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Prevention of post-surgical adhesion bands by local administration of frankincense n-hexane extract

Fatemeh Jamshidi-adegani [a], Saeid Vakilian [a], Juhaina Al-kindi [a], Najeeb Ur Rehman [b], Laila Alkalbani [c], Mohammed Al-Broumi [b], Nasar Al-Wahaibi [c], Asem Shalaby [d], Jamal Al-Sabahi [e], Ahmed Al-Harrasi [b, *], Sulaiman Al-Hashmi [a, **]

[a] Laboratory for Stem Cell & Regenerative Medicine, Natural and Medical Sciences Research Center, University of Nizwa, Nizwa, P. O. Box: 33, PC 616, Oman
[b] Natural Products Laboratory, Natural and Medical Sciences Research Center, University of Nizwa, Nizwa, P. O. Box: 33, PC 616, Oman
[c] Department of Allied Health Sciences, College of Medicine & Health Sciences, Sultan Qaboos University, P. O. Box: 35, Alkoudh.123, Oman
[d] Department of Pathology, College of Medicine & Health Sciences, Sultan Qaboos University, P. O. Box: 35, Alkoudh.123, Oman
[e] Central Instrument Laboratory, College of Agriculture and Marine Sciences, Sultan Qaboos University, Oman

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ABSTRACT

Background: and purpose: The formation of postoperative intra-abdominal adhesion band formation may lead to severe complications. This study aimed to evaluate the preventive effect of local administration of frankincense n-hexane extract (FHE) on the formation of postsurgical adhesion bands.
Materials and methods: FHE was extracted from the resin of a *Boswellia sacra* tree and its components were identified by gas chromatography-mass spectrometry (GC-MS). In an animal model, the expression levels of TNF-α and TGF-β1 cytokines after application of FHE were assessed to check the inflammatory and fibrotic cues, respectively.
Results: Following FHE compound analysis, *in vivo* experiments demonstrated that intraoperative local administration of FHE resulted in the prevention of adhesion band formation. The adhesion grades in the FHE-treated group were significantly lower than those in the negative control (NC) and the positive control (Interceed). The infiltration of inflammatory cells observed by histopathology revealed a significant anti-inflammatory potential of FHE. Furthermore, the gene expression results proved that significant suppression of TNF-α and TGF-β1 was responsible for its antiadhesion properties.
Conclusions: The study reported the potential of FHE as an ointment for the prevention of adhesion bands.

1. Introduction

One of the common postoperative complications following abdominal surgeries is the formation of adhesion bands.[1] These bands may lead to several clinical side effects such as female infertility, bowel obstruction, and chronic abdominal-pelvic pain.[2] Different approaches and strategies have been applied to prevent adhesion band formation with the fewest side effects.[3,4] Removing adhesion bands by surgical techniques such as mechanical separation might be associated with side effects, as they required an additional surgical intervention. However, using physical barriers against tissues coming into direct contact postoperatively, such as fluids, solids, and different types of gels, could prevent the problem.[4,5] Additionally, these barriers can be made of anti-adhesive

Abbreviations: FHE, Frankincense n-Hexane extract; GC-MS, gas chromatography-mass spectrometry; NC, negative control; PC, positive control.

* Corresponding author. Natural products Laboratory, Natural and medicinal science research center, University of Nizwa, Nizwa, P. O. Box: 33, PC 616, Oman.
** Corresponding author. Laboratory for stem cell & Regenerative Medicine, Natural and medical science research center, University of Nizwa, Nizwa, P. O. Box: 33, PC 616, Oman.
E-mail addresses: aharrasi@unizwa.edu.om (A. Al-Harrasi), sahashmi@unizwa.edu.om (S. Al-Hashmi).
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agents such as anticoagulants, anti-inflammatory agents, antibiotics and anti-fibrinolytic agents.[6] Among these physical barriers, Surgicel, Seprafilm, Interceed, and ePTFE have been approved by the FDA, and they have moderate effectiveness in the prevention of adhesion bands but their use is associated with some technical problems.[3,7] Interceed is a degradable oxidized cellulosic membrane that is clinically approved to act as a barrier for the prevention of adhesion band formation between adjoining surfaces.[8]

To find an appropriate preventive agent for adhesion band formation, the mechanism underlying their induction needs to be fully understood. The etiopathogenesis of adhesion bands is a complicated process involving oxidative stress, ischemia, infection, and inflammation.[9] The lack of equilibrium between the fibrinolytic system and coagulation leads to inappropriate healing of the damaged tissue.[10] This process handles the formation of adhesive bands within the abdominopelvic cavity.[3] Adhesion band is formed from cellular components including macrophages, red blood cells, mast cells, eosinophils, tissue debris, and fibroblasts accompanied with the extracellular matrix.[11] Following adhesion development, the cells are modified from polymorphonuclear leukocyte cells into predominated fibroblasts.[11] There are some factors which can mediate inflammatory and immune responses, tissue remodeling, and angiogenesis involving in the adhesion band development.[11] Among them, inflammatory and fibrotic factors play a critical role in the initiation of intra-abdominal adhesive band formation.[12] An increase in the secretion of inflammatory cytokines such as tumor necrosis factor α (TNF-α) is responsible for the migration of inflammatory and fibroblast cells, forming adhesion bands.[13,14] Additionally, profibrotic transforming growth factor β (TGF-β) plays a critical role in the formation of adhesion bands. This growth factor is released from activated fibroblasts and mesothelial cells in the peritoneal cavity.[15] The expression of fibrotic genes caused by activation of the TGF-β1/Smad signaling pathway is responsible for the improper healing of damaged tissue.[16] Therefore, TGF-β1 has been demonstrated to play a substantial role in fibrogenesis, resulting in adhesion band formation.[15]

Several preventive agents with anti-inflammatory and antifibrotic properties have been used to reduce postsurgical intra-abdomen adhesion formation.[17] Natural products have shown excellent bioactivities to prevent adhesion band. Sahbaz et al. found that pycnogenol, an extract from the bark of the French maritime pine is effective for the prevention of post-surgical adhesion band.[18] In another experiment, Liu et al. applied the aqueous extract of Bletilla striata to reduce adhesion band formation in a rat model.[19] Also, it has been reported by Roohbakhsh et al. that the aerial parts of Rosmarinus officinalis could be beneficial in the prevention of intra-abdominal adhesion band formation.[20] Zhao et al. presented that a formula consists of six plants (including peach kernel, Chinese rhubarb, radish seed, Corydalis yanhusuo, safflower, and Glauber's salt) eased the formation of adhesion band.[21] Moreover, it has been demonstrated that Bromelain, a crude extract of the pineapple, could diminish the adhesion formation via the reduction of inflammation, neovascularization, and fibrosis.[18]

Frankincense (also known as olibanum) is an aromatic resin derived from trees in the genus Boswellia with many reported medicinal applications, mainly in the treatment of inflammatory chronic diseases.[22] It has been also reported that frankincense resin as a folk remedy may treat inflammatory disorders, including rheumatoid arthritis, osteoarthritis, asthma, chronic bowel diseases, cancer, and cervical spondylosis.[23] The anti-inflammatory activity, molecular targets, and mechanism of action of Boswellia extracts and their active components are well documented.[24–28] Several compounds such as Incensole, Incensole acetate, 1-octanol, α-pinene, and linalool have been isolated from frankincense n-hexane extract (FHE) and have been demonstrated to have antifibrotic and anti-inflammatory activities.[26,29] In addition to its antifibrotic and anti-inflammatory properties, FHE can also act as a physical barrier.[6]

In the present study, the antiadhesion properties of FHE were compared with those of *Interceed* as a positive control in a mouse model. To the best of our knowledge, no reports have previously described the anti-adhesive effects of FHE as a result of its anti-inflammatory and antifibrotic activities. The aim of this study was to evaluate the adhesion bands prevention properties of FHE in comparison with the conventional treatment.

2. Materials and methods

2.1. Sample collection and preparation of FHE

Royal Hougari White (RHW) frankincense (*Boswellia sacra*) was collected from Hougar Mountain, Salalah in 2012 and authenticated by a taxonomist, Natural and Medical Sciences Research Center, University of Nizwa. The voucher specimen (RHW-02/2012) of the resin was deposited in the herbarium of the Natural & Medical Sciences Research Center, University of Nizwa, Oman. The air dried gum resin of frankincense (10 g) was ground to a fine powder and used for the extraction of FHE through a Soxhlet apparatus. The FHE was exhaustively extracted with 100% analytical grade n-hexane (250 mL) at 70 °C (with continuous and vigorous shaking) for 6 h and the n-hexane was evaporated under reduced pressure to yield a yellow semisolid residue (1.5 g).

2.2. Gas-chromatography-mass spectrometry (GC-MS) analysis

Gas chromatography-mass spectrometry (GC-MS) analysis was performed on a PerkinElmer Clarus 600 GC System fitted with an Rtx-5MS capillary column (30 mm × 0.25 mm I.D. 0.25 μm film thickness; maximum temperature of 350 °C) and coupled to a PerkinElmer Clarus 600 mass spectrometer.[30,31] Ultrahigh purity helium (99.9999%) was applied as the carrier gas at a constant flow rate of 1.0 mL/min. The injection, transfer line, and ion source temperatures were adjusted to 250, 260 and 260 °C, respectively. The ionizing energy was 70 eV. Electron multiplier (EM) voltage was attained from autotuning. All data were acquired by obtaining full-scan of mass spectra within the scan range 40–550 a.m.u. A 1 μL sample was injected with a split ratio of 10:1. The oven temperature program was 60 °C (held for 1 min) at a rate of 4 °C/min–260° (held for 4 min). The total run time was 50 min. The identification of unknown compounds was performed by matching the spectra with mass spectrum libraries (NIST 2011 v.2.3 and Wiley, 9th edition).[30,31]

2.3. Animal study

Twenty-one male CD-1 mice with weight between 25 and 30 g were housed in standard condition (12 h light, 12 h dark, temperature 20–25 °C, and humidity 55 ± 10%). The mice were hosted with a maximum of seven mice per cage and allowed to acclimatize for one week before starting the procedure. Water and food were available ad libitum.

2.4. Experimental design

In the present work, the surgical procedures were authorized by the Animal Ethics Committee, University of Nizwa. Twenty-one male CD-1 mice were randomly divided into three groups (7 mice per group) as follows: negative control (NC), surgical abrasion without any treatment (n = 7); positive control (PC), surgical

abrasion plus *Interceed* (n = 7); and frankincense n-hexane extract (FHE), surgical abrasion plus frankincense n-hexane extract (n = 7). This study is reported in accordance with the ARRIVE guidelines (Animals in Research: Reporting *In Vivo* Experiments).[32]

2.5. Surgical technique

The animals were kept at room temperature (20–25 °C) under standard light/dark conditions. The Hemadeh (1993) model was applied to create adhesive bands inside the abdomen.[33] This model caused a 100% rate of adhesions in our negative control mice. The surgical procedure was performed under general anesthesia induced by ketamine/xylazine (ketamine: 70 mg/kg; xylazine: 10 mg/kg) in combination. Animals were positioned in a supine position on a heating pad (37 °C). Then, the abdominal areas of the mice were shaved and sterilized with alcohol and povidone-iodine. By creating a 2 cm vertical midline incision in the skin, the abdomen was opened. The exposed cecum was then gently rubbed using cotton dental tampons at all surfaces until it was no longer glistening and hemorrhagic points were visible. Then, the cecum replaced in its anatomic position in the abdominal cavity. Before suturing the abdominal wall, a 1.5 × 1.5 cm piece of Interceed or 60 μL FHE/mice were applied between the abdominal wall and peritoneum in the PC and FHE groups, respectively. After 1 week, the animals were euthanized using an overdose of anesthesia, and their abdominal cavities were reopened. Postsurgical adhesion band formation was evaluated using two scoring systems presented by Zühlke et al. (1997) and Duran et al. (2003) (Table 1).[34,35]

2.6. Histologic evaluation

The adhesion bands and the peritoneal tissues around them were isolated from the other tissues and then fixed with 10% formalin and immersed in paraffin. Several sections were prepared using a rotary microtome followed by staining with hematoxylin and eosin. The inflammation degree was obtained using a semi-quantitative scoring system, as shown in Table 1.[36]

2.7. Quantitation of cytokine gene expression by real-time PCR

Total RNA was isolated from adhesion tissues by means of RNX-Plus Solution (Sinaclon, Iran). cDNA was synthesized with the aid of M-MuLV reverse transcriptase and random hexamer, according to the manufacturer's protocols (Thermo Fisher Scientific, USA). The expression levels of tumor necrosis factor α (TNF-α) and transforminggrowth factor-β1 (TGF-β1) were quantified by the real-time PCR. PCR amplification was performed using Maxima™ SYBR Green/Fluorescein qPCR Master Mix (Thermo Fisher Scientific), and B2M (Beta2-microglobulin) transcripts served as endogenous controls. All reactions were performed in triplicates. The results are described as the relative expression of the genes normalized to the relevant internal control. QuantStudio (Applied Biosystems by Thermo Fisher Scientific, USA) was used for quantitation of the mRNA transcript expression. The primer sequences are listed in Table 2.

2.8. Statistical analysis

All variables are represented as the mean ± SD. Differences between the adhesion and inflammation scores were evaluated by Kuskal-Wallis test and the significant difference ($p < 0.05$) between specific mean ranks was determined using one-way analysis of variance (ANOVA).

3. Results

3.1. Component analysis using gas chromatography-mass spectrometry (GC-MS)

Several compounds were identified by the comparison of fragmentation patterns in the GC/MS spectra with the literature.[30,31] These compounds, along with their retention time, peak area, and RI, are presented in Table 3. As the peak area represents the amount of each compound, it could be concluded that FHE contains a high proportion of β-amyrin (23.49%), retinoic acid, methyl ester (8.54%), *trans*-verbenol (8.10%), α-farnesene (5.69%), and betulin (3.42%).

3.2. In vivo morphological and histological assessment

Among the FHE treated mice, no infection, mortality, or changes in behavior were detected through the end of the experimental period, suggesting the biocompatibility of FHE. To evaluate the impact of treatment on the prevention of adhesion bands, the abdominal cavity was incised and assessed morphologically (Fig. 1). The intra-abdomen adhesion bands were scored according to the Zuhlke and Duran approaches (Fig. 2).

As stated in Fig. 2, the FHE treated group revealed fewer adhesion bands than the NC and PC groups. To quantify the adhesion band grades, scores were given by two surgeons blinded to the treatment group according to the Zuhlke and Duran methods. The adhesion Zuhlke score of the FHE group was significantly lower than that of the negative and positive controls, indicating decreased adhesion band formation in the FHE-treated mouse abdomen compared to the other groups. Regarding the Duran score, the FHE-treated mouse abdomen revealed a smaller adhesion band area, resulting in a remarkably lower score than that of the negative and positive controls.

As inflammation plays a vital role in the formation of adhesion bands, the infiltration of inflammatory cells was assessed and scored by two expert pathologists. As shown in Figs. 3 and 4, the infiltration of inflammatory cells around the peritoneal surface was observed in the NC group. Even though there were fewer inflammatory cells in the PO-treated group compared to NC group, the difference between their inflammation scores was not significant ($P > 0.05$). As shown in Fig. 3, there was less infiltration of inflammatory cells in the FHE-treated group than in the NC and PC

Table 1
Adhesion and inflammation scoring system.

Criteria	Adhesion bands morphology		Histopathology
Grade	Zühlke et al.	Duran et al.	Inflammation
0	No adhesion	No adhesion	Nill
1	Filmy adhesions: gentle, blunt dissection required to free adhesions	≤25% of area	Giant cells, occasional scattered lymphocytes, and plasma cells
2	Mild adhesions: aggressive blunt dissection required to free adhesions	25–50% of area	Giant cells with increased numbers of admixed lymphocytes, plasma cells, eosinophils, neutrophils
3	Moderate adhesions: sharp dissection required to free adhesions	50–100% of area	Many admixed inflammatory cells, micro abscesses present
4	Severe adhesions: not dissectible without damaging organs	_____	_____

Table 2
The primers and product lengths.

Gene	Forward primer	Reverse primer	Product length(bp)
TNF-α	TCTTCTCATTCCTGCTTGTG	ACTTGGTGGTTTGCTACG	199
TGF-β	ATTCCTGGCGTTACCTTGG	CCTGTATTCCGTCTCCTTGG	117
B2M	GCTATCCAGAAAACCCCTC	CCCGTTCTTCAGCATTTG	132

Table 3
GC-MS analysis of the n-hexane extract of frankincense.

Compounds	RT (min)	RI	%
β-Amyrin	54.302	3337	23.49
Retinoic acid, methyl ester	53.977	2528	8.54
trans-Verbenol	11.686	1162	8.10
α-Farnesene	10.115	1496	5.69
Betulin	44.55	1752	3.42
β-Elemen	18.274	1387	3.22
6-Isopropenyl-4,8a-dimethyl-3,5,6,7,8,8a-hexahydro-2(1H)-naphthalenone	34.83	1772	3.18
trans-3(10)-Caren-2-ol	10.386	1194	3.11
γ-Gurjunene	21.113	1469	3.05
1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-, (E)-	37.94	1555	2.65
Caryophyllene oxide	23.908	1575	1.89
Berbenone	12.64	1183	1.83
(−)-β-Bourbonene	18.036	1386	0.93
β-Eudesmol	25.88	1644	0.91
β-Myrcene	5.781	979	0.83
5β,14β-Androstane-17β-carboxylic acid, 3β,14-dihydroxy	47.898	2477	0.81
Eucalyptol	7.168	1020	0.78
L-Pinocarveol	10.863	1143	0.77
α-Pinene	4.816	931	0.76
Retinol	52.85	2453	0.71
Bornyl acetate	14.958	1269	0.63
trans-Pinocarvyl acetate	9.42	1281	0.62
α-Bulnesene	21.395	1508	0.59
Limonene oxide, *trans*-	8.717	1121	0.57
Myrtenol	12.282	1174	0.54
Isoborneol	11.231	1146	0.49
Murolan-3,9(11)-diene-10-peroxy	46.489	1729	0.49
α-Santalol	45.482	1669	0.48
Cycloeucalenyl acetate	53.24	2900	0.39
Bicyclo[2.2.1]heptane-3-methylene-2,2-dimethyl-5-ol acetate	15.998	1271	0.32
Aromandendrene	20.831	1439	0.24
Pregnan-20-one, 3β-hydroxy-	41.191	2254	0.24
(−)-Globulol	22.066	1580	0.15
cis-Z-α-Bisabolene epoxide	27.842	1704	0.13
α-Terpineol acetate	17.093	1322	0.12
Total 80.67			

RI = Retention indices; RT = Retention time (min); % = Percentage.

groups. Accordingly, the inflammation score assessment in Fig. 4 demonstrated a significant anti-inflammatory effect of FHE in comparison with NC and PC.

3.3. Quantitative gene expression by real-time PCR

The expression of candidate cytokine genes (TNF-α and TGF-β1) was evaluated in the FHE and PC groups compared to the NC group. The results showed that the expression levels of TNF-α and TGF-β were downregulated in the FHE group (0.044 and 0.334 times, respectively) and upregulated in the PC group (2.639 and 6.543 times, respectively) compared to the NC group, significantly. As illustrated in Fig. 5, FHE caused a statistically significant decrease in TNF-α and TGF-β1 mRNA levels ($p < 0.05$) compared to the positive control group.

4. Discussion

In this study, the effect of FHE on the prevention of intra-abdominal adhesion band has been assessed using an *in vivo* mouse model. We reported here that FHE significantly decreased the infiltration of inflammatory cells and inhibited the formation of postoperative adhesion bands. This is possibly resulted from the anti-inflammatory and antifibrotic properties of the active compounds.

Several compounds with anti-inflammatory activities have been found in FHE. α-Pinene has shown an interesting anti-inflammatory capacity by inhibiting COX-2 [29]. Additionally, β-eudesmol could decrease inflammation by regulating IL-6 levels.[37] A remarkable regulation of inflammation has been observed in endotoxin-shocked mice treated with betulin.[38] Additionally, α-santalol has been proven to be an anti-inflammatory agent that can diminish skin inflammation.[39,40] In addition, the anti-inflammatory effects of retinoic acid on IFN-γ-treated astrocytes have been found to be mediated by suppression of the JAK/STAT pathway.[41] Another study suggested an inflammation-regulatory effect of β-amyrin in a rat model of periodontitis.[42] Likewise, eucalyptol, caryophyllene oxide, 1,6,10-dodecatrien-3-ol, 3,7,11-trimethyl-, (E)-, and retinol have anti-inflammatory activities that may improve chronic pain treatment.[43–46] It is worth mentioning that the FHE has greater

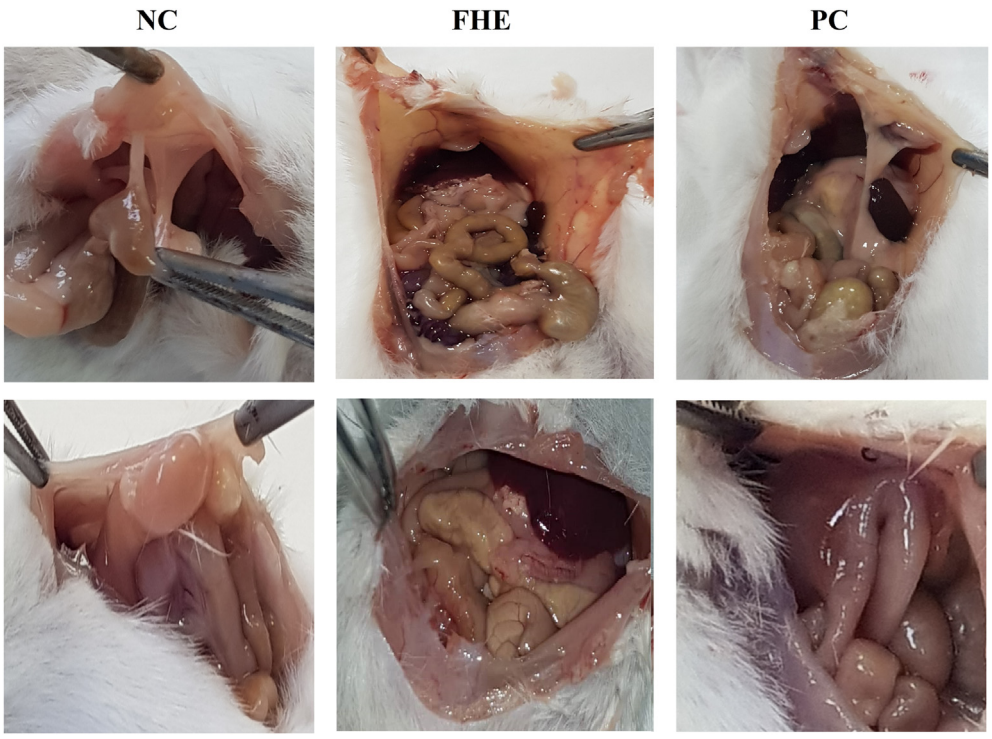

Fig. 1. The intraperitoneal adhesion formation in mice. NC: negative control group; FHE: frankincense n-hexane extract group; PC: positive control.

anti-inflammation properties than the water extract of frankincense.[47] This is possibly due to the low efficiency of liposoluble component isolation in water-based methodologies.[29]

In addition to the anti-inflammatory activities, antifibrotic effects could be beneficial for the prevention of intra-abdomen adhesion bands. The antifibrotic properties of caryophyllene oxide have been demonstrated by Eltahir et al. (2020) and Amiel et al. (2012).[17,48] Moreover, betulin could significantly inhibit the transduction signaling of TGF-β and NFκB/IκB, which are key players in adhesion band formation.[49] Likewise, the retinoic acid and β-amyrin available in FHE have exhibited strong antifibrotic activities.[50,51]

Due to the mentioned cues in the initiation of adhesion band formation, the recognized anti-inflammatory and antifibrotic compounds in FHE may be responsible for its intraabdominal adhesion preventive properties. According to the result, TNF-α, and TGF-β1 modulation involved in the intra-abdomen adhesion preventive properties of FHE. Kaidi et al. verified that the administration of anti-inflammatory drugs such as TNF-α blockers successfully reduced the rate of postoperative adhesion formation.[52] Moreover, among many cytokines, transforming growth factor- β1 (TGF-β1) has been documented to be associated with intraperitoneal adhesion development.[16] TGF-β1 may be the most important cytokine in adhesion band formation.[53] Both TGF-β1 and its receptor are increased in the peritoneal tissue and fluid after peritoneal surgery.[54] The suppression of TGF-β1 expression causes periostin inhibition, resulting in the prevention of adhesion band formation.[55]

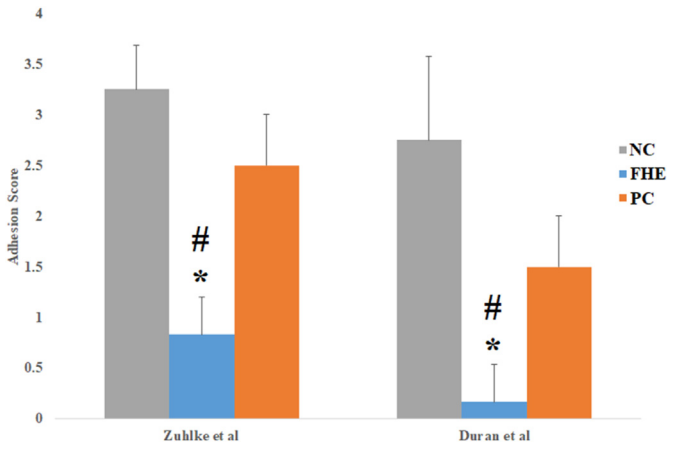

Fig. 2. Adhesion scores of each group using the Zulk and Duran scaling systems. * indicates a significant level compared to NC group at P value ≤ 0.05. # indicates a significant level compared to PC group at P value ≤ 0.05.

In line with our finding, breviscapine, a crude extract of Erigeron breviscapus, was reported to prevent the formation of post-surgical adhesion bands via the fibrotic and inflammatory pathways.[56] It has been revealed by Zhang et al. that breviscapine exerts antifibrotic and anti-inflammatory properties via the modulation of TNF-α, IL-18, and IL-6 in serum and TGF-β1, PAI-1, and connective tissue growth factor in the peritoneal fluid.[56] In the presented study, FHE could significantly reduce TNF-α, and TGF-β1 cytokines expression in tissue homogenates showing its anti-inflammatory and antifibrotic effects. On the other hand, in PC group, the expression levels of TNF-α, and TGF-β1 cytokines were highly expressed in comparison with the NC. This was in agreement with the reported result by Zhang et al.[57] Arora et al. have also revealed that Interceed induces an inflammatory response with foreign body giant cells, macrophages and eosinophils.[58] In summary, the presented results suggest that the anti-adhesive properties of FHE may occur through the regulation of TNF-α and TGF-β1. These findings support the preventive potency of FHE in the inhibition of post-surgical adhesion formation.

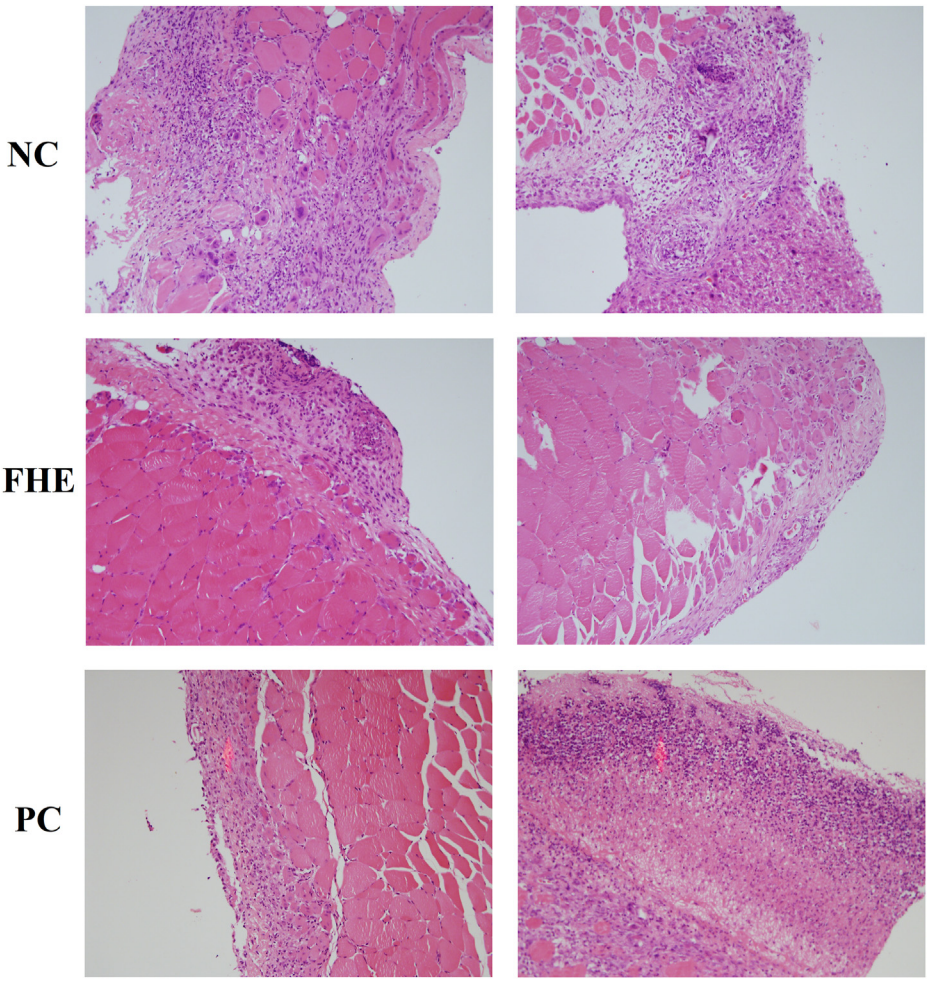

Fig. 3. Representative images of histological observations for NC, FHE, and PO. Scale bar: 100 μm.

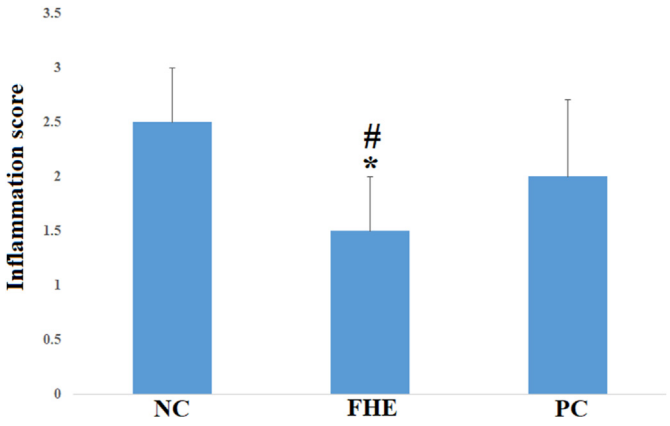

Fig. 4. Inflammation scores of the NC, FHE, and PC groups. * indicates a significant level compared to NC group at P value ≤ 0.05. # indicates a significant level compared to PC group at P value ≤ 0.05.

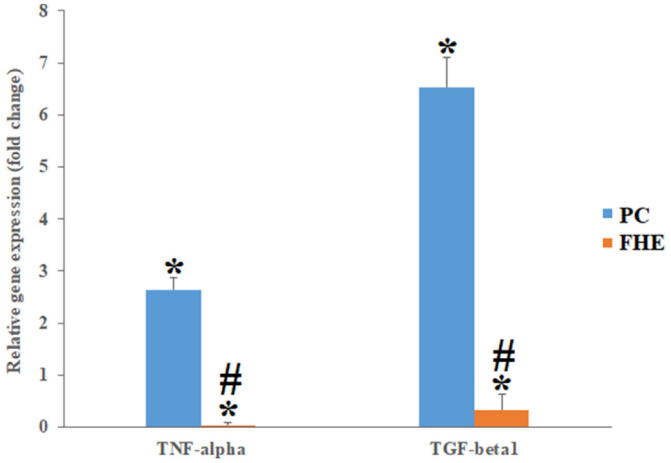

Fig. 5. Relative gene expression of TNF-α, and TGF-β1 in PC, and FHE compared to the NC. * indicates a significant level compared to the NC group at P value ≤ 0.05. # indicates a significant level compared to the PC group at P value ≤ 0.05.

5. Conclusion

This study concluded that FHE could prevent postsurgical adhesion formation. The results show that this effect may be mediated through its anti-inflammatory and antifibrotic activities. Further studies are warranted to assess other regulatory and inflammatory cytokines such as IL-1. IL-6, IL-10, and IL-17. Moreover, other factors such as oxidative stress, ischemia, and infection need to be addressed. Such studies might be helpful to identify the underlying pathways for the anti-adhesive properties of FHE and to develop a preventive agent for clinical applications.

Data statement

We declare here that all data belonging to the represented research are reproducible and clear. All the raw data are available for sharing upon request.

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Research involving animals

The animal experiments were performed according to the guidelines of the National Committee of Bioethics and the institutional Animal Ethics Committee (Approval ID: VCGSR, AREC/04/2020, Approval date: January 03, 2020). The animals were kept under standard laboratory conditions for housing, feeding, and breeding.

Author contributions

Fatemeh Jamshidi-adegani: Conceptualization, Methodology, Investigation, Writing-Original Draft, Saeid Vakilian: Methodology, Investigation, Writing-Review & Editing. Juhaina Al-kindi: Methodology, Investigation. Najeeb Ur Rehman: Investigation, Writing - Review & Editing. Laila Alkalbani: Investigation, Writing - Review & Editing, Mohammed Al-Broumi: Investigation, Nasar Al-Wahaibi: Methodology. Asem Shalaby: Investigation. Jamal Al-Sabahi: Investigation. Ahmed Al-Harrasi: Supervision, Funding acquisition, Writing - Review & Editing. Sulaiman Al-Hashmi: Conceptualization, Project administration, Writing-Original Draft, Supervision.

Declaration of competing interest

The authors declare no conflict of interest.

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References

1. Tabibian N, Swehli E, Boyd A, Umbreen A, Tabibian J. Abdominal adhesions: a practical review of an often overlooked entity. *Annals of Medicine and Surgery*. 2017;15:9–13.
2. Van Goor H. Consequences and complications of peritoneal adhesions. *Colorectal Dis*. 2007;9:25–34.
3. Boland GM, Weigel RJ. Formation and prevention of postoperative abdominal adhesions. *J Surg Res*. 2006;132(1):3–12.
4. Kamel RM. Prevention of postoperative peritoneal adhesions. *Eur J Obstet Gynecol Reprod Biol*. 2010;150(2):111–118.
5. Ahmad G, Thompson M, Kim K, et al. Fluid and pharmacological agents for adhesion prevention after gynaecological surgery. *Cochrane Database Syst Rev*. 2020;(7).
6. Fatehi Hassanabad A, Zarzycki AN, Jeon K, et al. Prevention of post-operative adhesions: a comprehensive review of present and emerging strategies. *Biomolecules*. 2021;11(7):1027.
7. Diamond MP, Wexner SD, Dizereg GS, et al. Adhesion prevention and reduction: current status and future recommendations of a multinational interdisciplinary consensus conference. *Surg Innovat*. 2010;17(3):183–188.
8. Wiseman DM, Trout JR, Franklin RR, Diamond MP. *Metaanalysis of the Safety and Efficacy of an Adhesion Barrier (Interceed TC7) in Laparotomy. Database of Abstracts of Reviews of Effects (DARE): Quality-Assessed Reviews [Internet]*. UK): Centre for Reviews and Dissemination; 1999.
9. Arjmand M-H. The association between visceral adiposity with systemic inflammation, oxidative stress, and risk of post-surgical adhesion. *Arch Physiol Biochem*. 2020:1–6.
10. Cheong Y, Laird S, Li T, Shelton J, Ledger W, Cooke I. Peritoneal healing and adhesion formation/reformation. *Hum Reprod Update*. 2001;7(6):556–566.
11. Fatehi Hassanabad A, Zarzycki AN, Jeon K, Deniset JF, Fedak PW. Post-operative adhesions: a comprehensive review of mechanisms. *Biomedicines*. 2021;9(8):867.
12. Hasdemir PS, Ozkut M, Guvenal T, et al. Effect of pirfenidone on vascular proliferation, inflammation and fibrosis in an abdominal adhesion rat model. *J Invest Surg*. 2017;30(1):26–32.
13. Başoğlu M, Kiziltunç A, Akcay F, Keleş S, Gündoğdu C, Durkaya Ö. Tumor necrosis Factor-\\alpha and interleukin-6 in peritoneal adhesion formation. *Turk J Med Sci*. 1998;28(3):253–258.
14. Tsutsui MSKIH, Jimbo KMM, Fujimoto EHJ. Blockade of tumor necrosis factor by etanercept prevents postoperative adhesion formation in mice. *Cell Physiol Biochem*. 2020;54:1041–1053.
15. Williams RS, Rossi AM, Chegini N, Schultz G. Effect of transforming growth factor β on postoperative adhesion formation and intact peritoneum. *J Surg Res*. 1992;52(1):65–70.
16. Gorvy DA, Herrick SE, Shah M, Ferguson MW. Experimental manipulation of transforming growth factor-β isoforms significantly affects adhesion formation in a murine surgical model. *Am J Pathol*. 2005;167(4):1005–1019.
17. Eltahir HM, Fawzy MA, Mohamed EM, Alrehany MA, Shehata AM, Abouzied MM. Antioxidant, anti-inflammatory and anti-fibrotic effects of Boswellia serrate gum resin in CCl4-induced hepatotoxicity. *Experimental and Therapeutic Medicine*. 2020;19(2):1313–1321.
18. Sahbaz A, Aynioglu O, Isik H, et al. Bromelain: a natural proteolytic for intra-abdominal adhesion prevention. *Int J Surg*. 2015;14:7–11.
19. Liu B, Zhang Q, Wu X, et al. Effect of Bletilla striata on the prevention of postoperative peritoneal adhesions in abrasion-induced rat model. *Evid base Compl Alternative Med*. 2019, 9148754.
20. Roohbakhsh Y, Rahimi VB, Silakhori S, et al. Evaluation of the effects of peritoneal lavage with rosmarinus officinalis extract against the prevention of postsurgical-induced peritoneal adhesion. *Planta Med*. 2020;86:405–414, 06.
21. Zhao M, Bian Y-Y, Yang L-L, et al. HuoXueTongFu formula alleviates intraperitoneal adhesion by regulating macrophage polarization and the SOCS/JAK2/STAT/PPAR-γ signalling pathway. *Mediat Inflamm*. 2019, 1769374.
22. Al-Harrasi A, Ali L, Hussain J, Rehman NU, Ahmed M, Al-Rawahi A. Analgesic effects of crude extracts and fractions of Omani frankincense obtained from traditional medicinal plant Boswellia sacra on animal models. *Asian Pacific journal of tropical medicine*. 2014;7:S485–S490.
23. Hamidpour R, Hamidpour S, Hamidpour M, Shahlari M. Frankincense (乳香 Rǔ Xiāng; Boswellia species): from the selection of traditional applications to the novel phytotherapy for the prevention and treatment of serious diseases. *Journal of traditional and complementary medicine*. 2013;3(4):221–226.
24. Shah BA, Qazi GN, Taneja SC. Boswellic acids: a group of medicinally important compounds. *Nat Prod Rep*. 2009;26(1):72–89.
25. Abdel-Tawab M, Werz O, Schubert-Zsilavecz M. Boswellia serrata. *Clin Pharmacokinet*. 2011;50(6):349–369.
26. Al-Harrasi A, Csuk R, Khan A, Hussain J. Distribution of the anti-inflammatory and anti-depressant compounds: Incensole and incensole acetate in genus Boswellia. *Phytochemistry*. 2019;161:28–40.
27. Al-Harrasi A, Hussain H, Csuk R, Khan HY. *Chemistry and Bioactivity of Boswellic Acids and Other Terpenoids of the Genus Boswellia*. Elsevier; 2018.
28. Ammon H. *Boswellic Acids and Their Role in Chronic Inflammatory Diseases. Anti-inflammatory Nutraceuticals and Chronic Diseases*. Springer; 2016:291–327.
29. Li X-J, Yang Y-J, Li Y-S, Zhang WK, Tang H-B. α-Pinene, linalool, and 1-octanol contribute to the topical anti-inflammatory and analgesic activities of frankincense by inhibiting COX-2. *J Ethnopharmacol*. 2016;179:22–26.
30. Rehman NU, Alsabahi JN, Bakht N, Khan A, Al-Harrasi A. Chemical constituents and biological activities of the oil from lycium shawii STEM. *Chem Nat Compd*. 2020:1–3.
31. Rehman NU, Hussain J, Ali S, et al. Chemical constituents of the essential oil of nepeta distans. *Chem Nat Compd*. 2020;56(1):159–160.
32. Kilkenny C, Browne WJ, Cuthill IC, Emerson M, Altman DG. Improving bioscience research reporting: the ARRIVE guidelines for reporting animal research. *PLoS Biol*. 2010;8(6), e1000412.
33. Hemadeh O, Chilukuri S, Bonet V, Hussein S, Chaudry IH. Prevention of peritoneal adhesions by administration of sodium carboxymethyl cellulose and oral vitamin E. *Surgery*. 1993;114(5):907–910.
34. Lorenz E, Zühlke H, Lange R, Savvas V. *Pathophysiology and Classification of Adhesions. Peritoneal Adhesions*. Springer; 1997:29–34.
35. Duran B, Cetin A, Guvenal T, Cetin M, Imir AG. Reduction of postoperative adhesions by N, O-carboxymethylchitosan and spermine NONOate in rats. *Exp Anim*. 2003;52(4):267–272.
36. Hooker GD, Taylor BM, Driman DK. Prevention of adhesion formation with use of sodium hyaluronate–based bioresorbable membrane in a rat model of ventral hernia repair with polypropylene mesh—a randomized, controlled study. *Surgery*. 1999;125(2):211–216.
37. Seo M-J, Kim S-J, Kang T-H, et al. The regulatory mechanism of β-eudesmol is through the suppression of caspase-1 activation in mast cell–mediated inflammatory response. *Immunopharmacol Immunotoxicol*. 2011;33(1):178–185.
38. Ci X, Zhou J, Lv H, Yu Q, Peng L, Hua S. Betulin exhibits anti-inflammatory activity in LPS-stimulated macrophages and endotoxin-shocked mice through an AMPK/AKT/Nrf2-dependent mechanism. *Cell Death Dis*. 2017;8(5):e2798. e2798.

39. Santha S, Dwivedi C. α-Santalol, a skin cancer chemopreventive agent with potential to target various pathways involved in photocarcinogenesis. *Photochem Photobiol*. 2013;89(4):919–926.
40. Bommareddy A, Brozena S, Steigerwalt J, et al. Medicinal properties of alpha-santalol, a naturally occurring constituent of sandalwood oil. *Nat Prod Res*. 2019;33(4):527–543.
41. Choi W-H, Ji K-A, Jeon S-B, et al. Anti-inflammatory roles of retinoic acid in rat brain astrocytes: suppression of interferon-γ-induced JAK/STAT phosphorylation. *Biochem Biophys Res Commun*. 2005;329(1):125–131.
42. Pinto SH, Pinto L, Cunha G, Chaves M, Santos F, Rao V. Anti-inflammatory effect of α, β-Amyrin, a pentacyclic triterpene from Protium heptaphyllum in rat model of acute periodontitis. *Inflammopharmacology*. 2008;16(1):48–52.
43. Juergens UR, Dethlefsen U, Steinkamp G, Gillissen A, Repges R, Vetter H. Anti-inflammatory activity of 1.8-cineol (eucalyptol) in bronchial asthma: a double-blind placebo-controlled trial. *Respir Med*. 2003;97(3):250–256.
44. Chavan M, Wakte P, Shinde D. Analgesic and anti-inflammatory activity of Caryophyllene oxide from Annona squamosa L. bark. *Phytomedicine*. 2010;17(2):149–151.
45. Shareef HK, Muhammed HJ, Hussein HM, Hameed IH. Antibacterial effect of ginger (Zingiber officinale) roscoe and bioactive chemical analysis using gas chromatography mass spectrum. *Orient J Chem*. 2016;32(2):20–40.
46. Wolf JE. Potential anti-inflammatory effects of topical retinoids and retinoid analogues. *Adv Ther*. 2002;19(3):109–118.
47. Banno N, Akihisa T, Yasukawa K, et al. Anti-inflammatory activities of the triterpene acids from the resin of Boswellia carteri. *J Ethnopharmacol*. 2006;107(2):249–253.
48. Amiel E, Ofir R, Dudai N, Soloway E, Rabinsky T, Rachmilevitch S. *β-Caryophyllene, a Compound Isolated from the Biblical Balm of Gilead (Commiphora Gileadensis), Is a Selective Apoptosis Inducer for Tumor Cell Lines. Evidence-Based Complementary and Alternative Medicine*. eCAM; 2012, 2012.
49. Szuster-Ciesielska A, Plewka K, Daniluk J, Kandefer-Szerszeń M. Betulin and betulinic acid attenuate ethanol-induced liver stellate cell activation by inhibiting reactive oxygen species (ROS), cytokine (TNF-α, TGF-β) production and by influencing intracellular signaling. *Toxicology*. 2011;280(3):152–163.
50. Xiao R, Yoshida N, Higashi Y, et al. Retinoic acids exhibit anti-fibrotic activity through the inhibition of 5-lipoxygenase expression in scleroderma fibroblasts. *J Dermatol*. 2011;38(4):345–353.
51. Thirupathi A, Silveira P, Nesi R, Pinho R. β-Amyrin, a pentacyclic triterpene, exhibits anti-fibrotic, anti-inflammatory, and anti-apoptotic effects on dimethyl nitrosamine–induced hepatic fibrosis in male rats. *Hum Exp Toxicol*. 2017;36(2):113–122.
52. Kaidi AA, Nazzal M, Gurchumelidze T, Ali MA, Dawe EJ, Silva YJ. Preoperative administration of antibodies against tumor necrosis factor-alpha (TNF-alpha) and interleukin-1 (IL-1) and their impact on peritoneal adhesion formation. *Am Surg*. 1995;61(7):569–572.
53. Uyama N, Tsutsui H, Wu S, et al. Anti-interleukin-6 receptor antibody treatment ameliorates postoperative adhesion formation. *Sci Rep*. 2019;9(1):1–14.
54. Wang G, Wu K, Li W, et al. Role of IL-17 and TGF-β in peritoneal adhesion formation after surgical trauma. *Wound Repair Regen*. 2014;22(5):631–639.
55. Takai S, Yoshino M, Takao K, Yoshikawa K, Jin D. Periostin antisense oligonucleotide prevents adhesion formation after surgery in mice. *J Pharmacol Sci*. 2017;133(2):65–69.
56. Zhang H, Song Y, Li Z, Zhang T, Zeng L. Evaluation of breviscapine on prevention of experimentally induced abdominal adhesions in rats. *Am J Surg*. 2016;211(6):1143–1152.
57. Zhang Y, Li X, Zhang Q, et al. Berberine hydrochloride prevents postsurgery intestinal adhesion and inflammation in rats. *J Pharmacol Exp Therapeut*. 2014;349(3):417–426.
58. Arora M, Jaroudi KA, Hamilton CJ, Dayel F. Controlled comparison of interceed and amniotic membrane graft in the prevention of postoperative adhesions in the rabbit uterine horn model. *Eur J Obstet Gynecol Reprod Biol*. 1994;55(3):179–182.