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THE COMBINE CREAM MIXTURE OF *NIGELLA SATIVA* AND *OLEA EUROPAEA* FASTEN THE BURN HEALING PROCESS WITH MINIMAL SCAR

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ABSTRACT

Background: *The healing process of burns is influenced by epithelial faster grown, VEGF expression (vascular endothelial growth factor) and the amount of collagen. Nigella sativaoil couldcure the second grade burn wound as effective as silver sulfadiazine cream. Olea europaea accelerates the formation of collagen, reepitelisation and enhances VEGF expression. The combination of both proved its potential in producing faster wound healing with minimal scarring.*

Methods: *This is a true experimental study using "posttest only control group design", including 40 malesWistar rats as subject, body weight ± 200 grams, and maintained at room temperature 280-320C, standard feed and ad libitum drink, adapted and acclimatized a week in Individual cages before treatment. All rats were induced burns in the back area with previous local anesthesia given. All rats are administered cream therapy immediately after burning induction, and repeated daily once for 5 days, and divided into 4 group with the treatment as follows; Group 1 were applied with Sulfadene® (silver sulfadiazine 1%), group 2 by Nigella sativa cream, Group 3 has given olive oil 5% and group 4 has given combination cream ofNigella sativa and olive oil 5%. On the 5th day of the study, each group was randomly assigned 5 rats to terminate the VEGF expression and counting the amount of fibroblast. The 21st day of the study, all groups were terminated and histopathologic examination of scar formation in scar burn areas measured in thickness with Optilab Pro® software.*

Results: *The highest number of fibroblast cells was consecutive group 1 (27.4), group 3 (26.7), group 2 (26.4), and the least group 4 (18.3).VEGF expression did not differ in all groups (Kruskal Wallis, p = 0.074).*

Conclusion:*The combination of Nigella sativa and Olea europea cream mixes speeds the healing of burns with scarring.*

Key words: *Nigella sativa, Oleaeurope, fibroblast, VEGF, burns,scar thickness*

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PENDAHULUAN

The management of complete burning wound/ plenary is to manage the burning wound that cures the wound itself and to minimize the scar tissue that might be occurred. Nowadays, with the esthetic demand for managing the burning wound with giving the antibiotic and liquid balance can only give a result to cure the burning wound itself, and it still cannot solve the problem from the esthetic view. One of the treatment is using the *Olive oil* and *Nigella sativa* which they belief can cure the wound in empirical way. This alternativeis cheaper and the ingredients are easier to get (Parakh, P.M., 2010). *Giving the Olive oil (Olea europaea)* has been tested for incision wound with reapplying it from extra virgin oil type, pure 100% *Olive oil* type, and *Olive pomace oil*, altogether will cure the wound faster and it can be seen from the third day of reapplying it(Tiffani Frezia Kartikaning Candra, no date). The compound inside the *Olive oil* will capable to increase the form of collagen, revitalization and showing the increasing of VEGF protein level in the third day and seventh day (Mehraein Fereshteh, Sarbishegi Maryam, 2012). Then the *Olive oil* mixed with honey and honey shell are capable to cover the wound with the average range 21.9 ± 2.23 days comparing with the group of *Silver sulfadiazine* that ranging 24.7 ± 2.39 days (Atiba, 2015).

The application of *Nigella sativa* oil has been able to increase the recovery for the chemical burn with HC1 80% reapplying it on the 20^{th} days, which almost similar with antibiotic (*Baneocin*®) on 18^{th} days and negative control on 26^{th} (OA, 2009). Giving the *Nigella sativa* oil cured the second degree of burning wound that can be showed from the covering wound area 81.20% compared with 63.31% control, potentially similar with Silver sulfadiazine 82.91% on the 12^{th} days (Surkhail P, Esmaily H, Baghaei A, Shafiee A, 2011). Referring to the research, giving the *Nigella sativa* oil only increased the cure process showed by shorting the timeof epithelium growing, more VEGF contents (Tiffani Frezia Kartikaning Candra, no date)(Mehraein Fereshteh, Sarbishegi Maryam, 2012), more collagen, but has potential for scar tissue because of the positive correlation between VEGF contents and the formed of scar tissues (Tiffani Frezia Kartikaning Candra, no date)(Atiba, 2015), compared with giving the *Nigella sativa* oil itself. The expectation of giving the *Nigella sativa* oil together with *Olive oil* ishopefully, to make the recovery time faster, less VEGF contents and less scar tissue result. Those two combinations are needs to be tested so it can give a result of faster recovery and more esthetic compared with giving the *nigella sativa* oil and *olive oil* separately.

The *Nigella sativa* contents of *flavonoid, tannin, saponin atsiri oil, steroid, terpenoid, alkaloid* and fat that can be extracted from the seed. The *Nigella sativa* defends the water content as the skin barrier and also act as immunostimulan directly to the wound that will increase the cell immune system (Wahyuni, 2004) (Aprilita, 2010). The *Nigella sativa* can stimulate macrophage activating factor (MAF) so it increases the function of macrophage which has role in cellular immune system. The *Nigella sativa* has saponin compound, flavonoid (Aprilita, 2010)(Mazandarani, 2015) which has role for helping the process of the wound recovery (angiogenesis) through VEGF. Another compound is zinc for boosting up recovery process and also as activator enzyme that is important in forming enzyme for proteinogenesis and the defense of the immune body. Zinc may also increase the process of fibroblast replication, synthetic collagen as well as tie up the crossing collagen. Thereby, the lack of Zinc will decrease the tensile strength and delay the covering wound process (Mazandarani, 2015).[14]

Olive oil contains 75% to 85% unsaturated fatty acids (especially oleat and linoleat acids) and 15% to 25% of saturated fatty acids (palmitic and stearic acids). The *Nigella sativa* and *Olive oil* belong to bioflavonoid, which has role as anti-proliferation (reduce proliferation) and works as collagen and fibroblast apoptosis, so it reduces the scar tissue (Mazandarani, 2015) (Najmeh Ebrahimia, Gholamreza Jahed Khaniki, Seyed Ali Keshavarz, Nabi Shariatifara, Ramin Nabizadeha, 2015)(Maha F El Goweini, 2015).

The used of *Silver Sulfadiazine* 1% (SSD 1%) is a standard medicine for burning wound treatment.[18] The main components of SSD cream are sulfa as bacterial static towards gram (+) and gram (-) bacterias. The other component is silver as also a bacteriostatic and has potential to penetrate on necrotic tissue. Thereby, the research needs to be done to prove the effectivities between the *Nigella sativa* with SSD 1%, *Olive oil* with SSD 1%, the combination between the *Nigella sativa* and *Olive oil*

with SSD 1%, as well as the combination between the *Nigella sativa* and *Olive oil* with the *Nigella sativa* and *Olive oil* it self. The result from the research hopefully becomes the solution for better burning wound treatment.

METHODS

This is a true experimental study using "posttest only control group design", including 40 malesWistar rats as subject, Obtained from the Unit of Experimental Animal Breeding of UGM, body weight ± 200 grams, and maintained at room temperature 280-320C, get standard feed and drink ad libitum, adapted and acclimatized a week in individual cages before treatment.

All rats were given local anesthecia and later induced burns in the back area. All rats were administered cream therapy immediately after burning induction, and repeated daily once for 5 days, and divided into 4 groups with the following treatment; Group 1 were applied with *Sulfadene*® (silversulfadiazine/SSD 1%), group 2 by *Nigella sativa cream*, Group3 has given *Olive oil* 5% and group 4 has given combination cream of *Nigella sativa* and *Olive oil* 5%.On the 5th day of the study, each group was randomly assigned 5 rat to termination and examination of the VEGF expression and counting the amount of fibroblast. The 21st day of the study, all groups were terminated and histopathologic examination was performed to assess the formation of scar tissue in the burns area.

Burns Induction

The burns induction was done on the back of Wistar rat that had previously been shaved 2 × 3 cm (Tiffani Frezia Kartikaning Candra, no date). The area was smeared with 5% xylocaine ointment and left for 5 minutes. Grade 3 burns were made by attaching a heated, 11 mm diameter nail, for one minute, to a spine structure with a 1x1x1 cm burn (Mehraein Fereshteh, Sarbishegi Maryam, 2012).

After being harmed with topical way, the group 1 has given SSD 1%, group 2 has given *Nigella sativa* cream 50%, group 3 has given *Olive oil* 5% and group 4 has given combination cream of *Nigella sativa* and *Olive oil* 5% with a flat application treatment using a sterile cotton bud once a day.

Making the cream

The extract cream of *Nigella sativa* made from the dried seed that resulting 448 grams, pureeing it using the blender machine added with 96% ethanol 800 ml, by soxhlet apparatus as the soxhlet appatus result extract 100% consentrate 52.2 grams, then making the cream using the basic cream that available at the medicine store, each 10 grams extract *Nigella sativa* added with 10 grams of basic cream so it became an extract *nigella sativa* with 50% consentration. The *olive oil* cream made from 1 gram *Oleaeuropea* that added by 19 gram basic cream, then mixed it together so become a 5% cream of *Olea europea*. The cream combination or contain mixture of *Nigella* and *Olea* cream is made by 1 gram of pure (100%) *olive oil*, 10 gram extract *Nigella sativa*, those are added by 19 gram basic cream, mixed it together until becoming a combination composition that consists of 50% consentrate *Nigella Sativa* and 5% consentrate *Olive oil* (Oleo europea).

Fibroblast Calculation, VEGF expression and Scar Thickness

Calculating fibroblast were performed on a skin preparation in the burn area excised along ± 2 mm from the outer margin of the wound for tissue processing and slide making with haematoxylin and eosin (H & E) staining. Fibroblast appears as an oval-shaped, oval-shaped cell located in the middle with a smooth granular cytoplasm, calculated at 400 × magnification in 5 field viewings, using the Olympus CX21 light microscope connectedto the Optilab pro® software.

VEGF was a growth factor in blood that plays a central role in the formation of endothelial cells and is an important parameter in angiogenesis in the wound healing process.On the 5th day, VEGF expression began to increase in the wounded tissue after a day post injury. VEGF levels were significantly increased compared with normal skin control at 3–5 days post-wound. Cellular VEGF expression was calculated on all fibroblasts expressing brownish color in the cytoplasm or cell nucleus, using Allred's method with 400x magnification in 5 field view, with Olympus CX 21 microscope connected Optilab Pro®.

The thickness of scar tissue is a measure of the

thickness of the natural defect tissue left behind by the wound healing process (in H & E painting, scarring appears dark red), measured by a sliding range in millimeters (mm) using the Olympus CX21 light microscope connected to connect with the Optilab pro software ®, 400x magnification in 5 field view.

RESULTS

The data of fibroblast was in normal distribution (Shapiro Wilk, p>0.05) with the homogeneity test was 0.140 (p>0.05) or homogeny, so the Oneway Anova has been accomplished (Table 1). The Oneway Annova showed minimum there was one treated group showed the average score of fibroblast which significantly different with other treated group, the score of p=0.000 (p<0.05).

Post Hoc LSD test as the continuing process from Oneway Annova test used to see the difference average score of fibroblast between two groups showed on table 2.

The differences of mean average score of fibroblastin group 1,2, and 3 resulted p>0.05 score showed that giving SSD 1% *Nigella sativa* 50% and *Oleo europa* 5% has equal influenced/affectivity level. Comparing with group 4 resulted p<0.05 score which showed that the combination between *Nigella sativa* 50% and *Oleo europa* 5% have different influenced comparing the treatment of the other group. There are some meaningful differences from each groups of the average score fibroblast resulted from the treatment.

The score of VEGF expression then later tested by spreading normality data with Shapiro Wilk (n=20) sample with p>0.05 result on group 1,2,3 which showed normal data distribution. On group 4 has resulted point p<0.5 which showed abnormal data distribution. The homogenates test has sig score 0.006 (p<0.05) meant the data is not homogeny. Based on that, the requirement of the test using Oneway anova cannot be accomplished, so non-parametric test *Kruskal Wallis* test can be used as the alternative. The result of *Kruskal Wallis* showed there is no significant difference of the average score of VEGF expression from all the four groups, the score of p=0.0074 (p>0.05). The VEGF expression in all treatment groups were not significant, because of that, it has proven that the treatment group on group 1 to 4 has equal influenced/affectivity toward the score of VEGF. The VEGF in group 4 has the lowest average score compared with the other groups, which means the combination between *Nigella sativa* 5% and *Oleo europa* 5% suppressed the VEGF expression on the fibroblast cell.

The thickness of scar tissue then later tested by

Tabel 1. DifferentialTest of the average number of fibroblasts, VEGF expression and scar thickness in all groups

Variables	Group				*p*
	G1	G2	G3	G4	
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	
Fibroblast	27.44 ± 1.29	26.40 ± 0.31	26.72 ± 0.67	18.32 ± 0.60	0.000*
VEGF	7.16 ± 0.26	6.96 ± 0.16	6.76 ± 0.21	5.48 ± 3.44	0.074**
Scar Thickness	262.69 ± 46.45	164.99 ± 31.16	145.87 ± 45.10	106.74 ± 19.41	0.002**

* Oneway Annova, p<0.05, significant
**Kruskal Wallis, p<0.05, significant

Tabel 2. Post hoc LSD for the amount of fibroblast

Group (I)	Group (II)	*p*	Description
Group 1	Group 2	0.058	not significant
	Group 3	0.176	not significant
	Group 4	0.000	significant
Group 2	Group 3	0.539	not significant
	Group 4	0.000	significant
Group 3	Group 4	0.000	significant

Tabel 3. The Mann Whitney test on the thickness scar tissue in all groups

Group (I)	Group (II)	*p*	Description
Group 1	Group 2	0.009	significant
	Group 3	0.009	significant
	Group 4	0.009	significant
Group 2	Group 3	0.347	not significant
	Group 4	0.016	significant
Group 3	Group 4	0.076	not significant

normal spreading data with *Shapiro Wilk* (n=20) samples with $p>0.05$ score result on the group 1, 2, and 4 which showed normal data distribution. On the group 3, taken $p<0.5$ score result which showed abnormal data distribution. The result from homogenate tested taken sig score 0.053 ($p>0.05$) which meant homogeny data. Based on that, the requirement test using one way anova test cannot be accomplished and using nonparametric *Kruskal Wallis* test as the other alternative. The result showed there was one minimum treatment group which showed the different significant average score of scar tissue thickness with other treatment group, with *p* score 0.002 ($p>0.05$). The *Mann Whitney* test as the continued process of the *Kruskal Wallis* test is use for knowing the difference average of score average of the scar tissue with thickness between two groups that can be showed on table 3.

Based on the table 3, showed the comparison of the thickness scar tissue score of group 1 to group 2, 3, and 4 taken score $p<0.05$ which meant giving SSD 1% has influenced differently compare with giving *Nigella sativa* 50% , *Oleo europa* 5% even though the combination between both of them, with the higher score of thickness.

The comparison between two groups compared with group 3 taken not significant results which meant the affectivity for giving *Nigella sativa* 50% compare with *Oleo europa* 5% had an equal mark/affectivity level. The scar tissue resulted by group 4 (combination between *Nigella sativa* 50% and *Oleo europa* 5%) pressed the expression of VEGF on the fibroblast cell and produced low scar tissue correlated with scar tissue from the result of low VEGF scar tissue and same also with scar tissue.

DISCUSSION

The highest average score resulted from the group 1 was *Silver Sulfadiazine* 1%, and known that the use of it was still as a standard medicine for burning wound.[18] The main component of SSD 1% cream is Sulfa as bacterial static towards the gram (+) and gram (-) bacterias. The other components are also silver as bacterial static and have potential to penetrate the necrotic tissue. In the healing process of burning wound, there are 3 phases; inflammation, proliferation and remodeling when it has given SSD cream 1% it will be vanished or reduced bacterial on the burning wound. In inflammation process, it will change the changing of vascular which influence by the size, amount, and permeability of the blood vessel and the cellular changed which caused chemotaxis to the lesion direction. In the proliferation process that it was clearly showed the forming of fibroblast component antibacterial from silver sulfadiazine compared with antibacterial from *Nigella sativa* and *Oleo europea* superior than silver sulfadiazine.

During the recovery wound process, VEGF was resulted by variants of cells, included keratinocytes, mast cell, macrophages, and fibroblast cell (especially). The producing of VEGF in a way to respond the injury has a big role in a lot of aspects in recovery process. During the acute process, VEGF will stimulate permeability of blood vessel and expression of adhesion molecule that will help to recruit some inflammation cells, the VEGF level can influence the level of covering wound/epithelium regrown, angiogenesis, the formation of granules tissue, and the strength of the recovery wound during the proliferation process. VEGF can also promote the form of scar tissue on a form of scar tissue formation in the phase of remodeling. On this research the VEGF expression will be examined from the fibroblast, not from mast cells, not from monocyte/macrophage. The average of VEGF came from the combination treatment of *Nigella sativa* 50% and *Oleo europea* 5%, giving intradermal oleuropein injection, the compound inside the *olive oil* can boost

up the collagen form, epithelium regrown and showed the increased of VEGF protein level on day 3rd and day 7th giving the *Nigella sativa* oil can cure the 2nd degrees burning wound, showed by the close of the wound area 81.2% compared with the control 63.31%, potentially similar with SSD (82.91%) on the 12th day. But when it added by *Nigella sativa* this can reduce VEGF expression on fibroblast.

The thinnest scar tissue resulted from the cream treatment of giving *Nigella sativa* 50% compared with *Oleo europa* 5% compared with the other three treatments *Nigella sativa* oil give a cure to a second degree burning wound, showed by the closure around the wound area 81.20% compare with control 63.31% has similar potential with Silver sulfadiazine 82.91% on the 12th day.

Burn wound healing is a complex dermal and epidermal mechanis such as inflammation, proliferation and collagen synthesis involve in healing process. In this study a full thickness 3th degree. Burn injury was induced surface by placing a heated soldering iron (nail was burned by stove fire, color of nail become red) in temperature more 2000C. The fact the healing process can be take place by itself, does not need any help or spontaneosuly, but the risk factor as infection delay in healing has brought to promote this process. Most important composition of *Nigella sativa* oil and *Olive oil* are flavonoid, saponin, but *olive oil* also have oleoropein. The combination between *Nigella sativa* and *olive oil* have pharmacological rich composition effect and more active antiinflammation, anti-bacterial and more active on scar reduced.

CONCLUSION

There are differences at the amount of fibroblast, VEGF expression and the thickness of scar tissue, between a group taken *Silver sulfadiazine* cream, the amount of fibroblast a group taken *Nigella sativa*, *Olive oil (Olea europaea)*, and the cream combination between *Nigella sativa* and *Olive oil (Oleo europaea)*. Combination cream or mixture of *Nigella sativa* and *Olive oil (Olea europaea)* which may cure process of the burns quickly and reducing less/thinnest scar tissue.

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