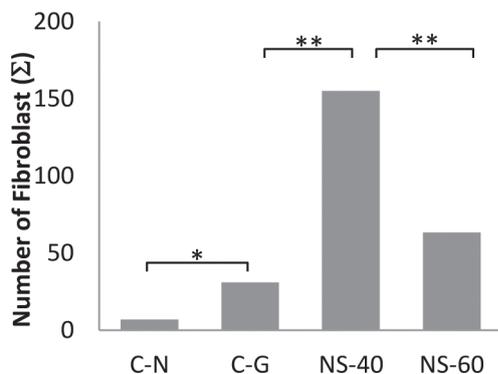


Figure 4. number of Fibroblast. Mann Withney: * p < 0.05; ** p > 0.05

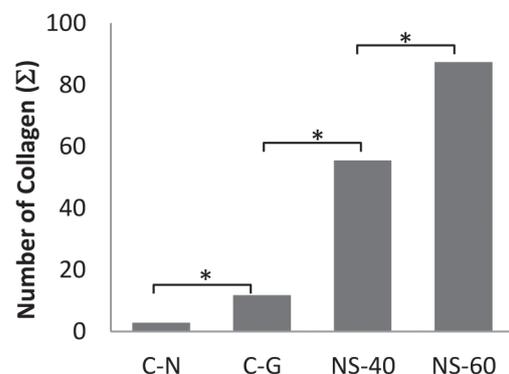


Figure 5. number of Collagen. Mann Withney: * p < 0.05; ** p > 0.05

of Pathology of Sultan Agung Islamic Hospital, after obtaining approval from the ethical committee FK Unissula Semarang.

RESULTS

After 7 and 14 days of treatment, the results and means were obtained as shown in figure and table 1.

The statistical analysis showed that the highest amount of fibroblasts were found in the NS-40 group followed by the NS-60, CG, and the lowest group in the CN group. The highest amount of collagen was found in the NS-60 group followed by the NS-40 group,

CG, and the lowest was the CN group. The result of Kurkall Wallis statistic analysis showed that there was a significant difference between the groups, p < 0.05.

Number of Fibroblasts

Mann Whitney statistical analysis showed that the number of fibroblasts in the NS-60 and CG groups was significantly higher than in the CN group, p < 0.05. While the number of fibroblasts in the NS-40 group tended to be significantly higher than CN, p = 0.06. The number of fibroblasts in the group of NS-60 and NS-40 compared to the CG group, although higher,

Mastuti, et al.

but not significant, $p > 0.05$. D emikian did the number of fibroblasts in the group of NS-40 NS-60, although higher, but not significant, $p > 0.05$ (figure 4).

Number of Collagen

In accordance with the results of statistical analysis Mann Whitney, the amount of collagen in the group of NS-60, NS-40, and CG were significantly higher than the CN group, $P < 0.05$. The amount of collagen in the NS-60 and NS-40 groups were significantly higher than in the CG group, $p < 0.05$. The same thing happened in the NS-60 group compared to the NS-40 group, $p < 0.05$ (figure 5).

DISCUSSION

These results indicate that administration of NS seed extract at a concentration of 40% proved to increase the number of fibroblasts in mice suffered from cuts and healing. The results of this study are supported by an invitro study conducted by Rachman *et al.* showed that *Nigella sativa* in vitro can increase the proliferation Human cells gingival fibroblasts (HGF) monolayer slices are created so that the closing wedge (Rahman *et al.*, 2014).

Healing effect caused by seed extract NS allegedly associated with the saponin contained in NS. Various studies have shown that saponins can help the process of wound yembuhan pen, so that the healing of wounds shorter duration. This is because s aponin have an effect in stimulating the Vascular Endothelial Growth Factor (VEGF), which can then be Activate Transforming Growth Factor beta (TGF- β) and Platelet Derived Growth Factor (PDGF) that resulted in the proliferation of fibroblasts (Scultz, 2003). Saponins may also act as immunomodulators that can induce macrophage cell activity, limfosit T, interleukin-1b, and Tumor Necrosis Factor [(TNF- α) (Suryadi, 2012)]. Furthermore, various s el in the immune system secrete growth factors and cytokines to accelerate wound healing (Surkhail *et al.*, 2011). Moreover, saponin is also proved able to stimulate the synthesis of fibroblasts by fibronectin (Froschle, 2004). This is in line with studies reported by Nova and colleagues. Studies conducted by Nova indicated that administration of nigela sativa extract proved to increase the number of fibroblasts and macrophages activity in healing wounds from rat tooth extraction (Nova *et al.*, 2013). In addition, N S also contains essential fatty acids such as linoleic acid and oleic acid to maintain the integrity of the epidermis as a barrier on the skin, maintaining the water content, and accelerate wound healing by enhancing the migration of immune cells to the wound tissue. Additionally, NS

containing flavonoid known to increase the amount of collagen and stimulate macrophage activity to trigger epithelialisation process, increase the production of extracellular matrix, growth factors, cytokines, and angiogenesis through the release of growth factors such as keratinocytes Growth Factor [(KGF) (Ambiga *et al.*, 2007)]. However, this study has a limitation that is not decisive where the active compound contained in the seed extract *Nigella sativa* that stimulates healing of cuts in mice faster. Therefore it is still require further research to determine the content.

In this study, the number of fibroblasts in the group of 40 tend to be more abundant NS- compared to the NS-60 group on day 7. It can be deduced that the number of fibroblasts in the NS-60 group were less a result of the wound healing process that lasts shorter, so many fibroblasts that have been converted into fibrosit then collagen. The statement is in accordance with the results of the examination of collagen at day 14 in the study, which showed that the amount of collagen in the NS-60 group were significantly higher than those in NS-40 and the group garamycin. Referring to the results of this study, then indirectly it can be said that the total collagen starts to increase at day 7, and gradually reached its peak at day 14.

CONCLUSION

Based on the results of this study as a whole it can be concluded that administration of black cumin seed extract ointment or *Nigella sativa* with a concentration of 40% and 60% in cuts for 7-14 days can increase the number of fibroblasts and collagen, resulting in a shorter healing cuts. *Nigella sativa* With a 60% better concentration in increasing the amount of fibroblasts and collagen than NS with concentrations of 40% and garamycin.

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