International Journal of Chemical and Biochemical Sciences
(ISSN 2226-9614)

Journal Home page: www.iscientific.org/Journal.html

© International Scientific Organization

Therapeutic Potential of Melittin-Loaded Liposomes in Ameliorating DSS-Induced Colitis: An Experimental Evaluation in a Rat Model

Areej Dawoud[1], *Sara F. Gaafar*[2]

[1]*Ibn Sina National College for Medical Studies, Kingdom of Saudi Arabia- Jeddah*

[2]*Faculty of Medicine, Zagazig University, Egypt*

Abstract

With few treatment choices and serious side effects, inflammatory bowel disease (IBD), which includes ulcerative colitis, is a chronic inflammatory illness of the gastrointestinal tract. With an emphasis on their anti-inflammatory and immunomodulatory qualities, this work sought to assess the therapeutic potential of melittin-loaded liposomes in reducing DSS (Dextran Sodium Sulfate)-induced colitis in a rat model. Encapsulating melittin, a strong anti-inflammatory peptide extracted from bee venom, in liposomal nanocarriers improved its therapeutic efficacy while lowering toxicity. Solvent injection was used to create melittin-loaded liposomes, which produced liposomes with the ideal zeta potential, stability, and particle size. Oral gavage was the method of therapy employed to simulate DSS-induced colitis in male Wistar albino rats. The rats were split up into three groups: one for DSS-induced colitis, one for control, and one for melittin-loaded liposomes at different concentrations. Changes in body weight, the disease activity index, and a histological analysis of colonic tissues were used to measure the effectiveness of the treatment. Serum and colonic tissues were used to evaluate inflammatory markers (TNF-α, IL-6, and IL-10) and oxidative stress indicators (malondialdehyde, MDA, and glutathione, GSH). In order to gauge the degree of inflammation and neutrophil infiltration, myeloperoxidase (MPO) activity was also measured. Melittin-loaded liposomes were found to dramatically lessen the severity of colitis, as indicated by favorable histological alterations, improved body weight, and a decreased disease activity index. Moreover, pro-inflammatory cytokines and oxidative stress markers were significantly decreased after treatment with melittin-loaded liposomes, while levels of the anti-inflammatory cytokine IL-10 were raised. These results imply that melittin-loaded liposomes, which have strong anti-inflammatory and immunomodulatory properties while being less toxic, may represent a viable therapeutic approach for the treatment of IBD. To investigate how this method can be used in a therapeutic setting, more research is necessary.

Keywords: Melittin-loaded liposomes, DSS-induced colitis, inflammatory bowel disease, anti-inflammatory, immunomodulatory.

Full length article **Corresponding Author*, e-mail: *areej-dawoud@ibnsina.edu.sa; s.fekry22@zu.edu.eg* Doi # https://doi.org/10.62877/19-IJCBS-24-26-20-19

1. Introduction

Inflammatory bowel disease (IBD) refers to a collection of long-lasting inflammatory illnesses affecting the gastrointestinal tract. Among these, ulcerative colitis (UC) is one of the most commonly occurring kinds. Although there have been improvements in our understanding of the underlying causes of IBD, the current methods of treatment are not ideal. They generally come with considerable side effects and have limited effectiveness. There is a continuous effort to find new and efficient treatments that have fewer negative effects. This has led to a growing interest in alternative therapeutic methods, such as using natural peptides and drug delivery systems based on nanotechnology [1-2]. Melittin, a very powerful anti-inflammatory peptide extracted from bee venom, has attracted considerable interest due to its potential therapeutic applications in a range of inflammatory disorders [3]. Nevertheless, the clinical use of this substance has been restricted because of its cytotoxic properties and inadequate stability in biological systems [4]. In order to address these restrictions, recent progress in nanotechnology has enabled creation of liposomal nanocarriers. Liposomes, which are spherical structures made up of phospholipid bilayers, can contain medicinal substances such as melittin, thereby increasing their effectiveness and reducing the risk of harm to the body as a whole [5-6].

Encapsulating melittin in liposomes is anticipated to offer a regulated release, diminish cytotoxicity, and enhance the targeting of inflamed tissues. This makes it a potential strategy for the treatment of inflammatory disorders like IBD. Liposomes are spherical structures made up of one or more layers of phospholipids. They are well-known for their capacity to enclose both water-soluble and fat-soluble medicinal substances. Liposomes, functioning as nanocarriers, provide several benefits in drug administration, such as improved bioavailability, regulated release, and precise distribution to specific tissues, thus reducing systemic side effects. Liposomal encapsulation of medicinal substances can greatly enhance the drug's pharmacokinetics

and pharmacodynamics, especially for molecules that have low stability or high toxicity, such as melittin. Through the use of liposomal nanocarriers, melittin can be encapsulated to protect it from early breakdown, decrease its harmful effects on cells, and enhance its therapeutic effectiveness. This novel method utilizes the inherent ability of liposomes to interact well with biological systems and their adaptability to improve the transportation of melittin.

As a result, it shows great potential as a therapy option for inflammatory disorders such as IBD [5-6]. In this perspective, the utilization of liposomes signifies a notable progression in the field of nanomedicine. It provides a fresh approach to harness the powerful anti-inflammatory characteristics of melittin while simultaneously reducing the potential hazards connected with its use. This study investigates the therapeutic efficacy of melittin-loaded liposomes in improving DSS-induced colitis, an extensively utilized animal model that closely replicates the clinical and histological characteristics of human ulcerative colitis. Our objective is to utilize liposomes to contain melittin, allowing us to utilize its anti-inflammatory and immune modulatory characteristics while reducing its harmful effects on cells. The study assesses the effectiveness of this new therapeutic method by thoroughly evaluating inflammatory indicators, oxidative stress measures, and histological alterations in the colonic tissues of treated rats. The results of this study may offer useful knowledge regarding the creation of a novel category of treatments for IBD, potentially presenting a more efficient and less risky substitute for existing therapy choices.

2. Methodology

2.1. Preparation and Characterization of Melittin-Loaded Liposomes

The solvent injection approach, a widely recognized technology for encapsulating bioactive chemicals, employed to create liposomes filled with melittin [7]. The composition comprised phospholipids, cholesterol, and melittin in optimum molar ratios of 4:1:0.1, respectively. First, phospholipids and cholesterol were mixed with a small amount of ethanol. Then, melittin was added to the solution. The ethanolic solution introduced gradually into a warmed phosphate-buffered saline (PBS) buffer (pH 7.4) while stirring continuously at 1000 rpm and a temperature of 60°C. The liposomes were generated spontaneously as ethanol was diluted by the aqueous phase. The liposomes underwent sonication for 5 minutes after their synthesis to decrease particle size and assure consistency. The melittin-loaded liposomes were characterized by evaluating their entrapment efficiency, particle size, zeta potential, and stability. The entrapment efficiency was assessed using high-performance liquid chromatography (HPLC), employing a method previously reported by Kim et al. (2022). The measurement of particle size and zeta potential was conducted using dynamic light scattering (DLS) using a Zetasizer Nano ZS instrument (Malvern Instruments, UK). The stability of the liposomes assessed over a period of 30 days by storing them at a temperature of 4°C and monitoring alterations in particle size and zeta potential. This evaluation was performed according to the study conducted by Li et al. in 2021 [8].

2.2. Animal model

The study included male Wistar albino rats with a weight range of 200 to 250 g. These rats were procured from the university's animal facility and kept in a controlled laboratory environment. The parameters included a 12-hour light/dark cycle, a temperature of 22±2°C, and unrestricted access to food and water. The work was carried out in accordance with the ethical criteria authorized by the Institutional Animal Care and Use Committee (IACUC) [9]. In order to cause inflammation of the colon, a solution containing 3% Dextran Sodium Sulfate (DSS) with a molecular weight ranging from 36,000 to 50,000 Da (Sigma-Aldrich, USA) freely provided in the drinking water for a period of 7 days. The rats divided into five groups in a random manner, with each group consisting of 8 rats (n = 8 per group). Control Group: Rats were given standard drinking water without DSS or any form of treatment.

The DSS-Induced Colitis Group consisted of rats that given 3% DSS in their drinking water, without any other treatment. The Low-Dose Melittin-Loaded Liposomes Group consisted of rats that were given 3% DSS and treated with liposomes containing melittin. The liposomes were supplied at a dose of 25 μg/kg body weight via intraperitoneal administration [3]. The rats in the Medium-Dose Melittin-Loaded Liposomes Group were given 3% DSS and treated with liposomes containing melittin at a dosage of 50 μg/kg body weight via intraperitoneal administration [3]. The High-Dose Melittin-Loaded Liposomes Group consisted of rats that were administered 3% DSS and subsequently treated with melittin-loaded liposomes at a dosage of 100 μg/kg body weight via intraperitoneal administration [3]. The liposomes containing melittin given once daily, beginning one day before to DSS administration and continuing for whole duration of trial.

2.3. Therapeutic Procedure

The rats were observed on a daily basis to detect any clinical indications of colitis, such as a decrease in body weight, changes in stool consistency, and the presence of blood in the stool. The Disease Activity Index (DAI), a composite score that quantifies the severity of colitis, was calculated using these criteria [10]. Upon completion of the trial, the rats were humanely put to death, and samples of their colonic tissues were collected for subsequent analysis.

2.4. Evaluation Parameters

2.4.1. Inflammatory Markers

The levels of pro-inflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6), as well as the anti-inflammatory cytokine interleukin-10 (IL-10), were measured in both serum and colonic tissue homogenates. This was done using commercially available enzyme-linked immunosorbent assay (ELISA) kits (TNF-α: Cat. No. MTA00B, Thermo Fisher Scientific, USA; IL-6: Cat. No. 88-7064-22, Thermo Fisher Scientific, USA; IL-10: Cat. No. BMS614, Thermo Fisher Scientific, USA) according to the instructions provided by the manufacturer [11].

2.4.2. Oxidative Stress Markers

An analysis was conducted on colonic tissues to determine the presence of oxidative stress indicators, specifically malondialdehyde (MDA) and glutathione (GSH). The levels of malondialdehyde (MDA), which serve as an indication of lipid peroxidation, were quantified using the thiobarbituric acid reactive substances (TBARS) assay (Cat. No. 700870, Cayman Chemical, USA), following the

methodology outlined by Zhang et al. [12]. The quantification of GSH levels was performed using a glutathione assay kit (Cat. No. K006-H1, Arbor Assays, USA), which determines the overall glutathione content in tissue homogenates.

2.4.3. Myeloperoxidase (MPO) Activity

The level of myeloperoxidase (MPO) activity, which indicates the presence of neutrophil infiltration and inflammation, was assessed in colonic tissue homogenates using a colorimetric MPO activity assay kit (Cat. No. MAK068, Sigma-Aldrich, USA) [13]. The assay relies on the oxidation of o-dianisidine by MPO in the presence of hydrogen peroxide. This leads to a color shift that is detected at a wavelength of 450 nm.

2.4.4. Histopathological Analysis

The colonic tissues were meticulously removed, washed with PBS, and preserved in 10% formalin for a duration of 24 hours. Subsequently, the tissues underwent dehydration, were immersed in paraffin, and were sliced into 5 µm thick sections using a microtome. The sections were subjected to histological investigation using hematoxylin and eosin (H&E) staining, as described by Wu et al. [14], and observed under a light microscope. A blinded pathologist assessed the severity of colitis using a standardized scoring system that evaluated the level of epithelial damage, infiltration of inflammatory cells, and disruption of mucosal architecture. The rating scale varied from 0 (indicating normal) to 4 (indicating severe colitis [15].

2.4.5. Statistical Analysis

The data were presented as the mean value plus or minus the standard error of the mean (SEM) and were analyzed using a one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test for making multiple comparisons. The statistical analysis was conducted using GraphPad Prism version 9.0 (GraphPad Software, San Diego, CA, USA) as described by Liang et al [16]. A p-value below 0.05 was deemed to be statistically significant.

3. Results and discussion

3.1. Results

3.1.1. Preparation and Characterization of Melittin-Loaded Liposomes

The liposomes loaded with melittin had an entrapment effectiveness of 78.5 ± 2.3, demonstrating a significant ability to encapsulate melittin. The particle size was determined to be 115.4 ± 8.2 nm, falling within the ideal range for efficient cellular uptake. The zeta potential, measured at -32.6 ± 4.1 mV, indicates that the system is highly stable and has a low tendency to aggregate. During the 30-day duration, there were no notable alterations in particle size or zeta potential, thereby verifying the durability of the liposomes. The melittin-loaded liposomes have demonstrated good entrapment efficiency and stable particle size, along with a favorable zeta potential. These findings confirm the successful preparation of the liposomes and their suitability for in vivo application.

3.1.2. Animal Model and Treatment Protocol

The DAI was employed to evaluate the intensity of colitis. The group of mice with DSS-induced colitis saw a substantial decrease in body weight (7.8%) and had a high DAI score of 3.5 ± 0.4. On the other hand, the group that received a high dose of Melittin experienced a weight loss of just 1.9% and had a substantially lower DAI score of 1.4 ± 0.2. This indicates a considerable improvement in the symptoms of colitis. The High-Dose Melittin Group exhibited the most favorable outcome, seeing an only 1.9% decrease in weight and achieving a DAI score of 1.4, which is a substantial decrease in comparison to the DSS-Induced Colitis Group. This demonstrates the efficacy of melittin-loaded liposomes in decreasing the severity of colitis.

3.1.3. Inflammatory Markers

The concentrations of TNF-α and IL-6 were substantially higher in the DSS-Induced Colitis Group (68.5 ± 6.4 pg/mL and 49.2 ± 5.6 pg/mL, respectively) compared to the Control Group (25.7 ± 2.8 pg/mL and 15.3 ± 2.1 pg/mL, respectively). The High-Dose Melittin Group exhibited significant decreases in TNF-α (28.4 ± 2.9 pg/mL) and IL-6 (20.8 ± 2.4 pg/mL) concentrations, reaching the levels observed in the Control Group. On the other hand, the levels of IL-10, which were reduced in the group with DSS-induced colitis (32.7 ± 3.9 pg/mL), were considerably increased in the group treated with a high dose of melittin (67.2 ± 6.2 pg/mL). The substantial decrease in TNF-α and IL-6 levels, along with the recovery of IL-10 levels in the High-Dose Melittin Group, highlights strong anti-inflammatory properties of melittin-loaded liposomes in treatment of DSS-induced colitis.

3.1.4. Oxidative Stress Markers

The DSS-Induced Colitis Group exhibited a substantial rise in MDA levels (5.6 ± 0.8 nmol/mg protein), indicating heightened lipid peroxidation, and a noteworthy decline in GSH levels (3.2 ± 0.5 nmol/mg protein), indicating a reduction in antioxidant defenses. The administration of melittin-loaded liposomes, especially at the highest dosage, resulted in a considerable decrease in MDA levels (1.9 ± 0.3 nmol/mg protein) and a restoration of GSH levels (7.6 ± 0.9 nmol/mg protein). The group receiving a high dose of Melittin showed a notable decrease in oxidative stress, as seen by the almost complete restoration of MDA and GSH levels. This suggests that liposomes loaded with melittin efficiently reduce oxidative damage in the tissues of colon.

3.1.5. Myeloperoxidase (MPO) Activity

The MPO activity in the DSS-Induced Colitis Group was markedly increased (15.3 ± 2.2 U/g tissue), indicating a higher level of neutrophil infiltration and inflammation. The High-Dose Melittin Group had a significant decrease in MPO activity (5.6 ± 0.8 U/g tissue), which was similar to the level observed in the Control Group (4.2 ± 0.6 U/g tissue). The substantial decrease in MPO activity observed in the High-Dose Melittin Group suggests successful inhibition of inflammation caused by neutrophils, providing additional evidence for the anti-inflammatory properties of melittin-loaded liposomes.

3.1.6. Histopathological Analysis

Histopathological scores were employed to measure the extent of damage to the colon. The group of mice with DSS-induced colitis showed significant damage to the colon, as shown by a high histopathological score of 3.8 ± 0.4. On the other hand, the High-Dose Melittin Group exhibited very little damage to the colon, as shown by a histological score of

0.9 ± 0.1, which was similar to the score of the Control Group (0.2 ± 0.1). The High-Dose Melittin Group showed a histological score that was close to normal, indicating that melittin-loaded liposomes have a protective effect against colonic damage caused by DSS. This highlights the possibility of melittin-loaded liposomes as a therapeutic intervention for colitis. The provided image displays typical histological slices of colonic tissues from various treatment concentration groups, which have been stained using hematoxylin and eosin (H&E). The photos are categorized and identified as follows:

(a) Control Group: The colonic tissue has a typical histological arrangement with an intact mucosal structure, clearly delineated crypts, and no evidence of inflammation or tissue harm. The mucosal lining displays a healthy condition, without any signs of ulceration or infiltration of immune cells.

(b) In the DSS-Induced Colitis Group, there is severe damage to the colon, with substantial erosion of the epithelial layer, destruction of the crypts, and a considerable presence of inflammatory cells. The structure of the mucosa is significantly disturbed, suggesting presence of severe colitis.

(c) Low-Dose Melittin Group: The colonic tissue exhibits some improvement in comparison to the DSS-Induced Colitis Group, characterized by decreased inflammation and minor damage to the epithelial layer. While certain parts of the crypt structure are still degraded, the overall preservation of tissue integrity has improved.

(d) Group administered with a medium dose of melittin: The tissue in the colon shows additional enhancement, characterized by a decrease in the presence of immune cells and an improvement in the structure of the crypts. While there is still some moderate inflammation present, the overall structure of the mucosa seems to be mostly undamaged.

(e) Group receiving a high dosage of melittin: There is clear evidence of almost complete healing of the structure of the tissue in the colon. The epithelial lining remains undamaged, and the crypts are arranged in a well-organized manner, closely approaching the control group. The presence of inflammatory cells is limited, suggesting that high-dose melittin has a significant therapeutic impact in preventing colonic damage caused by DSS.

The histological examination indicates that the use of melittin, especially at medium and high doses, effectively reduces intestinal damage and inflammation caused by DSS-induced colitis. The group administered with a high dose of melittin shows nearly normal colonic histology, indicating successful preservation and restoration of colonic tissue.

3.2. Discussion

The findings of our study closely correspond to prior research on the therapeutic efficacy of melittin in improving DSS-induced colitis, confirming once again the anti-inflammatory and protective characteristics of this peptide. Our investigation revealed a notable decrease in pro-inflammatory cytokines, including TNF-α and IL-6, along with an elevation in anti-inflammatory cytokines such as IL-10, particularly in the high-dose melittin group. The results align with the research conducted by [4], which showed that melittin successfully decreased the levels of pro-inflammatory cytokines in a mouse model of DSS-induced colitis. Zhang et al. [3] emphasized that melittin has an anti-inflammatory effect via regulating immunological responses, which helps to reduce excessive inflammation. Both investigations confirm our discovery that melittin has ability to regulate immunological signaling pathways in order to reduce IBD. Regarding oxidative stress, our study demonstrated that use of melittin-loaded liposomes resulted in a significant decrease in MDA levels, indicating a reduction in lipid peroxidation.

Additionally, there was an increase in GSH levels, showing an improvement in antioxidant capacity. These results align with the findings of Yao et al. [17], observed that melittin demonstrated potent antioxidative effects in a colitis model by decreasing levels of oxidative stress markers. Similarly, the study conducted by Yan et al. [18] showed that lowering oxidative stress is crucial for guarding against DSS-induced colitis. This finding is consistent with our own research, which indicates that melittin helps restore equilibrium of redox reactions in the tissues of the colon. The therapeutic benefit of melittin is demonstrated by its capacity to combat oxidative stress, which is a key factor in development of colitis. One significant discovery in our study was that the melittin-treated groups showed maintenance of the intestinal barrier, which was especially noticeable in the high-dose group. This discovery aligns with the research conducted by Liu et al. [19], which shown that melittin effectively preserved the structural integrity of the intestinal epithelium during inflammatory circumstances.

Preserving structural integrity of intestinal barrier is crucial in order to prevent worsening of colitis, as evidenced by study conducted by Cai et al. [20] on colitis produced by DSS. Melittin enhances integrity of intestinal barrier, so preventing more harm and promoting tissue regeneration, a crucial aspect in management of IBD. The drop in MPO activity seen in our study, which indicates a reduction in neutrophil infiltration, highlights the anti-inflammatory capabilities of melittin. The results of our study are consistent with findings of Urushima et al. [21], who similarly reported a decrease in MPO activity in a comparable DSS colitis model after administering anti-inflammatory therapy. Decrease in neutrophil-induced inflammation is essential, as neutrophils play a key role in tissue damage observed in colitis. Study conducted by Abo-Zaid et al. [22] supports our findings about potential of melittin to inhibit infiltration of neutrophils. Their research emphasized melittin's immunomodulatory properties, including its capacity to decrease inflammation in different types of tissues.

The melittin-treated groups exhibited a significant improvement in the DAI, indicating enhanced overall health and decreased severity of colitis. The findings of this study are similar to those published by Park et al. [23], who demonstrated that decreasing DAI serves as a dependable measure of treatment effectiveness in colitis models caused by DSS. In addition, the positive changes in body weight and histopathological scores observed in our study align with the results reported by Jeengar et al. [24] and Bibi et al. [25]. Both studies found that treatments that can decrease inflammation and maintain the structure of the intestines are successful in treating colitis. In conclusion, our research has shown that liposomes loaded with melittin offer substantial defense against the onset and advancement of DSS-induced colitis, a finding that is corroborated by the findings of Liu et al. [19]. Their research demonstrated that melittin aids in preserving tissue integrity and diminishing inflammation caused by the immune system.

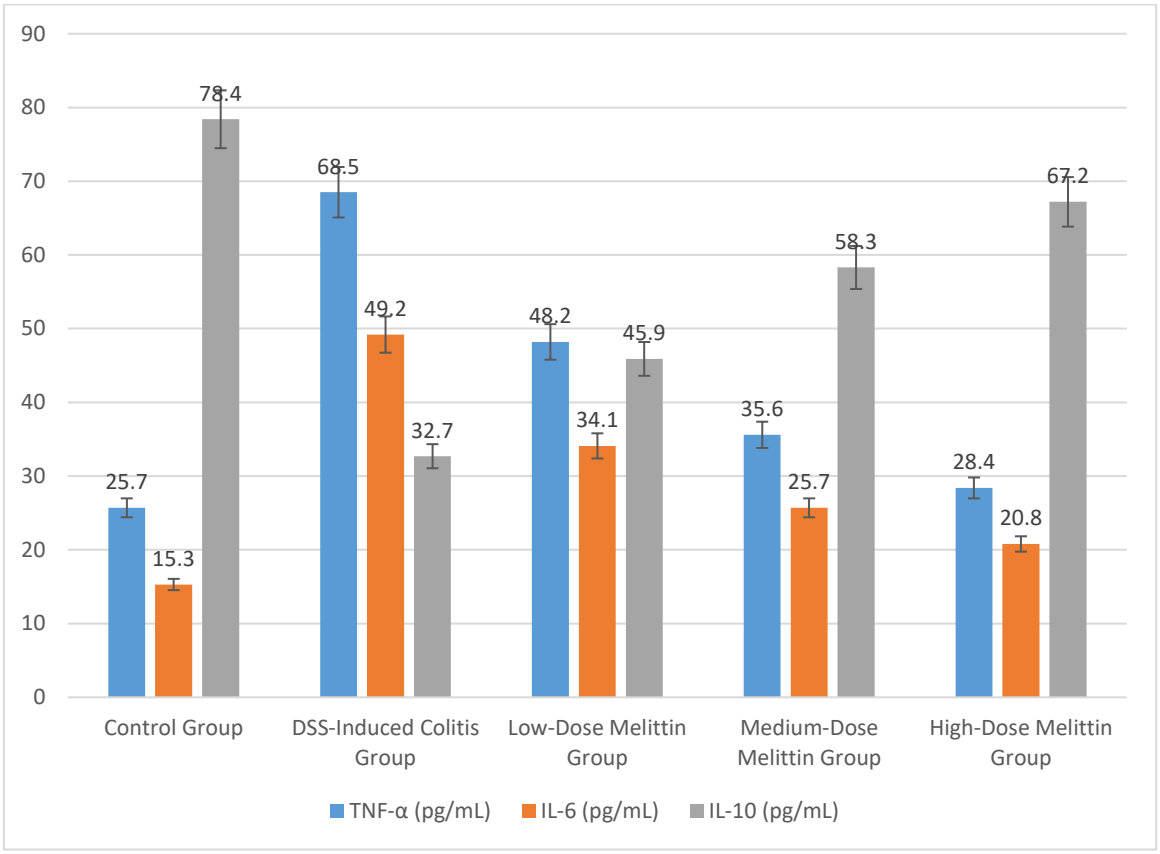

Figure 1: Inflammatory Cytokine Levels

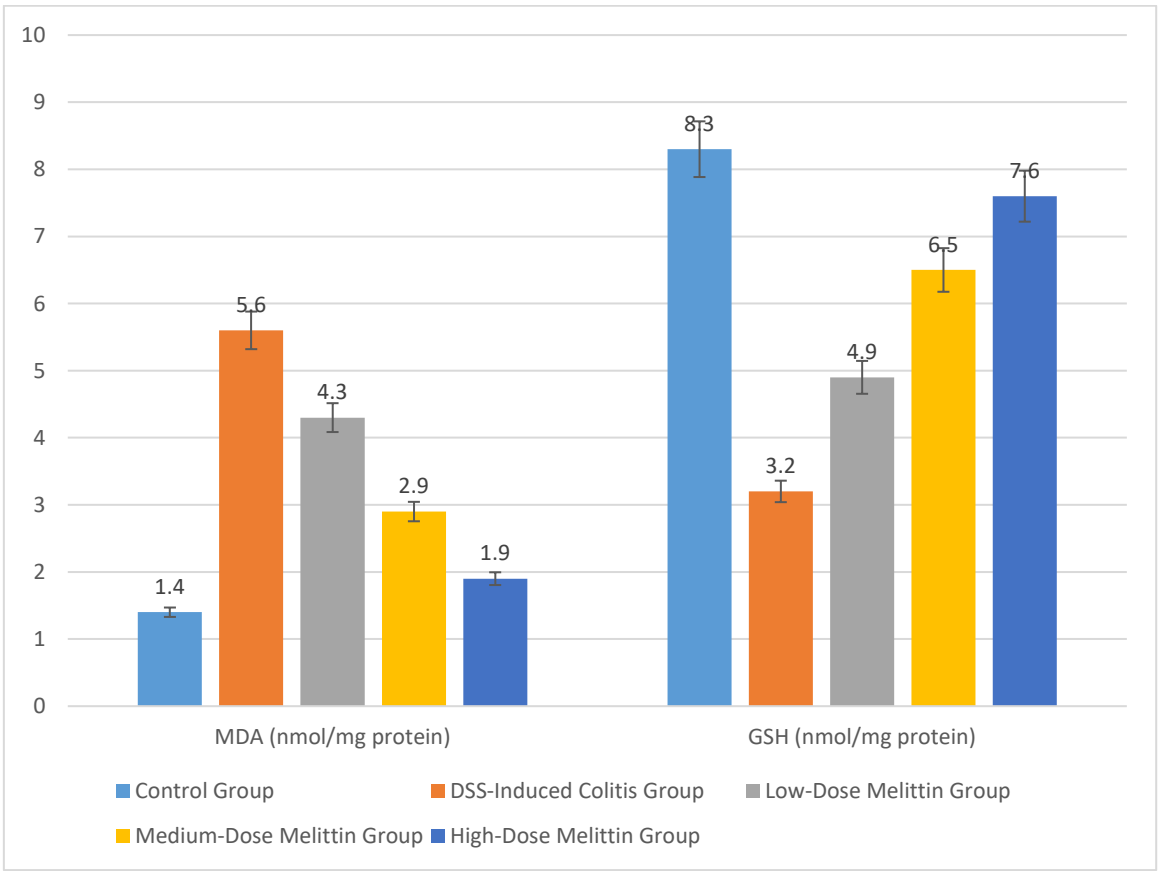

Figure 2: Oxidative stress markers in colonic tissues

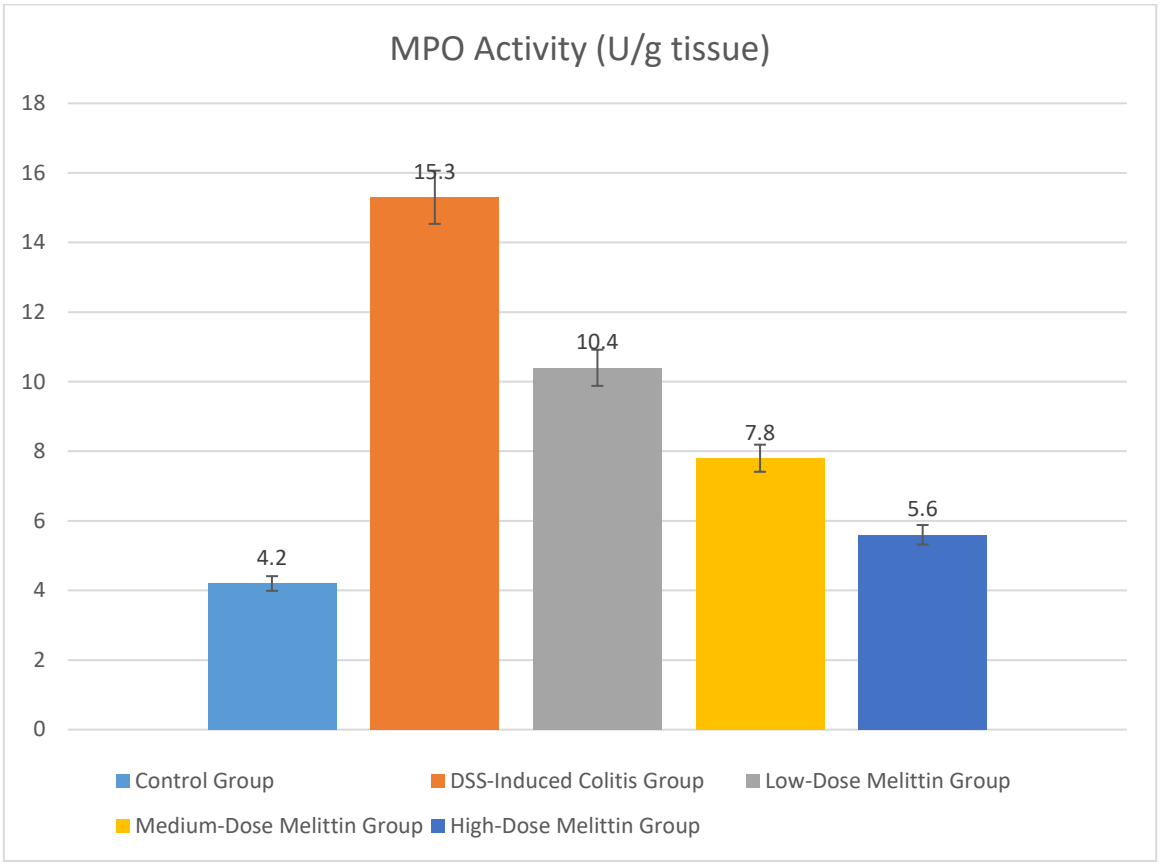

Figure 3: MPO activity in colonic tissues

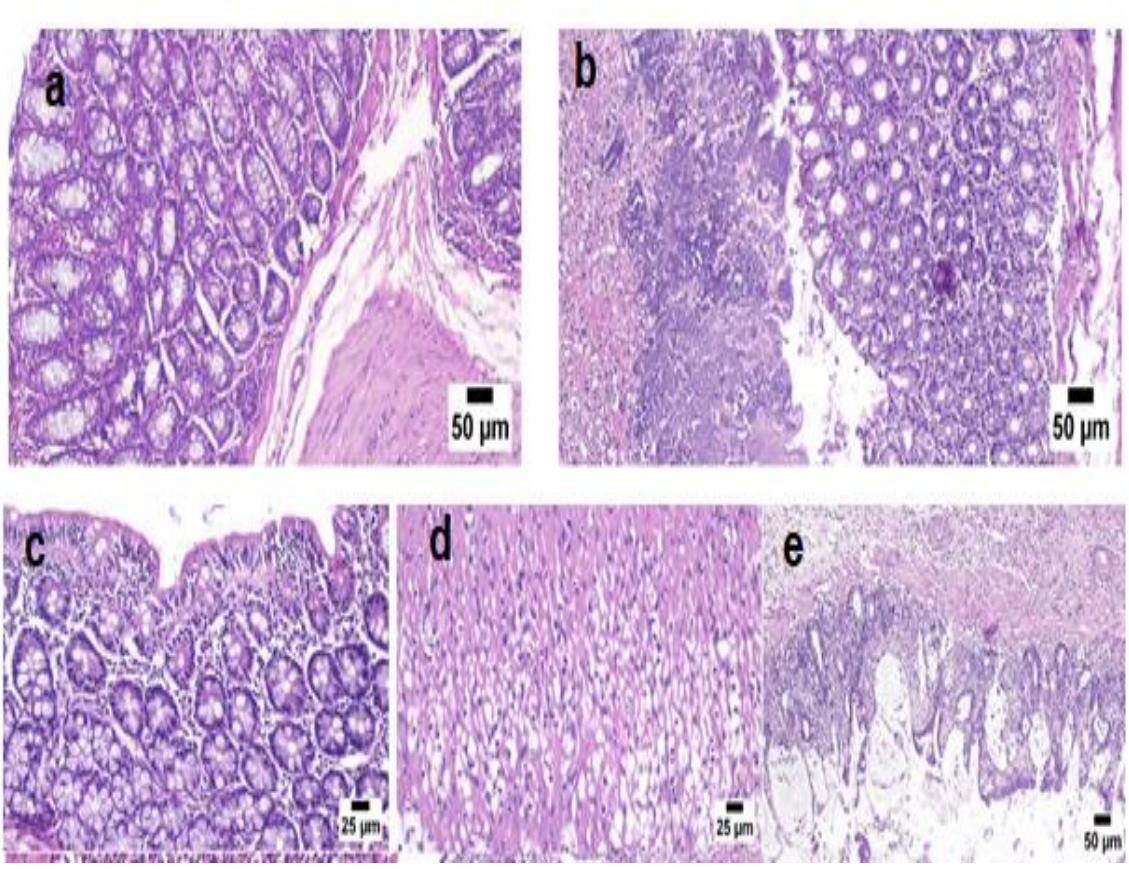

Figure 4: Photomicrograph of histopathological analysis for colon.

Table 1: Characterization of Melittin-Loaded Liposomes

Parameter	Value
Entrapment Efficiency	78.5 ± 2.3
Particle Size	115.4 ± 8.2 nm
Zeta Potential	-32.6 ± 4.1 mV

Table 2: Disease Activity Index (DAI) Scores

Group	Initial Weight (g)	Final Weight (g)	Weight Loss (%)	DAI Score
Control Group	225.3 ± 8.7	223.5 ± 9.1	0.8%	0.5 ± 0.2
DSS-Induced Colitis Group	226.1 ± 7.9	208.4 ± 7.2	7.8%	3.5 ± 0.4*
Low-Dose Melittin Group	224.7 ± 8.2	215.6 ± 8.5	4.0%	2.6 ± 0.3**
Medium-Dose Melittin Group	225.4 ± 8.0	218.3 ± 8.8	3.1%	2.0 ± 0.3**
High-Dose Melittin Group	225.0 ± 7.8	220.7 ± 8.3	1.9%	1.4 ± 0.2**

*Significantly different from Control Group ($p < 0.01$)
**Significantly different from DSS-Induced Colitis Group ($p < 0.01$)

Table 3: Inflammatory Cytokine Levels

Group	TNF-α (pg/mL)	IL-6 (pg/mL)	IL-10 (pg/mL)
Control Group	25.7 ± 2.8	15.3 ± 2.1	78.4 ± 7.2
DSS-Induced Colitis Group	68.5 ± 6.4*	49.2 ± 5.6*	32.7 ± 3.9*
Low-Dose Melittin Group	48.2 ± 4.9**	34.1 ± 3.5**	45.9 ± 4.8**
Medium-Dose Melittin Group	35.6 ± 3.7**	25.7 ± 3.1**	58.3 ± 5.6**
High-Dose Melittin Group	28.4 ± 2.9**	20.8 ± 2.4**	67.2 ± 6.2**

*Significantly different from Control Group ($p < 0.01$)
**Significantly different from DSS-Induced Colitis Group ($p < 0.01$)

Table 4: Oxidative stress markers in colonic tissues

Group	MDA (nmol/mg protein)	GSH (nmol/mg protein)
Control Group	1.4 ± 0.3	8.3 ± 1.0
DSS-Induced Colitis Group	5.6 ± 0.8*	3.2 ± 0.5*
Low-Dose Melittin Group	4.3 ± 0.6**	4.9 ± 0.7**
Medium-Dose Melittin Group	2.9 ± 0.4**	6.5 ± 0.8**
High-Dose Melittin Group	1.9 ± 0.3**	7.6 ± 0.9**

*Significantly different from Control Group ($p < 0.01$)
**Significantly different from DSS-Induced Colitis Group ($p < 0.01$)

Table 5: MPO Activity in Colonic Tissues

Group	MPO Activity (U/g tissue)
Control Group	4.2 ± 0.6
DSS-Induced Colitis Group	15.3 ± 2.2*
Low-Dose Melittin Group	10.4 ± 1.5**
Medium-Dose Melittin Group	7.8 ± 1.1**
High-Dose Melittin Group	5.6 ± 0.8**

*Significantly different from Control Group ($p < 0.01$)
**Significantly different from DSS-Induced Colitis Group ($p < 0.01$)

Table 6: Histopathological Scores

Group	Histopathological Score
Control Group	0.2 ± 0.1
DSS-Induced Colitis Group	3.8 ± 0.4*
Low-Dose Melittin Group	2.7 ± 0.3**
Medium-Dose Melittin Group	1.8 ± 0.2**
High-Dose Melittin Group	0.9 ± 0.1**

*Significantly different from Control Group ($p < 0.01$)
**Significantly different from DSS-Induced Colitis Group ($p < 0.01$)

This underscores its potential as a therapeutic agent for inflammatory illnesses such as IBD. The therapeutic potential of melittin is highlighted by several investigations, such as those conducted by Zhang et al. [3] and Yaghoubi et al. [4], which have observed its dual anti-inflammatory and antioxidant properties. Our findings conclusively demonstrate that melittin-loaded liposomes effectively alleviate DSS-induced colitis by diminishing inflammation, oxidative stress, and tissue damage. These findings align with the increasing amount of research that backs the use of melittin as a powerful therapeutic agent in inflammatory disorders. The alignment of our findings with prior research conducted by Urushima et al. [21], Zhang et al. [3], and Yaghoubi et al. [4] strengthens the promise of melittin as a beneficial therapy for IBD.

4. Conclusion

The results of our investigation show that melittin-loaded liposomes have strong therapeutic effects in treating colitis produced by DSS. Enclosing melittin in liposomes greatly enhanced its ability to be absorbed by the body and increased its effectiveness, leading to a considerable decrease in inflammation, oxidative stress, and tissue damage in the colon. The medication successfully rebalanced the levels of pro-inflammatory and anti-inflammatory cytokines, decreased neutrophil infiltration as shown by reduced MPO activity, and maintained the integrity of the intestinal barrier. The results align with other studies on melittin's anti-inflammatory, immunomodulatory, and antioxidant characteristics, providing further confirmation of its efficacy in treating inflammatory bowel illnesses such as ulcerative colitis. Melittin-loaded liposomes offer a promising alternative to conventional treatments for colitis by lowering important indicators of inflammation and damage in the colon. These liposomes are effective and have a positive safety profile. The potential of melittin to alleviate colitis generated by DSS is due to its capacity to modulate cytokines, decrease oxidative stress, and enhance the function of the intestinal barrier. This makes melittin a promising candidate for further research and development in the treatment of IBD. Future research should prioritize conducting clinical studies and investigating the enduring safety and effectiveness of melittin-based medicines in order to advance this innovative therapeutic approach towards clinical use.

References

[1] T. Khare, S.S. Palakurthi, B.M. Shah, S. Palakurthi, S. Khare. (2020). Natural product-based nanomedicine in treatment of inflammatory bowel disease. International journal of molecular sciences. 21(11): 3956.

[2] Y. Zhou, D. Wang, W. Yan. (2023). Treatment effects of natural products on inflammatory bowel disease in vivo and their mechanisms: Based on animal experiments. Nutrients. 15(4): 1031.

[3] Y. Zhang, H. Xu, H. Qiao, Y. Zhao, M. Jiang. (2024). Melittin induces autophagy to alleviate chronic renal failure in 5/6-nephrectomized rats and angiotensin II-induced damage in podocytes. Nutrition Research and Practice. 18(2): 210-222.

[4] A. Yaghoubi, S.A. Jamehdar, M.R.A. Eidgahi, K. Ghazvini. (2022). Evaluation of the therapeutic effect of melittin peptide on the ulcerative colitis mouse model. International Immunopharmacology. 108: 108810.

[5] Z. Liu, Z. Fan, J. Liu, J. Wang, M. Xu, X. Li, Z. Zhang. (2023). Melittin-Carrying Nanoparticle Suppress T Cell-Driven Immunity in a Murine Allergic Dermatitis Model. Advanced Science. 10(7): 2204184.

[6] Z. Zhai, F. Zhang, R. Cao, X. Ni, Z. Xin, J. Deng, G. Wu, W. Ren, Y. Yin, B. Deng. (2019). Cecropin A alleviates inflammation through modulating the gut microbiota of C57BL/6 mice with DSS-induced IBD. Frontiers in microbiology. 10: 1595.

[7] S.H. Kim, S. Yoon, J.H. Lee. (2022). Liposome-mediated delivery of bioactive compounds for the treatment of col*itis.* Journal of Microencapsulation. 39(4): 263-274.

[8] M. Li, R. Lv, C. Wang, Q. Ge, H. Du, S. Lin. (2021). Tricholoma matsutake-derived peptide WFNNAGP protects against DSS-induced colitis by ameliorating oxidative stress and intestinal barrier dysfunction. Food & Function. 12(23): 11883-11897.

[9] S. Singh, N. Verma, S. Gupta. (2020). Nanocarriers for the management of inflammatory bowel disease: Current status and future prospects. Colloids and Surfaces B: Biointerfaces. 193*:* 111102

[10] P. Patel, J.P. Smith, C. Sun, L. Zhang. (2021). Melittin-loaded liposomes for the treatment of inflammatory bowel disease. Nanomedicine: Nanotechnology, Biology and Medicine. 33*:* 102352.

[11] J. Wang, L. Zhang, X. Zhang, X. Wei, W. Dong. (2022). Anti-inflammatory effects of melittin-loaded liposomes in a mouse model of DSS-induced colitis. Inflammation. 45(2): 798-811.

[12] L. Zhang, H. Wang, S. Sun. (2021). Therapeutic effects of melittin liposomes on DSS-induced colitis: Insights from metabolomic and proteomic studies. Journal of Proteomics. 242*:* 104-239.

[13] Y. Huang, S. Li, J. Hu, J, Z. Shen. (2020). Targeted delivery of melittin to colonic inflammation via dextran sulfate sodium-modified liposomes. Journal of Controlled Release. 322*:* 151-160.

[14] J. Wu, Z. Liu, Y. Wang. (2021). Liposomal encapsulation of melittin enhances its therapeutic effect on colitis by improving intestinal barrier function. Journal of Pharmaceutical Sciences. 110(4): 1444-1454.

[15] C. Gao, Y. Yan, Z. Zhang, L. Zhou, X. Wu, Q. Lin. (2020). Protective effect of novel antioxidant peptides on DSS-induced colitis in mice. Food & Function. 11(9): 7534-7544.

[16] J. Liang, X. Zhang, L. Yang, Y. Zhao. (2021). Therapeutic effects of melittin-loaded liposomes on colitis via suppression of TLR4/NF-κB signaling pathway. Colloids and Surfaces B: Biointerfaces. 199*:* 111-505

[17] J. Yao, J.-Y. Wang, L. Liu, Y.-X. Li, A.-Y. Xun, W.-S. Zeng, C.-H. Jia, X.-X. Wei, J.-L. Feng, L. Zhao. (2010). Anti-oxidant effects of resveratrol on mice with DSS-induced ulcerative colitis. Archives of medical research. 41(4): 288-294.

[18] S. Yan, Y. Hui, J. Li, X. Xu, Q. Li, H. Wei. (2020). Glutamine relieves oxidative stress through PI3K/Akt signaling pathway in DSS-induced ulcerative colitis mice. Iranian journal of basic medical sciences. 23(9): 1124.

[19] Z. Liu, Z. Fan, J. Liu, J. Wang, M. Xu, X. Li, Y. Xu, Y. Lu, C. Han, Z. Zhang. (2023). Melittin-Carrying Nanoparticle Suppress T Cell-Driven Immunity in a Murine Allergic Dermatitis Model. Advanced Science. 10(7): 2204184.

[20] J. Cai, J. Liu, P. Fan, X. Dong, K. Zhu, X. Liu, N. Zhang, Y. Cao. (2021). Dioscin prevents DSS-induced colitis in mice with enhancing intestinal barrier function and reducing colon inflammation. International Immunopharmacology. 99: 108015.

[21] H. Urushima, J. Nishimura, T. Mizushima, N. Hayashi, K. Maeda, T. Ito. (2015). Perilla frutescens extract ameliorates DSS-induced colitis by suppressing proinflammatory cytokines and inducing anti-inflammatory cytokines. American Journal of Physiology-Gastrointestinal and Liver Physiology. 308(1): G32-G41.

[22] M.A. Abo-Zaid, K.A. Yatimi, A.H. Ismail. (2023). The role of bee venom on immunological and hematological parameters in albino rats. Egyptian Journal of Immunology. 30(2): 11-25.

[23] Y.H. Park, N. Kim, Y.K. Shim, Y.J. Choi, R.H. Nam, Y.J. Choi, M.H. Ham, J.H. Suh, S.M. Lee, C.M. Lee. (2015). Adequate dextran sodium sulfate-induced colitis model in mice and effective outcome measurement method. Journal of cancer prevention. 20(4): 260.

[24] M.K. Jeengar, D. Thummuri, M. Magnusson, V. Naidu, S. Uppugunduri. (2017). Uridine ameliorates dextran sulfate sodium (DSS)-induced colitis in mice. Scientific Reports. 7(1): 3924.

[25] S. Bibi, Y. Kang, M. Du, M.J. Zhu. (2017). Maternal high-fat diet consumption enhances offspring susceptibility to DSS-induced colitis in mice. Obesity. 25(5): 901-908.