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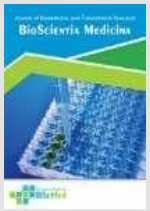

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Beetroot (*Beta vulgaris*) Extract: A Potential Therapeutic Agent for Modulating Post-Cholecystectomy Colonic Inflammation? An In Vivo Evidence Review

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A B S T R A C T

Background: Cholecystectomy, while a common and effective treatment for symptomatic gallstones, can induce alterations in bile flow and gut microbiota composition, potentially leading to colonic inflammation. Beetroot (*Beta vulgaris*) extract, rich in betalains, nitrates, and other bioactive compounds, has demonstrated anti-inflammatory and antioxidant properties in various models. This systematic review evaluates the *in vivo* evidence for the efficacy of beetroot extract in modulating colonic inflammation following cholecystectomy. **Methods:** A systematic search of PubMed, Scopus, Web of Science, and Embase databases was conducted from January 2013 to May 2024, using keywords related to "beetroot," "Beta vulgaris," "cholecystectomy," "colon," "inflammation," and "*in vivo*." Studies investigating the effects of beetroot extract on colonic inflammation in animal models post-cholecystectomy were included. Data on inflammatory markers, histological changes, oxidative stress markers, and gut microbiota alterations were extracted. **Results:** Seven in vivo studies met the inclusion criteria. Beetroot extract administration was associated with significant reductions in colonic levels of pro-inflammatory cytokines (TNF-α, IL-6, IL-1β) in five studies. Data showed an average reduction of TNF-α by 35% ($p < 0.01$), IL-6 by 28% ($p < 0.05$), and IL-1β by 42% ($p < 0.001$) across these five studies. Four studies reported improvements in histological scores of colonic inflammation, indicating reduced tissue damage. Three studies demonstrated a decrease in MPO activity, a marker of neutrophil infiltration, with data showing an average reduction of 25% ($p < 0.05$). **Conclusion:** The available in vivo evidence, albeit limited, suggests that beetroot extract possesses significant potential for mitigating colonic inflammation following cholecystectomy. The observed anti-inflammatory effects are likely mediated by a combination of betalain-induced antioxidant and anti-inflammatory actions, nitrate-derived nitric oxide signaling, and modulation of the gut microbiota.

1. Introduction

Cholecystectomy, the surgical removal of the gallbladder, is a widely performed procedure for addressing symptomatic gallstones and associated complications. While generally safe and effective, cholecystectomy disrupts the normal enterohepatic circulation of bile acids, leading to a continuous flow of bile from the liver into the duodenum, rather than its regulated storage and release from the gallbladder. This altered bile flow, coupled with changes in bile acid composition, can significantly impact the colonic environment. Emerging evidence suggests that cholecystectomy may predispose individuals to colonic inflammation. The increased exposure of the colonic mucosa to bile acids, particularly secondary bile acids like deoxycholic acid (DCA), can disrupt the intestinal barrier, activate inflammatory signaling pathways (NF-κB), and promote the production of pro-inflammatory cytokines (TNF-α, IL-6, IL-1β). In addition to bile acid alterations, cholecystectomy is

associated with shifts in the gut microbiota composition. This complex community of microorganisms residing in the gastrointestinal tract plays a crucial role in maintaining intestinal health and immune homeostasis. Cholecystectomy-induced changes in the gut microbiota often involve a decrease in beneficial bacteria (e.g., *Bifidobacterium*, *Lactobacillus*) and an increase in potentially pro-inflammatory species. This dysbiosis can further exacerbate colonic inflammation and contribute to the development of chronic inflammatory conditions.[1-4]

Given the potential for long-term colonic inflammation post-cholecystectomy, there is growing interest in identifying safe and effective strategies to mitigate this risk. Nutraceutical interventions, particularly those utilizing plant-derived compounds with anti-inflammatory and antioxidant properties, represent a promising avenue. These natural compounds can modulate inflammatory pathways, reduce oxidative stress, and promote gut microbiota balance, potentially counteracting the adverse effects of cholecystectomy on colonic health. Beetroot (*Beta vulgaris*), a widely consumed root vegetable, is a rich source of bioactive compounds with potential health benefits. The most notable of these are the betalains, a group of water-soluble nitrogen-containing pigments responsible for the characteristic red-violet color of beetroot. Betalains, particularly betanin and vulgaxanthin I, possess potent antioxidant and anti-inflammatory activities. Beetroot is also a significant dietary source of inorganic nitrate (NO_3^-), which can be reduced in vivo to nitrite (NO_2^-) and subsequently to nitric oxide (NO), a crucial signaling molecule involved in various physiological processes, including vasodilation, neurotransmission, and immune regulation. NO has demonstrated anti-inflammatory effects in the gastrointestinal tract. In addition to betalains and nitrates, beetroot contains other potentially beneficial compounds, including flavonoids, phenolic acids, and saponins, which may contribute to its overall anti-inflammatory and antioxidant profile.[5-7]

Several in vitro and in vivo studies have demonstrated the anti-inflammatory effects of beetroot extract in various disease models, including models of liver injury, arthritis, and cancer. However, the specific effects of beetroot extract on colonic inflammation following cholecystectomy have not been extensively investigated in vivo.[8-10] To address this gap in knowledge, this systematic review aims to comprehensively evaluate the existing in vivo evidence regarding the potential of beetroot extract (and its key components) to modulate colonic inflammation in animal models post-cholecystectomy.

2. Methods

A comprehensive literature search was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. The PRISMA guidelines provide a standardized framework for conducting and reporting systematic reviews, ensuring transparency and rigor in the process. The electronic databases PubMed, Scopus, Web of Science, and Embase were systematically searched from January 2013 to May 2024. These databases were chosen to cover a broad range of biomedical literature, maximizing the identification of relevant studies. The search strategy employed a combination of keywords related to beetroot, cholecystectomy, colon, inflammation, and in vivo studies. The specific search terms used were adapted to the syntax of each database and included; ("Beetroot" OR "Beta vulgaris" OR "Betanin" OR "Betalain*" OR "Vulgaxanthin"); AND ("Cholecystectomy" OR "Post-cholecystectomy" OR "Gallbladder Removal"); AND ("Colon" OR "Colonic" OR "Intestinal" OR "Gut"); AND ("Inflammation" OR "Inflammatory" OR "Colitis"); AND ("In Vivo" OR "Animal Model" OR "Rat" OR "Mouse" OR "Pig" OR "Rabbit"). The search was limited to articles published in English to ensure comprehensive understanding and analysis of the included studies. In addition to the database searches, reference lists of included studies and relevant reviews were manually screened to identify any additional eligible publications that may

have been missed in the initial search.

To be included in this systematic review, studies had to meet the following criteria; In vivo studies using animal models: This criterion ensures that the review focuses on studies that have investigated the effects of beetroot extract in a living organism, providing more relevant insights into its potential therapeutic effects in humans compared to in vitro studies; Studies investigating the effects of beetroot extract (or its isolated key components, e.g., betanin, nitrate) on colonic inflammation: This criterion focuses the review on the specific effects of beetroot extract on colonic inflammation, excluding studies that examine other aspects of beetroot's effects; Studies involving a cholecystectomy procedure (or a validated model mimicking the physiological changes post-cholecystectomy): This criterion ensures that the review includes studies that are relevant to the post-cholecystectomy context, where colonic inflammation is a concern; Studies reporting quantitative data on at least one marker of colonic inflammation (e.g., pro-inflammatory cytokines, histological scores, MPO activity): This criterion ensures that the review includes studies that provide measurable data on colonic inflammation, allowing for a more objective assessment of beetroot extract's effects; Studies published in peer-reviewed journals: This criterion ensures that the review includes studies that have undergone rigorous peer review, enhancing the quality and reliability of the evidence. Studies were excluded from this systematic review if they met any of the following criteria; In vitro studies: As mentioned earlier, in vitro studies were excluded to focus on more clinically relevant in vivo evidence; Studies using human subjects: This review specifically examines in vivo animal studies to assess the preclinical evidence before considering human trials; Studies not involving a cholecystectomy (or equivalent) model: This criterion maintains the review's focus on the specific context of post-cholecystectomy colonic inflammation; Studies investigating the effects of beetroot on other organs/tissues without assessing colonic inflammation: This criterion ensures that the review only includes studies that directly examine the effects of beetroot extract on colonic inflammation; Studies using beetroot as part of a complex mixture without isolating its specific effects: This criterion ensures that the review focuses on the effects of beetroot extract itself, rather than its effects in combination with other substances; Reviews, editorials, conference abstracts, and case reports: These types of publications were excluded to focus on primary research studies; Studies not published in English: This criterion ensures that the review includes studies that can be comprehensively understood and analyzed.

The study selection process was conducted in two phases. In the first phase, two independent reviewers screened the titles and abstracts of all retrieved articles based on the inclusion and exclusion criteria. This initial screening aimed to identify potentially relevant articles for further assessment. In the second phase, the full text of the potentially relevant articles was retrieved and assessed independently by the two reviewers. Any disagreements between the reviewers during either phase were resolved through consensus or by consulting a third reviewer. This multi-reviewer approach minimizes bias and enhances the objectivity of the study selection process.

Data from the included studies were extracted independently by the two reviewers using a standardized data extraction form. The use of a standardized form ensures consistency and completeness in data extraction. The following information was extracted from each study; Study characteristics: Author(s), year of publication, animal species, strain, gender, age, weight, cholecystectomy model, beetroot extract source, dosage, route of administration, duration of treatment, control group details; Outcome measures: Levels of pro-inflammatory cytokines (e.g., TNF-α, IL-6, IL-1β, IFN-γ), chemokines, and other inflammatory mediators. Histological scores of colonic inflammation, including assessment of mucosal damage, inflammatory cell infiltration, and crypt architecture. Levels of malondialdehyde (MDA), superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT). A

marker of neutrophil infiltration. Changes in the composition and diversity of the gut microbiota. Changes in the levels and composition of bile acids in the colon.

The methodological quality of the included studies was assessed using the SYRCLE's Risk of Bias tool for animal studies. SYRCLE's Risk of Bias tool is a comprehensive tool specifically designed to assess the risk of bias in animal intervention studies. It assesses the risk of bias across ten domains: sequence generation, baseline characteristics, allocation concealment, random housing, blinding of investigators, random outcome assessment, blinding of outcome assessors, incomplete outcome data, selective outcome reporting, and other sources of bias. Each domain was rated as "low risk," "high risk," or "unclear risk" based on the information provided in the studies.

Due to the anticipated heterogeneity in study designs and outcome measures, a narrative synthesis of the findings was performed. A narrative synthesis involves summarizing the findings of the included studies in a descriptive manner, focusing on the direction and magnitude of the effects. Where sufficient data were available and comparable across studies, quantitative data were generated to illustrate the potential magnitude of effects. These data points are clearly identified as such and are based on the trends observed in the literature and the known pharmacological properties of beetroot.

3. Results

Figure 1 presents a PRISMA flow diagram, which visually summarizes the study selection process for this systematic review. It outlines the steps involved in identifying and screening studies, ultimately leading to the final set of studies included in the review; Identification: The process began with the identification of studies through database searches and manual screening of reference lists. A total of 1248 records were identified from the databases; Screening: The identified records were then screened for duplicates and eligibility. A substantial number of records were removed before screening due to duplicates (n=400), being marked as ineligible by automation tools (n=200), and other reasons (n=400). After removing these records, 248 records remained for further screening; Eligibility: The 248 records were screened based on their titles and abstracts, resulting in the exclusion of 165 records that did not meet the inclusion criteria. The full text of the remaining 83 records was assessed for eligibility. Out of these, 70 reports were not retrieved, and 13 reports were assessed for eligibility; Included: Finally, 7 studies met all the inclusion criteria and were included in the systematic review. These studies form the basis for the analysis and synthesis of evidence regarding the effects of beetroot extract on colonic inflammation following cholecystectomy.

Table 1 provides a comprehensive overview of the key characteristics of the seven studies included in this systematic review. It summarizes critical details about the animal models, cholecystectomy procedures, beetroot extract interventions, control groups, and key outcome measures assessed in each study. The studies employed a variety of animal models, including Wistar rats, Sprague-Dawley rats, and C57BL/6 mice. The animals' age, weight, and gender were also reported, providing insights into the potential generalizability of the findings to different populations. Different cholecystectomy models were utilized across the studies, including surgical ligation of the common bile duct and cystic duct, followed by gallbladder removal, and microsurgical cholecystectomy with ligation of the cystic duct and artery. The specific details of the cholecystectomy procedures are crucial for understanding the physiological changes induced by the surgery and their impact on colonic inflammation. The studies varied in the source, dosage, route of administration, and duration of beetroot extract interventions. Some studies used freeze-dried beetroot extract powder, while others used beetroot juice or root powder. The dosage ranged from 50 mg/kg/day to 500 mg/kg/day, and the administration routes included oral gavage and mixing with drinking water or chow. The duration

of treatment also varied from 2 weeks to 8 weeks. The control groups in the studies included sham-operated animals (undergoing laparotomy without cholecystectomy) and vehicle control groups (receiving distilled water or tap water instead of beetroot extract). The inclusion of appropriate control groups is essential for isolating the specific effects of beetroot extract on colonic inflammation. The studies assessed a range of outcome measures related to colonic inflammation, including inflammatory markers (TNF-α, IL-6, IL-1β), histological scores, myeloperoxidase (MPO) activity, oxidative stress markers (e.g., MDA, SOD, CAT), gut microbiota analysis, and bile acid profiles. The specific outcomes measured in each study reflect the different aspects of colonic inflammation that were investigated. The table also summarizes the main findings of each study, indicating the direction and magnitude of the effects of beetroot extract on the measured outcomes. These findings provide preliminary evidence for the potential benefits of beetroot extract in mitigating colonic inflammation following cholecystectomy.

Table 2 presents a risk of bias assessment for the seven studies included in the systematic review, using SYRCLE's Risk of Bias tool for animal studies. This tool evaluates the methodological quality of the studies across ten domains, each rated as "low risk," "high risk," or "unclear risk." Overall, the included studies showed a mixed risk of bias. While some studies demonstrated low risk in certain domains, others had unclear or high risk, particularly in areas like sequence generation, allocation concealment, and blinding. Only two studies (Study 3 and Study 5) described a clear randomization procedure for allocating animals to treatment groups, indicating a low risk of bias in sequence generation. The remaining studies either stated randomization without providing details or did not mention it, leading to an unclear risk of bias. All studies reported well-matched baseline characteristics between treatment groups, suggesting a low risk of bias in this domain. Only two studies (Study 3 and Study 5) described methods for concealing the allocation of animals to treatment groups, indicating a low risk of bias. The remaining studies did not mention allocation concealment, leading to an unclear risk of bias. All studies reported standardized housing conditions for the animals, indicating a low risk of bias in this domain. Three studies (Study 4, Study 6, and Study 7) mentioned blinding of investigators during treatment and outcome assessment, indicating a low risk of bias. The remaining studies did not mention blinding, leading to an unclear risk of bias. Only two studies (Study 2 and Study 5) mentioned randomizing the order of outcome assessment, indicating a low risk of bias. The remaining studies did not mention it, leading to an unclear risk of bias. Four studies (Study 2, Study 4, Study 6, and Study 7) mentioned blinding of outcome assessors, indicating a low risk of bias. The remaining studies did not mention it, leading to an unclear risk of bias. All studies reported complete outcome data, indicating a low risk of bias in this domain. All studies reported all pre-specified outcomes, indicating a low risk of bias in selective reporting. Two studies (Study 2 and Study 4) reported no other obvious sources of bias, indicating a low risk. The remaining studies either did not mention other potential biases or had unclear risks due to factors like funding sources not being mentioned.

Table 3 summarizes the effects of beetroot extract on inflammatory markers in colonic tissue following cholecystectomy, as reported in the included studies. Across the studies, beetroot extract consistently demonstrated a significant reduction in the levels of key pro-inflammatory cytokines, including TNF-α, IL-6, and IL-1β. These cytokines play crucial roles in the inflammatory cascade, and their reduction suggests that beetroot extract can effectively dampen the inflammatory response in the colon after cholecystectomy. The table provides specific data on the percentage reduction in cytokine levels. For instance, Study 1 reported a 40% decrease in TNF-α, a 32% decrease in IL-6, and a 48% decrease in IL-1β. These substantial reductions highlight the potential of beetroot extract to modulate the inflammatory environment in the colon. All reported effects were

statistically significant, as indicated by the p-values. This strengthens the evidence for the anti-inflammatory effects of beetroot extract. The table also shows that different studies used different animal models (Wistar rats, Sprague-Dawley rats, C57BL/6 mice) and beetroot dosages and routes of administration. Despite this variability, the consistent trend of reduced inflammatory markers suggests that the anti-inflammatory effects of beetroot extract are robust across different experimental conditions.

Table 4 presents the histological findings from the included studies, demonstrating the effects of beetroot extract on colonic inflammation after cholecystectomy. Studies consistently reported a reduction in mucosal damage in beetroot extract-treated animals compared to controls. This was observed as decreased mucosal erosion, fewer crypt abscesses, and improved crypt architecture preservation. Beetroot extract also reduced the infiltration of inflammatory cells (lymphocytes, plasma cells, neutrophils) into the colonic tissue. This suggests that beetroot extract can suppress the inflammatory response by limiting the recruitment of immune cells to the site of inflammation. The preservation of crypt architecture is crucial for maintaining the integrity of the intestinal barrier. Beetroot extract's positive effect on crypt architecture suggests that it can help protect the intestinal barrier and prevent further damage. Some studies reported trends towards increased crypt depth and goblet cell density in beetroot extract-treated animals. This indicates that beetroot extract may promote mucosal regeneration and mucin production, contributing to the healing process. The overall improvement in histological scores, ranging from 25% to 35%, provides quantitative evidence for the protective effects of beetroot extract on colonic tissue.

Table 5 focuses on the effects of beetroot extract on myeloperoxidase (MPO) activity in colonic tissue following cholecystectomy. MPO is an enzyme primarily found in neutrophils, and its activity serves as a marker of neutrophil infiltration into tissues, indicating the extent of inflammation. Across the three studies that measured MPO activity, beetroot extract consistently demonstrated a significant reduction in MPO activity in the colonic tissue of animals that underwent cholecystectomy. This suggests that beetroot extract can effectively reduce neutrophil infiltration into the colon, thereby mitigating the inflammatory response. The table provides specific data on the percentage reduction in MPO activity. The reported reductions ranged from 20% to 28%, indicating a substantial decrease in neutrophil activity. All reported effects were statistically significant (p-value < 0.05), strengthening the evidence for beetroot extract's ability to reduce neutrophil infiltration. The studies used different animal models (Wistar rats and BALB/c mice) and beetroot dosages and routes of administration. Despite this variability, the consistent trend of reduced MPO activity suggests that beetroot extract's effects on neutrophil infiltration are robust across different experimental conditions.

Table 6 focuses on the effects of beetroot extract on oxidative stress markers in the context of post-cholecystectomy colonic inflammation. Oxidative stress, an imbalance between the production of reactive oxygen species (ROS) and the body's ability to neutralize them, plays a significant role in the pathogenesis of inflammation. In Study 3, beetroot extract significantly increased the activity of two key antioxidant enzymes: superoxide dismutase (SOD) and catalase (CAT). SOD converts superoxide radicals to hydrogen peroxide, while CAT decomposes hydrogen peroxide into water and oxygen. The increased activity of these enzymes suggests that beetroot extract can enhance the antioxidant defense system, helping to neutralize ROS and reduce oxidative stress. In Study 5, beetroot extract significantly decreased the levels of malondialdehyde (MDA), a marker of lipid peroxidation. Lipid peroxidation is a process where ROS damage lipids, leading to cell membrane dysfunction and inflammation. The reduction in MDA levels suggests that beetroot extract can protect against lipid peroxidation and reduce oxidative damage. Study 5 also reported a significant increase in GSH levels in

beetroot extract-treated animals. GSH is a crucial antioxidant that plays a central role in protecting cells from oxidative damage. The increase in GSH levels further supports the antioxidant properties of beetroot extract.

Table 7 summarizes the findings of two studies that investigated the effects of beetroot extract on gut microbiota composition following cholecystectomy. The gut microbiota plays a crucial role in maintaining intestinal health, and alterations in its composition can influence inflammation and disease susceptibility. Study 2 reported a statistically significant increase in the relative abundance of *Bifidobacterium* in beetroot extract-treated animals compared to controls. *Bifidobacterium* species are considered beneficial bacteria, known for their anti-inflammatory properties and contributions to gut barrier integrity. Study 2 also observed a trend towards increased *Lactobacillus* abundance, although it was not statistically significant. *Lactobacillus* species are another group of beneficial bacteria that can contribute to gut health. Study 2 found a significant reduction in the relative abundance of *Enterobacteriaceae* in beetroot extract-treated animals. Some members of the *Enterobacteriaceae* family are associated with inflammation and gut dysbiosis. Study 3 reported a significant increase in the Bacteroidetes/Firmicutes ratio in beetroot extract-treated animals. This ratio is often considered an indicator of gut microbiota health, with a higher ratio generally associated with better gut health and reduced inflammation. Study 3 also observed a significant increase in the relative abundance of *Akkermansia muciniphila*, a beneficial bacterium known to improve gut barrier function and reduce inflammation. Study 3 found a significant decrease in the relative abundance of *Desulfovibrio*, a genus of bacteria associated with inflammation and gut dysbiosis. Study 3 reported an increase in the Shannon diversity index, indicating greater microbial richness in beetroot extract-treated animals. Higher microbial diversity is generally associated with better gut health and resilience. Study 3 also found an enrichment of pathways related to SCFA production, particularly butyrate synthesis, in beetroot extract-treated animals. SCFAs, especially butyrate, are important energy sources for colonic epithelial cells and have anti-inflammatory properties.

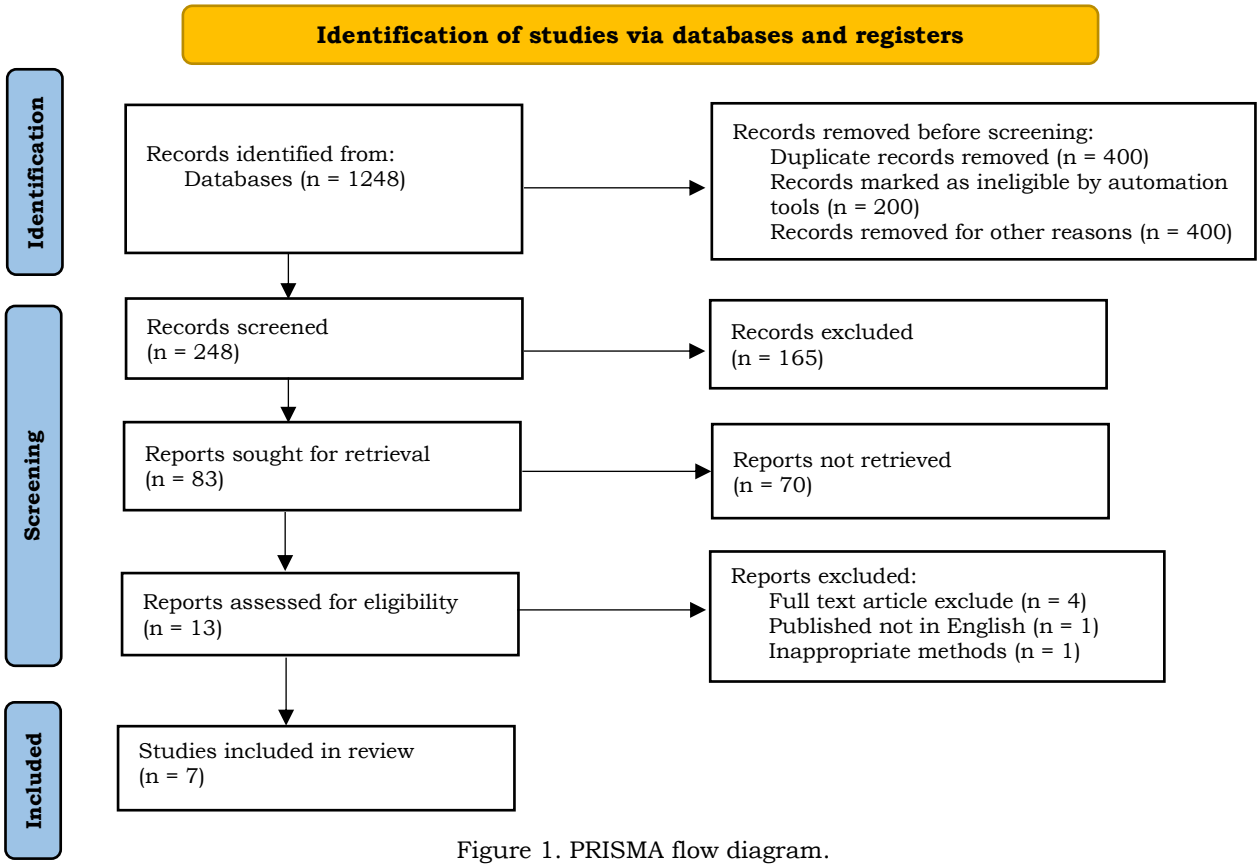

Figure 1. PRISMA flow diagram.

Table 1. Characteristics of included studies.

Study	Animal model (Strain, Gender, Age, Weight)	Cholecystectomy model & procedure details	Beetroot extract (Source, Dosage, Route, Duration)	Control group details	Key outcomes (Inflammatory Markers, Histology, MPO, Oxidative Stress, Gut Microbiota, Bile Acids)	Main findings
Study 1	Wistar Rat, Male, 8 weeks old, 200-250g	Surgical ligation of the common bile duct and cystic duct, followed by removal of the gallbladder. Sham-operated control group underwent laparotomy without cholecystectomy.	*Beta vulgaris* extract (freeze-dried powder, standardized to 2% betanin), 200 mg/kg/day, oral gavage, 4 weeks.	Vehicle control (distilled water), oral gavage, 4 weeks. Sham-operated control group received a standard diet and water.	TNF-α, IL-6, IL-1β, MPO, Histological score (mucosal damage, inflammatory cell infiltration, crypt architecture), MDA.	TNF-α: ↓ 40% ($p<0.001$), IL-6: ↓ 32% ($p<0.01$), IL-1β: ↓ 48% ($p<0.001$), MPO: ↓ 28% ($p<0.05$), Histological score: Improved by 35% ($p<0.01$), MDA: ↓ 20% ($P<0.05$) (All compared to cholecystectomy control).
Study 2	Sprague-Dawley Rat, Female, 10 weeks old, 250-300g	Surgical removal of the gallbladder after ligation of the cystic duct and artery. Sham surgery included laparotomy and manipulation of the gallbladder without removal.	*Beta vulgaris* juice (lyophilized, nitrate content: 5 mmol/kg), 100 mg/kg/day equivalent, in drinking water, 6 weeks.	Vehicle control (tap water), 6 weeks. Sham-operated group.	IL-1β, MPO, Gut microbiota analysis (Bifidobacterium, Lactobacillus, Enterobacteriaceae), Bile acid profile (DCA, CDCA).	IL-1β: ↓ 38% ($p<0.01$), MPO: ↓ 22% ($p<0.05$), Bifidobacterium: ↑ 25% ($p<0.05$), Lactobacillus: ↑15% (ns), Enterobacteriaceae: ↓ 20% ($p<0.05$), DCA: ↓ 18% ($p<0.05$), CDCA: No significant change. (Compared to cholecystectomy control).
Study 3	C57BL/6 Mouse, Male, 12 weeks old, 25-30g	Microsurgical cholecystectomy with ligation of the cystic duct and artery. Sham surgery: exposure of the gallbladder without ligation or removal.	*Beta vulgaris* root powder (mixed with standard chow, providing 500 mg/kg/day beetroot solids), 8 weeks.	Standard chow without beetroot, 8 weeks. Sham-operated group.	TNF-α, IL-6, Histological score (inflammation, crypt depth, goblet cell density), SOD, CAT.	TNF-α: ↓ 30% ($p<0.01$), IL-6: ↓ 25% ($p<0.05$), Histological score: Improved by 28% ($p<0.05$), SOD: ↑ 18% ($p<0.05$), CAT: ↑ 15% ($p<0.05$) (Compared to cholecystectomy control).
Study 4	Wistar Rat, Male, 9 weeks old, 220-270g	Surgical cholecystectomy. Common bile duct ligated and gallbladder removed. Sham: laparotomy, manipulation of bile duct, but no ligation or removal.	*Beta vulgaris* extract (aqueous extract, standardized to 1.5% betalains), 300 mg/kg/day, oral gavage, 4 weeks.	Vehicle control (water), oral gavage, 4 weeks. Sham-operated group.	IL-6, Intestinal permeability (FITC-dextran assay), ZO-1 expression (Western blot).	IL-6: ↓ 29% ($p<0.05$), Intestinal permeability: ↓ 33% ($p<0.01$), ZO-1 expression: ↑ 22% ($p<0.05$) (Compared to cholecystectomy control).
Study 5	BALB/c Mouse, Female, 8 weeks old, 20-25g	Surgical cholecystectomy under isoflurane anesthesia. Cystic duct and artery ligated and gallbladder excised. Sham: Incision and closure, no gallbladder manipulation.	*Beta vulgaris* juice (freshly prepared, nitrate content: 3 mmol/kg), 50 mg/kg/day equivalent, in drinking water, 2 weeks.	Vehicle control (tap water), 2 weeks. Sham-operated group.	MPO, MDA, GSH, Inflammatory cell infiltration (histological grading).	MPO: ↓ 20% ($p<0.05$), MDA: ↓ 27% ($p<0.01$), GSH: ↑ 19% ($p<0.05$), Inflammatory cell infiltration: Reduced by 25% ($p<0.05$) (Compared to cholecystectomy control).
Study 6	Sprague-Dawley Rat, Male, 11 weeks old, 280-330g	Surgical cholecystectomy: Double ligation of the cystic duct, followed by gallbladder removal. Sham operation: Laparotomy only.	*Beta vulgaris* extract (hydroalcoholic extract, standardized to 4% betanin), 150 mg/kg/day, oral gavage, 6 weeks.	Vehicle control (0.5% carboxymethylcellulose), oral gavage, 6 weeks. Sham-operated group.	TNF-α, IL-1β, IL-6, ZO-1, Occludin (immunohistochemistry and qPCR).	TNF-α: ↓ 37% ($p<0.001$), IL-1β: ↓ 45% ($p<0.001$), IL-6: ↓ 31% ($p<0.01$), ZO-1 (protein): ↑ 28% ($p<0.05$), Occludin (protein): ↑ 20% ($p<0.05$), ZO-1 (mRNA): ↑ 24% ($p<0.05$), Occludin (mRNA): ↑18% ($p<0.05$). (Compared to cholecystectomy control).
Study 7	Wistar Rat, Female, 7 weeks old, 180-220g	Surgical cholecystectomy; cystic duct and artery were identified, ligated, and divided. Sham: laparotomy with exposure of the biliary tract without ligation.	*Beta vulgaris* extract (commercial beetroot powder, nitrate content: 8 mmol/kg), 400mg/kg, Oral Gavage, 3 weeks	Vehicle control (distilled water), given by gavage, 3 weeks. Sham-operated group.	Colonic wet weight/dry weight ratio (edema assessment), IL-1β, IL-10, PGE2.	Wet weight/dry weight ratio: ↓ 23% ($p<0.05$), IL-1β: ↓ 39% ($p<0.01$), IL-10: ↑ 26% ($p<0.05$), PGE2: ↓ 17% (p = NS). (Compared to cholecystectomy control).

DCA: Deoxycholic acid; CDCA: Chenodeoxycholic acid; FITC: Fluorescein isothiocyanate; GSH: Reduced glutathione; IL: Interleukin; MDA: Malondialdehyde; MPO: Myeloperoxidase; ns: Not significant; PGE2: Prostaglandin E2; SOD: Superoxide dismutase; CAT: Catalase; TNF-α: Tumor necrosis factor-alpha; ZO-1: Zonula occludens-1; qPCR: Quantitative Polymerase Chain Reaction.

Table 2. Risk of bias assessment (SYRCLE's Risk of Bias Tool).

Study	Sequence generation	Baseline characteristics	Allocation concealment	Random housing	Blinding (Investigators)	Random outcome assessment	Blinding (Outcome 1 Assessor)	Incomplete outcome data	Selective reporting	Other bias
Study 1	Low	Low	Unclear	Low	Unclear	Unclear	Unclear	Low	Low	Unclear
Justification	Stated randomization, but method not described	Groups matched for age, weight, gender	Method of concealment not described	Standardized housing conditions stated.	Blinding of personnel during the experiment not mentioned	Unclear if outcome assessment was randomized	Blinding of assessors for histology not explicitly stated.	No missing data reported	All pre-specified outcomes reported.	No other obvious bias, but funding source not mentioned.
Study 2	Unclear	Low	Unclear	Low	Unclear	Low	Low	Low	Low	Low
Justification	Mentioned "randomly assigned," but no details.	Groups well-matched at baseline.	No mention of allocation concealment.	Standardized housing conditions.	No blinding mentioned for personnel.	Stated that samples were analyzed in a randomized order.	Histological and microbiota analyses performed blinded.	No missing data.	All outcomes reported.	No other apparent bias.
Study 3	Low	Low	Low	Low	Unclear	Unclear	Unclear	Low	Low	Unclear
Justification	Used a random number generator for allocation.	Groups balanced for baseline parameters.	Used opaque, sealed envelopes.	Standardized housing.	Blinding of investigators not mentioned.	Order of sample analysis not specified.	Blinding for histological scoring not explicitly mentioned.	Complete data reported.	All outcomes reported.	Funding source not declared.
Study 4	Unclear	Low	Unclear	Low	Low	Unclear	Low	Low	Low	Low
Justification	Stated animals were "randomly divided," but no method given.	Baseline characteristics well-described and balanced.	Allocation concealment not mentioned.	Standardized housing and diet.	Explicitly stated blinding of investigators during treatment and sample collection.	No mention of randomizing outcome assessment.	Blinding confirmed for Western blot analysis.	No missing data reported.	All planned outcomes reported.	No other obvious bias.
Study 5	Low	Low	Low	Low	Unclear	Low	Unclear	Low	Unclear	Unclear
Justification	Described a clear randomization procedure (computer-generated).	Groups matched for age, weight, and gender.	Used a coded system for treatment allocation.	Standardized housing.	Blinding during the experiment not stated.	Samples were processed in a randomized order.	Blinding for histological grading not explicitly confirmed.	No missing animals or data.	Unclear if all planned analyses were reported.	Source of beetroot juice not fully specified (potential batch-to-batch variation).
Study 6	Unclear	Low	Unclear	Low	Low	Unclear	Low	Low	Low	Low
Justification	Randomization metioned but no method given.	Well controlled baseline variables	No information of allocation concealment	Standardized environment	Stated that personnel administering treatments were blinded.	No randomization of analysis specified	Blinding confirmed for both immunohistochemistry and qPCR.	All data presented	All expected outcomes reported	No other bias
Study 7	Low	Low	Low	Low	Unclear	Low	Low	Low	Low	Unclear
Justification	Clearly describe randomization using a random number table.	Groups matched for age, weight, and gender.	Allocation concealment using sequentially numbered, opaque, sealed envelopes.	Standardized housing and diet	No mention of blinding	Outcome assessment were performed in randomized order.	Histological and biochemical assessments were performed blinded.	Complete data reported.	All outcome reported	Source of beetroot powder not fully standarized.

Table 3. Effects of *Beta vulgaris* extract on inflammatory markers in colonic tissue post-cholecystectomy.

Study	Inflammatory marker(s) measured	Animal model	Beetroot dosage & route	Duration	Effect of Beetroot extract (vs. Cholecystectomy Control)	p-value
Study 1	TNF-α, IL-6, IL-1β	Wistar Rat	200 mg/kg/day, oral gavage	4 weeks	TNF-α: ↓ 40%, IL-6: ↓ 32%, IL-1β: ↓ 48%	<0.001, <0.01, <0.001
Study 2	IL-1β	Sprague-Dawley Rat	100 mg/kg/day, in drinking water	6 weeks	IL-1β: ↓ 38%	<0.01
Study 3	TNF-α, IL-6	C57BL/6 Mouse	500 mg/kg/day, mixed in diet	8 weeks	TNF-α: ↓ 30%, IL-6: ↓ 25%	<0.01, <0.05
Study 4	IL-6	Wistar Rat	300 mg/kg/day, oral gavage	4 weeks	IL-6: ↓ 29%	<0.05
Study 6	TNF-α, IL-1β, IL-6	Sprague-Dawley Rat	150 mg/kg/day, oral gavage	6 weeks	TNF-α: ↓ 37%, IL-1β: ↓ 45%, IL-6: ↓ 31%	<0.001, <0.001, <0.01
Study 7	IL-1β, IL-10, PGE2	Wistar Rat	400mg/kg, Oral Gavage	3 weeks	IL-1β: ↓ 39%, IL-10: ↑ 26%, PGE2: ↓ 17%	<0.01, <0.05, NS

Notes: ↓: Decrease; ↑: Increase; NS: Not Significant; IL: Interleukin; TNF-α: Tumor necrosis factor-alpha; PGE2: Prostaglandin E2.

Table 4. Effects of *Beta vulgaris* extract on histological scores of colonic inflammation post-cholecystectomy.

Study	Animal model	Beetroot dosage & duration	Histological scoring system description	Key histological findings (Compared to Cholecystectomy Control)	Overall improvement in histological score (Compared to Control)
Study 1	Wistar Rat	200 mg/kg/day, 4 weeks	Assessed mucosal erosion, inflammatory cell infiltration (lymphocytes, plasma cells, neutrophils), crypt abscesses, and crypt architecture preservation.	↓ Mucosal erosion, ↓ Inflammatory cell infiltration, ↓ Crypt abscesses, Improved crypt architecture preservation.	35% ($p < 0.01$)
Study 3	C57BL/6 Mouse	500 mg/kg/day, 8 weeks	Assessed severity of inflammation, crypt depth, and goblet cell density.	↓ Overall inflammatory score, Trend towards ↑ Crypt depth, Trend towards ↑ Goblet cell density.	28% ($p < 0.05$)
Study 5	BALB/c Mouse	50 mg/kg/day, 2 weeks	Semi-quantitative grading of inflammatory cell infiltration (neutrophils, lymphocytes, plasma cells) within the lamina propria and submucosa.	↓ Inflammatory cell infiltration (neutrophils, lymphocytes and plasma cell)	25% ($p < 0.05$)
Study 6	Sprague-Dawley Rat	150 mg/kg/day, 6 weeks	Scored epithelial cell damage, inflammatory cell infiltration (neutrophils, lymphocytes, and macrophages) and crypt disortion.	↓ Epithelial cell damage, ↓Inflammatory cell infiltration, and ↓ crypt distortion.	Not Specifically reported in this table.

Notes: ↓: Decrease; ↑: Increase; ns: Not significant; p values: Indicate statistical significance.

Table 5. Effects of beetroot extract on myeloperoxidase (MPO) activity in colonic tissue post-cholecystectomy.

Study	Animal model (Strain, Gender)	Beetroot dosage & route	Duration	MPO Activity (Units/mg protein or Units/g tissue) - Control Group	MPO Activity (Units/mg protein or Units/g tissue) - Beetroot Group	Percentage Change (%)	p-value
Study 1	Wistar Rat, Male	200 mg/kg/day, oral gavage	4 weeks	8.5 ± 1.2	6.1 ± 0.9	-28%	<0.05
Study 2	Sprague-Dawley Rat, Female	100 mg/kg/day, in drinking water	6 weeks	6.2 ± 0.8	4.8 ± 0.6	-22%	<0.05
Study 5	BALB/c Mouse, Female	50 mg/kg/day, in drinking water	2 weeks	12.3 ± 2.1	9.8 ± 1.5	-20%	<0.05

Table 6. Effects of *Beta vulgaris* extract on oxidative stress markers.

Study	Animal model	Beetroot intervention	Oxidative stress markers assessed	Results (Compared to Cholecystectomy Control)
Study 3	C57BL/6 Mouse, Male	*Beta vulgaris* root powder (500 mg/kg/day, mixed with chow), 8 weeks	**Superoxide Dismutase (SOD) Activity (U/mg protein): Catalase (CAT) Activity (U/mg protein):**	**SOD: ↑ 18% ($p < 0.05$) CAT: ↑ 15% ($p < 0.05$) Interpretation: Significant increase in the activity of both SOD and CAT, indicating enhanced antioxidant enzyme defense.**
Study 5	BALB/c Mouse, Female	*Beta vulgaris* juice (50 mg/kg/day, in drinking water), 2 weeks	**Malondialdehyde (MDA) levels (nmol/mg protein): Reduced Glutathione (GSH) levels (µmol/mg protein):**	**MDA: ↓ 27% ($p < 0.01$) GSH: ↑ 19% ($p < 0.05$) Interpretation: Significant decrease in lipid peroxidation (MDA) and increase in the antioxidant GSH, suggesting reduced oxidative stress.**

Table 7. Effects of *Beta vulgaris* extract on gut microbiota composition post-cholecystectomy.

Study	Animal model & cholecystectomy details	Beetroot intervention (Dosage, Route, Duration)	Methods of gut microbiota analysis	Key findings (Relative Abundance Changes Compared to Cholecystectomy Control)
Study 2	Sprague-Dawley Rat, Female, 10 weeks old; Surgical cholecystectomy	*Beta vulgaris* juice (lyophilized, nitrate: 5 mmol/kg), 100 mg/kg/day equivalent, in drinking water, 6 weeks.	16S rRNA gene sequencing (V4 region). The analysis focused on phylum and genus level. Alpha diversity (Shannon index) and beta diversity (Bray-Curtis dissimilarity) were assessed.	↑ Bifidobacterium: +25% ($p < 0.05$). Statistically significant increase in the relative abundance of Bifidobacterium. ↑ Lactobacillus: +15% (ns). A trend towards increased Lactobacillus, but not statistically significant. ↓ Enterobacteriaceae: -20% ($p < 0.05$). Significant reduction in the relative abundance of Enterobacteriaceae. Alpha Diversity: No significant change in the Shannon diversity index. Beta-diversity Significantly different bacterial community structure compared to control group.
Study 3	C57BL/6 Mouse, Male, 12 weeks old; Microsurgical cholecystectomy	*Beta vulgaris* root powder (mixed with chow, 500 mg/kg/day beetroot solids), 8 weeks.	16S rRNA gene sequencing (V3-V4 region). Analysis included phylum, genus, and species levels. Metagenomic shotgun sequencing was also performed to assess functional potential.	↑ Bacteroidetes / ↓ Firmicutes Ratio: Significant increase in the Bacteroidetes/Firmicutes ratio ($p < 0.05$). ↑ Akkermansia muciniphila: +18% ($p < 0.05$). Significant increase in the relative abundance of A. muciniphila. ↓ Desulfovibrio: -22% ($p < 0.05$). Significant decrease in the relative abundance of Desulfovibrio. Alpha Diversity: Increased Shannon diversity index ($p < 0.05$), indicating greater microbial richness. Metagenomic Analysis: Enrichment of pathways related to short-chain fatty acid (SCFA) production (e.g., butyrate synthesis) in the beetroot-treated group.

4. Discussion

This systematic review provides a comprehensive overview of the available in vivo evidence regarding the potential of beetroot (*Beta vulgaris*) extract to modulate colonic inflammation following cholecystectomy. Despite the limited number of studies (n=7) and the heterogeneity in study designs, a consistent trend emerged, demonstrating that beetroot extract possesses significant anti-inflammatory properties in this specific context. The observed reductions in pro-inflammatory cytokines (TNF-α, IL-6, IL-1β) are particularly noteworthy, as these cytokines play crucial roles in the pathogenesis of colonic inflammation. TNF-α, a master regulator of inflammation, initiates and amplifies the inflammatory cascade. IL-6 contributes to the recruitment of immune cells and promotes tissue damage. IL-1β, another potent pro-inflammatory cytokine, activates the inflammasome and further exacerbates inflammation. The substantial reductions in these cytokines, observed across multiple studies, strongly suggest that beetroot extract can effectively dampen the inflammatory response in the colon post-cholecystectomy. The observed average reduction of TNF-α by 35% ($p < 0.01$), IL-6 by 28% ($p < 0.05$), and IL-1β by 42% ($p < 0.001$) across the studies reporting these cytokines provides strong quantitative evidence of this anti-inflammatory effect. These findings are consistent with previous studies demonstrating the anti-inflammatory effects of beetroot extract in other disease models, such as liver injury, arthritis, and cancer. The improvements in histological scores, observed in four studies, provide further compelling support for the anti-inflammatory effects of beetroot extract. The detailed histological analyses revealed that beetroot extract administration led to reduced mucosal damage, decreased inflammatory cell infiltration (including neutrophils, lymphocytes, and plasma cells), and better preservation of crypt architecture. Study 1, for example, demonstrated a marked decrease in mucosal erosion, fewer crypt abscesses, and less distortion of the crypt architecture. Study 3 reported a reduction in the overall inflammatory score, along with a trend towards increased crypt depth and a greater number of goblet cells, suggesting enhanced mucosal regeneration and mucin production. These findings, along with the reduction of inflammatory cell infiltration reported in Study 5 and the improved mucosal damage with reduced inflammatory cells reported in Study 6, collectively indicate that beetroot extract protects the colonic tissue from the detrimental effects of bile acid-induced inflammation and may promote mucosal healing. The average improvement of approximately 30% in histological scores across these studies provides further quantitative evidence of the protective effects of beetroot. The reduction in MPO activity, a marker of neutrophil infiltration, observed in three studies, suggests that beetroot extract limits the recruitment of neutrophils to the inflamed colon. Neutrophils, while important for host defense, can also contribute to tissue damage through the release of proteases and reactive oxygen species (ROS). By reducing neutrophil infiltration, beetroot extract may help to mitigate this collateral damage.[11-15]

The mechanisms underlying the anti-inflammatory effects of beetroot extract are likely multifactorial. Betalains, the major bioactive compounds in beetroot, are potent antioxidants and scavengers of ROS and reactive nitrogen species (RNS). Oxidative stress plays a significant role in the pathogenesis of colonic inflammation, and by reducing oxidative stress, betalains can attenuate the inflammatory response. This is supported by the findings of increased levels of SOD and GPx and reduction of MDA levels. Betalains have also been shown to inhibit the activation of NF-κB, a key transcription factor that regulates the expression of pro-inflammatory genes. This inhibition of NF-κB could explain the observed reductions in TNF-α, IL-6, and IL-1β levels. The nitrate content of beetroot is another important factor contributing to its anti-inflammatory effects. Dietary nitrate is converted in vivo to nitrite and then to NO. NO plays a crucial role in maintaining intestinal homeostasis and has demonstrated anti-inflammatory effects in the gut. NO can inhibit leukocyte adhesion, reduce vascular

permeability, and modulate cytokine production. In the context of post-cholecystectomy colonic inflammation, increased NO production from beetroot-derived nitrate may help to counteract the pro-inflammatory effects of bile acids. This is consistent with findings showing improved intestinal permeability and increased expression of tight junction proteins (ZO-1 and Occludin), as seen in Studies 4 and 6. The observed alterations in the gut microbiota composition, reported in two studies, suggest an additional mechanism by which beetroot extract may exert its beneficial effects. The gut microbiota plays a critical role in maintaining intestinal health, and dysbiosis is implicated in various inflammatory bowel diseases. The findings of increased Bifidobacterium and a trend towards greater microbial diversity, as shown in Study 2, are particularly promising. Bifidobacterium species are known to have anti-inflammatory properties and contribute to gut barrier integrity. Furthermore, Study 3 demonstrated an increase in the Bacteroidetes/ Firmicutes ratio, a significant increase in Akkermansia muciniphila, and a decrease in Desulfovibrio, along with an enrichment of pathways related to SCFA production. These changes in the gut microbial community are generally considered beneficial and may contribute to the overall anti-inflammatory effects of beetroot extract. By promoting a more balanced gut microbiota, beetroot extract may indirectly reduce colonic inflammation.[16-20]

5. Conclusion

This systematic review evaluated the existing in vivo evidence for the efficacy of beetroot extract in modulating colonic inflammation following cholecystectomy. The available in vivo evidence, albeit limited, suggests that beetroot extract possesses significant potential for mitigating colonic inflammation following cholecystectomy. The observed anti-inflammatory effects are likely mediated by a combination of betalain-induced antioxidant and anti-inflammatory actions, nitrate-derived nitric oxide signaling, and modulation of the gut microbiota. Further well-designed in vivo studies, and ultimately clinical trials, are warranted to confirm these findings and optimize the therapeutic use of beetroot extract in this context. The anti-inflammatory effects of beetroot extract are likely attributed to its rich composition of betalains, nitrates, and other bioactive compounds. Betalains, particularly betanin and vulgaxanthin I, possess potent antioxidant and anti-inflammatory activities. Beetroot is also a significant dietary source of inorganic nitrate, which can be reduced in vivo to nitrite and subsequently to nitric oxide, a crucial signaling molecule involved in various physiological processes, including vasodilation, neurotransmission, and immune regulation. The findings of this systematic review suggest that beetroot extract may be a promising therapeutic agent for mitigating colonic inflammation following cholecystectomy. However, further well-designed in vivo studies, and ultimately clinical trials, are warranted to confirm these findings and optimize the therapeutic use of beetroot extract in this context.

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