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The Effect of Vitamin E Administration on the Profile of Hypoxia-Inducible Factor-1 Alpha (Hif-1α) and Vascular Endothelial Growth Factor (VEGF) in Intraperitoneal Adhesion

Taufik Sudirman[1,2], Mochammad Hatta[3], Prihantono Prihantono[4], Agussalim Bukhari[5], Tjahjadi Robert Tedjasaputra[6], Warsinggih[4], Ibrahim Labeda[4], Burhanuddin Bahar[5], Hendry Lie[2]

[1]Doctoral Program, Faculty of Medicine, Universitas Hasanuddin, Makassar, Indonesia

[2]Department of Surgery, Faculty of Medicine, Universitas Pelita Harapan, Tangerang, Indonesia

[3]Department of Molecular Biology and Immunology for Infectious Disease, Universitas Hasanuddin, Makassar, Indonesia

[4]Department of Surgery, Faculty of Medicine, Universitas Hasanuddin, Makassar, Indonesia

[5]Department of Nutritional Sciences, Faculty of Medicine, Universitas Hasanuddin, Makassar, Indonesia

[6]Department of Internal Medicine, Division of Gastroenterology, Siloam Hospital Lippo Village, Tangerang, Indonesia

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ABSTRACT

Introduction: Intraperitoneal adhesions are a common complication following surgical procedures, often leading to significant morbidity and healthcare costs. These adhesions are associated with hypoxia-induced pathways, including the upregulation of hypoxia-inducible factor-1 alpha (HIF-1α) and vascular endothelial growth factor (VEGF), which play critical roles in inflammation and angiogenesis during tissue repair. Vitamin E, known for its antioxidant properties, has been proposed as a potential therapeutic agent to mitigate adhesion formation. This study aims to explore the effects of vitamin E administration on the mRNA expression of HIF-1α and VEGF genes, their protein levels, and the subsequent impact on intraperitoneal adhesion formation in an animal model.

Methods: This experimental study utilized 20 three-month-old Wistar strain Rattus norvegicus rats, divided into four groups: (1) sham-operated group, (2) placebo group, (3) intraperitoneal administration of vitamin E combined with olive oil, and (4) oral administration of vitamin E. Blood samples were collected on day one (pre-laparotomy), day three, and day fourteen to measure the mRNA expression of HIF-1α and VEGF genes. On day fourteen, a relaparotomy was performed to collect adhesion tissue for macroscopic and microscopic evaluation. Statistical analysis was conducted to compare the outcomes across the groups, with a significance level set at $p < 0.001$.

Results: The results demonstrated that intraperitoneal administration of vitamin E combined with olive oil significantly inhibited the increase in HIF-1α and VEGF mRNA expression and protein levels while preventing the formation of intraperitoneal adhesions compared to the sham and placebo groups ($p < 0.001$). Oral administration of vitamin E also suppressed the elevation of HIF-1α and VEGF expression and protein levels but was less effective than intraperitoneal administration. Nevertheless, both routes of administration showed significant anti-adhesion effects compared to the control groups.

Conclusion: Intraperitoneal administration of vitamin E combined with olive oil exhibits a potent anti-adhesion effect in Rattus norvegicus by reducing the incidence and severity of intraperitoneal adhesions. This effect is mediated through the suppression of HIF-1α and VEGF gene expression and protein levels, highlighting the therapeutic potential of this combination in mitigating post-surgical adhesion formation. While oral vitamin E also shows promise, its efficacy is comparatively lower than intraperitoneal administration. These findings warrant further investigation in clinical settings to validate their translational potential.

Keywords: *Intraperitoneal adhesion, vitamin E, HIF-1α mRNA Expression, HIF- 1α Levels, VEGF mRNA, Expression, VEGF Levels.*

1. INTRODUCTION

Intraperitoneal adhesions are a common complication following abdominal surgery, with an incidence rate of approximately 93–100% after upper abdominal laparotomy and 67–93% after lower abdominal laparotomy.[1] Approximately 15–18% of adhesion cases resulting from laparotomies require surgical intervention. Among all cases of small bowel obstruction, 65–75% are caused by peritoneal adhesions, while 32% of acute intestinal obstruction cases are also attributed to peritoneal adhesions.[1] By definition, adhesions refer to the accumulation of fibrous tissue within a cavity such as the peritoneum, pericardium, or pleura, resulting from injury to the membrane lining of that cavity.[2] Another definition describes intraperitoneal adhesions as bands of vascularized and innervated tissue that connect or bind intraperitoneal organs, which are normally separated.[2] The causes of peritoneal adhesions include intra-abdominal infections, surgical trauma, ischemia, thermal injury, and immune reactions to foreign bodies. Peritoneal trauma can disrupt blood flow to the injured area, increase vascular permeability, and trigger inflammatory cell exudation, leading to fibrin matrix formation.[1]

Vitamin E is one of several agents extensively studied for its potential in preventing peritoneal adhesions. As a lipid-soluble vitamin found in all cell membranes, vitamin E exhibits antioxidant, anti-inflammatory, anticoagulant, and antifibroblastic properties. These potentials suggest that vitamin E may provide beneficial effects in adhesion prevention. However, evidence regarding the efficacy of vitamin E administration in preventing intra-abdominal or peritoneal adhesions remains inconclusive.[1] Vitamin E functions as an antioxidant and protects biological membranes from free radical-induced damage. It shields unsaturated fatty acids in phospholipid membranes. Peroxyl radicals react 1,000 times faster with vitamin E than with unsaturated fatty acids, forming tocopheroxyl radicals. Subsequently, these tocopheroxyl radicals interact with other antioxidants, such as vitamin C, which regenerate tocopherol. For instance, vitamin E is crucial in protecting red blood cell membranes, which are rich in polyunsaturated fatty acids, from oxidative damage. Additionally, vitamin E protects circulating lipoproteins, particularly oxidized low-density lipoprotein (LDL), which plays a significant role in the development of atherosclerosis. Oxidized LDL is more readily taken up by macrophages compared to non-oxidized LDL, forming foam cells that adversely affect endothelial cells and may cause vasoconstriction. High doses of vitamin E (1,600 mg/day) protect LDL from oxidation. Although still controversial, some epidemiological studies suggest that vitamin E may protect against cardiovascular diseases, though the underlying mechanisms remain unclear. Beyond its antioxidant effects, direct actions on vascular endothelium, smooth muscle cells, or blood coagulation are suspected to play a role. Vitamin E regulates vascular smooth muscle cell proliferation, induces vasodilation, and inhibits both platelet activation and leukocyte adhesion. It also protects beta-carotene from oxidation. Certain dietary substances, such as selenium, sulfur-containing amino acids, and coenzyme Q, can substitute for vitamin E.[3]

Under normoxic conditions, the HIF-1α subunit has a very short half-life, and cells continuously synthesize and degrade HIF-α proteins.[4] However, under hypoxic conditions, the degradation of HIF-1α is inhibited.[5] The enzymatic hydroxylation of two prolyl residues (Pro402 and Pro564) facilitates oxygen interaction with the HIF-1α subunit, a process occurring in the oxygen-dependent degradation domain (ODDD).[6] This oxygen-dependent hydroxylation regulates interaction with the von Hippel-Lindau tumor suppressor protein (pVHL), a component of the ubiquitin E3 ligase complex that targets HIF-1α for proteolysis via the ubiquitin-proteasome pathway.[7] Under hypoxic conditions, prolyl hydroxylase-domain (PHD) enzymes are suppressed, preventing HIF-1α degradation and allowing its accumulation. Subsequently, HIF-1α translocates to the nucleus and dimerizes with HIF-1β. The heterodimeric HIF transcriptional complex then binds to hypoxia-responsive elements (HRE) in the promoter or enhancer sequences of target genes.[8]

Vascular endothelial growth factor (VEGF) is a specific endothelial mitogen in vitro and an angiogenic trigger in various in vitro models.[9] The concept of VEGF emerged in 1939 when Ide et al. postulated the existence of a factor stimulating blood vessel growth in tumors, based on robust neovascularization responses.[10] VEGF mRNA expression is induced by low oxygen levels, with HIF-1 acting as the primary mediator under hypoxic conditions.[11] Similar to HIF-1α, the tumor suppressor gene VHL plays a critical role in regulating VEGF. The mitogenic activity of endothelial cells produced by renal carcinoma cells with VHL mutations can be neutralized using antibodies against VEGF.[12] Thus, it can be concluded that the VHL protein regulates VEGF and other hypoxia-induced genes.

2. METHODS

This study employed an experimental laboratory design with pre- and post-tests conducted on 20 white Rattus norvegicus strain Wistar rats to investigate the effects of vitamin E administration on the expression of HIF-1α and VEGF genes in the formation of intraperitoneal adhesions. The research was conducted between August and October 2023 at the Molecular Biology and Immunology Laboratory in the Microbiology Department of the Faculty of Medicine, Hasanuddin University, Makassar, South Sulawesi, Indonesia.

The subjects of the study were two-month-old male Wistar rats weighing between 170 and 300 grams, sourced from the animal laboratory of the Molecular Biology and Immunology Department at Hasanuddin University. Healthy rats were included, while those that refused to eat or died before the final blood sample collection were excluded. The sample size consisted of 20 rats divided into four groups of five rats each. Ethical approval was obtained from the Ethics Committee of

the Faculty of Medicine, Hasanuddin University, and the study adhered to standard guidelines for the use and care of laboratory animals. Prior to treatment, the rats were housed in cages measuring 60 x 30 x 30 cm³, maintained at a constant temperature of 28°C, humidity of 40–60%, and a 12-hour light-dark cycle. They were provided with standard pellet feed and water ad libitum.

The materials used included 20 white rats, cages, rat feed, feeding and drinking containers, 70% alcohol solution, 10% povidone iodine solution, sterile gauze, gauge sponges, ketamine, diazepam, sulfas atropine, vitamin E, olive oil, distilled water, adhesive plaster, sterile gloves, syringes of various sizes, silk thread 3/0, surgical blades, kits for mRNA HIF-1α and ELISA assays for HIF-1α and VEGF, qPCR/RT-PCR equipment, and a constant-temperature incubator. The rats were randomly divided into four groups: Group 1 (Sham) received no treatment, Group 2 (Control) received 5 ml of olive oil as a placebo, Group 3 (Intervention) received 10 mg of vitamin E dissolved in 5 ml of olive oil administered intraperitoneally, and Group 4 (Intervention) received 10 mg of vitamin E orally.

All rats underwent laparotomy with a 4 cm midline incision, during which a 2x2 cm section of the left parietal peritoneum was excised below the transversus abdominis muscle. After the procedure, the parietal peritoneum and skin were sutured with 3/0 silk thread. Blood samples were collected from the tail vein on day 1 (pre-laparotomy), day 3, and day 14 to measure mRNA expression of HIF-1α and VEGF genes, as well as their protein levels. On day 14, a relaparotomy was performed with a 4 cm incision above the previous wound to obtain biopsy samples of adhesion tissue measuring 0.5 x 0.5 cm.

Nucleic acid extraction was performed using 100 µl of venous blood mixed with 900 µl of a lysis solution containing guanidium thiocyanate, Tris-HCl, EDTA, and Triton X-100. The mixture was centrifuged at 12,000 rpm, and the sediment was resuspended in a diatom suspension. Further washing steps were carried out with solutions containing guanidium thiocyanate, 70% ethanol, and acetone. The final product was eluted in TE buffer and stored at -80°C before qPCR analysis. Specific primers were designed for HIF-1α, VEGF, and GAPDH as a housekeeping gene. The qPCR conditions included an initial reverse transcription step at 48°C for 30 minutes, followed by PCR activation at 92°C for 5 minutes, and 29 cycles of denaturation at 92°C for 15 seconds and annealing at 53°C for 30 seconds. SYBR Green-based qRT-PCR was performed using a CFX Connect System (Bio-Rad Laboratories, USA). A standard curve was generated using Ct values, and relative gene expression was calculated using the 2-ΔΔCt method.

Protein levels of HIF-1α and VEGF were measured using enzyme-linked immunosorbent assays (ELISA). Serum samples were prepared and assayed in duplicate according to the manufacturer's instructions. Standards and samples were added to microplate wells, incubated for 2 hours at room temperature, and washed with PBS. Streptavidin-HRP conjugate was added, followed by another 2-hour incubation and washing. Substrate solution containing TMB was added, and the reaction was stopped with H2SO4 after 20 minutes. Absorbance was measured at 450 nm using an ELISA reader, and protein concentrations were expressed in pg/ml.

Statistical analysis was performed using IBM SPSS version 25. The Kolmogorov-Smirnov test was used to assess data normality, and Levene's test was applied to evaluate variance homogeneity. Parametric tests were used for normally distributed data, while non-parametric tests were applied for non-normal data. Differences in mRNA expression of HIF-1α and VEGF genes, protein levels, and adhesion tissue between days 1, 3, and 14 were analyzed using one-way ANOVA. Post-hoc Bonferroni tests were conducted to compare variables on days 3 and 14 using dependent t-tests. The relationship between microscopic and macroscopic adhesion scores was analyzed using the Kruskal-Wallis test. Correlations between mRNA and protein levels of HIF-1α and VEGF with adhesion scores were evaluated using Pearson's or Spearman's rank correlation tests.

3. RESULT

Expression of HIF-1α mRNA, HIF-1α Levels, VEGF mRNA Expression, and VEGF Levels Before Laparotomy (Day 1)

The results of mRNA expression of HIF-1α, VEGF, and protein levels of HIF-1α and VEGF are summarized in **Table 1**. There were no statistically significant differences among the groups for any variable before laparotomy ($p > 0.05$). The mean values across all samples were as follows:

- Mean HIF-1α mRNA expression: 7.999 Fc
- Mean HIF-1α protein level: 1160.525 pg/mL
- Mean VEGF mRNA expression: 6.215 Fc
- Mean VEGF protein level: 4478.505 pg/mL

After laparotomy and specific treatments according to each group, measurements were taken on day 3. Statistically significant differences were observed among the groups for all variables ($p < 0.001$). The most significant reduction was seen in the Olive Oil + Vitamin E Intraperitoneal group, which had the lowest variable values compared to other groups. This indicates that intraperitoneal administration of olive oil and vitamin E significantly reduced HIF-1α and VEGF expression and protein levels compared to the sham group, olive oil group, and oral vitamin E.

The overall increase in HIF-1α mRNA expression was 2.112 Fc, HIF-1α protein levels increased by 575.555 pg/mL, VEGF mRNA expression increased by 1.989 Fc, and VEGF protein levels increased by 4101.545 pg/mL (all $p < 0.001$). Expression of HIF-1α mRNA, HIF-1α Levels, VEGF mRNA Expression, and VEGF Levels on Day 14 Post-Laparotomy

After 14 days of treatment, measurements were taken again on day 14 (**table 3**). Statistically significant differences were observed among the groups for all variables ($p < 0.001$). A linear increase in HIF-1α mRNA expression, HIF-1α protein levels, VEGF mRNA expression, and VEGF protein levels was observed in the sham and olive oil groups, while a slower increase was noted in the oral vitamin E group and especially in the Olive Oil + Vitamin E Intraperitoneal group. The overall increases were as follows:

- HIF-1α mRNA expression: 1.676 Fc
- HIF-1α protein levels: 414.115 pg/mL
- VEGF mRNA expression: 1.384 Fc
- VEGF protein levels: 3382.215 pg/mL (all $p < 0.001$).

Table 1. Results of Gene Expression Analysis of mRNA HIF-1α, HIF-1α Levels, mRNA VEGF Expression, and VEGF Levels on the First Day Before Laparotomy Surgery

Variable		Sham (Mean ± SD)	Olive Oil (Mean ± SD)	Olive Oil + Vit E (Mean ± SD)	Vit E Oral (Mean ± SD)	P-Value
mRNA (Fc)	HIF-1α	7.6438 ± 0.73391	8.2598 ± 0.63291	7.8898 ± 0.49376	8.1934 ± 0.77933	0.459
HIF-1α (pg/mL)	Level	1141.3 ± 186.55	1138.5 ± 151.01	1289.62 ± 155.13	1072.68 ± 123.68	0.825
mRNA (Fc)	VEGF	6.3618 ± 0.50817	6.349 ± 0.71707	6.0628 ± 0.78325	6.0852 ± 0.62015	0.2
VEGF (pg/mL)	Level	4253.84 ± 1642.91	4888.6 ± 564.72	3736.14 ± 683.69	5035.44 ± 693.55	0.185

Statistical analysis was performed using ANOVA test.

Table 2. Results of Gene Expression Analysis of mRNA HIF-1α, mRNA VEGF, HIF-1α Levels, and VEGF Levels on the 3rd Day After Laparotomy Surgery

Variable		Sham (Mean ± SD)	Olive Oil (Mean ± SD)	Olive Oil + Vit E (Mean ± SD)	Vit E Oral (Mean ± SD)	P-Value
mRNA (Fc)	HIF-1α	10.629 ± 0.513	10.356 ± 0.488	9.347 ± 0.114	10.105 ± 0.355	<0.001
HIF-1α (pg/mL)	Level	1855.06 ± 114.842	1848.8 ± 132.5	1541.9 ± 70.292	1698.56 ± 40.723	<0.001
mRNA (Fc)	VEGF	8.619 ± 0.155	9.938 ± 0.668	7.520 ± 0.240	7.736 ± 0.243	<0.001
VEGF (pg/mL)	Level	9774.32 ± 761.656	9849.76 ± 995.068	7206.9 ± 560.671	7589.22 ± 432.252	<0.001

Statistical analysis was performed using ANOVA test.

Table 3. Results of Gene Expression Analysis of mRNA HIF-1α, HIF-1α Levels, mRNA VEGF Expression, and VEGF Levels on the 14th Day After Laparotomy Surgery

	Mean (SD) – Sham	Mean (SD) – Olive Oil	Mean (SD) – Olive Oil + Vitamin E	Mean (SD) – Vitamin E Oral	P-Value
mRNA HIF-1α Expression (Fc)	13.429 (SD = 0.655)	12.553 (SD = 0.783)	9.771 (SD = 0.301)	11.386 (SD = 0.303)	<0.001
HIF-1α Level (pg/mL)	2567.5 (SD = 68.233)	2543.28 (SD = 230.85)	1605.06 (SD = 89.844)	1884.94 (SD = 65.487)	<0.001
mRNA VEGF Expression (Fc)	10.435 (SD = 0.354)	10.409 (SD = 0.516)	8.045 (SD = 0.746)	9.458 (SD = 0.204)	<0.001
VEGF Level (pg/mL)	15802.22 (SD = 1407.52)	14936 (SD = 1945)	7266.68 (SD = 491.485)	9844.16 (SD = 262.023)	<0.001

*Statistical analysis was performed using ANOVA.

Dynamic Profile of HIF-1α mRNA Expression and Protein Levels

The dynamic profile of HIF-1α mRNA expression and protein levels is illustrated in **Figure 2**. The highest increase in HIF-1α mRNA expression and protein levels was observed in the sham and olive oil groups, while the oral vitamin E group showed a slower increase, and the Olive Oil + Vitamin E Intraperitoneal group demonstrated the least increase.

Post hoc analysis revealed that the Olive Oil + Vitamin E Intraperitoneal group had significantly lower HIF-1α mRNA expression compared to the sham group (mean difference = 1.282 Fc, $p < 0.001$) and the oral vitamin E group (mean difference = 0.758 Fc, $p < 0.001$). This suggests that intraperitoneal administration of olive oil and vitamin E effectively reduced HIF-1α mRNA expression.

Table 4. Results of mRNA HIF-1α Gene Expression Examination Before (Day 1) and After Surgery (Day 3)

Group	mRNA HIF-1α Gene Expression Before Surgery (H1) (Fc)	mRNA HIF-1α Gene Expression After Surgery (H3) (Fc)	Mean Difference (Fc)	P-Value
Group 1 (Sham)	7.644 (SD = 0.734)	10.629 (SD = 0.513)	2.985 (SD = 0.964)	0.001
Group 2 (Olive oil)	8.260 (SD = 0.633)	10.356 (SD = 0.488)	2.096 (SD = 0.687)	0.001
Group 3 (Olive oil + Vitamin E Intraperitoneal)	7.89 (SD = 0.494)	9.347 (SD = 0.114)	1.457 (SD = 0.48)	0.001
Group 4 (Oral Vitamin E)	8.193 (SD = 0.779)	10.105 (SD = 0.355)	1.911 (SD = 0.771)	0.003

Table 5. Post Hoc Analysis of mRNA Gene Expression Differences on Day 3 Among Groups

Main Variable	Comparison Variable	Mean Difference in mRNA Expression (Fc)	P-Value
Sham	Olive oil	0.273	0.414
	Olive oil + Vitamin E	1.282	<0.001

	Vitamin E oral	0.524	0.97
Olive oil	Sham	0.273	0.414
	Olive oil + Vitamin E	1.01	0.002
	Vitamin E oral	0.251	0.378
Olive oil + Vitamin E	Sham	1.282	<0.001
	Olive oil	1.01	0.002
	Vitamin E oral	0.758	0.002
Vitamin E oral	Sham	0.524	0.97
	Olive oil	0.251	0.378
	Olive oil + Vitamin E	0.758	0.002

Note: Post Hoc paired t-test using the Bonferroni method.

Table 6. mRNA HIF-1α Gene Expression During Treatment (Day 3) and Post-Treatment (Day 14)

Group	mRNA HIF-1α Expression After Surgery (H3) (Fc)	mRNA HIF-1α Expression After Surgery (H14) (Fc)	Mean Difference	P-Value
Group 1 (Sham)	10.629 (SD = 0.513)	13.429 (SD = 0.655)	2.80 (SD = 0.978)	0.002
Group 2 (*Olive oil*)	10.356 (SD = 0.488)	12.553 (SD = 0.783)	2.197 (SD = 0.717)	0.001
Group 3 (*Olive oil + Vitamin E Intraperitoneal*)	9.347 (SD = 0.114)	9.771 (SD = 0.301)	0.424 (SD = 0.227)	0.007
Group 4 (*Vitamin E Oral*)	10.105 (SD = 0.355)	11.386 (SD = 0.303)	1.282 (SD = 0.412)	0.001

Note: Statistical test used: **paired t-test**.

Table 7. Post Hoc Analysis of Differences in mRNA HIF1-α Gene Expression After Treatment on Day 14 Among Groups

Main Variable	Comparison Variable	Mean Difference in mRNA Expression (Fc)	P-Value
Sham	*Olive oil*	0.876	0.092
	Olive oil + Vitamin E	3.658	<0.001
	Vitamin E oral	2.042	<0.001
Olive oil	Sham	0.876	0.092
	Olive oil + Vitamin E	2.782	<0.001
	Vitamin E oral	1.167	0.015
Olive oil + Vitamin E	Sham	3.658	<0.001

Main Variable	Comparison Variable	Mean Difference in mRNA Expression (Fc)	P-Value
	Olive oil	2.782	<0.001
	Vitamin E oral	1.616	<0.001
Vitamin E oral	Sham	2.042	<0.001
	Olive oil	1.167	0.015
	Olive oil + Vitamin E	1.616	<0.001

Note: Post Hoc paired T-test using the **Bonferroni** method.

Table 8. Differences in HIF-1α Levels Before (Day 1) and After Laparotomy (Day 3)

Group	HIF-1α Level Before Surgery (H1) (pg/mL)	HIF-1α Level After Surgery (H3) (pg/mL)	Mean Difference (pg/mL)	P-value
Group 1 (sham)	1141.3 (SD = 186,545)	1855.06 (SD = 114,842)	-713.76 (SD = 131,464)	<0.001
Group 2 (Olive oil)	1138.5 (SD = 151,012)	1848.8 (SD = 132.5)	-710.3 (SD = 206,757)	<0.001
Group 3 (Olive oil + Vit E intraperitoneal)	1289.62 (SD = 144,134)	1541.9 (SD = 70.29)	-252.28 (SD = 133,942)	0.007
Group 4 (Vit E oral)	1072.68 (SD = 123,679)	1698.56 (SD = 40,723)	-625.88 (SD = 141,679)	<0.001

Table 9. Post Hoc Analysis of Mean Differences in HIF-1α Levels During the Treatment Period (Day 3) Between Groups

Main Variable	Comparison Variable	Mean Difference (pg/mL)	P-value
Sham	Olive oil	6.26	0.938
	Olive oil + Vitamin E	313.16	<0.001
	Vitamin E oral	156.5	0.021
Olive oil	Sham	6.26	0.938
	Olive oil + Vitamin E	306.9	0.002

Main Variable	Comparison Variable	Mean Difference (pg/mL)	P-value
	Vitamin E oral	150.24	0.042
Olive oil + Vitamin E	Sham	313.16	<0.001
	Olive oil	306.9	0.002
	Vitamin E oral	156.66	0.003
Vitamin E oral	Sham	156.5	0.021
	Olive oil	150.24	0.042
	Olive oil + Vitamin E	156.66	0.003

Table 10. Differences in Mean HIF-1α Levels After Laparotomy (Day 3) and After the Experiment Period (Day 14)

Group	HIF-1α Level Before Surgery (H3) (pg/mL)	HIF-1α Level After Surgery (H14) (pg/mL)	Mean Difference (pg/mL)	P-value
Group 1 (sham)	1855.06 (SD = 114,842)	2567.5 (SD = 68,233)	-712.44 (SD 139,872)	= <0.001
Group 2 (Olive oil)	1848.8 (SD = 132.5)	2543.28 (SD = 230,848)	-694.48 (SD 210,234)	= <0.001
Group 3 (Olive oil + Vit E intraperitoneal)	1541.9 (SD = 70.29)	1605.06 (SD = 89,844)	-63.16 (SD 81,311)	= 0.079
Group 4 (Vit E oral)	1698.56 (SD = 40,723)	1884.94 (SD = 65,487)	-186.38 (SD 92,023)	= 0.005

Table 11. Post Hoc Analysis of Mean Differences in HIF-1α Levels During the Research Period Between Experimental Groups

Main Variable	Comparison Variable	Mean Difference (pg/mL)	P-value
Sham	Olive oil	24.22	0.828
	Olive oil + Vitamin E	962.44	<0.001

Main Variable	Comparison Variable	Mean Difference (pg/mL)	P-value
	Vitamin E oral	682.56	<0.001
Olive oil	Sham	24.22	0.828
	Olive oil + Vitamin E	938.22	<0.001
	Vitamin E oral	658.34	<0.001
Olive oil + Vitamin E	Sham	962.44	<0.001
	Olive oil	938.22	<0.001
	Vitamin E oral	279.88	<0.001
Vitamin E oral	Sham	682.56	<0.001
	Olive oil	658.34	<0.001
	Olive oil + Vitamin E	279.88	<0.001

Dynamic Profile of VEGF mRNA Expression and Protein Levels

The highest increase in VEGF mRNA expression and protein levels was observed in the sham group, followed by the olive oil group. Both the oral vitamin E group and the Olive Oil + Vitamin E Intraperitoneal group showed significantly lower increases in VEGF mRNA expression and protein levels.

Table 12. Results of mRNA VEGF Gene Expression Before (Day 1) and After Surgery (Day 3)

Group	mRNA VEGF Gene Expression Before Surgery (H1) (Fc)	mRNA VEGF Gene Expression After Surgery (H3) (Fc)	Mean Difference	P-value
Group 1 (sham)	6,3618 (SD = 0.50817)	8,619 (SD = 0.155)	2.257 (SD = 0.487)	<0.001
Group 2 (Olive oil)	6,349 (SD = 0.71707)	9,938 (SD = 0.668)	2.589 (SD = 1.160)	0.004
Group 3 (Olive oil + Vit E intraperitoneal)	6,0628 (SD = 0.78325)	7,520 (SD = 0.240)	1.457 (SD = 0.819)	0.008
Group 4 (Vit E oral)	6,0852 (SD = 0.62015)	7,736 (SD = 0.243)	1.651 (SD = 0.736)	0.004

Table 13. Post Hoc Analysis of mRNA VEGF Gene Expression Differences After Treatment (Day 3) Between Groups

Main Variable	Comparison Variable	Mean Difference in mRNA Expression (Fc)	P-value
Sham	Olive oil	0.320	0.328
	Olive oil + Vitamin E	1.099	<0.001
	Vitamin E oral	0.883	<0.001
Olive oil	Sham	0.320	0.328
	Olive oil + Vitamin E	1.418	0.002
	Vitamin E oral	1.203	0.005
Olive oil + Vitamin E	Sham	1.099	<0.001
	Olive oil	1.418	0.002
	Vitamin E oral	0.215	0.196
Vitamin E oral	Sham	0.883	<0.001
	Olive oil	1.203	0.005
	Olive oil + Vitamin E	0.215	0.196

Table 14. mRNA VEGF Gene Expression During Treatment (Day 3) and After Treatment (Day 14)

Group	mRNA VEGF Gene Expression After Surgery (H3) (Fc)	mRNA VEGF Gene Expression After Surgery (H14) (Fc)	Mean Difference	P-value
Group 1 (sham)	8,619 (SD = 0.155)	10,435 (SD = 0.354)	1,816 (SD = 0.370)	<0.001
Group 2 (Olive oil)	9,938 (SD = 0.668)	10,409 (SD = 0.516)	1,471 (SD = 0.348)	<0.001
Group 3 (Olive oil + Vit E intraperitoneal)	7,520 (SD = 0.240)	8,045 (SD = 0.746)	0.525 (SD = 0.537)	0.047

Group	mRNA VEGF Gene Expression After Surgery (H3) (Fc)	mRNA VEGF Gene Expression After Surgery (H14) (Fc)	Mean Difference	P-value
Group 4 (Vit E oral)	7,736 (SD = 0.243)	9,458 (SD = 0.204)	1,722 (SD = 0.093)	<0.001

Table 15. Post Hoc Analysis of mRNA VEGF Gene Expression Differences During the Research Period Between Experimental Groups

Main Variable	Comparison Variable	Mean Difference in mRNA Expression (Fc)	P-value
Sham	Olive oil	0.262	0.925
	Olive oil + Vitamin E	2.390	0.001
	Vitamin E oral	0.977	0.001
Olive oil	Sham	0.262	0.925
	Olive oil + Vitamin E	2.364	<0.001
	Vitamin E oral	0.951	0.007
Olive oil + Vitamin E	Sham	2.390	0.001
	Olive oil	2.364	<0.001
	Vitamin E oral	1.413	0.006
Vitamin E oral	Sham	0.977	0.001
	Olive oil	0.951	0.007
	Olive oil + Vitamin E	1.413	0.006

Table 16. Differences in VEGF Levels Before Laparotomy (Day 1) and After Laparotomy (Day 3)

Group	VEGF Level Before Surgery (H1) (pg/mL)	VEGF Level After Surgery (H3) (pg/mL)	Mean Difference (pg/mL)	P-value
Group 1 (sham)	4253.84 (SD = 1642.91)	9774.32 (SD = 761,656)	-5420.48 (SD =	<0.001

Group	VEGF Level Before Surgery (H1) (pg/mL)	VEGF Level After Surgery (H3) (pg/mL)	Mean Difference (pg/mL)	P-value
			1111,498)	
Group 2 (Olive oil)	4888.6 (SD = 564.72)	9849.76 (SD = 995,068)	-4961.16 (SD = 1076,698)	<0.001
Group 3 (Olive oil + Vit E intraperitoneal)	3736.14 (SD = 683.69)	7206.9 (SD = 560,671)	-3470.76 (SD = 1043,381)	<0.001
Group 4 (Vit E oral)	5035.44 (SD = 693.55)	7589.22 (SD = 432,252)	-2553.78 (SD = 544,522)	<0.001

Table 17. Post Hoc Analysis of Mean Differences in VEGF Levels During Treatment (Day 3) Between Groups

Main Variable	Comparison Variable	Mean Difference in VEGF Levels (pg/mL)	P-value
Sham	Olive oil	175.44	0.762
	Olive oil + Vitamin E	2467.42	<0.001
	Vitamin E oral	2085.1	<0.001
Olive oil	Sham	175.44	0.762
	Olive oil + Vitamin E	2642.86	<0.001
	Vitamin E oral	2260.54	0.002
Olive oil + Vitamin E	Sham	2467.42	<0.001
	Olive oil	2642.86	<0.001
	Vitamin E oral	382.32	0.262
Vitamin E oral	Sham	2085.1	<0.001
	Olive oil	2260.54	0.002
	Olive oil + Vitamin E	382.32	0.262

Table 18. Differences in Mean VEGF Levels After Laparotomy (Day 3) and After the Experiment Period (Day 14)

Group	VEGF Level Before Surgery (H3) (pg/mL)	VEGF Level After Surgery (H14) (pg/mL)	Mean Difference (pg/mL)	P-value
Group 1 (sham)	9774.32 (SD = 761,656)	15802.22 (SD = 1407,52)	-6127.9 (SD = 1651,585)	<0.001
Group 2 (Olive oil)	9849.76 (SD = 995,068)	14936 (SD = 1945)	-5086.24 (SD = 1285,352)	<0.001
Group 3 (Olive oil + Vit E intraperitoneal)	7206.9 (SD = 560,671)	7266.68 (SD = 491,485)	-59.78 (SD = 249,776)	0.310
Group 4 (Vit E oral)	7589.22 (SD = 432,252)	9844.16 (SD = 262,023)	-2254.94 (SD = 466,484)	<0.001

Table 19. Post Hoc Analysis of Mean Differences in VEGF Levels During the Experiment Period (Day 14) Between Groups

Main Variable	Comparison Variable	Mean Difference in VEGF Levels (pg/mL)	P-value
Sham	Olive oil	866.22	0.443
	Olive oil + Vitamin E	8535.54	<0.001
	Vitamin E oral	5958.06	<0.001
Olive oil	Sham	866.22	0.443
	Olive oil + Vitamin E	7669.32	<0.001
	Vitamin E oral	5091.84	<0.001
Olive oil + Vitamin E	Sham	8535.54	<0.001
	Olive oil	7669.32	<0.001
	Vitamin E oral	2577.48	<0.001
Vitamin E oral	Sham	5958.06	<0.001
	Olive oil	5091.84	<0.001

Main Variable	Comparison Variable	Mean Difference in VEGF Levels (pg/mL)	P-value
	Olive oil + Vitamin E	2577.48	<0.001

Post hoc analysis indicated that the Olive Oil + Vitamin E Intraperitoneal group had significantly lower VEGF protein levels compared to the sham group (mean difference = 8535.54 pg/mL, $p < 0.001$) and the olive oil group (mean difference = 7669.32 pg/mL, $p < 0.001$). The oral vitamin E group also showed significantly lower VEGF protein levels compared to the sham group (mean difference = 5958.06 pg/mL, $p < 0.001$) and the olive oil group (mean difference = 5091.84 pg/mL, $p < 0.001$).

Macroscopic and Microscopic Examination of Adhesion Tissue

Macroscopic and microscopic examinations were conducted on day 14. The highest adhesion formation was observed in the sham and olive oil groups, characterized by fibrous tissue connecting the intestinal lining to the peritoneum. Histopathological findings revealed dense fibrotic tissue with numerous blood vessels, granulation tissue containing fibroblast proliferation, capillaries, and inflammatory cell infiltration in the extracellular matrix.

Table 20. Distribution of Subjects Based on Macroscopic Grades and Groups

Group	Macroscopic					P-value
	0	**1**	**2**	**3**	**4**	
Sham	0	0	0	1	4	0,003
Olive oil	0	1	0	1	3	
Olive oil + Vitamin E intraperitoneal	3	0	1	1	0	
Vitamin E oral	0	2	1	2	0	

*Statistical analysis using the Kruskal-Wallis test.

Table 21. Distribution of Subjects Based on Microscopic Grades and Groups

Group	Microscopic					P-value
	0	**1**	**2**	**3**	**4**	
Sham	0	0	0	4	1	0,02
Olive oil	0	1	2	2	0	
Olive oil + Vitamin E intraperitoneal	3	1	0	1	0	
Vitamin E oral	0	1	3	1	0	

Kruskal-Wallis tests revealed statistically significant differences in both macroscopic and microscopic adhesion scores ($p = 0.003$ and $p = 0.02$, respectively). The Olive Oil + Vitamin E Intraperitoneal group showed the lowest incidence of adhesions, with 60% of samples showing no adhesions.

Correlation Between Variables

Pearson correlation analysis revealed significant correlations between all variables. Notably, strong correlations were observed between HIF-1α mRNA expression and VEGF mRNA expression ($r = 0.864$, $p < 0.001$) and between HIF-1α mRNA expression and HIF-1α protein levels ($r = 0.880$, $p < 0.001$). Similar correlations were noted between VEGF mRNA expression and HIF-1α protein levels ($r = 0.911$, $p < 0.001$).

Table 22. Correlation Test Between Variables mRNA HIF-1α, mRNA VEGF, HIF-1α Levels, and VEGF Levels

Variable Pair	R Coefficient	95% CI for R		P-value
		Lower	Upper	
mRNA HIF-1α – mRNA VEGF	.864	.682	.945	<0.001
mRNA HIF-1α – HIF1α	.880	.717	.952	<0.001
mRNA HIF-1α – VEGF	.911	.785	.965	<0.001
mRNA HIF-1α – Microscopic	.718	.403	.880	<0.001
mRNA HIF-1α – Macroscopic	.821	.595	.927	<0.001
mRNA VEGF – HIF1α	.909	.779	.964	<0.001
mRNA VEGF – VEGF	.864	.682	.945	<0.001
mRNA VEGF – Microscopic	.740	.442	.891	<0.001
mRNA VEGF – Macroscopic	.813	.578	.923	<0.001
HIF1α – VEGF	.950	.875	.980	<0.001
HIF1α – Microscopic	.699	.371	.872	<0.001
HIF1α – Macroscopic	.862	.677	.944	<0.001
VEGF – Microscopic	.695	.365	.870	<0.001
VEGF – Macroscopic	.858	.669	.943	<0.001
Microscopic – Macroscopic	.878	.713	.951	<0.001

4. DISCUSSION

The research findings indicate that intraperitoneal administration of vitamin E in combination with olive oil effectively reduces the mRNA expression and protein levels of HIF-1α and VEGF, which are key factors in the regulation of angiogenesis and fibrosis. These mechanisms of angiogenesis and fibrosis play a significant role in the formation of postoperative adhesions, where increased expression of HIF-1α triggers tissue hypoxia, leading to the activation of VEGF. As a primary factor in the formation of new blood vessels, VEGF enhances vascular permeability and fibroblast proliferation, contributing to the development of more extensive and thicker adhesions. Consequently, inhibiting these two factors becomes a crucial target in preventing adhesion formation. Statistical analysis reveals that the group receiving the combination of

intraperitoneal vitamin E and olive oil experienced a significant reduction in gene expression and protein levels compared to the sham group and the groups receiving only oral olive oil or oral vitamin E ($p < 0.001$). This suggests that intraperitoneal administration of vitamin E has a stronger effect than oral administration in inhibiting the HIF-1α and VEGF pathways. The higher efficacy of the intraperitoneal route is likely due to the direct distribution of vitamin E to the traumatized peritoneal area post-surgery, allowing for better absorption and a broader surface area, thus achieving faster effects in reducing pro-angiogenic and pro-fibrotic factors that support adhesion formation.

Macroscopic evaluation results show a significant difference between the treatment and control groups regarding the severity of adhesion formation. In the intraperitoneal vitamin E group, 60% of subjects did not develop any adhesions, while the remainder exhibited only grade 1 and 3 adhesions, indicating that the formed adhesions were generally mild and easily detachable. Conversely, in the control group, 80% of subjects developed grade 3 adhesions, meaning the formed adhesions were quite strong and difficult to remove, while the remaining 20% developed grade 4 adhesions, signifying very tight adhesions that merged with surrounding tissues. These differences confirm that vitamin E plays a role in reducing the severity of adhesions by suppressing factors contributing to their formation.

Furthermore, microscopic evaluation demonstrated that adhesion tissues in the treatment group were thinner and less abundant compared to the control group. In the control group, there was an increased proliferation of fibroblasts, enhanced deposition of the extracellular matrix, and heightened vascularization of the adhesion tissue. On the other hand, in the group receiving intraperitoneal vitamin E, fewer active fibroblasts were observed, with a more organized extracellular matrix and minimal blood vessel formation. This indicates that vitamin E exerts a protective effect on postoperative peritoneal tissues by suppressing fibroblast proliferation and reducing collagen deposition and inflammatory factors involved in adhesion formation.

Additionally, correlation tests conducted in this study revealed a significant relationship between HIF-1α mRNA expression and VEGF mRNA expression, as well as HIF-1α and VEGF protein levels. The correlations found in this study reinforce the hypothesis that HIF-1α expression regulates VEGF expression, which subsequently induces adhesion tissue formation through increased angiogenesis mechanisms. With the inhibition of HIF-1α expression by vitamin E, VEGF expression also decreased, ultimately leading to reduced adhesion tissue formation. Reduced angiogenesis due to decreased VEGF can result in diminished oxygen and nutrient supply to fibroblasts involved in adhesion formation, making the formed adhesions more minimal and less stable compared to the control group. These findings confirm that vitamin E has a protective effect in preventing postoperative adhesions through the inhibition of the HIF-1α and VEGF pathways. Besides acting as a potent antioxidant capable of neutralizing free radicals generated from surgical trauma, vitamin E also exhibits anti-inflammatory effects that suppress the activation of pro-inflammatory factors such as TNF-α and NF-κB, known to regulate HIF-1α. Thus, vitamin E not only directly suppresses the expression of pro-angiogenic factors but also reduces inflammatory stimulation contributing to adhesion formation.

This study provides strong evidence that a combination of vitamin E and olive oil, particularly through intraperitoneal administration, can significantly reduce the level and severity of postoperative adhesions. However, further research is needed to explore the optimal dosage that can provide maximal protective effects, as well as long-term studies to assess potential side effects of prolonged vitamin E administration. Therefore, the results of this study offer a solid scientific basis for considering vitamin E as a potential therapy in preventing postoperative adhesions, which can be further developed for broader clinical applications.

The findings of this study align with previous studies demonstrating the effectiveness of vitamin E in preventing adhesions through various biological mechanisms. One relevant study found that vitamin E administration could reduce adhesion formation in a rat model with uterine trauma.[13] This study highlighted the significant role of vitamin E's antioxidant effect in suppressing inflammatory processes resulting from tissue trauma, ultimately reducing the stimulation of pro-inflammatory factors like TNF-α and IL-1β. With the reduction of these inflammatory factors, fibroblast activation and excessive collagen production can also be suppressed, leading to minimal adhesion formation. Additionally, Atilgan et al. reported that vitamin E administration not only decreased the number of formed adhesions but also reduced their severity, consistent with the findings of this study.

Another study supporting these findings is by Portilla et al. (2004), which reported that intraperitoneal administration of vitamin E had the ability to decrease the intensity of peritoneal adhesions, both macroscopically and microscopically.[14] The study showed that the group receiving vitamin E experienced a significant reduction in adhesions compared to the control group, especially concerning the thickness of adhesion tissue, the number of active fibroblasts, and extracellular matrix deposition. This effect is suspected to be related to vitamin E's ability to inhibit lipid peroxidation caused by postoperative oxidative stress, which, if uncontrolled, can trigger increased production of pro-inflammatory cytokines and the expression of factors promoting adhesions like HIF-1α and VEGF. Therefore, the results from Portilla et al.'s study further strengthen the evidence that vitamin E has potential as a therapeutic agent in preventing adhesions by targeting inflammatory and oxidative mechanisms involved in the adhesion process.[14]

Moreover, research by Yetkin et al. (2009) also supports these findings, showing that the effectiveness of vitamin E in

preventing adhesions is comparable to sodium hyaluronate/carboxymethylcellulose (HA/CBMC), the standard therapy for postoperative adhesion prevention.[15] HA/CBMC is known to function by forming a physical barrier between traumatized tissues, thereby reducing the likelihood of adhesions. The fact that vitamin E demonstrates similar effectiveness to HA/CBMC indicates that its anti-adhesion mechanism is not solely derived from antioxidant and anti-inflammatory effects but may also involve reducing adhesive interactions between traumatized tissues. Thus, this study reinforces the potential of vitamin E as an alternative therapy in preventing postoperative adhesions, especially for patients who may not receive HA/CBMC-based therapy due to cost or availability issues.

Nevertheless, although the results of this study align with several previous studies, there are discrepancies with research conducted by Zaffarin et al. (2020).[16] In their study, it was found that plasma tocotrienol content after intraperitoneal administration was lower compared to oral or parenteral administration. This suggests that the effectiveness of vitamin E in preventing adhesions might be influenced by the route of administration, with oral or parenteral administration potentially being more optimal in increasing plasma tocotrienol levels. Zaffarin et al. also noted that the bioavailability of tocotrienol is lower compared to tocopherol, so its effectiveness in adhesion prevention likely depends on the form of vitamin E used.[16]

These differences can be explained through the metabolism and distribution mechanisms of vitamin E in the body. Tocotrienol and tocopherol have structural differences that affect how they are absorbed and distributed in tissues. Tocotrienol tends to have a shorter half-life compared to tocopherol and is metabolized more quickly in the liver, resulting in lower availability in systemic circulation. This can lead to variations in effectiveness depending on the route of administration. In this study, intraperitoneal administration of vitamin E with olive oil showed more significant effects compared to oral administration, likely due to direct distribution to the traumatized peritoneal area. However, findings from Zaffarin et al. indicate that in terms of systemic bioavailability, oral or parenteral administration may be more optimal in increasing plasma vitamin E levels over a longer duration.[16]

The results of this study support the hypothesis that vitamin E has potential as an anti-adhesion agent through the inhibition of HIF-1α and VEGF expression. Both factors play crucial roles in regulating angiogenesis and fibrosis, contributing to postoperative adhesion formation. Vitamin E acts as a potent antioxidant that can inhibit lipid peroxidation and oxidative stress resulting from postoperative inflammation. Uncontrolled oxidative stress can increase the production of reactive oxygen species (ROS), which in turn activates the HIF-1α pathway under hypoxic conditions. HIF-1α serves as a primary transcription factor that induces VEGF expression, subsequently triggering fibrous tissue and adhesion formation by enhancing fibroblast proliferation and tissue vascularization. Therefore, by suppressing HIF-1α expression, vitamin E also directly decreases VEGF expression, thereby inhibiting angiogenesis and excessive adhesion formation.

Additionally, vitamin E is known to possess anti-inflammatory effects that contribute to preventing adhesions. Some studies indicate that vitamin E can inhibit the activation of the transcription factor NF-κB, which is involved in the production of pro-inflammatory cytokines such as TNF-α and IL-6. These cytokines play a role in recruiting immune cells to the trauma site and increasing fibroblast activation, thereby accelerating adhesion formation. By inhibiting the NF-κB pathway, vitamin E can reduce excessive inflammation and suppress fibroblast activity contributing to adhesion formation. Moreover, the effect of vitamin E in reducing pro-inflammatory factor production may also contribute to accelerated wound healing without causing excessive scar tissue formation.

Despite the effectiveness of vitamin E in suppressing postoperative adhesions, several challenges and controversies remain regarding its long-term efficacy. One major discrepancy between this study and others is the limited bioavailability of vitamin E through the intraperitoneal route. Research by Zaffarin et al. (2020) showed that plasma tocotrienol content after intraperitoneal administration was lower compared to oral or parenteral routes.[16] This raises questions about whether the effectiveness of intraperitoneally administered vitamin E can be sustained over a longer period or if its effects are temporary due to limited systemic distribution. The bioavailability of vitamin E is also influenced by its chemical form, with tocotrienol having a shorter half-life and being metabolized more quickly than tocopherol. Therefore, the effectiveness of vitamin E in preventing adhesions may depend on its specific form, as well as the dosage and route of administration.

Furthermore, although vitamin E has proven effective in inhibiting adhesions, further studies are needed to explore the possibility of combining it with other agents that could enhance its effectiveness. Some studies suggest that combining vitamin E with other agents like curcumin, resveratrol, or hyaluronic acid may provide stronger protective effects against adhesions. Curcumin, for instance, possesses potent anti-inflammatory and antioxidant properties and may work synergistically with vitamin E in suppressing pro-inflammatory cytokine production and reducing oxidative stress. Hyaluronic acid, on the other hand, has been widely used as an anti-adhesion agent functioning by forming a protective layer between traumatized tissues. Combining vitamin E with hyaluronic acid may offer additional protection by inhibiting adhesions through two different mechanisms: the antioxidant and anti-inflammatory effects of vitamin E and the mechanical effects of hyaluronic acid in preventing direct contact between traumatized tissues.

Another factor to consider is the potential side effects of high-dose vitamin E administration, especially when used over the long term. Some studies indicate that high doses of vitamin E can disrupt the body's antioxidant system balance, potentially increasing oxidative stress under certain conditions. Additionally, large amounts of vitamin E can interfere with vitamin K

metabolism, which is essential for blood coagulation, thereby increasing the risk of bleeding. Therefore, although this study shows that vitamin E has significant potential as an anti-adhesion agent, further research is needed to determine the optimal dose that maximizes benefits without causing adverse side effects.

5. CONCLUSION

This study demonstrates that vitamin E plays a role in reducing the incidence and severity of postoperative adhesions following laparotomy, particularly when administered intraperitoneally in combination with olive oil, which influences the regulation of the transcription factor HIF-1α. Intraperitoneal vitamin E also regulates soluble HIF-1α levels to prevent adhesion formation in animal models, with a similar effect observed on VEGF, where vitamin E combined with olive oil suppresses VEGF transcription factor expression and regulates soluble VEGF levels, thereby inhibiting adhesion formation. The results further indicate that intraperitoneal administration of vitamin E with olive oil leads to a greater reduction in HIF-1α and VEGF mRNA expression as well as HIF-1α and VEGF protein levels compared to oral administration, with statistically significant differences confirming the superior efficacy of the intraperitoneal route in preventing postoperative adhesions. Additionally, a correlation was found between the macroscopic and microscopic adhesion grades and the expression levels of HIF-1α and VEGF mRNA, as well as HIF-1α and VEGF protein levels, highlighting the role of vitamin E in regulating the HIF-1α and VEGF pathways that contribute to adhesion formation. While this study provides strong evidence of vitamin E's effectiveness, some potential side effects must be considered, including the risk of disrupting antioxidant balance and affecting inflammatory regulation, such as increased TNF-α levels, along with possible digestive disturbances, allergic reactions, and long-term impacts on vitamin D metabolism due to high doses of vitamin E and excessive olive oil intake. The study's strengths lie in its comprehensive evaluation methods, including macroscopic and microscopic grading based on Zühlke's system, molecular analysis of HIF-1α and VEGF mRNA expression using qPCR, and protein quantification via ELISA, providing a more precise molecular approach. Additionally, robust statistical analyses—including Kolmogorov-Smirnov normality tests, Levene's homogeneity test, and data evaluation through one-way ANOVA, independent t-tests, and Kruskal-Wallis tests—which also demonstrated vitamin E's effectiveness in adhesion prevention. However, some limitations must be acknowledged, including the need for further research to determine the optimal vitamin E dosage for maximal anti-adhesion effects, the relatively small sample size of only 20 Wistar rats divided into four groups of five, which limits generalizability, and the short study duration of just 14 days post-treatment, restricting the ability to assess long-term effects. Future studies are necessary to explore vitamin E's efficacy, safety, and mechanisms in preventing postoperative adhesions more comprehensively.

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