

Comparative Effects of Intraperitoneal Natural and Biological Agents on Postoperative Peritoneal Adhesion Formation: An Experimental Rat Study

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ABSTRACT

Aim: Postoperative peritoneal adhesions are driven by inflammatory and oxidative pathways, and effective prevention remains challenging. This study evaluated the comparative effects of intraperitoneal natural and biological agents on adhesion formation in a rat model.

Material and Methods: Fifty-six male Wistar rats were randomly assigned to eight groups (n=7 per group). A standardized cecal abrasion model was used to induce adhesions. Treatment groups received intraperitoneal synovial fluid, honey, water-extracted propolis, vitamin E, olive oil, or vitamin E plus olive oil. The alcohol group was excluded due to early mortality. On postoperative day 14, adhesions were evaluated macroscopically (Nair score), histopathologically (fibrosis, inflammation, vascular proliferation), and biochemically (IL-6, glutathione, HSP-70).

Results: Macroscopic adhesion scores differed significantly among groups (p<0.001), with the lowest scores observed in the vitamin E plus olive oil group and the highest in the propolis group. Vitamin E plus olive oil and olive oil alone significantly reduced adhesion compared with control and propolis (p<0.001). Fibrosis scores were significantly lower in the vitamin E group compared with propolis (p=0.040). IL-6 levels were lower in the honey and synovial fluid groups compared with propolis (p=0.001), while glutathione levels were higher in the synovial fluid group (p=0.031). HSP-70 levels were lower in the honey group compared with control (p=0.011).

Conclusion: Vitamin E and olive oil, particularly in combination, were associated with the greatest reduction in macroscopic adhesion formation. Honey and synovial fluid demonstrated favorable anti-inflammatory and antioxidant profiles, whereas water-extracted propolis did not provide protective benefit. Further studies are required to clarify optimal dosing and translational applicability.

Keywords: Peritoneal adhesions, honey, propolis, vitamin E, olive oil, synovial fluid, interleukin-6, glutathione, HSP-70, rat model.

INTRODUCTION

Postoperative peritoneal adhesions remain one of the most prevalent and clinically challenging complications of abdominal surgery. Despite improvements in minimally invasive techniques and perioperative management, adhesions continue to occur in a substantial proportion of patients undergoing laparotomy (1,2). Their consequences include small bowel obstruction, chronic abdominal pain, infertility in women, and increased technical difficulty during reoperations. Beyond individual morbidity, adhesion-related complications impose significant economic burdens on healthcare systems due to recurrent hospitalizations, prolonged operative times, and increased surgical complexity (3,4). Thus, effective and safe prevention of postoperative adhesions remains an unresolved clinical priority.

The pathogenesis of peritoneal adhesions is initiated by surgical trauma to the mesothelial lining, leading to a disruption of the delicate equilibrium between fibrin deposition and fibrinolysis. Tissue injury triggers an inflammatory cascade characterized by the release of cytokines, growth factors, and reactive oxygen species (5). During the early postoperative period, fibrin-rich exudates

form temporary bridges between adjacent peritoneal surfaces. Under physiological conditions, adequate fibrinolytic activity resolves these bridges within 72 hours. However, when inflammation and oxidative stress suppress fibrinolysis, persistent fibrin matrices facilitate fibroblast proliferation, extracellular matrix deposition, neovascularization, and ultimately permanent fibrous adhesion formation. Among the mediators involved, interleukin-6 (IL-6) serves as a central pro-inflammatory cytokine, while heat shock protein-70 (HSP-70) reflects cellular stress responses (6,7). Conversely, glutathione (GSH) plays a protective role by limiting oxidative injury and modulating inflammatory signaling (8).

Various pharmacological and mechanical strategies have been explored to prevent adhesion formation, including anti-inflammatory agents, anticoagulants, fibrinolytics, antioxidants, and bioresorbable barrier materials. Hyaluronic acid-based membranes have demonstrated partial efficacy by physically separating traumatized peritoneal surfaces during the critical healing window (3,9). Nevertheless, no intervention has consistently achieved universal effectiveness, and concerns regarding

cost, availability, and potential side effects continue to limit widespread adoption. Consequently, attention has shifted toward biologically active natural substances that may provide combined mechanical, anti-inflammatory, and antioxidant effects.

Natural compounds such as honey and propolis have been extensively investigated for their antimicrobial, immunomodulatory, and antioxidant properties (10). Honey has been shown to downregulate pro-inflammatory cytokines and reduce oxidative stress, whereas propolis contains flavonoids and phenolic acids with documented antioxidant activity (11). Similarly, olive oil—particularly due to its polyphenolic constituents—and vitamin E are recognized for their strong free radical scavenging capacity and potential antifibrotic effects (12). In addition to these natural agents, synovial fluid contains hyaluronic acid and glycosaminoglycans that contribute to lubrication, tissue repair, and modulation of inflammatory processes. Given the established role of hyaluronic acid-based barriers in adhesion prevention, intraperitoneal synovial fluid may exert both mechanical separation and biological modulation of adhesion formation (3,13,14).

Although several of these agents have been evaluated individually in experimental settings, comparative data integrating macroscopic, histopathological, and biochemical parameters remain limited. A direct head-to-head experimental comparison may provide valuable insights into their relative effectiveness and underlying mechanisms.

We hypothesized that agents with strong antioxidant and anti-inflammatory properties—particularly vitamin E and olive oil, alone or in combination—would demonstrate superior efficacy in reducing postoperative adhesion formation compared with control and propolis-treated groups. Furthermore, we postulated that effective anti-adhesive agents would be associated with lower IL-6 and HSP-70 levels and higher GSH concentrations, reflecting attenuation of inflammatory and oxidative stress pathways. Therefore, the present study aimed to evaluate and compare the effectiveness of intraperitoneally administered synovial fluid, honey, propolis, vitamin E, and olive oil in a rat model of postoperative peritoneal adhesion, using comprehensive macroscopic, histopathological, and biochemical assessments.

MATERIAL AND METHOD

Study Design and Ethical Approval

This experimental animal study was designed as a randomized, controlled *in vivo* investigation and reported in accordance with the ARRIVE 2.0 guidelines. The study protocol, primary and secondary outcome measures, and statistical analysis plan were predefined prior to initiation of the experiment.

All procedures were conducted at the Experimental Animals Application and Research Center of Düzce University following approval by the Institutional Animal Care and Use Committee (Approval No: 2019/10/2). The study was performed in compliance with national regulations governing laboratory animal experimentation and adhered to the principles outlined in the Guide for the Care and Use of Laboratory Animals. All efforts were made to minimize animal suffering and reduce the number of animals used.

Animals and Experimental Conditions

A total of 56 adult male Wistar albino rats weighing between 250 and 300 grams were included in the study. Male animals were specifically selected to eliminate hormonal variability related to the estrous cycle, which may influence inflammatory and fibrotic responses.

The animals were housed in polypropylene cages under standardized environmental conditions with a controlled temperature of 23–25°C, relative humidity of 50–60%, and a 12-hour light/dark cycle. Standard pellet diet and water were provided *ad libitum*. Food was withheld for 10–12 hours prior to surgery to reduce the risk of aspiration, while water access was maintained.

Animals were acclimatized for one week prior to the experimental procedures.

Sample Size Determination

Sample size planning was performed based on previous experimental adhesion studies demonstrating moderate-to-large differences in macroscopic adhesion scores. Assuming an effect size sufficient to detect clinically meaningful differences with 80% statistical power at an alpha level of 0.05, a minimum of six animals per group was estimated to be sufficient. To account for potential perioperative mortality and experimental variability, seven animals were allocated to each group.

Randomization and Group Allocation

Following acclimatization, the animals were randomly allocated to eight experimental groups, with seven rats assigned to each group. Randomization was performed using a computer-generated random sequence to ensure unbiased allocation. Group assignments were concealed until the time of surgical intervention to minimize allocation bias.

The experimental groups consisted of a control group, synovial fluid group, honey group, propolis group, vitamin E group, olive oil group, vitamin E plus olive oil combination group, and an alcohol group. Each animal received the assigned intraperitoneal intervention immediately following induction of peritoneal injury.

In the alcohol group, intraperitoneal administration of 70% ethanol resulted in acute postoperative mortality in all animals within the first 24 hours. Due to complete mortality and ethical considerations, this group was discontinued and excluded from subsequent statistical analyses. Following this observation, alcohol-based propolis preparation was abandoned, and water-extracted propolis was used in order to eliminate alcohol-related toxicity and ensure experimental safety.

Induction of Peritoneal Adhesion Model

All surgical procedures were performed under sterile conditions. Following induction of general anesthesia with intraperitoneal ketamine (90 mg/kg) and xylazine (10 mg/kg), a 4 cm midline laparotomy was performed. The cecum was gently exteriorized and subjected to standardized serosal abrasion using sterile dry gauze until uniform petechial hemorrhage was observed. The corresponding anterior abdominal wall peritoneum was similarly traumatized to create opposing injured surfaces and ensure reproducible adhesion formation (Figure 1). Care was taken to apply consistent mechanical pressure and duration of abrasion in all animals to standardize the degree of peritoneal injury.



Figure 1. The cecum and anterior abdominal wall were traumatized with gauze.

Intraperitoneal Interventions

Immediately following induction of injury, the assigned agents were administered intraperitoneally. The control group received no intraperitoneal agent, while the synovial fluid group received 1 mL of sterile, filtered synovial fluid obtained from a large animal joint under aseptic conditions. The honey group received 1 mL of a 10% sterile-filtered honey solution prepared under laboratory conditions. The propolis group received 1.25 mL (equivalent to 90 mg) of water-extracted (alcohol-free) propolis solution, specifically prepared to eliminate alcohol-related toxicity and ensure biocompatibility. The vitamin E group received 0.5 mL vitamin E solution (75 mg), the olive oil group received 5 mL of sterile-filtered pure olive oil, and the vitamin E + olive oil group received a combination of 0.5 mL vitamin E (75 mg) and 4.5 mL olive oil. All agents were applied directly over the traumatized peritoneal surfaces before abdominal closure. The administered volumes differed between groups due to the distinct physicochemical properties and viscosity of each agent, as well as previously reported experimental protocols, and were selected to ensure adequate peritoneal surface coverage while avoiding volume-related complications. The abdominal wall was closed in two layers using 3/0 polyglactin sutures for the fascia and 3/0 polypropylene sutures for the skin. Postoperative analgesia was provided using fentanyl citrate (0.02 mg/kg, subcutaneously) administered twice within the first 24 hours, and no antibiotics were administered to avoid confounding inflammatory responses.

Postoperative Follow-Up

Animals were monitored daily for signs of distress, infection, wound dehiscence, or behavioral abnormalities. Body weight and general activity were observed throughout the 14-day follow-up period.

The 14-day interval was selected to allow sufficient time for maturation of fibrous adhesions.

Macroscopic Evaluation of Adhesions

On postoperative day 14, relaparotomy was performed under the same anesthesia protocol. An inverted U-shaped incision was made to avoid disrupting adhesions.

Macroscopic adhesions were evaluated using the Nair Adhesion Scoring System (Grade 0–4). Adhesion scoring was performed by two independent investigators blinded to group allocation. In cases of disagreement, consensus was reached through joint evaluation.

Histopathological Assessment

Adhesion tissues were excised and fixed in 10% neutral buffered formalin. After routine paraffin embedding, 4- μ m sections were stained with hematoxylin-eosin.

Fibrosis, inflammatory cell infiltration, and vascular proliferation were graded semi-quantitatively on a scale from 0 to 3 (0 = none, 1 = mild, 2 = moderate, 3 = severe). Histopathological evaluation was performed by a blinded pathologist unaware of group assignments.

Biochemical Analysis

At the time of relaparotomy, 2 mL of intracardiac blood was collected. Serum samples were obtained by centrifugation at 3000 rpm for 10 minutes and stored at -80°C until analysis.

Serum interleukin-6 (IL-6) levels were measured using a commercially available enzyme-linked immunosorbent assay (ELISA) kit according to the manufacturer's instructions.

Heat shock protein-70 (HSP-70) levels were quantified using ELISA-based methods.

Glutathione (GSH) levels were measured spectrophotometrically using an enzymatic colorimetric assay based on reduction of DTNB (5,5'-dithiobis-2-nitrobenzoic acid).

All measurements were performed in duplicate to ensure analytical reliability.

Statistical Analysis

Statistical analysis was performed using SPSS version 22 (IBM Corp., Armonk, NY, USA). Normality of data distribution was assessed using the Shapiro–Wilk test.

Since most variables did not demonstrate normal distribution, non-parametric tests were applied. Group comparisons were conducted using the Kruskal–Wallis test. When overall significance was detected, pairwise comparisons were performed using Dunn's post hoc test with Bonferroni correction.

Continuous variables were presented as median (interquartile range) and minimum–maximum values. A p -value < 0.05 was considered statistically significant.

RESULTS

Initially, eight experimental groups were established, each consisting of seven animals ($n = 56$). Due to complete mortality in the alcohol group within the first postoperative day, this group was excluded from further analysis. Consequently, statistical evaluation was performed on seven groups ($n = 49$).

The results were analyzed under three categories: macroscopic adhesion scores, histopathological findings, and biochemical parameters.

Macroscopic adhesions were evaluated using the Nair adhesion scoring system. Dense adhesions corresponding to grades 3 and 4 were predominantly observed in the control and propolis groups. These high-grade adhesions were not observed in the vitamin E, olive oil, vitamin E + olive oil, honey, or synovial fluid groups. Conversely, low-grade adhesions (grades 0–1) were more frequently observed in the vitamin E- and olive oil-treated groups.

Kruskal–Wallis analysis demonstrated a statistically significant difference in macroscopic adhesion scores among the groups (Table 1). Post hoc Dunn testing revealed that the vitamin E + olive oil group had significantly lower adhesion scores compared with both the

control and propolis groups ($p < 0.001$). The olive oil group also demonstrated significantly reduced adhesion scores compared with the control and propolis groups ($p < 0.001$). In addition, the vitamin E group showed significantly lower adhesion scores than the propolis group ($p < 0.001$). According to the median rank distribution, the highest macroscopic adhesion scores were observed in the propolis group, whereas the lowest scores were observed in the vitamin E + olive oil group (Figure 2).

Grade 3 fibrosis was most frequently detected in the propolis group and was also observed in the control group. Grade 0 fibrosis was predominantly observed in the honey and vitamin E groups. Kruskal–Wallis analysis revealed a statistically significant difference in fibrosis scores among the groups (Table 1). Post hoc analysis showed a significant reduction in fibrosis in the vitamin E group

compared with the propolis group ($p = 0.04$). No other pairwise comparisons reached statistical significance. Consistent with these findings, the highest median rank fibrosis scores were observed in the propolis group, whereas the lowest scores were observed in the vitamin E group (Figure 2).

Grade 3 inflammatory infiltration was observed most frequently in the vitamin E, vitamin E + olive oil, and control groups. Grade 0 inflammation was most commonly observed in the honey group. Although differences in median rank values were noted among the groups, the overall comparison using the Kruskal–Wallis test did not demonstrate a statistically significant difference in inflammatory infiltration scores ($p = 0.067$) (Table 1, Figure 2).

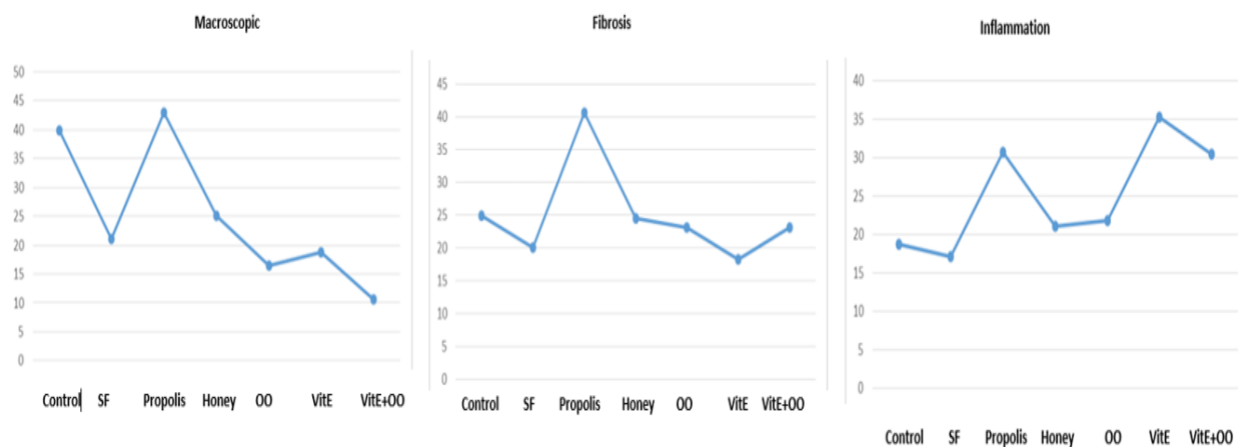


Figure 2. Median rank distributions of macroscopic adhesion, fibrosis, and inflammatory infiltration scores among the study groups. OO: Olive oil; VitE: Vitamin E; VitE+OO: Vitamin E + olive oil; SF: Synovial fluid. (From left to right, the graphs show macroscopic adhesion, fibrosis, and inflammatory infiltration scores. OO: Olive oil; VitE: Vitamin E; VitE+OO: Vitamin E + olive oil; SF: Synovial fluid.)

Table 1. Comparison of macroscopic, histopathological, and biochemical parameters among experimental groups.

Parameter	Control	Synovial Fluid	Honey	Propolis	Vitamin E	Olive Oil	Vitamin E + Olive Oil	p-value
Macroscopic Adhesion Score (Nair)	3 (3–4) [2–4]	2 (1–2) [1–3]	2 (1–2) [0–3]	4 (3–4) [3–4]	1 (1–2) [0–2]	1 (1–2) [0–2]	0 (0–1) [0–1]	<0.001
Fibrosis Score (0–3)	2 (1–2) [1–3]	1 (1–2) [0–2]	1 (0–1) [0–2]	3 (2–3) [2–3]	0 (0–1) [0–2]	1 (1–2) [0–2]	1 (0–1) [0–2]	0.040
Inflammation Score (0–3)	2 (2–3) [1–3]	1 (1–2) [0–2]	1 (0–1) [0–2]	2 (1–2) [1–3]	3 (2–3) [2–3]	2 (1–2) [1–3]	2 (1–2) [1–3]	0.067
Vascular Proliferation (0–3)	1 (0–1) [0–2]	1 (0–1) [0–2]	0 (0–1) [0–1]	2 (1–2) [1–2]	1 (1–2) [0–2]	1 (0–1) [0–2]	1 (0–1) [0–2]	0.118
IL-6 (pg/mL)	68 (60–75) [55–82]	45 (38–52) [34–58]	40 (35–46) [30–52]	92 (85–101) [78–110]	58 (50–64) [45–70]	54 (48–60) [44–66]	50 (44–58) [40–63]	0.001
Glutathione (μmol/L)	4.8 (4.3–5.2) [4.0–5.6]	6.2 (5.8–6.8) [5.5–7.2]	5.5 (5.0–6.0) [4.6–6.4]	3.9 (3.5–4.2) [3.2–4.6]	5.8 (5.2–6.3) [4.9–6.8]	5.6 (5.0–6.1) [4.8–6.5]	6.0 (5.4–6.6) [5.1–7.0]	0.031
HSP-70 (ng/mL)	3.8 (3.4–4.2) [3.1–4.6]	3.1 (2.8–3.4) [2.5–3.7]	2.6 (2.3–2.9) [2.0–3.2]	3.5 (3.1–3.8) [2.9–4.1]	3.3 (3.0–3.6) [2.7–3.9]	3.2 (2.9–3.5) [2.6–3.8]	3.0 (2.7–3.3) [2.4–3.6]	0.011

OO, Olive oil; VitE, Vitamin E; SF, Synovial fluid; IL-6, Interleukin-6; HSP-70, Heat shock protein-70; IQR, Interquartile range. Values are presented as median (IQR) [minimum–maximum]. *p-values were derived from the Kruskal–Wallis test.

Grade 0 vascular proliferation was most frequently observed in the control, synovial fluid, and honey groups.

No grade 3 vascular proliferation was identified in any group.

Despite variation in median rank values, with the propolis group demonstrating the highest vascular proliferation scores and the honey group the lowest scores, the Kruskal–Wallis test did not reveal statistically significant differences among the groups (Table 1, Figure 3).

On postoperative day 14, serum interleukin-6 (IL-6), glutathione (GSH), and heat shock protein-70 (HSP-70) levels were analyzed. Kruskal–Wallis analysis demonstrated a statistically significant difference in IL-6 levels among the groups (Table 1). Post hoc Dunn testing showed that the honey group had significantly lower IL-6 levels compared with the propolis group ($p = 0.001$).

Similarly, the synovial fluid group demonstrated significantly lower IL-6 levels compared with the propolis group ($p = 0.001$). No additional statistically significant differences were observed. Median rank distributions are presented in Figure 3.

Analysis of GSH levels revealed a statistically significant difference between the synovial fluid and propolis groups ($p = 0.031$), with higher GSH levels observed in the synovial fluid group (Table 1). No other significant pairwise differences were identified. Median rank distributions are shown in

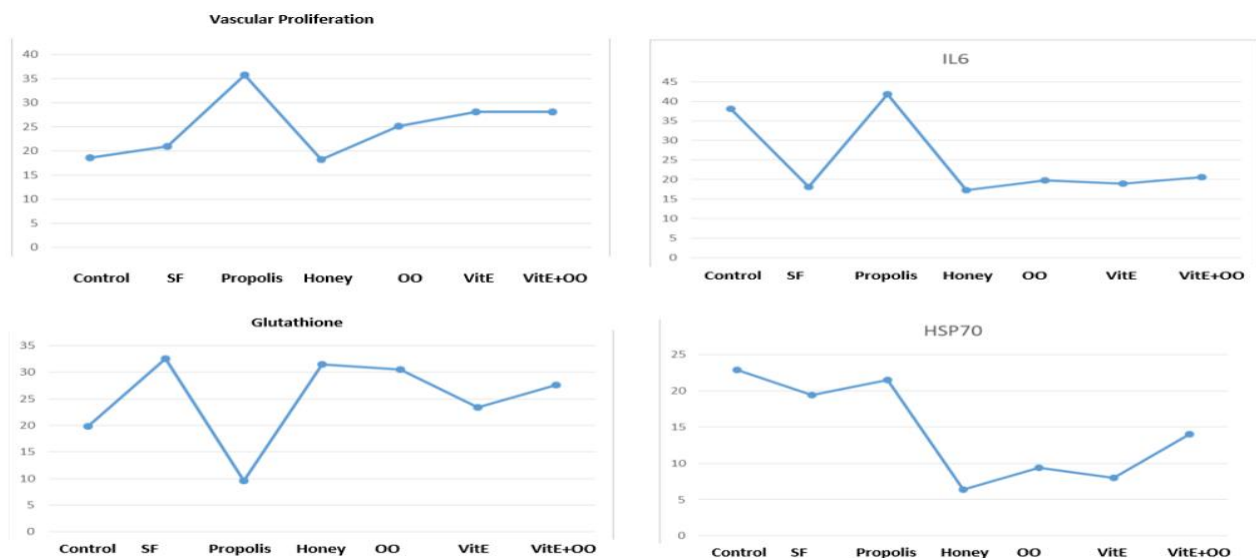


Figure 3. Median rank distributions of vascular proliferation and biochemical marker levels among the study groups. (Upper left: vascular proliferation; upper right: interleukin-6; lower left: glutathione; lower right: heat shock protein-70. OO: Olive oil; VitE: Vitamin E; VitE+OO: Vitamin E + olive oil; SF: Synovial fluid.)

DISCUSSION

Postoperative peritoneal adhesions remain a significant surgical challenge despite decades of experimental and clinical research. The present study provides a comparative evaluation of several biologically active and naturally derived agents using a standardized rat model and an integrated assessment approach incorporating macroscopic, histopathological, and biochemical parameters. The findings demonstrate that vitamin E and olive oil, particularly when administered in combination, were associated with the most pronounced reduction in macroscopic adhesion formation, whereas water-extracted propolis did not confer protective benefit and was associated with higher inflammatory and oxidative stress markers.

Adhesion formation is fundamentally driven by an imbalance between fibrin deposition and fibrinolysis during the early postoperative period. Excessive inflammatory activation and oxidative stress suppress fibrinolytic pathways and promote fibroblast proliferation and extracellular matrix deposition (7,15). In this context, the significant reduction in macroscopic adhesion scores observed in the vitamin E and olive oil groups may be attributed to their well-documented antioxidant and anti-inflammatory properties. Vitamin E functions as a lipid-soluble free radical scavenger, protecting cellular membranes from oxidative injury and modulating

inflammatory signaling cascades (16,17). Similarly, olive oil contains polyphenolic compounds such as oleuropein, which exhibit anti-inflammatory and antifibrotic activity. The combined administration of vitamin E and olive oil resulted in the lowest adhesion scores, suggesting a potential synergistic interaction between mechanical barrier effects and biochemical modulation of oxidative stress (12,18).

Interestingly, while the macroscopic benefit of vitamin E and olive oil was pronounced, histopathological parameters such as inflammation and vascular proliferation did not consistently demonstrate statistically significant reductions. This discrepancy may indicate that macroscopic adhesion severity is influenced not only by cellular inflammatory intensity but also by surface lubrication and mechanical separation during the critical early healing phase. Olive oil, in particular, may act partly as a transient physical barrier, limiting direct contact between injured peritoneal surfaces and thereby reducing adhesion maturation independent of deep histological remodeling (19).

In contrast to expectations based on its reported antioxidant and immunomodulatory properties, water-extracted propolis demonstrated the highest macroscopic adhesion scores and was associated with elevated IL-6 levels and reduced glutathione concentrations. These findings suggest that the formulation, solubility profile, or dosing strategy may

critically influence its biological effects (20). Propolis is a complex resinous compound whose activity depends on flavonoid concentration, extraction method, and bioavailability. Water-based extraction may reduce the concentration of lipophilic active components, potentially diminishing its antioxidant capacity. Furthermore, the elevated IL-6 levels observed in this group indicate enhanced inflammatory activation, which may have contributed to increased fibrosis and adhesion formation (21).

Honey demonstrated a different biological profile. Although macroscopic and histological improvements were modest, honey was associated with significantly lower IL-6 and HSP-70 levels. This suggests that honey may exert systemic anti-inflammatory effects without necessarily translating into substantial structural reduction of adhesion bands within the 14-day observation period. It is plausible that higher concentrations or prolonged follow-up could reveal more pronounced structural benefits (22). Synovial fluid, containing hyaluronic acid and glycosaminoglycans, exhibited moderate macroscopic improvement and was associated with higher glutathione levels and lower IL-6 compared with the propolis group. Hyaluronic acid-based barrier agents are known to reduce adhesion formation by creating temporary physical separation during mesothelial regeneration. The biochemical profile observed in the synovial fluid group supports a potential dual mechanism involving both mechanical protection and modulation of oxidative stress (23).

The biochemical findings provide additional insight into the mechanisms underlying adhesion formation. Elevated IL-6 levels were consistently associated with higher macroscopic adhesion severity, reinforcing the central role of inflammatory cytokine activation in adhesion pathogenesis. Similarly, lower glutathione levels in the propolis group highlight the importance of oxidative stress in promoting fibrotic remodeling. HSP-70, as a cellular stress marker, was highest in the control group and lowest in the honey group, suggesting that reduced cellular stress responses may accompany anti-inflammatory modulation (24,25).

Taken together, the results suggest that effective adhesion prevention may require both mechanical separation of injured surfaces and suppression of inflammatory and oxidative pathways. The combination of vitamin E and olive oil appears to address these mechanisms simultaneously, which may explain its superior macroscopic performance in this experimental model.

These findings may contribute to bridging traditional and complementary medicine practices with evidence-based experimental research, particularly in regions where natural products are widely used in clinical and non-clinical settings. In addition, the lack of protective effect observed with water-extracted propolis in the present study may be partly explained by the extraction method, as aqueous formulations may contain lower concentrations of lipophilic bioactive compounds compared to alcohol-based extracts.

Several limitations of the present study should be acknowledged. First, this was an experimental animal study conducted in a standardized rat model, and extrapolation of the findings to human clinical practice should be approached with caution. Although the cecal abrasion model is widely accepted for studying

postoperative peritoneal adhesions, it does not fully replicate the complexity of human abdominal surgery, which may involve infection, ischemia, foreign materials, and variable surgical techniques.

Second, the sample size within each group was relatively limited. While sufficient to detect significant differences in macroscopic adhesion scores, more subtle histopathological or biochemical differences may have remained undetected due to limited statistical power.

Third, only a single dosage and a single time point (14 days) were evaluated. Adhesion formation is a dynamic process, and earlier or later assessments may yield different results. In addition, dose-response relationships were not investigated, particularly for honey and propolis, whose biological activity may depend strongly on concentration and formulation.

Fourth, differences in the administered intraperitoneal volumes between groups may have influenced the mechanical barrier effect, potentially contributing to the observed macroscopic outcomes independent of the intrinsic biological activity of the agents.

Fifth, the propolis preparation used in this study was water-extracted. The biological activity of propolis varies considerably depending on extraction method and chemical composition. Therefore, the observed lack of benefit may be formulation-dependent rather than an inherent inefficacy of propolis itself.

Finally, although IL-6, glutathione, and HSP-70 were evaluated as markers of inflammation and oxidative stress, additional molecular analyses—such as assessment of fibrinolytic activity, TGF- β signaling, or matrix metalloproteinases—could have provided deeper mechanistic insight into adhesion pathophysiology.

Future studies incorporating different dosages, longer follow-up periods, standardized volume protocols, and expanded molecular profiling are warranted to clarify the translational relevance of these findings.

CONCLUSION

In this experimental rat model of postoperative peritoneal adhesion, intraperitoneal administration of vitamin E and olive oil—particularly in combination—was associated with the most pronounced reduction in macroscopic adhesion formation. Synovial fluid and honey demonstrated partial anti-inflammatory and antioxidant effects, whereas water-extracted propolis did not confer protective benefit and was associated with increased inflammatory and oxidative stress markers.

These findings suggest that effective adhesion prevention may require a multimodal approach involving both mechanical separation of injured peritoneal surfaces and modulation of inflammatory and oxidative pathways. The observed superiority of the vitamin E and olive oil combination may reflect a synergistic interaction between barrier function and biochemical activity.

Although these results are promising, they should be interpreted with caution due to the experimental design. Further well-designed experimental and clinical studies are required to determine optimal formulations, standardized dosing strategies, and long-term safety profiles before translation into routine clinical practice can be considered.

DECLARATIONS

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interest.

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Use of AI Tools: The authors declare that no Artificial Intelligence (AI) tools were used in the creation of this article.

REFERENCES

- Moris D, Chakedis J, Rahnemai-Azar AA, Wilson A, Hennessy MM, Athanasiou A, et al. Postoperative abdominal adhesions: clinical significance and advances in prevention and management. *J Gastrointest Surg*. 2017;21(10):1713-22. doi:10.1007/s11605-017-3488-9.
- Ghobrial S, Ott J, Parry JP. An overview of postoperative intraabdominal adhesions and their role on female infertility: a narrative review. *J Clin Med*. 2023;12(6):2263. doi:10.3390/jcm12062263.
- Fatehi Hassanabad A, Zarzycki AN, Jeon K, Dundas JA, Vasanthan V, Deniset JF, et al. Prevention of post-operative adhesions: a comprehensive review of present and emerging strategies. *Biomolecules*. 2021;11(7):1027. doi:10.3390/biom11071027.
- Pados G, Venetis CA, Almaloglou K, Tarlatzis BC. Prevention of intra-peritoneal adhesions in gynaecological surgery: theory and evidence. *Reprod Biomed Online*. 2010;21(3):290-303. doi:10.1016/j.rbmo.2010.04.021.
- Arung W, Meurisse M, Detry O. Pathophysiology and prevention of postoperative peritoneal adhesions. *World J Gastroenterol*. 2011;17(41):4545-53. doi:10.3748/wjg.v17.i41.4545.
- Liao J, Li X, Fan Y. Prevention strategies of postoperative adhesion in soft tissues by applying biomaterials: Based on the mechanisms of occurrence and development of adhesions. *Bioact Mater*. 2023;26:387-412. doi:10.1016/j.bioactmat.2023.02.026.
- Liu Z-Y, Li R-F, Qin H-Y, Ma P-F. Postoperative adhesion formation: the role of peritoneal macrophages and targeting therapy. *Front Immunol*. 2025;16:1601642. doi:10.3389/fimmu.2025.1601642.
- Forman HJ, Zhang H, Rinna A. Glutathione: overview of its protective roles, measurement, and biosynthesis. *Mol Aspects Med*. 2009;30(1-2):1-12. doi:10.1016/j.mam.2008.08.006.
- Teo ZY, Senthilkumar SD, Srinivasan DK. Nanotechnology-based therapies for preventing post-surgical adhesions. *Pharmaceutics*. 2025;17(3):389. doi:10.3390/pharmaceutics17030389.
- Wagh VD. Propolis: a wonder bees product and its pharmacological potentials. *Adv Pharmacol Sci*. 2013;2013:308249. doi:10.1155/2013/308249.
- Ahmed S, Sulaiman SA, Baig AA, Ibrahim M, Liaqat S, Fatima S, et al. Honey as a potential natural antioxidant medicine: an insight into its molecular mechanisms of action. *Oxid Med Cell Longev*. 2018;2018:8367846. doi:10.1155/2018/8367846.
- Bucciantini M, Leri M, Nardiello P, Casamenti F, Stefani M. Olive polyphenols: antioxidant and anti-inflammatory properties. *Antioxidants (Basel)*. 2021;10(7):1044. doi:10.3390/antiox10071044.
- Papakonstantinou E, Roth M, Karakiulakis G. Hyaluronic acid: a key molecule in skin aging. *Dermatoendocrinol*. 2012;4(3):253-8. doi:10.4161/derm.21923.
- Saad B. Immunomodulatory and anti-inflammatory properties of honey and bee products. *Immuno*. 2025;5(2):19. doi:10.3390/immuno5020019.
- Liang P, Zhao C, Peng X, Ye T, Deng L, Wang L, et al. Hydrogels strategies for organ adhesions: from mechanism to application. *J Nanobiotechnology*. 2026;24(1):122. doi:10.1186/s12951-025-03989-5.
- Losada-Barreiro S, Sezgin-Bayindir Z, Paiva-Martins F, Bravo-Díaz C. Biochemistry of antioxidants: mechanisms and pharmaceutical applications. *Biomedicines*. 2022;10(12):3051. doi:10.3390/biomedicines10123051.
- Traber MG, Atkinson J. Vitamin E, antioxidant and nothing more. *Free Radic Biol Med*. 2007;43(1):4-15. doi:10.1016/j.freeradbiomed.2007.03.024.
- Karković Marković A, Torić J, Barbarić M, Jakobić B, Braća C. Hydroxytyrosol, tyrosol and derivatives and their potential effects on human health. *Molecules*. 2019;24(10):2001. doi:10.3390/molecules24102001.
- Schwingshackl L, Christoph M, Hoffmann G. Effects of olive oil on markers of inflammation and endothelial function—a systematic review and meta-analysis. *Nutrients*. 2015;7(9):7651-75. doi:10.3390/nu7095356.
- Ayad AS, Benchaabane S, Daas T, Smaghe G, Loucif-Ayad W. Propolis stands out as a multifaceted natural product: meta-analysis on its sources, bioactivities, applications, and future perspectives. *Life (Basel)*. 2025;15(5):764. doi:10.3390/life15050764.
- Šuran J, Cepanec I, Mašek T. Propolis extract and its bioactive compounds—from traditional to modern extraction technologies. *Molecules*. 2021;26(10):2930. doi:10.3390/molecules26102930.
- Olas B. Honey and its phenolic compounds as an effective natural medicine for cardiovascular diseases in humans? *Nutrients*. 2020;12(2):283. doi:10.3390/nu12020283.
- Glinkowski W, Śladowski D, Tomaszewski W, Group P-IS. Molecular mechanisms and therapeutic role of intra-articular hyaluronic acid in osteoarthritis: a precision medicine perspective. *J Clin Med*. 2025;14(8):2547. doi:10.3390/jcm14082547.
- Hu Q, Xia X, Kang X, Song P, Liu Z, Wang M, et al. A review of physiological and cellular mechanisms underlying fibrotic postoperative adhesion. *Int J Biol Sci*. 2021;17(1):298-306. doi:10.7150/ijbs.54403.
- Awonuga AO, Belotte J, Abuanzeh S, Fletcher NM, Diamond MP, Saed GM. Advances in the pathogenesis of adhesion development: the role of oxidative stress. *Reprod Sci*. 2014;21(7):823-36. doi:10.1177/1933719114522550.