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Partial healing effects of St. John's wort oil on the rat excisional wound model

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ABSTRACT

Objective: St. John's wort (SJW) oil (Hypericum perforatum) has been used for its immunomodulatory and anti-inflammatory effects. Several studies have shown the efficacy of SJW on wound healing. The aim of this study is to assess the effectiveness of SJW using a combination of biochemical, histopathological and laser Doppler evaluations.

Materials and Methods: Sixteen young Wistar albino rats were used as case and control groups (having 8 in each group). After anesthesia protocol, 6 mm punch biopsy was taken from six separate sites on the rats' dorsal skin. Three wounds were stitched (closed wounds); three wounds were left as they were (open wounds). SJW oil was administered topically to case group once a day for 14 days. Controls did not receive any treatment.

Results: There was no statistical difference in blood perfusion between the groups. No statistical difference was present between the groups in GPx (glutathione peroxidase)values. Rat MDA (malonyldialdehyde) values were higher in the case group compared to the control group. SJW oil was found to be beneficial and effective within some histological parameters.

Conclusion: SJW may be an effective salve within some parameters. Nevertheless, this judgment is uncertain due to the low sample size. We encourage further studies on this promising natural medicine.

Keywords: Antiinflammatory, fibroblast, glutathione peroxidase, St. John's wort, wound

1. INTRODUCTION

St. John's wort (SJW), also known as *Hypericum perforatum (H. perforatum),* has been used for immunomodulatory, anti-inflammatory and antiseptic purposes for centuries. Its sedative and antidepressive effects are also known in psychiatric science [1-3].

Wound healing begins from the moment the wound is formed and consists of three main phases: inflammation, proliferation, and maturation [4]. After a wound is formed, the first reaction of the body is vasoconstriction and activation of platelets. Then various inflammatory cells such as neutrophils, mast cells and T cells flow to the injured area. Angiogenesis occurs when the inflammatory phase ends. Endothelial proliferation, migration, and branching occur for the formation of new vessels in angiogenesis. As new blood vessels form, fibroblasts proliferate and attack the clot to form granulation tissue that can contract. Some fibroblasts turn into myofibroblasts, allowing the wound edges to converge. Dividing fibroblasts store an extracellular matrix and transform the microenvironment of the wound from an inflamed state to a growth phase[5].

There are several studies showing the activity of SJW on wound healing. The active ingredient of SJW, *H. perforatum*, contains many active ingredients such as biapigenin, hypericin, flavonoids, and hyperforin. *H. perforatum* exerts its anti-inflammatory effect mainly with hyperforins and pseudohypericin compounds. This compound's anti-inflammatory effect triggers the migration of fibroblasts and collagen storage and shortens healing time[6].

Oxidant substances act by destroying the DNA, lipids and membranes of cells. When looking specifically at wound healing, DNA damage affects a wound's proliferation phase by causing collagen degradation. Antioxidants protect cells and tissues against the harmful effects of reactive oxygen species (ROS). Superoxide dismutase and glutathione peroxidase are

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aid wound healing because these antioxidants play an important role in enzymatically inactivating ROS[7].

St. John's wort is widely used, but its clinical benefit has not been demonstrated in appropriate conditions and in such a way as to evaluate several parameters simultaneously. This study was conducted to study the curative effectiveness of SJW on experimental animals by examining macro and micro factors within specific healing parameters.

2. MATERIALS and METHODS

2.1. Study Design and Ethical Approval

This animal experiment protocol was approved by the Local Ethics Committee for Animal Experimentation, University of Health Sciences, Istanbul Mehmet Akif Ersoy Animal Experiments Production and Experimental Research Center (Approval number: 2019/11 on 17.05.2019)

Sixteen female young Wistar albino rats, weights ranging from 220-250 grams, were used in this study. The rats were housed in separate sterile cages under standard conditions with a temperature of 26 ± 2 °C and a 12 hour day-night cycle. Standard rodent food and ad libitum water were provided.

The rats were randomly divided equally into two groups: Group A (St. John's wort treatment group) and Group B (control group). The dorsal skin of the rats in both Group A and B were shaved and cleaned with 70% isopropyl alcohol before being wounded with a 6 mm punch biopsy instrument at six separate sites 1 cm apart from the midline and from each other. Intraperitoneal anesthesia was administered with 7 mg/kg xylazine and 35 mg/kg ketamine before surgical wound formation. The wounds of each rat were numbered from 1 to 6. Wounds numbered 1, 3 and 5 were left as they were (non-stitched=open wounds); and wounds number 2, 4 and 6 were stitched with 4/0 vicryl sutures and defined as closed wounds (sutured wounds).

St. John's wort oil was administered topically to Group A, once a day for 14 days of treatment. The administration of the oil started right after surgical wound formation. *St. John's extract oil was provided by Zadevital ®, Konya, Turkey*. The animals in Group B did not receive any treatment.

Rats in both groups were inspected during the whole process for any possible infections, toxicity or side effects.

Punch biopsy was obtained from both open and closed wounds for histopathologic examination. These samples were evaluated to determine polymorphonuclear nuclei leukocytes (PMN) infiltration, angiogenesis, collagen and reepithelization on the 7th, 14th, and 21st days of the experiment. Another punch biopsy was taken from a separate wound for the evaluation of superoxide dismutase, glutathione peroxidase, malonyldialdehyde (SOD, GPx, and MDA) by using ELISA on the 14th day. In addition, both the open and sutured wounds of Group A and Group B were evaluated by PeriScan PIM 3 System Laser Doppler Blood Perfusion Imager (Perimed. Järfälla, Sweden) in order to calculate perfusion on the 0th, 7th, 14th and 21st days. The rats were photographed on the 0th, 7th, 14th and 21st days.

2.2. Evaluation of the Wounds

The PeriScan PIM 3 system was used to evaluate the wounds with laser Doppler. This system measures blood flow at the microcirculation level without touching the tissue, then transfers the data to computer software. Two-dimensional mapping can thus be done easily. In this study, blood flow was evaluated in healing tissue using the PeriScan PIM 3 system.

Antioxidant capacity was evaluated with superoxide dismutase (SOD) and glutathione peroxidase (GPX), while malondialchehyche (MDA) was used as an oxidative marker. Tissue samples obtained by punch biopsy were used for ELISA method evaluation of these items on day 14. Standard OD (optical density) and concentration values (ng / ml) were evaluated by an optic reader at 450 nm. Sunred Biotechnology (Shanghai, China) provided Ready-ELISA kits. (Catalogue numbers of MDA, GPX and SOD respectively: 201-11-0157/ 201-11 – 1705/ SOD: 201-11-0169).

Neovascularization, inflammation, fibroblast activation levels and granulation of both groups were evaluated histopathologically by a pathologist blinded for this study. The pathologist applied a specific scale for histopathological grading in this experiment. After fixing the tissues in paraffin, 4 µm tissue pieces with a thickness of 4 meters were cut. The histological evaluation was performed under a light microscope. All prepared slides were scanned using a digital pathology system (3D Histech company, P250 – Flash III Digital Scanner, 20x), and microscopic photos were taken using special software (3D Histech company, CaseViewer program, tiff format and 300 dpi). Microscopic areas involving neovascularization, inflammation, granulation and fibroblast regeneration were calculated with this software. Ratios of the above-mentioned parameters were proportioned over the entire tissues. According to this software, the proportioned structures were divided into 5 subgroups. Level 1: 0-20%, Level 2: 21-40%, Level 3: 41-60%, Level 4: 61-80%, Level 5: 81-100% of the total tissue.

Statistical Analysis

SPSS 22.0 Statistical package program was used for statistical analysis. Certain comparisons were made to examine the effects of St. John's wort oil of control and treatment groups on subjects. The conformity of the values of the subjects to the normal distribution was tested by the Shapiro Wilk method. Parametric tests (independent t-test) were applied on values that are suitable for normal distribution. Nonparametric tests (Mann-Whitney U test) were used to assess values that were not suitable for normal distribution. In addition, Chi-Square analysis was made for comparison of categorical data. Comparison results in 95%; it was evaluated at a significance level of $p < 0.05$.

3. RESULTS

PeriScan PIM 3 System Laser Doppler Blood Perfusion Imager was used to calculate perfusion of the wounded rats.

Open Wounds

There was no statistically significant difference between Group A (St. John's wort, 66.71 ± 10.80) and Group B (control, 61.83 ± 4.60) on day 0 in subjects with open wounds (p> 0.05). On the 7th day, there was no statistically significant difference between the A and B groups (p> 0.05, 79.17 ± 11.18 and 74.68 + 10.98, respectively). There was no statistically significant difference in groups A and B on day 14 (p> 0.05, 77.76 ± 18.07 and 80.95 + 20.11, respectively). On day 21, no statistically significant difference was found between the control (78.91 ± 7.63) and St. John's Wort (69.00 ± 17.29) group types (p> 0.05).

Closed Wounds

On day 0, there was no statistically significant difference between A (76.88 ± 14.80) and B (75.99 ± 14.80) groups (p> 0.05). There was no statistically significant difference between the A (78.43 ± 8.63) and B (81.35 ± 9.97) groups in the measurement values on the 7th day (p> 0.05). There was no statistically significant difference between A (78.03 ± 16.22) and B (75.17 ± 13.61) groups on day 14 (p> 0.05). On the 21st day, there was no statistically significant difference between the groups (A: 63.52 ± 9.74, B: 69.64 ± 8.57) (p> 0.05).

RAT GPX

Open wounds

There was no statistically significant difference in the standard OD values between Group A (0.38 ± 0.16) and Group B (0.52 ± 0.16) (p> 0.05). In addition, the concentration (ng/ml) values did not differ statistically between Group A (5.27 ± 2.48) and Group B (21.78 ± 8.54) (p> .05).

Closed wounds

For the standard OD values, there was no statistically significant difference between Group A (0.40 ± 4.69) and Group B (0.40 ± 0.097) (p> 0.05). In addition, the concentration (ng/ml) values were not statistically different between Group B (15.66 ± 4.69) and Group A (5.27 ± 2.48) (p> 0.05).

RAT MDA

Open wounds

There was no statistically significant difference between Group A (0.27 ± 0.11) and Group B (0.24 ± 0.11) in terms of standard OD values (p> 0.05). Concentration (ng/ml) values were also not statistically different between Group A (5.27 ± 2.48) and Group B (4.82 ± 2.53) (p> 0.05).

Closed wounds

A statistically significant difference was found between Group A (0.36 ± 0.053) and Group B (0.15 ± 0.029) in the standard OD values (p = 0.002; p <0.05). In this case, it was found that the MDA values were higher in subjects belonging to Group A. There was also a statistically significant difference in the concentration (ng / ml) values between Group A (7.35 ± 1.66) and B (2.84 ± 0.35) (p = 0.002; p <0.05). MDA values were higher in the Group A.

Table 1. *PeriScan PIM 3 System Laser Doppler Blood Perfusion Imager-Evaluation of Blood Perfusion*

Open	**Group type**			
	B (n=3 wounds)	**A (n=3)**	**t**	**p**
Day 0	61.83±4.60	66.71±10.80	-0.721	0.511
Day 7	74.68±10.98	79.17±11.18	-0.496	0.646
Day 14	80.95±20.11	77.76±18.07	0.204	0.848
Open	**Group type**			
	B (n=3)	**A (n=3)**	**U**	**p**
Day 21	78.91±7.63	69.00±17.29	2.000	0.400
Closed	**Group type**			
	B(n=3	**A (n=3)**	**t**	**p**
Day 0	75.99±14.80	76.88±14.80	-0.076	0.943
Day 7	81.35±9.97	78.43±8.63	0.383	0.721
Day 14	75.17±13.61	78.03±16.22	-0.234	0.826
Closed	**Group type**			
	B (n=3)	**A (n=3)**	**U**	**p**
Day 21	69.64±8.57	63.52±9.74	2.000	0.400

p<0.05

Table 2. *Determination of SOD, GPX, and MDA by ELISA method*

Rat_GPX	**Group Type**			
Open	**B (n=6)**	**A (n=6)**	**t**	**p**
Standard OD	0.52±0.16	0.38±0.16	2.013	0.090
Concentration(ng/ml)	21.78±8.54	14.29±2,56	2.057	0.086
Closed	**B (n=6)**	**A (n=6)**	**t**	**p**
Standard OD	0.40±0.097	0.40±4.69	0.156	0.879
Concentration (ng/ml)	15.66±4.69	15.19±2.76	0.210	0.838
Rat_MDA	**Group Type**			
Open	**B (n=6)**	**A (n=6)**	**t**	**p**
Standard OD	0.24±0.11	0.27±0.11	-0.360	0.726
Concentration (ng/ml)	4.82±2.53	5.27±2.48	-0.311	0.763
Closed	**B (n=6)**	**A (n=6)**	**U**	**p**
Standard OD	0.15±0.029	0.36±0.053	0.000	0.002*
Concentration (ng/ml)	2.84±0.35	7.35±1.66	0.000	0.002*
Rat_SOD	**Group Type**			
Open	**B (n=6)**	**A (n=6)**	**t**	**p**
Standard OD	0.49±0.051	0.31±0.068	5.083	0.000*
Concentration (ng/ml)	8.54±0.879	5.69±1.04	5.109	0.000*
Closed	**B (n=6)**	**A (n=6)**	**t**	**p**
Standard OD	0.64±0.21	0.26±0.037	4.133	0.008*
Concentration (ng/ml)	11.41±4.10	4.97±0.54	3.811	0.012*

**p<0.05*

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RAT SOD

Open wounds

A statistically significant difference was found between Group A (0.31 ± 0.068) and Group B (0.49 ± 0.051) in the standard OD values (p = 0.000; p <0.05). There was also a statistically significant difference in the concentration (ng / ml) values between Group A (5.69± 1.04) and Group B (8.54 ± 0.879) (p = 0.000; p <0.05). SJW has not been found to be effective within this parameter.

Closed wounds

There was a statistically significant difference between Group A (0.26 ± 0.037) and Group B (0.64 ± 0.21) in terms of the standard OD values (p = 0.008; p <0.05). There was also a statistically significant difference between Group A (4.97 ± 0.54) and Group B (11.41 ± 4.10) groups for concentration (ng/ml) values (p = 0.012; p <0.05). SJW has not been found to be effective within this parameter.

Histopathogical results

Open wounds

There was no statistically significant difference between the inflammation and granulation levels on the 7th day in open wounds depending on group type (p> 0.05).

According to day 14 values, a statistically significant difference was found in inflammation levels depending on group type (p = 0.000; p <0.05). When this difference was examined, the first level inflammation rate was higher in the control group than the first level inflammation in Group A. In Group A, 2nd and 3rd level inflammation rates were higher than in Group B. There was also a relationship between neovascularization levels and group type. According to these values, there was a statistically significant difference in neovascularization levels depending on group type (p = 0.001; p <0.05). While 1st level neovascularization was high in Group B, 2nd level neovascularization was dominant in Group A. In addition, there was a statistically significant difference in fibroblast levels depending on group type (p = 0.000; p <0.05). While 1st and 3rd levels fibroblasts were higher in Group B, 2nd level fibroblasts were higher in Group A.

According to day 21 values, it was observed that there was a statistically significant relationship between inflammation levels and group type (p = 0.000; p <0.05). The first-level inflammation rate in the control group was higher than in Group A, whereas 2nd level inflammation was higher in Group A. There was no statistically significant relationship between neovascularization and granulation levels and group type (p> 0.05). There was a statistically significant difference between fibroblast levels depending on group type (p = 0.000; p <0.05). The ratio of first – and second-level fibroblasts in Group Bwas higher than in Group A. In Group A, the rate of third-level fibroblasts was higher than Group B.

Table 3. Histopathological Evaluation of the Open Wounds

Day	Open	Group type				
		Level	**B (n=8)**	**A (n=8)**	Chi square	p
Day 7	Inflammation	2	0(0%)	1(12.5%)	1.067	0.302
		3	8(100%)	7(87.5%)		
		Level	**B (n=8)**	**A (n=8)**	Chi square	p
	Granulation	2	0(0%)	1(12.5%)	1.067	0.302
		3	8(100%)	7(87.5%)		
		Level	**B (n=8)**	**A (n=8)**	Chi square	p
Day 14	Inflammation	1	8(100%)	0(0(0%)	16.000	0.000*
		2	0(0%)	7(87.5%)		
		3	0(0%)	1(12.5%)		
		Level	**B (n=8)**	**A (n=8)**	Chi square	p
	Neovascularization	1	7	0(0%)	12.444	0.001*
		2	1(12.5%)	8(100%)		
		Level	**B (n=8)**	**A (n=8)**	Chi square	p
	Fibroblast	1	1(12.5%)	0(0%)	16.000	0.000*
		2	7(87.5%)	0(0%)		
		3	0(0%)	8(100%)		
		Level	**B (n=8)**	**A (n=8)**	Chi square	p
Day 21	Inflammation	1	8(100%)	1(12.5%)	12.444	0.000*
		2	0(0%)	7(87.5%)		
		Level	**B (n=8)**	**A (n=8)**	Chi square	p
	Neovascularization	1	1(12.5%)	0(0%)	1.067	0.302
		2	7(87.5%)	8(100%)		
		Level	**B (n=8)**	**A (n=8)**	Chi square	p
	Granulation	1	1(12.5%)	0(0%)	1.874	1.000
		2	7(87.5%)	7(87.5%)		
		3	0(0%)	1(12.5%)		
		Level	**B (n=8)**	**A (n=8)**	Chi square	p
	Fibroblast	1	1(12.5%)	0(0%)	16.000	0.000*
		2	7(87.5%)	0(0%)		
		3	0(0%)	8(100%)		

*p<0.05

Closed wounds

There was no statistically significant relationship between neovascularization and granulation levels and group type according to 7th-day values (p> 0.05).

When values were examined again on the 14th day, a statistically significant difference was found between neovascularization levels depending on group type (p = 0.000; p <0.05). The 1st level neovascularization rate in Group A was higher than in the control group, whereas 2nd level neovascularization was higher in the control group. There is no statistically significant

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difference between the granulation and fibroblast levels on day 14 depending on the group type (p> 0.05).

According to day 21 values, there is a statistically significant difference between inflammation levels depending on group type (p = 0.000; p <0.05). The 1st level inflammation rate was higher in Group A compared to the control group. However, the 2nd level inflammation rate was higher in the control group. There is a statistically significant difference between neovascularization levels depending on group type (p = 0.001; p <0.05). The rate of first-level neovascularization in Group A was higher compared to the control group. In the control group, the rate of second-level neovascularization was higher compared to Group A. There was also a significant relationship between granulation levels depending on group type on day 21 (= 0.000; p <0.05). While the 1st level granulation rate was higher in the control group, 2nd level granulation was found to be higher in Group A. There was no statistically significant difference between fibroblast levels depending on group type (p> 0.05).

Table 4. *Histopathological Evaluation of the Closed Wounds*

Day	Closed	Group type				
		Level	**B(n=8)**	**A (n=8)**	Chi square	p
Day 7	Neovascularization	2	0(0%)	1(12.5%)	1.067	0.302
		3	8(100%)	7(87.5%)		
		Level	**B (n=8)**	**A (n=8)**	Chi square	p
	Granulation	2	0(0%)	1(12.5%)	1.067	0.302
		3	8(100%)	7(87.5%)		
		Level	**B (n=8)**	**A (n=8)**	Chi square	p
Day 14	Neovascularization	1	0(0%)	8(100%)	16.000	0.000*
		2	8(100%)	0(0%)		
		Level	**B (n=8)**	**A(n=8)**	Chi square	p
	Granulation	1	0(0%)	2(25%)	2.286	0.467
		2	8(100%)	6(75%)		
		Level	**B (n=8)**	**A (n=8)**	Chi square	p
	Fibroblast	2	1(12.5%)	1(12.5%)	0.000	1.000
		3	7(87.5%)	7(87.5%)		
		Level	**B (n=8)**	**A (n=8)**	Chi square	p
Day 21	Inflammation	1	0(0%)	8(100%)	16.000	0.000*
		2	8(100%)	0(0%)		
		Level	**B (n=8)**	**A (n=8)**	Chi square	p
	Neovascularization	1	1(12.5%)	8(100%)	12.444	0.001*
		2	7(87.5%)	0(0%)		
		Level	**B (n=8)**	**A (n=8)**	Chi square	p
	Granulation	1	8(100%)	0(0%)	16.769	0.000*
		2	0(0%)	7(87.5%)		
		3	0(0%)	1(12.5%)		
		Level	**B (n=8)**	**A (n=8)**	Chi square	p
	Fibroblast	2	1(12.5%)	1(12.5%)	0.000	1.000
		3	7(87.5%)	7(87.5%)		

**p<0.05*

No adverse effects, toxic effects, or infections were observed in rats in either the control or treatment groups.

4. DISCUSSION

A wound is formed by a physical or infective process in the anatomical and physiological structure of the skin, which causes a set of inflammatory events. Wound healing and the effect of St John's wort oil as a salve have been much discussed in the literature. However, no study was found in which several parameters were co-examined. Our experiment differs from previous studies by showing that SJW may not be effective in every phase and parameter of wound healing. Unlike findings presented in the literature, we did not detect any improvement

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in GPx, SOD, and MDA values in our study. However, we did find that St. John's wort oil may have a positive effect on the neovascularization healing phase. This substance may be effective in the epithelialization and contraction phases as well, but larger series may be required to verify our hypothesis.

Inflammation is the result of complex events. Even a simple inflammatory change is the result of various, interrelated biochemical events and cellular activities. Each successive stage of inflammation depends on the functioning of regulatory and inhibitory systems that prevent excessive response to the stimulus that initiated the event. During development of the event, active cells, pharmacological substances, cell surface adhesion molecules, and receptors all change. It should be considered that the cells or mediators involved in inflammation are the results of dynamic, interlinked changes[8,9].

During wound healing, many growth factors, cytokines, enzymes, and other various molecules are secreted from local tissues to promote recuperation. Fibroblast growth factor 2 (FGF-2) and vascular endothelial growth factor (VEGF) are necessary for stimulating angiogenesis and forming granulation tissue[3,9]. Disruption of the balance between free oxygen radicals and antioxidants fighting against them has been associated with tissue damage, oxidative stress, and retardation of wound healing[10].

The effective use of St. John's wort oil may be due to its stimulating effect on keratinocyte differentiation, collagen production, and fibroblast motility, as well as its anti-inflammatory and antimicrobial properties. Other compounds including hyperforin, hypericin, and their derivatives like SJW have also been found to be useful in the treatment of skin abrasions, ulcers and burns[11]. Therefore, our research used a rat model to test this hypothesis for the first time by evaluating the effect of St John's wort extract on wound healing, inflammation, free oxygen radicals, and perfusion.

Antioxidant enzymes effective in preventing or repairing the damage of free oxygen radicals include catalase (CAT), glutathione peroxidase (GPx), and superoxide dismutase (SOD) [12]. In many studies examining the effect of St. John's wort extract on the release of pro-inflammatory cytokines, it was observed that IL-6 synthesis decreased with inhibition of the release of substance P[13,14]. The antioxidant effect of St John's wort extract has also been demonstrated in many in vivo and in vitro experiments. This extract protects many cell types against extensive free oxygen radical injury[15-17]. It is reported that the antioxidant effect acts by directly reducing ROS due to the flavonoids contained in St John's wort extract[16], an outcome made possible by increasing the gene expression of the main antioxidant enzymes[18]. Thus, it is reasonable to evaluate antioxidant enzymes such as CAT, SOD, and GSH in granulation tissue[10].

In our analysis, GPx, SOD, and MDA levels were measured on the 14th day. These levels were not found to be in keeping with expected values. One of the reasons may be that St. John's wort oil was used directly without a carrier such as a liposome. A second reason may be related to whether SJW is effective. Although studies prove the positive curative effect of SJW, our findings in antioxidant parameters are not correlated in this study design. However, in an excisional mouse wound model made by Han et al., GPx and SOD levels measured on the 7th and 14th days were found to be higher in the hypericum perforatum group compared to the control group. Also, MDA levels were found to be higher in the control group[19]. Large-scale studies are required to elucidate conflicting data.

In both acute and chronic wounds, the activity of enzymatic antioxidants such as superoxide dismutase, calatase and glutathione peroxidase decreases, since excessive amounts of antioxidants are released when high oxidative stress occurs. In addition, this high oxidative stress load causes a decrease in non-enzymatic antioxidants such as glutathione and vitamins C and E[20]. It has been reported that the phytochemicals contained in SJW extract include substances such as vitamin C, flavonoids (such as quercetin and kaempferol), and carotene [2]. Furthermore, it can be said that these substances may have been effective in increased epithelization and wound contraction on the 14th day in the open wound group. However, this must be confirmed by further research.

This study could not demonstrate the effects of St John's wort total extract formula on the healing of epithelial wounds and wound closure. For example, wound perfusion was not statistically different between the control and SJW group. However, histopathologically, 14th day neovascularization levels were higher in the treatment group (open wounds). The incompatibility between the two parameters may be due to the periscan laser system being user-dependent. In addition, although the fact that it causes improvement within some of the parameters mentioned above cannot be denied, St. John's wort oil still needs further investigation. We are optimistic this herbal extract, which is an ethnomedical drug, is likely to reveal new wound treatment options in studies designed to test its healing properties on humans.

Compliance with the Ethical Standards

Conflict of Interest: The authors have no potential conflicts of interest to disclose.

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REFERENCES

[1] Belwal T, Devkota HP, Singh MK, et al. St. John's Wort (Hypericum Perforatum) in Nonvitamin and Nonmineral Nutrirional Supplements. Nabavi SM, Silva AS. Elsevier Inc.; 2019. Chapter 3.40. https://doi.org/10.1016/C2016-0-03546-5

[2] Klemow KM, Bartlow A, Crawford J, Kocher N, Shah J, Ritsick M. Medical Attributes of St. John's Wort (Hypericum

perforatum). In: Benzie IFF, Wachtel-Galor S, editors. Herbal Medicine: Biomolecular and Clinical Aspects. 2nd ed. Boca Raton (FL): CRC Press/Taylor & Francis; 2011. Chapter 11. PMID: 22593920

[3] Barrientos S, Brem H, Stojadinovic O, et al. Clinical application of growth factors and cytokines in wound healing. Wound Repair Regen 2014;22:5:569-78. doi:10.1111/wrr.12205

[4] Topal Hançer A, Yılmaz Pınar. Editöre mektup: Balın Yara İyileşmesi Üzerine Etkisinin Değerlendirilmesi. Bozok Tıp Derg 2019;9:1:152-9. doi:10.16919/bozoktip.399768

[5] Rodrigues M, Kosaric N, Bonham CA, et al. Wound healing: A cellular perspective. Physiol Rev 2019;99:1:665-706. doi:10.1152/physrev.00067.2017

[6] Gunpinar S, Kilic OA, Duran I, et al. Tosun M, Firat T, Soyler G. Evaluation of the Effect of Topical Hypericum perforatum Oil on Excisional Palatal Wound Healing in Rabbits. J Investig Surg 2020;33:1:49-58. doi:10.1080/08941.939.2018.1474980

[7] Abd Jalil MA, Kasmuri AR, Hadi H. Stingless bee honey, the natural wound healer: A review. Skin Pharmacol Physiol 2017;30:2:66-75. doi:10.1159/000458416

[8] Trautinger F, Schwarz T, Luger T. Cytokines in dermatotherapy. J Dermatolog Treat 1992;3:3:143-152. doi:10.3109/095.466.39209088710

[9] Morelli T, Neiva R, Nevins ML, et al. Angiogenic biomarkers and healing of living cellular constructs J Dent Res. 2011;90:4:456-62. doi:10.1177/002.203.4510389334

[10] Murthy S, Gautam MK, Goel S, et al. Evaluation of in vivo wound healing activity of Bacopa monniera on different wound model in rats. Biomed Res Int 2013;2013. doi:10.1155/2013/972028

[11] Yücel A, Kan Y, Yesilada E, Akın O. Effect of St.John's wort (Hypericum perforatum) oily extract for the care and treatment of pressure sores; a case report. J Ethnopharmacol 2017;196:236-41. doi:10.1016/j.jep.2016.12.030

[12] Grundmann O, Lv Y, Kelber O, et al. Mechanism of St. John's wort extract (STW3-VI) during chronic restraint stress is mediated by the interrelationship of the immune, oxidative defense, and neuroendocrine system. Neuropharmacology 2010;58:4-5:767-73. doi:10.1016/j.neuropharm.2009.12.014

[13] Fiebich BL, Höllig A, Lieb K. Inhibition of substance P-induced cytokine synthesis by St. John's wort extracts. Pharmacopsychiatry 2001;34: 1:26-8. doi:10.1055/s-2001-15462

[14] DeRose V, Robbins R, Snider R, et al. Substance P increases neutrophil adhesion to bronchial epithelial cells. J Immunol 1994;152:3:1339–46

[15] Lu YH, Du C Bin, Liu JW, et al. Neuroprotective effects of Hypericum perforatum on trauma induced by hydrogen peroxide in PC12 cells. Am J Chin Med 2004;32:3:397-405. doi:10.1142/S0192415X04002053

[16] Silva B, Oliveira PJ, Dias A, et al. Quercetin, kaempferol and biapigenin from hypericum perforatum are neuroprotective against excitotoxic insults. Neurotox Res 2008;13:3-4:265-79. doi:10.1007/BF03033510

[17] Zou Y, Lu Y, Wei D. Antioxidant activity of a flavonoid-rich extract of Hypericum perforatum L. in vitro. J Agric Food Chem 2004;52:16:5032-39. doi:10.1021/jf049571r

[18] Sánchez-Reus MI, Gómez del Rio MA, Iglesias I, et al. Standardized Hypericum perforatum reduces oxidative stress and increases gene expression of antioxidant enzymes on rotenone-exposed rats. Neuropharmacology 2007;52:2:606-16.doi:10.1016/j.neuropharm.2006.09.003

[19] Han MC, Durmuş AS, Sağliyan A, et al. Effects of Nigella sativa and Hypericum perforatum on wound healing. Turkish J Vet Anim Sci 2017;41:1:99-105. doi:10.3906/vet-1603-26

[20] Bilgen F, Ural A, Kurutas EB, Bekerecioglu M. The effect of oxidative stress and Raftlin levels on wound healing. Int Wound J 2019;16:5:1178-84. doi:10.1111/iwj.13177