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The Effects of Opium Seed Oil Massage on Oxidant-Antioxidant Status and Biochemical Parameters

Haşhaş Yağı Masajının Oksidan-Antioksidan Statü ile Bazı Biyokimyasal Parametreler Üzerine Etkilerinin Araştırılması

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ABSTRACT

In this study, it was aimed to examine the effects of oxidant-antioxidant status and massage applicati on swith opium oil on some blood values. After the study, the question of whether opium oil can be used as aromatrapic massage oil was sought to be answered. Thirty healthy women between the ages of 18-25 participated in thestudy. Ten of the participants were divided as control group, 10 as vaseline group and 10 as poppy oil group. Vaseline and poppy oil groups received general massage for an average of 30-40 minutes 3 days a week for 8 weeks. Blood was taken 4 times from all groups. The fatty acids of the opium oil to be used before the massage were analyzed. Covariance analysis was used by making normality distribution in statistical analysis. Tukey-Kramer test was used for group differences. The level of significance was set at *p* .05. There were statistically significant differences in biochemical and hematological values between the groups (*p* <.05). As a result, it has been observed that massage with opium oil increases HDL values and increases the Dopamine hormone. it was seen that it caused a significant decrease in WBC, MPV and Basophil level samong hematological parameters, significantly decreased the MDA level in the Vaseline group, and the massage application with petroleum jelly significantly reduced the level of DNA damage. New research is needed to use opium oil as a massage oil.

Keywords: Opium oil, Massage, Aroma therapy, Hematology, Treatment, Oxidative stres

ÖZ

Bu araştırma da oksidan-antioksidan statü ile haşhaş yağı ile yapılan masaj uygulamaların bazı kan değerleri üzerine etkisinin incelenmesi amaçlanmıştır. Araştırma öncesi gerekli izinler alınmıştır. Araştırmaya yaşları 18-25 yaş arası olan 30 sağlıklı kadın katıldı. Katılımcıların 10'u kontrol grubu, 10'u vazelin grubu ve 10'u da haşhaş yağı grubu olarak ayrıldı. Vazelin ve haşhaş yağı gruplarına 8 hafta boyunca haftada 3 gün ortalama 30-40 dakika genel masaj uygulandı. Tüm gruplardan 4 kez kan alındı. Alınan kan değerlerinden biyokimyasal, hematolojik parametreler, DNA hasarı, bazı vitamin değerleri incelenmiştir. İstatistiksel analizlerde normallik dağılımı yapılarak Kovaryans analizi kullanılmıştır. Grup farklılıkları için Tukey-Kramer testine bakıldı. Anlamlılık düzeyi *p* ,05 olarak belirlendi. Gruplar arası biyokimyasal ve hematolojik değerlerde istatistiksel olarak farklılıklar görülmüştür (*p* <,05). Sonuç olarak afyon yağı ile yapılan masajın HDL değerlerini yükselttiği ve Dopamin hormonunu arttırdığı gözlemlenmiştir. Hematolojik parametrelerden WBC, MPV ve Bazofil düzeyinde önemli bir azalmaya neden olduğu, vazelin grubunda MDA düzeyini önemli ölçüde azalttığı, birlikte vazelinle gerçekleştirilen masaj uygulamasının DNA hasar düzeyini önemli ölçüde azalttığı görülmüştür. Haşhaş yağının masaj yağı olarak kullanılması için yeni araştırmalara ihtiyaç vardır.

Anahtar Kelimeler: Haşhaş yağı, masaj, Aromaterapi, Hematoloji, Tedavi, Oksidatif stres.

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Introduction

Throughout history, both medicinal and recreational use of opium has attracted more attention and other forms of use have been overshadowed. In fact, in the regions where opium is grown, the use of its seeds, oil and pulp is so intertwined with daily life that today it is the basic element of the food and nutrition culture of those regions (Arslan et al., 2000). Seeds should also be used since opium cultivation is economical, The seeds and the oil obtained from the seeds do not have narcotic properties (Azcan at al., 2004; Kapoor, 1995). The valuable seed oil is widely used as edible cooking oil and in the production of high-quality paints, varnishes and cosmetics. Its pulp is used as animal feed. Oil contents vary significantly depending on the origin and color of the seeds. Reported oil content values of seeds range from 41.4-49.1% in India, 47.0-53.0% in Pakistan and 44.0-57.0% in Turkey (Azcan et al., 2004). The fatty acid compositions of oils vary greatly even in seed samples from the same region. Determined the fatty acid compositions of opium seeds produced in Turkey by GC/MS and found the main components to be 56.4-69.2% linoleic and 16.1-19.4% oleic and 10.6-16.3% palmitic depending on the color of the seeds (Azcan et al., 2004). The composition of opium oil contains 62-72% linoleic acid and 15-20% oleic acid, which are unsaturated fatty acids and 4.8-9.5% palmitic acid and 2-2.9% stearic acid which are saturated fatty acids. The oil has a laxative effect internally and a skin nourishing effect externally. It is used as massage oil in aromatherapy. It is an oil rich in omega fatty acids. In addition, its seeds contain vitamins and minerals that are beneficial to human health (Arslan, 2009). Complaints such as acne, itching, allergies, and increased body hair are generally reported due to the use of baby oil, olive oil and massage oils in massage practice classes. Based on this information, this study aims to investigate the effects of opium oil massage on oxidant-antioxidant status and some biochemical parameters.

Methods

A total of 30 healthy volunteer women between the ages of 18 and 25 participated in this research at Afyon Kocatepe University Sports Sciences massage parlour. Before the research, ethics committee approval was received from Osmangazi University Clinical Research ethics committee with decision no. 05 dated 08.06.2016. Verbal consent was obtained from all participants who participated in the study. In the study, participants were given a classical Swedish massage, also known as manual hand massage. Before the study, the participants were questioned about their use of any medication, history of surgery, injury, etc., and whether they were taking antioxidant supplements. Participating women were given arm, neck, leg, back, and superficial abdominal massage (optionally) during the menstrual period.

Table 1.
Distribution of participant groups

Groups	N	Application protocols
Control Group (C)	10	Massage was not applied.
Liquid Vaseline Massage Group (VM)	10	Classic Swedish massage, known as manual hand massage, was applied to the participants in the VM group, 3 sessions a week (Monday-Wednesday-Friday) for 8 weeks, for a total of 24 sessions, using Liquid Vaseline (30-40 minutes).
Opium Oil Massage Group (PM)	10	Classic Swedish massage, known as manual hand massage, was applied to the participants in the PM group, 3 sessions a week (Monday-Wednesday-Friday) for 8 weeks, for a total of 24 sessions, using opium oil (30-40 minutes).

Table 1 shows distribution of participant groups. Blood samples were taken from the participants in all groups a total of 4 times: on the 1st week, 1 day before and 1 day after the 1st session, on the 4th week after the 12th session, and on the 8th week after the 24th session.

Taking Blood Samples: Blood samples were taken from the antecubital vein by an expert in this field, in accordance with the technique. Blood samples were collected into EDTA and serum tubes. Some of the blood samples are reserved for hematological parameters. Serum and plasma of the remaining blood samples were removed in accordance with the technique and placed in Eppendorf tubes and stored at -80 °C until the day of analysis.

Hematological parameters, biochemical parameters (Vit D, Vit E, ALT, AST, CK, total protein, Lactic Acid, Glucose, Cholesterol, HDL, LDL, Cortisol, Seratonin, Dopamine) and markers of Oxidant-Antioxidant status (Malondialdehyde (MDA), Reduced Glutathione (GSH), Total Antioxidant Status (TAS), Total Oxidant Status (TOS), Protein oxidation (PCO), DNA damage (8-OHdG), NOx) were investigated in the blood samples.

Determination of the Fatty Acids Composition of Opium Oil

Table 2.
Gas Chromatography/Mass Spectrometry (GC/MS) Conditions

System: Agilent 7890B GC 5977B Mass Selective Dedector System
Column: Agilent HP-Innowax (60 m, 0.25 mm inner diameter, 0.25 µm film thickness)
Injection Temperature: 250°C
Ion source temperature: 230°C
Ionisation mode: EI
Electron energy: 70 ev
Mass range: 35- 450 m/z
Temperature programme: 60°C (10 min), 4°C/min. 220°C (10 min) 1°C/min 240°C (20 min), Total 100 min

Determination of Vitamin E and D amounts in Opium Oil

Vitamin E and D amounts of opium oil were determined with the Waters Acquity UPC2 system, which was carried out at AÜBİBAM. Detection and quantification of the substance was made according to mass spectrum (QDA). A 5% solution of the sample was prepared with tert-Butyl methyl ether and 2 µL was injected into the system.

Table 3.
Chromatographic Conditions for Determination of Vitamin E and D Amount of Opium Oil

System: WatersAcquity UPC2
Column: WatersAcquity UPC2 C18 1.9µm (3.0x150 mm)
Column Temperature: 40 ºC
Mobile phase: % 98 CO2- % 2 ACN 1.0mL/dk
Back pressure: 1650 psi
Injection Temperature: 15ºC
Detector: 1) PDA 3 Size: 210-500 nm scanning Probe temp: 600ºC
Size: 294 nm Ion source temperature: 100ºC
QDA: Electron energy 15 V
Capillary voltage: 0.8kV
Mass range: 100-650
Ionisation mode: Positive
Carrier solvent MeOH0.3mL/min

Hematological Parameters

Hematological analyzes were carried out at Afyon Health Sciences University, Faculty of Medicine, Department of Biochemistry. Measurement of hematological parameters was performed on the Mindray BC-6800 hematology analyzer using Mindray brand commercial kits (Mindray Bio-Medical Electronics Co., Ltd., Shenzhen, China).

Biochemical Parameters

Vit D, Vit E, ALT, AST, CK, Total protein, Lactic Acid, Glucose, Cholesterol, HDL, LDL, Cortisol, Seratonin, Dopamine levels in blood samples were carried out at Afyon Health Sciences University Faculty of Medicine Department of Biochemistry.

Statistical analysis

All data in the study were analyzed with a mixed model (PROC MIXED) within the SAS package program (version 9.4, SAS Institute, Cary, NC). While the random effect is determined as the participant (person) in the model, fixed effects application were determined as time and application × time interaction. The values of each parameter determined before the study were selected as covariants for the relevant parameter. Additionally, Body mass index (BMI) was determined as a covariate for each subject. Since no statistical difference was detected between the groups between the subject ages ($p<0.05$), it was not included in the model as a covariateBoth covariates and their interactions with each other were included in the model and if it did not affect the model ($p<.10$), it was removed from the model by step wise back ward elimination as reported by Firkins et al., (2001). Degrees of freedom were calculated with the BW (Between-within degrees of freedom) command under the MODEL subcommand of the SAS program. Distribution of the data was checked with Kolmogorov-Smirnov and Shapiro-Wilk tests under the PROC UNIVARIATE command. Logarithmic transformation was applied to data that was not normally distributed. In addition, residual values have been normalized (stundentized residuals) to ensure normalization in all data. Marginal values (outliers) in the residual values of the observations were determined as <-4 and >4. Once an outlier was identified, it was removed from the analysis. As reported by Çetin and Bek (2019) the covariance structure of the model was chosen as the Exponent of Distance (SP POW) for samples with equal numbers but unequal intervals over time. the PDIFF command was used in the SAS program as a post hoc test to determine which group caused the difference. Tukey-Kramer correction was applied to the obtained significance values. In the tables, data are expressed as least squares mean (LSMEANS) ± SEM. In the analyses, the significance level was taken as $p<.05$.

Results

When the fatty acid content of opium oil was examined in the study, the highest rate was 67.7% linolenic acid. This was followed by oleic acid (18:1) with 19.2%; ω-9 and palmitic acid (16:0) with 8.1%.

Table 4
Fatty Acids Composition of Opium Oil

No	Compound *	Relative Percentage (%)
1	Hexadecanoic acid (= Palmitic acid); (16:0)	8.1
2	Octadecanoic acid (=Stearic acid); (18:0)	2.6
3	(Z)-9-Octadecenoic acid (=Oleic acid); (18:1); ω-9	19.2
4	(E)-9-Octadecenoic acid (=Elaidic acid); (18:1); ω-9	0.9
5	(Z,Z)-9,12-Octadecadienoic acid (=Linoleic acid); (18:2);ω-6	67.7
6	(Z,Z,Z)- 9,12,15-Octadecatrienoic acid (=linolenic acid); (18:3); ω-6	0.5
7	Eicosanoic acid (=Arachidic acid); (20:0)	0.2
8	Docosanoic acid (=Behenic acid); (22:0)	0.3
Total		**99.5**

* ≥ % 0.2

Table 4 shows fatty acids composition of opium oil. According to the vitamin E and vitamin D analysis results of the opium oil used in the study, it was determined that the sample contained 0.16% (mg/100mg) Vitamin E. Vitamin D was not detected in the sample.

Table 5
Hematological Parameters

Parameter	Experimental Groups				Values		
	Control	Vaseline	Opium	SEM	Application	Time	Application*Time
WBC (x10⁹/L)	6,1911[ab]	6,8260[a]	5,7759[b]	0,3291	0,0244	0,5218	0,8322
MCV	86,6333	86,7163	88,7758	2,2550	0,7603	0,764	0,8670
MON (x10⁹/L)	0,4006	0,4370	0,4021	0,02482	0,5610	0,6207	0,7807
MON%	6,3454	6,8613	6,5398	0,3479	0,5735	0,6965	0,8560
HGB	13,4654	13,1252	12,8281	0,2588	0,3314	0,3482	0,0890
HCT (%)	42,2260	41,4401	40,1108	0,6285	0,0603	0,1762	0,3983
PLT (x10⁹/L)	245,88	216,19	251,52	15,3555	0,4819	0,5064	0,3868
MPV (fL)	11,1277[a]	10,4918[ab]	10,1572[b]	0,2089	0,0413	0,0001	0,1255
PDW	15,0133	14,5025	14,6659	0,1686	0,3969	<.0001	0,1111
PCT (%)	0,2603	0,2343	0,2561	0,01559	0,5974	0,6790	0,1045
BASO	0,0394[a]	0,03718[a]	0,02515[b]	0,003566	0,0350	<.0001	0,4353
BASO%	0,6014	0,5832	0,4182	0,06126	0,1191	<.0001	0,6785
EOS	0,1872	0,1486	0,1348	0,02951	0,7341	0,6070	0,4685
EOS%	2,4112	2,0890	1,4493	0,3514	0,2208	0,9360	0,2396
LYM (x10⁹/L)	2,4205	2,0248	1,9363	0,1380	0,0519	0,2138	0,2297
LYM%	38,1424	31,6447	33,2295	1,6508	0,1677	0,8073	0,2908
MCH	27,5242	27,2197	28,4303	0,9113	0,6309	0,9320	0,8546
MCHC	31,5446	31,6757	31,9697	0,3434	0,7284	0,6414	0,6014
NEUT	3,1895	4,1049	3,5696	0,2381	0,1732	0,9141	0,9018
RDW_CV	14,0397	13,6266	13,5061	0,3264	0,5584	0,6474	0,5019
RDW_SD	41,9277	40,6495	41,7879	0,7511	0,4655	0,4866	0,6503

[a,b]:Different letters in the same line represent statistically significant differences. WBC: White Blood Cell Count; MCV: Mean Corpuscular Volume; MON: Monocyte Count; MON%: Monocyte Percentage; HGB: Hemoglobin; HCT (%): Hematocrit; PLT: Platelet Count; MPV (fL): Mean Platelet Volume; PDW: Platelet Distribution Width; PCT (%): Plateletcrit; BASO: Basophil Count; BASO%: Basophil Percentage;EOS: Eosinophil Count; EOS%: Eosinophil Percentage; LYM (x10⁹/L): Lymphocyte Count; LYM%: Lymphocyte Percentage; MCH: Mean Corpuscular Hemoglobin; MCHC: Mean Corpuscular Hemoglobin Concentration; NEUT: Neutrophil Count; RDW_CV: Red Cell Distribution Width - Coefficient of Variation; RDW_SD: Red Cell Distribution Width - Standard Deviation.

When the Leukocyte Count (WBC) was examined, it was found to be 6.19 x109/L in the control group, 6.82 x109/L in the vaseline group and 5.77 x109/L in the opium group. When all periods of the study are evaluated together and looked at overall, there is a significant difference in WBC value between the groups (p=.02). While both treatment groups showed no difference compared to the control; The Vaseline group had a significantly higher WBC value than the Opium group. Although there is a total effect of the application according to all times of the study, the change in WBC values over time is not significant (p=.52). Additionally, since the changes over time were not significant in the study, there was no application × time interaction (p=.83) as expected.

Table 6
Biochemical parameters

Parameter	Experimental Groups				Values		
	Control	Vaseline	Opium	SEM	Application	Time	Application*Time
MDA (nmol/ml)	54,8022[a]	39,8763[b]	45,4922[ab]	3,7173	0,0320	0,2418	0,1139
GSH (µmol/L)	25,3221	24,7031	25,7865	0,6065	0,6988	<.0001	<.0001
NO (nmol/ml)	3,6566	5,4132	3,9974	0,7711	0,4627	0,8995	0,0675
TAS (mmolTroloxEquiv./L)	0,8037	0,7299	0,6922	0,03437	0,0980	0,1121	0,1849
TOS (µmol H2O2Equiv./L)	10,9616	9,5512	9,4228	0,4877	0,0737	0,0002	0,0001
PC (ng/mL)	13,5978[b]	25,4294[ab]	32,5159[a]	3,5301	0,0147	0,7801	0,0562
OHdG (ng/mL)	3,1738[a]	1,6733[b]	2,6395[ab]	0,3724	0,018	0,120	0,870
AST (U/L)	17,8124	16,3076	15,7722	0,5196	0,0830	0,0245	0,0342
ALT (U/L)	12,5158	12,5288	11,3745	0,5586	0,2875	0,3187	0,3763
Total Cholesterol (mg/dl)	144,96	145,42	148,02	2,7944	0,0854	0,2091	0,1896
HDL (mg/dl)	55,2937[ab]	53,5804[b]	56,8504[a]	0,8532	<.0001*	0,0819	0,1739
LDL (mg/dl)	95,6786	94,1450	99,8762	2,7378	0,3423	0,4666	0,3995
Glucose (mg/dl)	77,6745	82,7381	80,5191	1,3057	0,0766	0,0777	0,2647
Cortisol (µg/dl)	13,9122	13,9044	11,548	0,7800	0,3888	0,3825	0,1403
Dopamine (ng/L)	174,7700	160,4000	218,4300	11,4301	0,0733	0,0020	0,0226
Serotonin (ng/ml)	70,6384	94,8680	108,86	4,3528	0,1125	0,1734	0,0010
CK (U/L)	78,2759	89,6772	86,1048	8,3913	0,5675	0,4778	0,9382
Creatinine (mg/dl)	0,6873a	0,6314b	0,6397b	0,01598	0,0392	0,0047	0,0773
Lactate (mg/dl)	49,8702b	58,775a	57,0188b	2,2949	0,0359	<.0001	<.0001
VitE (nmol/ml)	29,1296	30,0494	30,8623	1,1892	0,6110	0,1942	0,0651
VitD (ng/ml)	11,7317	13,0202	12,3657	0,6842	0,4774	0,0002	<.0001

[a,b]:Different letters in the same line represent statistically significant differences.*p <,05. MDA: Malondialdehyde; GSH: Glutathione; NO: Nitric Oxide; : Total Antioxidant Status; TOS :Total Oxidant Status; PC :Protein Carbonyl; OHdG : 8-Hydroxy-2'-deoxyguanosine; AST : Aspartate Aminotransferase; ALT :Alanine Aminotransferase HDL: High-Density Lipoprotein Cholesterol; LDL: Low-Density Lipoprotein Cholesterol;CK: Creatine Kinase; VitE :Vitamin E; VitD : Vitamin D

When malondialdehyde (MDA) levels, one of the lipid peroxidation indicators measured in our study, were examined, It was found to be 54.80 nmol/ml in the control group, 39.87 nmol/ml in the vaseline group and 45.49 nmol/ml in the opium group. While serum MDA concentration decreased significantly in the vaseline group compared to the control group (p=.03), the opium group was similar to the other two groups. While serum MDA concentration decreased significantly in the vaseline group compared to the control group (p=.03); The opium group was similar to the other two groups.

When protein carbonyl (PCO) levels, which are one of the markers that reflect oxidative stress status well as a complication of experimental diabetes, are examined, It was found to be 13.59 ng/mL in the control group, 25.42 ng/mL in the vaseline group and 32.51 ng/mL in the opium seed group. Although the application showed a significant difference on PC (p=.01), there was no time (p=.78) and application × time interaction (p=.05). While PC concentration increased significantly in the opium group compared to the control group (p=.01), the vaseline group was similar to the other two groups.

When this marker, used as a measure of DNA damage, was examined, it was found to be 3.17 ng/mL in the control group, 1.67 ng/mL in the vaseline group and 2.63 ng/mL in the opium seed group. The application showed a significant difference on OHdG (p=.01). While OHdG concentration decreased significantly in the vaseline group compared to the control group; The opium group was similar to the control group. It was not observed that the values fluctuated over time in both the control group and the application groups.

Discussion

Opium (Papaver somniferum L.) has been cultivated since ancient times because it is rich in oil. The opium obtained from the seeds and scraped seeds are capsules. Alkaloids from opium capsules and straw are widely used in the pharmaceutical industry, its seeds are widely used in various bakery products (Bernath, 1998; Singh et al., 1998). It is recognized that it significantly changes people's biochemistry, both immediately after massage sessions and throughout massage therapy treatment periods (Field et al., 2005). There are many studies in the literature examining the effects of massage on biochemical parameters and the effects of acute exercises on oxidative stress (Karabulut et al., 2013).

Özkan and Baydar (2006), determined the fatty acid composition of Opium (papaversomniferum l.) seeds in different colors in the following ranges: 70.94-73.15% linoleic acid, 13.56-14.61% oleic acid, 10.68-12.15% palmitic acid, 1.13-1.97% stearic. acid and 0.29-0.70% linolenic acid. In our study, the highest fatty acid content of opium oil, which is preferred as massage oil, was 67.7% linolenic acid ω-6. This was followed by oleic acid (18:1) with 19.2%; It was followed by palmitic acid (16:0) with ω-9 and 8.1%. However, while the vitamin E level of the opium oil used in the study was determined as 0.16% (mg/100mg), Vitamin D was not detected.

Our data obtained in the study show that opium oil, which is preferred as massage oil, contains high amounts of omega 6 fatty acids. The main active ingredient and source of ω-6 fatty acids taken in sufficient amounts through diet is linoleic acid (LA). As a result of the metabolism of linoleic acid, dihomo-gamma-linoleic acid (DGLA) and arachidonic acid are formed. Omega-6 fatty acids have been shown to protect skin health and regulate body temperature and water loss. Due to excessive amounts of omega-6 fatty acids in the blood, arteriosclerosis, thrombosis, rheumatoid arthritis or vision problems occur. The effects of omega-6 fatty acids on health can generally be listed as "inflammatory, hyperalgesic, thrombotic, mitogenic" (Watkins, 1991).

The ratio of omega-6 and omega-3 fatty acids in the body is very important. It has been reported that omega-3 fatty acids can inhibit lipid peroxidation in OA (osteoarthritis) by acting as antioxidants (Tayyebi-Khosroshahi et al., 2010; Sakata et al., 2015). However, it has been stated that the benefits of omega-3 fatty acids are affected by omega-6 fatty acids, which have pro-inflammatory properties. The reason for this is that eicosanoids synthesized from omega-3 fatty acids compete with omega-6 fatty acids and have opposing functions. The level of both fatty acids in the blood is determined by the intake from the foods consumed, so it is important to maintain the ratio balance between the intake of omega fatty acids, the ideal ratio is approximately 1-4:1. In ideal diet, the ratio is desired to be between 5:1 and 10:1 (Simopoulos, 2008). However, in today's world, due to the increase in the consumption of vegetable oils such as margarine, omega-6 intake has increased and this ratio has changed between 10:1 and 50:1 (Turan et al., 2013). Nowadays, it has been reported that changes in the society's diet may cause inflammation and oxidative stress, causing a tendency for this rate to increase (Calder, 2012; Simopoulos, 2016; Patterson et al., 2012; Yang et al., 2016).

Oxidative stress describes the disruption of the prooxidant-antioxidant balance in the body and tissues in favor of prooxidants. The formation of reactive oxygen species, known as prooxidants, is a natural consequence of normal aerobic life. A portion of ROS is needed for the development of normal cell function, provided that the oxidation of each molecule returns to the reduced state. The existence and development of cells in oxygen-containing environments is not possible

without powerful antioxidant enzymes and non-enzyme antioxidant systems. Prooxidants, which are constantly formed in aerobic life, must be regularly absorbed by antioxidants and balanced by consumption. Otherwise, oxidative damage occurs and pathophysiological events may occur with its accumulation. Excessive ROS production can overwhelm the body's natural antioxidant defense system, causing lipid peroxidation and damage to DNA and cell membranes (Sies et al., 2016; Mukhoirotin, 2020).

Yang et al. (2016) found that MDA levels increased as the ratio of omega-6/omega-3 fatty acids increased. To achieve an optimal ratio between the intake of omega-6 and omega-3 fatty acids, he emphasized the importance of increasing the consumption of dietary sources of omega-3 fatty acids (Angelia et al., 2019). Tourtas et al., (2011) reported that both omega-3 and omega-6 fatty acids have antioxidant effects on human TM (TrabecularMeshwork) cells exposed to oxidative stress. They stated that omega-6 fatty acids have a stronger suppressive antioxidative effect. As a result, they argued that a combined treatment could maximize the protective effect. Both ω-3 and ω-6 fatty acids are important for human health. Studies draw attention to the importance of taking these fatty acids in a certain ratio and maintaining the balance between them. In the analysis of the opium oil used in our study, it comes to mind that the high level of ω-6 fatty acid may have an effect on the changes in the parameters explained below.in our study, MDA, GSH, NO, TAS, TOS, PC and OHdG levels, which are oxidative stress markers, were investigated. The data revealed that there was no statistical change in NO and TAS levels between all 3 groups, neither in terms of application time nor time x application interaction. However, when the MDA levels of the groups are considered, it is seen that the MDA levels of the control group are numerically higher than the other groups. The data obtained showed that massage application significantly reduced MDA levels, however, massage application with opium oil did not change MDA levels and was at the level of the control group. This revealed that the massage applied in the study significantly reduced the lipid peroxidation level, while the massage applied with opium oil did not affect lipid peroxidation. Veiskaramyan et al. (2021) in a study they conducted on acute coronary syndrome patients, stated that aromatherapy with Melissa essential oil had a positive effect on stress and hemodynamic parameters. When the PC levels of the groups are examined, it is seen that the PC levels of the control group are numerically lower than the other groupsThe data obtained show that the massage application did not statistically change the PC level, but it was at the level of the control group. However, it has been observed that massage with opium oil increases PC levels statistically. This shows that the massage application with opium oil increases the protein oxidation level, but the massage application using vaseline as a lubricant does not affect the protein oxidation levels statistically. Additionally, PC levels did not differ over time or in terms of application x time interaction. OHdG levels differed between groups, and the decrease in the vaseline group compared to the control group was statistically significant. On the other hand, there is a similarity between the control group and the opium group, and the numerical decrease in the opium group is not statistically significant. These results revealed that massage with vaseline had a significant reducing effect on DNA damage in the subjects, while massage with opium seed caused a numerical decrease that was not statistically significant.

Changes in total cholesterol, HDL and LDL cholesterol levels are lipid profile indicators. In our study, no statistical change was found between groups in total cholesterol and LDL levels. In addition, a statistical difference was detected between the groups in the levels of HDL cholesterol, which has an important role in the reverse transport of cholesterol to the liver and has antioxidant, anti-inflammatory and endothelial function regulating effects (Kızılaslanoğlu & Güven, 2011) and the HDL level in the opium group increased significantly compared to the vaseline group. This reveals that massage with opium oil significantly increases the HDL level. In addition, HDL levels did not differ depending on time nor in terms of application x time interaction.

White blood cells (WBC) are divided into 5 main groups; these are phagocytic polymorphonuclear leukocytes (neutrophils), eosinophils, basophils, mononuclear phagocytes (monocytes) and lymphocytes (Bello, 2001). When WBC values were examined in our study; There was a significant difference in WBC values between groups. While both treatment groups showed no difference compared to the control; The Vaseline group had a significantly higher WBC value than the Opium group. However, the smallest numerical value among the groups was recorded in the opium group. Although there is a total effect of the application according to all times of the study, the change in WBC values over time is not significant. In addition, since time-dependent changes were not significant in the study, no application × time interaction was found as expected. In addition, there were statistically significant differences between the groups in basophil levels and MPV values, which are indicators of average platelet volume. Considering all periods of the study in total, the MPV value in the Control group was significantly higher than in the Opium; The Vaseline group was similar to the other two groups. It has been

observed that massage using opium oil significantly reduces the MPV level. When the basophil level is examined; It was observed that the basophil levels of the opium group were significantly lower than both the control and vaseline groups.

Conclusion and Recommendation

In this study, it was aimed to investigate the effects of Opium Oil Massage on Oxidant-Antioxidant Status and Some Biochemical Parameters. As a result, when the situation is evaluated based on the hematological and biochemical parameters evaluated in the research, massage application with opium oil caused a significant decrease in the hematological parameters WBC, MPV and Basophil levels and did not cause a significant change in the level of MDA, one of the lipid peroxidation indicators evaluated in the study, and massage applied with opium oil did not affect lipid peroxidation, however, it has been found that massage with vaseline significantly reduces MDA levels.

It can be said that the 0mega 3 and Omega 6 acids in opium oil do not have an antioxidative effect by reducing the MDA level. At the same time, although it was revealed that it significantly increased the protein oxidation level, massage application using vaseline as a lubricant did not statistically affect the protein oxidation levels, although it reduced the OHdG levels numerically below the control group levels, this decrease was not significant, however, it was observed that the massage application with petroleum jelly significantly reduced the level of DNA damage. It was observed that the average HDL level decreased significantly (opium oil statistically increased the HDL average value compared to the vaseline group) compared to the control group members who did not receive massage and the subjects who received massage with vaseline. This may be thought to be due to the high fatty acid content in opium oil. It showed that massage had a significant reducing effect on DNA damage in the application group, and that massage with opium oil caused a numerical decrease that was not statistically significant. It was observed that opium oil statistically increased the HDL average value compared to the vaseline group. This may be thought to be due to the high fatty acid content in opium oil. The same decrease, which caused a significant decrease in creatinine levels, was also seen in the massage application with vaseline. It was observed that there was an increase in the average value of dopamine hormone in the group receiving opium oil massage, although it was not statistically significant compared to the other groups.

As a result, it has been observed that massage with opium oil increases HDL values and increases the Dopamine hormone. In addition, no special analgesic effect was found. In order to use opium oil as massage oil, more comprehensive studies are needed to determine the effects of massage applications with opium oil on metabolic mechanisms.

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Katılımcı Onamı: Çalışmaya katılan tüm katılımcılardan sözlü onam alınmıştır.
Hakem Değerlendirmesi: Dış bağımsız.
Yazar Katkıları: Fikir-SGB; Tasarım-SGB, YO; Denetleme-YO, SGB, ÜD; Kaynaklar-MD, SGB, AFF; Veri Toplanması ve/veya İşlemesi YO, SGB; Analiz ve/ veya Yorum-AFF, MD, YO, SBG; Literatür Taraması- AFF, MD, YO, SBG; Yazıyı Yazan; AFF, MD, YO, SBG
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