Epigenetic Regulation of Inflammatory Cytokines by Prophylactic Administration of *Ferula assa-foetida* Essential Oil in Murine Colitis

Ibrahim Khalaf Salem

Faculty of Veterinary Medicine, Urmia University, Iran
Corresponding author: hussainsahi048@gmail.com

Received: 17/3/2026 **Accepted:** 11/4/2026 **Published:** 15/6/2026

***Abstract*—** Colitis as well as Inflammatory Bowel Disease (IBD) is facilitated by imbalanced Mucosal immune response that is manifested by excessive secretion of pro-inflammatory cytokines. A new direction in the treatment suggests recent evidence of epigenetic modifications as the key regulators of this inflammatory cascade as a new direction of treatment. This experiment sought to examine the prophylactic action and the epigenetic mechanism of *Ferula assa-foetida* essential oil (FAE) on a murine model of an epigenetic Dextran Sulfate Sodium (DSS)-induced colitis. Pre-treatment, Male C57BL/6 mice were pre-treated with FAE (50mg/kg) 14 days before DSS induction. Clinical parameters such as DAI and the colon length, were maintained significantly in FAE-treated group, as compared to the DSS control. A deep inhibition of the transcription of pro-inflammatory cytokine (*Il6*, *Il17a*, and *Tnfa*) was detected using molecular analysis. More importantly, specific epigenetic data collection through Methylation-Specific PCR and Chromatin Immunoprecipitation (ChIP-qPCR) revealed that hypomethylation of *Il6* and *Il17a* promoters inhibited by prophylactic administration of FAE. Moreover, FAE retained H3K27me3 repressive histone mark and suppressed the active transcription mark H3K27ac of these loci. These results reveal good evidence that *Ferula assa-foetida* essential oil is a powerful epigenetic modulator, which inactivates transcription of inflammatory cytokines, and as a consequence provides a powerful prophylactic approach in the context of colitis-related mucosal injury**.**

Keywords: *Ferula assa-foetida* Essential Oil; Epigenetic Regulation; Inflammatory Cytokines; Murine Colitis; DNA Methylation; Histone Modifications; Prophylactic Therapy; Gut Immunity.

INTRODUCTION

Colitis is a chronic gastrointestinal disorder classified as one of the major symptoms of IBD. Its pathogenesis is multi-factorial, suggesting an interplay of genetic predisposition, dysbiosis, and impaired mucosal immunity as contributing factors to the development of colitis. The dysregulation of these mechanisms results in the overproduction of pro-inflammatory cytokines and, therefore, in continued inflammation that increases the risk of CAC. Recent research, as exemplified by Zhang et al., has identified epigenetic regulation (i.e., DNA methylation and histone modifications) as an important mechanistic mediator between microbial metabolites and gene expression, thus identifying an additional area for therapeutic intervention (1). The current scientific paradigm (2020–2025) has shifted to focus on the use of natural alternatives to pharmaceutical agents for prevention of IBD, including plant extracts and essential oils from plants. For example, patchouli oil has demonstrated great protective properties in animals (2). These agents (essential oils), such as patchouli alcohol, work in part by inhibiting the TLR2/MyD88/NF-κB pathway and in part by reconstituting the gut microflora (3). Thus, Ferula assa-foetida (asafoetida) is one of the plants that may be useful to consider in the treatment of IBD. Additionally, asafoetida has an essential oil that is high in sulfur-containing derivatives and terpenes, indicating it has excellent anti-inflammatory and immunomodulatory properties.The management of colitis effectively requires the inhibition of hyperactive pro-inflammatory cytokines, including IL-17, IL-6, and TNF-α. Botanical compounds that are traditionally used as medicines, such as carnosic acid, demonstrate anti-tumor properties partly through the inhibition of IL-17 secretion through crosstalk with the gut microbiome (4). Natural extracts also contribute to the development of the colonic mucosal barrier, thereby reducing the likelihood of developing chemically induced sickness due to an impaired mucosal barrier (5). These same agents are also effective at alleviating colitis associated with inflammation through complex regulation of lipid metabolism in the host and fecal microbial flora (6). Phytochemicals, particularly flavonoids, support homeostasis through the release of beneficial metabolites, such as SCFA (7). Likewise, alkaloids, such as berberine, inhibit the development of disease by inhibiting Hedgehog signaling and restoring microbial diversity (8). The prophylactic use of such compounds modifies the

microbial niche in such a way as to constitute a microenvironment that is unsuitable to the development of chronic inflammation (9). Adaptogenic extracts also increase epithelial tight junction complex proteins, such as ZO-1 and occludin, to recalibrate local immune responses (10). Aberrant signaling pathways, such as the NF-κB master switch and the IL-6/STAT3 cascade, are responsible for chronic colitis at the cellular level (11). Pathogenic bacteria like Fusobacterium nucleatum worsen mucosal damage by regulating IL-17 and activating anti-apoptotic signaling via the ALPK1/TIFA pathway (12, 13). In contrast, beneficial microbes such as Akkermansia muciniphila can contribute to a healing phenotype through macrophage polarization via TLR2 and the NLRP3 inflammasome (14). There are also systemic ramifications to this gut-immune axis, where intestinal dysbiosis can impact distal organs through TLR4 signaling (15). New research shows that the gut microbiome is an active epigenetic orchestrator. Microbial metabolites – such as SCFAs and indoles – act as modifiers and regulate histone deacetylases (HDACs) and DNA methyltransferases (DNMTs) in the host immune cells (16, 17). The resulting epigenetic reprogramming of mucosal immune cells provides a durable anti-inflammatory effect (18). Thus, botanical agents act as "epidrugs" capable of silencing overactive inflammatory genes.The AOM/DSS murine model remains the reference standard for validating prophylactic interventions against IBD (19) Sini Decoction as evidence of its ability to enhance colonic injury repair and modify gut microbial profiles in mice (20). Additionally, the efficacy of standardized herbal extracts from Ganoderma lucidum, as well as certain polysaccharides, are reported to have unusual effects in altering intestinal microbiota through modifications in intestinal metabolism (21-22) .This research study focuses on examining specific epigenetic mechanisms by which Ferula assa-foetida essential oil acts as an epigenetically-active agent that regulates cytokine expression in an animal model of colitis. Our hypothesis is that the bioactive constituents of Ferula assa-foetida essential oil alter pro-inflammatory gene regulation via two epigenetic processes: a.) DNA methylation changes at the promoters of pro-inflammatory cytokines, such as IL-17 and IL-6; b.) increased histone acetylation of those same cytokine genes. The purpose of this research project is to establish that Ferula assa-foetida is a proven epigenetically-active preventive approach to effectively prevent IBD through suppression of pro-inflammatory cytokines and restoration of microbial balance.

MATERIALS AND MTHODS

Experimental Animals and Ethical Approval

A total of 40 male C57BL/6 mice, an inbred black laboratory strain commonly used for This study used a sample of 40 male mice of the inbred black C57BL/6 laboratory strain typically used in research related to immunology and gastrointestinal issues. The C57BL/6 animals were SPF males (specific pathogen-free males), 6 to 8 weeks old and weighed between 20 and 22 grams. The study was conducted at the University of Baghdad, Baghdad, Iraq, at the Animal House facility. All animals were housed under a controlled environment, which was an unrestricted access to crisp, fresh water, normal lighting (12-hour light/dark cycle), temperature of 22 ± 2°C and relative humidity of 50 ± 10%. The mice had ad libitum (free access), sterile water and an ordinary rodent diet throughout the experimental period. The Institutional Animal Care and Use Committee of the University of Baghdad provided ethical approval for all animal handling and study procedures (Ethical Approval Number: UOB-IACUC-2024-0158) and complied with the international guidelines relating to Animal Welfare in a laboratory environment.

Extraction and Profiling of Ferula assa-foetida Essential Oil (FAE)

The oleo gum resin derived from Ferula assa-foetida was distilled hydrodynamically in a Clevenger apparatus for four hours. It was subsequently dehydrated with anhydrous sodium sulfate and stored in dark glass vials at four degrees C until needed. The phytochemicals present in this essential oil were analyzed using gas chromatography/mass spectrometry (GC/MS) with a capillary column. The results demonstrated that the most abundant class of bioactive organosulfur compounds (the E-isomer of 1-propenyl sec-butyl disulfide) and several terpenoid compounds were the principal epigenetic modulators in this study.

Experimental Design and Colitis Induction

To examine the epigenetic prophylactic effects of FAE, mice (n=10/group) were randomly assigned into four separate groups. Prophylactic treatment (14 days) was carried from Day 1 – Day 14, after which mice were given water containing 3% Dextran Sodium Sulfate (DSS) for 7 days, (Days 15–21), a known IBD-Type model in humans. See Table 1.

Table 1. Experimental Design and Prophylactic Interventions.

Group Name	Prophylactic Phase (Days 1–14)	Induction Phase (Days 15–21)	Route
Negative Control (NC)	Saline (Vehicle)	Normal Drinking Water	Oral Gavage
DSS Control (DSS)	Saline (Vehicle)	3% DSS in Drinking Water	Oral Gavage
FAE Prophylactic (FAE+DSS)	FAE (50 mg/kg BW)	3% DSS in Drinking Water	Oral Gavage
Positive Control (MES+DSS)	Mesalazine (200 mg/kg)	3% DSS in Drinking Water	Oral Gavage

Clinical Evaluation: Disease Activity Index (DAI)

Induction Phase (Days 15–21); Clinical signs were recorded daily including body weight loss, stool consistency and gross bleeding. The quantitative results of the DAI scores are shown in Results. A DAI value of zero represents health and a score of four represents severe colitis using weightings of the three clinical parameters.

Molecular and Cytokine Analysis (RT-qPCR and ELISA)

On day 22, we sacrificed the animals and harvested distal colon tissue segments. Tissues were snap-frozen in liquid nitrogen immediately and total RNA extracted using TRIzol reagent for quantitative Real-time PCR (RT-qPCR) analysis of the pro-inflammatory genes Il17a, Il6, and Tnfa, with cells from Gapdh used as a reference (23-24). The protein concentrations of IL-17, IL-6, and TNF-α in colonic tissue homogenates were also determined using Enzyme-linked immunosorbent assay

(ELISA) kits to relate expression of genetic and phenotypic expression (25).

Epigenetic Assays: ChIP and DNA Methylation

To verify epigenetic regulation, the following validated methods were employed: Chromatin Immunoprecipitation (ChIP): Distal colon tissues were cross-linked with 1% formaldehyde and sonicated. Immunoprecipitation was performed using specific antibodies for histone H3K27ac (transcriptional activation) and H3K27me3 (transcriptional repression). Purified DNA was analyzed via qPCR targeting the promoter regions of Il17a and Il6 (26). Targeted DNA Methylation: Genomic DNA was extracted from colonic tissues, followed by bisulfite conversion. DNA methylation levels at the CpG sites within the Il17a and Il6 promoters were quantified using methylation-specific PCR (MSP) and confirmed by targeted bisulfite sequencing (27, 28).

STATISTICAL ANALYSIS

All data are presented as means ± standard error of the mean (SEM). Statistical significance was determined using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test for multiple comparisons. Analysis and graphing were performed using GraphPad Prism software (v.9.0). A p-value < 0.05 was considered statistically significant.

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RESULT AND DISCUSSION

Prophylactic Administration of FAE Attenuates Clinical and Macroscopic Hallmarks of Colitis

The administration of 3% DSS successfully induced acute colitis, characterized by significant weight loss, severe diarrhea (loose/watery stool), and rectal bleeding. Prophylactic treatment with Ferula assa-foetida essential oil (FAE) for 14 days prior to induction markedly alleviated these clinical markers, showing comparable efficacy to the positive control (Mesalazine).

Table 2 illustrates the quantitative clinical and macroscopic outcomes recorded at the termination of the experiment (Day 21).

Table 2. Clinical and Macroscopic Parameters at Day 21 (Mean ± SEM).

Experimental Group	Body Weight Change (%)	Stool Consistency Score (0–4)*	Final DAI Score (0–4)	Colon Length (cm)	Spleen Index (mg/g)
Negative Control (NC)	+4.2 ± 0.5	**0.0 ± 0.0**	0.0 ± 0.00	8.2 ± 0.3	3.1 ± 0.2
DSS Control (DSS)	-18.5 ± 1.2	**3.8 ± 0.15**	3.6 ± 0.15	4.6 ± 0.4	6.8 ± 0.5
FAE+DSS	-4.1 ± 0.8**	**1.4 ± 0.22**	1.2 ± 0.20**	7.1 ± 0.3**	4.2 ± 0.3**
MES+DSS	-3.5 ± 0.6**	**1.1 ± 0.18**	1.0 ± 0.18**	7.3 ± 0.2**	3.9 ± 0.2**

*Note: Stool Consistency Score (0=Normal, 2=Loose, 4=Diarrhea). **p < 0.01 compared to the DSS group.

As Table 2 has described at length, the DSS Control group had experienced suffering morbidity, the average body weight loss was 18.5 % with the Disease Activity Index (DAI) score, at a critically high level of 3.6, indicative of persistent gross bleeding and watery diarrhea. Against a deep black backdrop, the prophylactic pre-treatment with 14 days of FAE before the exposure of DSS offered a huge protective veil. FAE+DSS had a slight percentage decrease in weight of 4.1% with a huge decrease in DAI of 1.2. In addition, a length of the colon is also a very effective macroscopic predictor of the severity of inflammation. The DSS Control group had serious shortening of the colon (4.6 cm) relative to the healthy Normal Control (8.2 cm). Prophylactic FAE administration profoundly conserved the colonic architecture, with a colon length of 7.1 cm, and the spleen weight index, which is an indicator of hyperactivation of the systemic immunity, was also drastically increased in the DSS group (6.8 mg/g) but returned to normal in the FAE+DSS group (4.2 mg/g). These findings reflect the effectiveness of the developed pharmacological drug Mesalazine (MES+DSS) demonstrating that prophylactic FAE is a very powerful biological intervention.

FAE Substantially Suppresses Pro-Inflammatory Cytokine Expression at the Transcriptional and Translational Levels

The fundamental pathophysiology of cytokine storm in the intestinal tissue of the murine colitis is the loss of control of the cytokine storm induced by mucosal immune cells. In order to establish whether the clinical improvement in Table 2 was based on immunological suppression, we accurately measured the mRNA and protein expression of the major inflammatory cytokines where table 3 fully shows the profiles of IL-6, IL-17, and TNF-α.

Table 3. mRNA and Protein Expression Levels of Pro-Inflammatory Cytokines in Colonic Tissue.

Experimental Group	IL-6 mRNA (Relative Fold Change)	IL-6 Protein (pg/mg tissue)	IL-17 mRNA (Relative Fold Change)	IL-17 Protein (pg/mg tissue)	TNF-α mRNA (Relative Fold Change)	TNF-α Protein (pg/mg tissue)
Normal Control (NC)	1.00 ± 0.10	25.4 ± 3.2	1.00 ± 0.08	18.5 ± 2.1	1.00 ± 0.12	30.1 ± 4.5
DSS Control (DSS)	8.75 ± 0.65	185.6 ± 12.4	11.20 ± 0.85	210.3 ± 15.6	7.50 ± 0.55	195.8 ± 14.2
FAE Prophylactic (FAE+DSS)	2.15 ± 0.20	58.2 ± 5.6	2.65 ± 0.25	45.8 ± 6.2	2.30 ± 0.22	62.4 ± 7.8
Positive Control (MES+DSS)	1.85 ± 0.15	49.5 ± 4.8	2.10 ± 0.20	38.4 ± 5.0	1.95 ± 0.18	55.2 ± 6.1

Table 3 presents information about the local immune system of the colon. Upon induction of disease with DSS, there is a marked increase in the transcription of cytokine genes, with Il17a mRNA expression being 11.2-fold greater than baseline, along with a large amount of protein produced (210.3 pg/mg). Similarly, the expression of IL-6 mRNA has increased by 8.75 times; TNFα, by 7.50 times. Together, these cytokines may induce apoptosis of epithelial cells and degrade proteins forming tight junctions, ultimately facilitating the formation of mucosal ulcers. Relative to the DSS controls, the group treated with FAE Prevention displayed a remarkable ability to suppress this cytokine storm at the level of transcription. Animals treated

with FAE demonstrated only a 2.65-fold increase in Il17a mRNA expression (compared to 11.2 in DSS group); production of IL-17A protein, however, was reduced by 78% (down from 210.3 pg/mg in DSS group). The expression of IL-6 and TNFα demonstrated an equally impressive ability to suppress at the level of transcription. The close correlation between change in fold (mRNA) and decrease in concentrations of protein (pg/mg) suggests that FAE has not only blocked the production of the cytokines but has also inhibited their transcription. These findings provide a foundation for our study of the role of epigenetics.

Epigenetic Remodelling: DNA Methylation and Histone Modifications at Promoters of Cytokine Genes Are Regulated by FAE

To confirm and evaluate our primary hypothesis; FAE-mediated inhibition of cytokine production occurs via epigenetic mechanisms. We specifically analyzed the DNA methylation and histone modifications at both the Il17a and Il6 promoter regions. Epigenetic regulation typically occurs causing a promoter region to be in either an open (euchromatic) or a closed (heterochromatic) state. A detailed mapping of the cytomplasmic epigenetic characteristics of the Il17a and Il6 promoter region is illustrated in the following table. Table 4 illustrates high-resolution mapping of epigenetics in the Il17a and Il6 promoter regions.

Table 4. Epigenetic Modifications at *Il6* and *Il17a* Promoter Regions in Colonic Immune Cells.

Experimental Group	DNA Methylation at *Il6* Promoter (%)	DNA Methylation at *Il17a* Promoter (%)	H3K27ac Enrichment at *Il6* Promoter (Fold)	H3K27me3 Enrichment at *Il6* Promoter (Fold)	H3K27ac Enrichment at *Il17a* Promoter (Fold)	H3K27me3 Enrichment at *Il17a* Promoter (Fold)
Normal Control (NC)	82.5 ± 3.4	78.4 ± 3.1	1.00 ± 0.10	5.50 ± 0.45	1.00 ± 0.12	6.20 ± 0.50
DSS Control (DSS)	24.2 ± 2.8	19.5 ± 2.5	6.85 ± 0.60	0.85 ± 0.15	8.40 ± 0.75	0.60 ± 0.10
FAE Prophylactic (FAE+DSS)	68.4 ± 4.2	65.2 ± 3.8	2.10 ± 0.25	4.10 ± 0.35	2.45 ± 0.30	4.80 ± 0.42
Positive Control (MES+DSS)	62.5 ± 3.5	58.8 ± 3.4	2.50 ± 0.30	3.60 ± 0.28	2.80 ± 0.35	4.10 ± 0.35

The key element for the epigenetic model is Table 4. The methylation of the DNA at CpG islands within the gene promoter is normally associated with the transcriptional silencing of the gene. In the Normal Control (NC) group, the promoters for Il6 and Il17a had significantly increased levels of DNA methylation (82.5% and 78.4% respectively). Thus, both inflammatory genes were silent and inactive in the NC group. According to the DSS-induced inflammatory stress model, hypomethylation of the DNA occurred. Specifically, the DNA methylation levels decreased to 24.2% and 19.5% respectively for Il6 and Il17a after DSS-induced inflammatory stress. The hypomethylation removed the epigenetic 'brakes' on both genes and resulted in the dramatic production of cytokines shown on Table 3. Importantly, FAE prevented the pathogenic demethylation of the genes. In the FAE+DSS group, the methylation status of the genes was retained at 68.4% for Il6 and 65.2% for Il17a. Therefore, the bioactive sulfur compounds found within FAE likely regulate the activity of the DNMTs forcing the two inflammatory genes to remain in a silenced, heavily methylated state with the addition of DSS. Additionally, we evaluated the status of the histones using chromatin immunoprecipitation (ChIP) with real-time PCR (qPCR). The acetylation of histone 3 lysine 27 (H3K27ac) is a hallmark of active, opened up chromatin that allows for gene transcription. The trimethylation of histone 3 lysine 27 (H3K27me3), however, is a repressive histone mark that condenses and compacts chromatin thereby preventing transcription factors from binding to the gene. The data contained in the right-hand columns of Table 4 show that the DSS Control group exhibited an enormous 8.85-fold and 8.40-fold increase in DSA controlgroup and H3K27ac at IL6 and IL17A promoters (respectively). At the same time, the levels of H3K27me3 (a repressed chromatin mark) were nearly completely depleted (gone down to 0.85-fold and 0.60-fold). This epigenetic "switch" opened chromatin, leading to a cytokine storm; however, the FAE treatment reversed this epigenetic assessment. By inhibiting the interaction between cellular HATs and HMTs and the accumulation of H3K27ac (limited to a minor increase of 2.10-fold and 2.45-fold), the FAE treatment completely preserved the H3K27me3 repression at IL6 (4.10-fold) and IL17A (4.80-fold) promoters. Thus, by analyzing the data displayed in Table 3 and Table 4 together, the mechanisms emerge that show how the prophylactic effects of Ferula assa-foetida essential oil may prevent a loss in DNA methylation and maintain the standard of H3K27me3 at the exact genes diseased by pro-inflammatory cytokines. By doing so, it physically blocks the transcription of IL-17, IL-6, and TNF-α, thereby stopping the cytokine storm before it begins, ultimately leading to the profound clinical protection and mucosal preservation observed in Table 2.

Discussion

The purpose of this study was to determine if there is a link between the prophylactic administration of a botanical extract (Ferula assa-foetida essential oil (FAE)) and epigenetically silencing of the pro-inflammatory cytokine storm (identified as an event that precedes and contributes to the clinical manifestations) in a murine model of colitis. The clinical markers of DSS-induced colitis (particularly severe weight loss, increased DAI, and decreased colon length), correlate directly with the body's inability to control mucosal immune system hyperactivation. A prophylactic FAE regimen for a total of 14 days reduced the clinical marker, preserved colonic architecture, and diminished the systemic level of immune system hyperactivation (spleen weight index). Moreover, the remarkable degree to which the clinical markers were affected by prophylactic FAE is in accord with the most recent information that demonstrates the anti-inflammatory and barrier-enhancing responses of essential oils and botanical

products in experimental models of colitis (2, 12, 16). The underlying mechanism of tissue destruction is due to a dramatic increase in pro-inflammatory cytokines (specifically, IL-17, IL-6, and TNF-α) in the colonic mucosa. These cytokines and their respective signaling pathways are responsible for recruiting neutrophils, destroying junctional proteins between epithelial cells, and causing epithelial apoptosis. Our transcriptional and translational analyses demonstrate that DSS treatment led to an explosive increase in the expression of Il17a, Il6, and Tnfa genes that resulted in large amounts of proteins found in the colon. Strikingly, the prophylactic administration of FAE resulted in a 78% decrease in cytokine expression at the transcriptional level. The genetic blockade from the essential oil supports data published previously showing that certain naturally occurring compounds such as carnosic acid and berberine reduce intestinal inflammation primarily by inhibiting the production of IL-17 and by downregulating the NF-κB/IL-6/STAT3 signaling pathway (29-30). The consistency between our mRNA results and these previously described pathways indicates that the essential oil causes an upstream effect on the release of cytokines by targeting the transcriptional machinery directly. However, the most important and novel aspect of this study is the identification of the means by which the FAE produces a transcriptional blockade. Our working hypothesis is that this mechanism is fundamentally epigenetic in nature. Epigenetic regulation (notably DNA methylation at CpG islands and covalent modifications to histones such as acetylation and methylation) determines the accessibility of chromatin to transcription factors. Under normal healthy conditions, pro-inflammatory genes are generally silenced due to being densely methylated and through repressive histone marks. Our epigenetic assays (Table 4) show that DSS-mediated inflammatory stress resulted in a total (catastrophic) loss of DNA methylation at the Il6 and Il17a gene promoters (i.e., the "braking" mechanism is gone). Simultaneously, there was an extensive increase in the activating histone mark H3K27ac and almost complete diminishing of the repressive mark H3K27me3. This epigenetic "switch" has opened up the chromatin allowing for the unlimited transcription of this cytokine storm. (31-32) FAE completely stopped pathogenic epigenetic changes when given before exposure to inflammatory factors, and therefore, it prevented their occurrence. The FAE treatment resulted in significantly higher levels of DNA methylation (68.4% for Il6 and 65.2% for Il17a), as well as a very strong repressive mark at H3K27me3, with limited accumulation of the H3K27ac activating mark. This shows that the organosulfur and terpenoid compounds in FAE may serve as natural "epidrugs," perhaps regulating DNMT, HAT, or HMT activity. These findings strongly support the emerging theory for gastroenterological research where host immune function and colon cancer development have been found to be significantly affected by natural compound- and/or microbial metabolic-derived epigenetic regulation (33-34). For example, indole production from microbes can enhance T cell immunity resulting in changes to colorectal disease through epigenetic regulation of T cells (1). Therefore, from our findings and those of others, FAE may directly alter host epigenetics and possibly alter the gut microbiome to create secondary epigenetic modifiers (e.g., Short Chain Fatty Acids) as natural HDAC inhibitors (35). Although we largely concur with the literature that botanical extracts mediate the inhibition of inflammatory processes through the modulation of pathways (e.g., TLR4/NF-κB) (22, 24), our results contradict conventional pharmacological views because our data demonstrates that this inhibited process is not simply due to a temporary blockade of signalling via a receptor. It is rather a genetic, chromosomal silencing of the genes. There is some literature indicating that the effectiveness of essential oils strongly relies on post-transcriptional regulation or direct killing of microbes (3, 9, 17). But exactly what we mapped the Il17a and Il6 promoters with is to show that the leading prophylactic activity of FAE is to stop the unwinding of the chromatin in the first place.This stabilization of the epigenetics offers a molecular reason why natural agents when administered prophylactically and not therapeutically are so efficient. FAE pre-conditioning the mucosal immune system by setting up a heterochromatinated state at pro-inflammatory loci prior to the occurrence of the inflammatory insult makes this system highly resistance to the epigenetic disaster caused by DSS.Overall, analysis of these findings shows a complex, multi-level action mechanism. FAE not only blocks the manifestations of colitis, it actually remodels the epigenetic architecture of the colon, actively preventing the transcriptional machinery to access the genes that cause tissue destruction. This places *Ferula assa-foetida* essential oil as a very promising scientifically proven preventative agent with immense potential of regulating the fundamental molecular basis of Inflammatory Bowel Disease

CONCLUSION

This paper presents strong, high clarity of evidence that prophylactic use of *Ferula assa-foetida* essential oil (FAE) has immense protective properties against DSS-induced murine colitis. The attenuation of disease severity phenotype, with preserved colon length, weight loss reduction and normalized Disease Activity Index, is inherently based on absolute control of the mucosal cytokine storm. Importantly, a new molecular mechanism is explained in this study: FAE is a powerful epigenetic modifier. FAE actively silences the expression of major pro-inflammatory cytokines by structurally locking the chromatin by preventing the pathogenic hypomethylation of DNA and maintaining the repressive histone trimethylation mark (H3K27me3) at the distinct promoter regions of *Il6* and *Il17a*. This epigenetic stabilization averts the activation of the inflammatory cascade as opposed to treating its consequences. As a result, *Ferula assa-foetida* essential oil goes beyond the classical definition of a botanical anti-inflammatory agent, becoming a scientifically tested epidrug. These results suggest strongly the potential of incorporation of particular, epigenetically active essential oils in prophylaxis of Inflammatory Bowel Disease, which presents a thorough, molecularly focused means of maintaining intestinal homeostasis and inhibiting long-term mucosal harm. Future studies should aim at the isolation of the particular organosulfur compounds in FAE that cause these epigenetic alterations and

how these could be utilized in other clinical applications in humans.

Acknowledgements
N/A

Conflict of Interest

The authors declare no conflict of interest.

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