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A systematic review of polyphenols therapeutic and preventive role in cholelithiasis

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ABSTRACT

Introduction: Gallstone disease (GSD) or cholelithiasis has become prevalent in recent years and can increase the risk of gallbladder cancers. This systematic review study investigates the effects of polyphenols in preventing and treating GSD and its associated complications.
Methods: An extensive search of the available literature was performed for articles published since 5/20/2024 using multiple databases, including PubMed, Scopus, Web of Science, Embase, and Cochrane Library, using polyphenols, phenolic compounds, gallstones, and cholelithiasis-related keywords. After screening the records, data were extracted, and the most important outcomes were reviewed.
Results: Polyphenols reduce the levels of ABCG5/8 in the liver and decrease the expression of Niemann-Pick C1-like 1 (NPC1L1) and sterol regulatory element-binding protein 2 (SREBP2) in the intestine. Additionally, the upregulation of the CYP7A1 gene may help regulate various genes, enzymes, and proteins, potentially leading to GSD. They also reduce the cholesterol saturation index (CSI), bile salts, phospholipids, lipid peroxidation, and malondialdehyde (MDA) levels. They also increase glutathione (GSH) levels and alleviate edema in the gallbladder wall. Additionally, polyphenols inhibit the action of 5-Lipoxygenase (a lipid mediator of inflammation), nuclear factor kappa B (NF-κB), peroxisome proliferator-activated receptor-γ (PPAR-γ), and cyclooxygenase-2 (COX-2). These actions contribute to their antioxidant and anti-inflammatory properties in relation to GSD. They also prevented GSD by modulating gut microbiota and improving histopathological changes.
Conclusion: Polyphenols have potentially protective effects against GSD incidence. Further clinical studies are necessary before they can be used in clinical settings.

Implication for health policy/practice/research/medical education:
The findings of this systematic review indicate that polyphenols may play a protective role in both the prevention and management of GSD. These insights have significant implications for health policy, clinical practice, research, and medical education. Policymakers in healthcare should consider incorporating dietary recommendations rich in polyphenols into public health strategies aimed at reducing the prevalence of GSD. Clinicians may also explore dietary counselling or supplements as potential non-invasive interventions.

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Introduction

The incidence of gallstone disease (GSD), also known as cholelithiasis, has significantly increased in developed and developing countries in recent years (1,2). Most patients are asymptomatic, and these stones are often diagnosed incidentally. Gallstones are mainly composed of cholesterol, bile, and bilirubin (3). Patients who do experience biliary colic symptoms typically complain of right upper quadrant abdominal pain after eating fatty or spicy meals; they also have feelings of nausea, vomiting, and discomfort in the upper abdomen (4). GSD can potentially increase the risk of gallbladder and bile duct cancer (5). Studies indicate that age, female gender, use of hormonal contraceptives, genetic predisposition, higher

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body mass index, diabetes, non-alcoholic fatty liver, gallbladder motility impairment, excessive liver cholesterol secretion, and accelerated growth of cholesterol crystals are the most important risk factors on GSD occurrence (2,6). Moreover, patients with GSD are at higher risk of developing diseases such as cardiovascular disease and cancer (7,8).

Various strategies are implemented for the prevention and treatment of GSD. For example, traditional Chinese medicine (TCM) offers a holistic approach to preventing and treating GSD through herbal medicine, acupuncture, dietary recommendations, and lifestyle adjustments (9,10). Cholecystectomy (gallbladder removal) is a common and highly effective treatment, especially for symptomatic GSD (11). Polyphenols may help prevent GSD through their potent antioxidant properties. They help reduce oxidative stress and inflammation in the liver and gallbladder, thereby protecting against cellular damage that can lead to the formation of gallstones. Additionally, polyphenols regulate cholesterol metabolism by decreasing its absorption in the intestine and modulating its synthesis in the liver, ultimately lowering the risk of cholesterol-based stones (5,12,13).

Numerous studies have shown that medicinal plants and their derivatives (such as polyphenols and alkaloids) positively affect various diseases, including gastrointestinal disorders (14-20). Polyphenols as natural compounds in plants, are known for their antioxidant properties, and play a significant role in health and wellness, with potential benefits for managing GSD (21). To the best of our knowledge, there has been a review study on the effects of polyphenols in preventing and treating GSD. Only one study investigated their effect on cholesterol metabolism through bile acid biosynthesis, which showed satisfactory results (13). Due to the limitations of the studies conducted in this field, the present study is a comprehensive review of the effects of polyphenols in preventing and treating GSD.

Materials and Methods

Search strategy

For the electronic search, a comprehensive systematic review was undertaken for articles published since May 20, 2024, and based on the study objective, all relevant studies before this date were reviewed. Multiple databases were used in this systematic review to ensure broad coverage, including PubMed/MEDLINE, Embase, Web of Science, Cochrane Library, and Scopus. Finally, Google Scholar was checked to enhance search accuracy. These databases were selected to maximize relevant study retrieval, while search terms included both general and specific polyphenol and gallstone-related keywords. This approach aimed to reduce selection bias by ensuring that studies were retrieved without database or keyword limitations.

The following main and Medical Subject Headings (MeSH) keywords were used to search: (("polyphenols" OR "phenolic compound" OR "provinols" OR "resveratrol" OR "silymarin" OR "curcumin" OR "quercetin" OR "rutin" OR "genistein" OR "daidzein" OR "fisetin" OR "baicalein" OR "luteolin" OR "catechins" OR "epigallocatechin-3-gallate" OR "ellagic acid" OR "hesperidin" OR "chrysin") AND ("gallstone" OR "gallstone disease" OR "cholelithiasis" OR "cholesterol gallstones" OR "pigment gallstones")). We also reviewed the reference lists of relevant articles and previous systematic reviews to supplement our searches. We refined our search iteratively until all relevant publications were included. The peer-reviewed publications were imported into EndNote X8 (8 November 2016, Thomson Reuters) to identify and remove duplicate publications.

Selection criteria

According to our inclusion criteria, all experimental and clinical studies that directly investigated the impact of various polyphenols on the prevention and treatment of GSD (cholesterol GSD, pigment GSD, or mixed) were considered for this systematic review. The PICO (Patient/Population, Intervention, Comparison, and Outcome) elements were defined as follows:

- Population: Patients/animals/cells affected by GSD
- Intervention: Administration of polyphenols
- Comparison: Other treatment and no intervention
- Outcome: Prevention and attention to the GSD and its complications.

Our exclusion criteria were composed of pregnant women or patients with malignant tumors for clinical studies, systematic reviews, meta-analyses, case reports, reviews, studies without original data, abstract-only publications, conference poster publications, unpublished study protocols, studies published in languages other than English, and letters to editors.

Screening process

Two researchers screened the titles and abstracts of the publications following specific inclusion and exclusion criteria. They identified potential studies that met the inclusion criteria and obtained full-text publications. The same researchers then individually assessed the full-text articles to determine their eligibility. If disagreements arose during the review process, they were resolved through discussion with a third team member.

Data extraction

In this review, a standardized form was used to gather essential information. This included details such as study characteristics (author, publication year), design, subject details (patients/animals/cells), intervention specifics (type, dosage, duration), outcomes, proposed mechanisms, and safety considerations.

Quality assessment

To assess the methodological quality of the included

studies, we used SYRCLE's risk of bias (RoB) tool, which was designed for animal studies. The evaluation focused on key domains: selection bias (random sequence generation, baseline characteristics), performance bias (allocation concealment, random housing), detection bias (blinding of caregivers and investigators, random outcome assessment), attrition bias (incomplete data), reporting bias (selective outcome reporting), and other biases (funding sources and conflicts of interest). A "yes" indicates a low risk of bias, "no" indicates a high risk, and "unclear" denotes insufficient information for evaluation (22).

Narrative synthesis

The data synthesis process involved compiling a narrative summary of the results from the included studies. Variations in study design, outcomes, and methodologies were carefully considered to prevent selective reporting that could distort conclusions.

Reporting guidelines

We adhered to the 2020 Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines to ensure comprehensive, transparent, and accurate reporting of our systematic review process. Moreover, we included a PRISMA flow diagram in the final report to illustrate the number of studies identified, screened, and included or excluded at each stage, enhancing clarity and transparency.

Results

Search results

The PRISMA flowchart depicting the search strategy is shown in Figure 1. The initial electronic search included 1308 titles/abstracts. Of these, 238 articles were excluded because of duplicate titles. Additionally, six titles/abstracts were excluded for not containing the desired data (23-28), non-English languages (29), and the full text was not available (30,31). After the final screening, 13 studies were included in this study and analyzed (5,32-43).

Description of the included studies

Most of the studies that examined the effects of polyphenols on GSD were conducted *in vivo* and *in vitro*. Two studies had a clinical trial design (32,38). The included studies indicated that polyphenols had promising effects on GSD prevention and treatment through various mechanisms (Table 1).

Bias assessment results

The potential for bias was evaluated and classified as low, high, or unclear according to established criteria. We

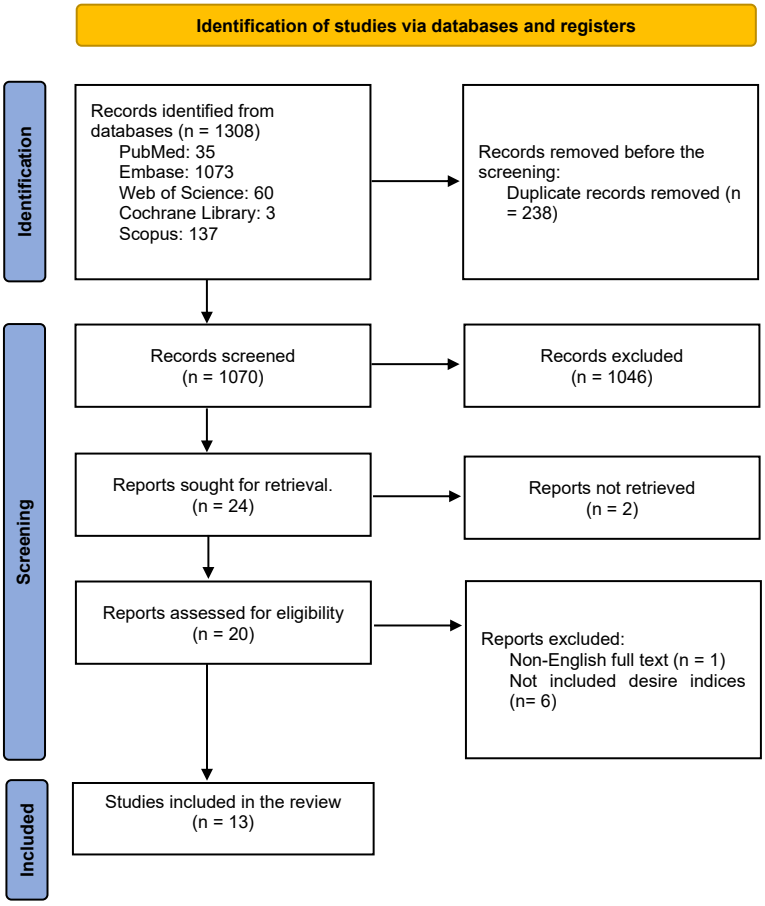

Figure 1. PRISMA flowchart for including studies in the systematic review.

Table 1. Characteristics of included studies in this systematic review

Lead author	Type of polyphenols	Study design	Studied subjects (Patients/ Animals/cells)	Experimental approach (Dosage/Duration)	Main outcomes
Nassuato (32)	Silibinin	Clinical, *in vivo,* and *in vitro*	Sprague-Dawley rats and cholecystectomies patients	50-100 mg/kg body weight i.p. (7 days) for rats and 420 mg per day for 30 days administered for GSD and cholecystectomy patients	Total liver cholesterol content indicated no significant changes. ↓ Biliary lipids and the bile saturation index.
Hussain (a) (33)	Curcumin	*In vivo*	Albino Swiss strain mice and hamsters	Feeding with 0.5% curcumin or capsaicin 5 mg/100g for 6 weeks	⊥ The occurrence of cholesterol GSD ↓ The concentrations of biliary cholesterol and cholesterol/ phospholipid ratios.
Hussain (b) (34)	Curcumin	*In vivo*	Weanling male Swiss strain mice	Feeding with 0.5% curcumin or 5 mg% capsaicin for 5 or 10-week	⊥ The incidence of cholesterol GSD ↓ The biliary cholesterol and phospholipids.
Panjehshahin (35)	Curcumin	*In vivo*	Female rats	0.5% diet curcumin extract for 10 weeks	↓ Cholesterol concentration (Inhibited cholesterol crystals and aggregation of microcrystals.
Shan (36)	Epigallocatechin-3-gallate	*In vivo*	C57BL/6 mice	40 mg kg^{-1} d^{-1} and 80 mg kg^{-1} d^{-1}, i.g for 6-weeks	⊥ The expression of NF-kB expression and the PPARγ ↑ The CYP7A1 gene ↓ The serum TC, VLDL, and TG
Shubha (37)	Curcumin	*In vivo*	Male albino mice	0.015% capsaicin/0.2% curcumin/0.015% capsaicin + 0.2% curcumin. for ten weeks	↓ Biliary cholesterol ↑ Phospholipid and lipid peroxidation. ↓ The activities of hepatic glutathione reductase, glutathione-S-transferase, and ascorbic acid.
Marciani (38)	Curcumin	Clinical trial	Male or female	80 mg curcumin with other different food ingredients and assessed after 65 min	↓ Gall bladder volume ↑ Gallbladder emptying
Li (a) (39)	Curcumin	*In vivo*	C57BL6 mice	Curcumin at 500 or 1000 mg/kg and/or piperine at 20 mg/kg administered for four weeks	⊥ The formation of gallstones ↓ The expression of NPC1L1 and SREBP2 at both mRNA and protein levels and blood lipid and bile cholesterol levels.
Ren (40)	Glansreginin A, ellagic acid, gallic acid, methyl gallate, and ethyl gallate	*In vivo*	C57BL/6 mice	0.052 mg/mL of glansreginin A, 0.050 mg/mL of ellagic acid, 0.042 mg/mL of ethyl gallate, 0.027 mg/mL of methyl gallate, and 0.030 mg/mL of gallic acid	↓ ABCG5/8 in the liver and NPC1L1 levels in the intestine.
Li (b) (41)	Curcumin	*In vitro*	Human colorectal adenocarcinoma cell line	5-50 µM for 24-48 h	↓ The levels of mSREBP-2 by directly blocking the production of S1P mRNA and protein.
Peker (42)	Ellagic acid	*In vivo*	Adult female rats	100 mg/kg/d for ten days	⊥ Intra-abdominal adhesion formation caused by GSD in rats
Hong (5)	Curcumin	*In vivo* and *in vitro*	Male Syrian golden hamsters and Human colorectal adenocarcinoma cell line	0.1% w/w for 12 weeks in hamsters and 12 µmol/L in cells for 24 h	Modulating gut microbiota, short-chain fatty acid production. ↓ The expression of intestinal NPC1L1 in hamsters and cholesterol absorption in cancerous cells.
Fang (43)	Glansreginin A and gallic acid	*In vivo* and *in vitro*	Sprague-Dawley rats and gallbladder tissue	1 mM of glansreginin A and gallic acid were added into gastric and intestinal juice and incubated for 45 min	↓ The occurrence of GSD, the levels of unconjugated bilirubin, calcium, inflammation, edema, congestion of gallbladder tissue, and infiltration of inflammatory cells.

↑: Increase; ↓: Reduce; ⊥: Inhibit; NF-κB; Nuclear factor kappa B; PPARγ: Proliferator-activated receptor gamma; TG; Triglyceride, TC: Total cholesterol; VLDL; Very low-density lipoprotein; NPC1L1: Niemann-Pick C1-Like 1; COX-2: Cyclooxygenase-2; SREBP-2: Sterol regulatory element-binding protein 2; TRPV1: Transient receptor potential vanilloid 1; CYP7A1: Cytochrome P450 family 7 subfamily A member 1; GSD: Gallstone disease.

assessed the quality of the animal studies by employing SYRCLE's risk of bias tool. The results were displayed using a color-coded system: green cells represent a low risk of bias, red cells indicate a high risk of bias, and yellow cells reflect insufficient information or an unclear risk of bias (Table 2).

Discussion

This systematic review aimed to investigate the biological effects of polyphenols on the prevention and treatment of GSD. Table 1 shows polyphenols play a crucial role in preventing and treating GSD through various mechanisms that reduce cholesterol saturation and enhance bile composition. Additionally, polyphenols lower biliary cholesterol concentrations and improve the cholesterol/phospholipid ratio, contributing to the reduction of cholesterol crystallization and microcrystal aggregation, critical in gallstone development.

Polyphenols provide a multifaceted approach to managing GSD through their intricate interactions within cholesterol metabolism, the modulation of inflammatory processes, and the regulation of gut microbiota. These biological actions address the formation of gallstones and may mitigate complications associated with inflammation, presenting a holistic and non-invasive therapeutic strategy.

In this systematic review, in each section, we first discuss the pathophysiology of GSD and then explain the mechanisms of polyphenols on GSD as follows. Polyphenols are known for their antioxidant properties and potential health benefits, including antioxidant and anti-inflammatory, antihypertensive, antimicrobial, and immunomodulatory effects (44,45). The most important mechanisms of polyphenols in preventing the occurrence of GSD are as follows:

Regulation of gene expression, enzyme and protein

Regulation of the CYP7A1 gene

Polyphenols may affect the activity of genes involved in bile acid production, cholesterol transport, and lipid metabolism, offering a comprehensive approach to preventing the formation of gallstones. Cholesterol is transported to the liver via high-density lipoprotein (HDL) particles and converted into bile acids. It suggests polyphenols may increase bile acid excretion and reduce total and LDL cholesterol. Three primary mechanisms for enhancing bile acid excretion are proposed: Firstly, upregulating the expression of the enzyme CYP7A1. The CYP7A1 gene encodes cholesterol 7α-hydroxylase, an enzyme critical for synthesizing bile acids from cholesterol. Secondly, it downregulated the expression of intestinal bile acid transporters and finally influenced the gut microbiota (13). Shan et al. and Pi et al. in their study reported that EGCG (40 mg kg^{-1} d^{-1} and 80 mg kg^{-1} d^{-1}, i.g, for 6 weeks) and capsaicin (Diet containing 0.015% capsaicin for 8 weeks) upregulated in mice the expression of the CYP7A1 gene (36,46). Polyphenols have been found to primarily stimulate the expression of the CYP7A1 gene through the LXRα pathway in human cells. This process involves signaling through FXR, NF-KB, and ERK (13).

Modulating ABCG5/8

The proteins encoded by the ABCG5 and ABCG8 genes control the release of cholesterol into bile and eliminate sterols in the biliary tree. Changes in these genes, such as mutations or polymorphisms, can result in higher cholesterol levels in bile, which can encourage the creation of gallstones (47). Various genetic factors affect the formation of cholesterol and bilirubin gallstones. Genetic variations in specific proteins such as ABCG8,

Table 2. The SYRCLE's risk of bias tool for animal studies

Study	A	B	C	D	E	F	G	H	I	J	Score
Nassuato (32)	High	Low	Unclear	Unclear	High	High	High	Low	Low	High	3
Hussain (a) (33)	High	Low	Unclear	Unclear	High	High	High	Low	Low	High	3
Hussain (b) (34)	Low	Low	Unclear	Unclear	High	Low	Low	Low	Low	High	6
Panjehshahin (35)	High	Low	Unclear	Unclear	High	High	Unclear	Low	Low	High	3
Shan (36)	Low	Low	Unclear	Unclear	High	High	Unclear	Low	Low	High	4
Shubha (37)	High	Low	Unclear	Unclear	Unclear	High	Low	Low	Low	High	4
Li (a) (39)	High	Low	Unclear	Unclear	High	High	High	Low	Low	Low	4
Ren (40)	Low	Low	Unclear	Unclear	High	Unclear	High	Low	Low	Unclear	4
Peker (42)	High	Low	Unclear	Unclear	Low	High	Low	Low	Low	Unclear	5
Hong (5)	Low	Low	Unclear	Unclear	High	Unclear	High	Low	Low	Low	5
Fang (43)	Low	Low	Unclear	Unclear	High	Unclear	High	Low	Low	Unclear	4

Low risk, High risk, Unclear. A: Sequence generation; B: Baseline characteristics; C: Allocation concealment; D: Random housing; E: Blinding of caregivers/ investigators; F: Random outcome assessment; G: Blinding of outcome assessor; H: Incomplete outcome data; I: Selective outcome reporting; J: Other bias. A scale from 1 to 10 was used to evaluate the quality of the studies, with higher scores reflecting better methodological quality in the articles. The analysis of the risk of bias in the studies revealed that 32% were categorized as low risk, 42% as high risk, and 26% as unclear.

ABCG5, ABCB4, and ABCB11, as well as genes from the apolipoprotein family like ApoB100 and ApoE, and members of the MUC family, play a significant role in influencing the formation of cholesterol gallstones. However, the formation of bilirubin gallstones is associated with genetic variations in heavily expressed hepatic genes, particularly UGT1A1, ABCC2 (MRP2), ABCC3 (MRP3), CFTR, and MUC, in addition to genetic abnormalities related to hemolytic anemias and disorders affecting erythropoiesis (48). Ren et al in their study indicated that glansreginin A, ellagic acid, gallic acid, methyl gallate, and ethyl gallate reduced the levels of ABCG5/8 in the liver and NPC1L1 in the intestine in C57BL/6 mice (40).

Downregulated the expression of NPC1L1 and SREBP2

NPC1L1 is a transmembrane protein critical in cholesterol absorption within the intestine. It is primarily situated in the small intestine's enterocytes and the liver's hepatocytes. NPC1L1 facilitates cholesterol intake from the intestinal lumen into enterocytes. It is also involved in the liver's reuptake of cholesterol from bile. Inhibition of NPC1L1 reduces cholesterol absorption, resulting in decreased plasma cholesterol levels (49). High cholesterol absorption via NPC1L1 can lead to increased cholesterol levels in bile, promoting the formation of cholesterol gallstones (50). Moreover, SREBP2, a transcription factor, is critical in regulating gene expression related to cholesterol biosynthesis and uptake. It is activated in response to low intracellular cholesterol levels. It increases the expression of genes involved in cholesterol synthesis, including HMG-CoA reductase and uptake of LDL in receptors (51). Elevated SREBP2 activity can lead to heightened cholesterol levels in the liver and bile, contributing to the supersaturation of bile with cholesterol and ultimately leading to the formation of gallstones (52). Several included studies indicated that various polyphenols, including curcumin, glansreginin A, ellagic acid, gallic acid, methyl gallate, and ethyl gallate, can reduce the expression of intestinal NPC1L1 and SREBP2 at both mRNA and protein levels. They are crucial regulators of cholesterol absorption and synthesis and regulators of cholesterol metabolism and homeostasis (5,39,40).

Modulation of cholesterol metabolism, solubilization, and excretion

Bile lipids comprise a combination of bile salts, phospholipids, cholesterol that is not esterified, and bilirubin conjugates. The primary problem in cholesterol GSD arises from the overproduction of hepatic cholesterol in bile, sometimes accompanied by reduced production of bile salts and phospholipids (53). Other factors contributing to GSD pathogenicity include reduced movement, inflammation mediated by the immune system, hepatic hypersecretion of cholesterol, supersaturated bile, and excessive mucin production. Additionally, reduced movement in the intestines leads to increased synthesis of "secondary" bile salts by anaerobic microflora (53,54). The majority of GSD diagnosed in Western nations consists of cholesterol. This occurrence is frequently due to imbalanced cholesterol levels in the body. GSDs are hardened deposits found in the gallbladder or bile ducts. They comprise cholesterol monohydrate crystals, calcium bilirubinate, mucin, and protein aggregates, forming solid conglomerates within the biliary tree (55). Furthermore, the development of GSD is associated with high levels of cholesterol in the bile, which can be evaluated by calculating the CSI. It is typical to assess this using a CSI derived from the mole percent (mol%) values of the three primary lipids in gallbladder bile and the proportion of bile acids, cholesterol, and phospholipids (56). In individuals with GSD, the increased CSI in the bile in the gallbladder is mainly due to reduced bile acids, and the lack of bile acids is the most important cause of bile supersaturation (57). Polyphenols can impact the composition of bile by reducing the CSI. This helps to maintain cholesterol dissolved in bile, preventing the formation of gallstones by crystallization (13). Nassuato et al, in their study, revealed that biliary lipid was decreased after silibinin administration, and the bile saturation index attenuated (32). Polyphenols may impact lipid metabolism by reducing serum cholesterol levels and aiding in cholesterol excretion. For example, curcumin has been shown to regulate enzymes involved in cholesterol synthesis and uptake (5). In addition, by preventing lipid peroxidation, polyphenols can reduce the formation of cholesterol monohydrate crystals, a key step in gallstone formation (37). Polyphenols can potentially boost the removal of bile acids and lower overall and LDL cholesterol levels in animals and laboratory studies. Nonetheless, the precise mechanism behind this process has yet to be extensively examined. Three main ways to enhance the excretion of bile acids are increasing the production of Cholesterol 7 Alpha-Hydroxylase (CYP7A1), decreasing intestinal bile acid transporters' expression, and modifying the gut microbiota composition (13).

Polyphenols antioxidant properties

The total antioxidant capacity in the diet was found to have a negative correlation with the occurrence of GSD (58). The findings highlight the advantages of consuming anti-inflammatory diets high in antioxidants, which could lower the occurrence of gallstones in American adults (59). On the other hand, the pathophysiology of GSD could be related to increased free radicals and oxidative stress, resulting from higher quantities of lipid and protein oxidation products and lower levels of antioxidant enzymes and vitamins (60). Incorporating a substantial intake of dietary antioxidants from plant-based sources as a constituent of a comprehensive and balanced diet may provide health benefits by mitigating oxidative damage within the body (58,61). Antioxidant defense mechanisms exhibit a decreased presence in individuals

with gallbladder disease, characterized by an aggravation of pathological conditions in the gallbladder wall and circulation (62). For example, studies have shown that vitamin C deficiency and α-tocopherol, a well-known antioxidant micronutrient, can increase the risk of GSD development. This is because they disrupt the production of free radicals involved in forming gallstones, impacting bile's protein and lipid content. Moreover, the over-secretion of mucin, a glycoprotein by epithelial cells in the gallbladder stimulated by oxygen radicals, has been linked to the destabilization of cholesterol and the creation of gallstones (60,63,64). Patients who have been diagnosed with asymptomatic cholelithiasis display increased levels of lipid peroxidation and decreased antioxidant capacity. Additionally, individuals with asymptomatic cholelithiasis and signs of metabolic syndrome show even higher lipid peroxidation levels and lower antioxidant capacity than those with just asymptomatic cholelithiasis (65). The result of lipid peroxidation was significantly higher in subjects with cholelithiasis compared with healthy controls, while the antioxidant enzyme paraoxonase-1 (PON1) activity was lower. Among cholelithiasis patients, higher malondialdehyde (MDA) levels were associated with fasting blood glucose, triglyceride, and total cholesterol and negatively associated with HDL-C and PON-1 activity. These correlations were not observed in the control group. Abnormal changes in lipid parameters leading to oxidation may contribute to decreased PON-1 activity (66).

The combined use of dietary curcumin and capsaicin did not show an additional effect in reducing the occurrence of cholesterol gallstones in mice. However, it enhanced the liver's antioxidant enzyme glutathione reductase activity more effectively in the lithogenic condition. This emphasizes the potential of these spice compounds to preserve liver health (37). Polyphenols with anti-inflammatory and antioxidant properties can decrease adhesion formation (42).

Polyphenols' anti-inflammatory properties

Inflammation has a significant role in the progression of GSD, and it could be a consequence of GSD. It influences the formation of gallstones, the motility of the gallbladder, and the progression to acute or chronic conditions (67). The mean of high-sensitivity C-reactive protein (hs-CRP) serum levels and dietary inflammatory index are associated with GSD incidence in women (68). A study revealed a significant increase in the lower two quartiles of the Dietary Inflammatory Index, which signifies adherence to an anti-inflammatory diet, in subjects following the GSD (Generic Statistical Diet) compared to those in the higher quartiles, which indicates a proinflammatory dietary pattern (69).

Inflammation induces alterations in calcium sensitization within arterial smooth muscle cells by desensitizing calcium stores and disrupting the functionality of calcium channels in the cell membrane. Furthermore, inflammation reduces the production of contractile proteins, such as F-actin, in smooth muscle cells, which may account for the observed decrease in excitation-contraction coupling sensitivity (70). Hydrophobic bile salts can exacerbate inflammatory processes by diffusing through the mucosa and influencing the generation of reactive oxygen species (ROS) by gallbladder smooth muscle. This phenomenon may occur through direct effects on smooth muscle cells or by augmenting the presence of inflammatory cells in the gallbladder wall (71). Gallbladder inflammation (cholecystitis) can occur due to GSD blocking the bile duct. Fang et al, in their study, administered 1 mM of glansreginin A, and gallic acid was added into gastric and intestinal juice and incubated for 45 min. They found a reduction in inflammation, edema, and congestion of gallbladder tissue and a decrease in the infiltration of inflammatory cells (43).

A research study examined the essential oils from *N. sativa* seeds and *S. aromaticum* buds, explicitly focusing on eugenol and *p*-cymene, targeting the 5-lipoxygenase (5-LOX) protein. This protein plays a crucial role as an enzymatic catalyst in transforming arachidonic acid into leukotrienes known for their involvement in inflammation. The study observed that Zileuton binds to the active site of the 5-LOX enzyme, indicating its potential to competitively inhibit leukotriene synthesis by acting against arachidonic acid (12). Treatment of mice liver tissues with EGCG (40 mg kg^{-1} d^{-1} and 80 mg kg^{-1} d^{-1}, i.g, for 6 weeks) inhibited the protein expression of NF-κB in the nucleus. They enhanced the protein expression of peroxisome proliferator-activated receptor-γ (PPAR-γ). These transduction pathways may be involved in mediating cholesterol metabolism (36). So, these inhibitions can reduce the inflammatory gallstone tissue that increases gallstone formation.

Modulating gut microbiota by polyphenols

The gut microbiota has an essential impact on the main chemical processes responsible for creating cholesterol in the liver, generating secondary bile acids, and circulating bile acids between the intestines and the liver. Maintaining a proper equilibrium between cholesterol and bile acids is essential for preventing GSD (72). The gut microbiota may contribute to cholelithogenesis through several mechanisms. The gut microbiota, rich in Desulfovibrionales, can increase the presence of 7α-dehydroxylation bacteria, which convert primary bile acids to secondary bile acids. This process regulates the FXR-CYP7A1 pathway, leading to an increase in taurodeoxycholic acid and a decrease in tauro-β-muricholic acid (73). Moreover, lipopolysaccharides can upregulate mucins via transarterial chemoembolization/transforming growth factor-α/epidermal growth factor receptor pathway and E-prostanoid receptor 4/p38 mitogen-activated protein kinases pathway. Gram-

positive bacteria can increase mucin 4 production, which can impact calcification. Some bacteria also generate exogenous GUS, which results in the breakdown of bilirubin diglucuronides and speeds up the formation of calcium bilirubinate. *Helicobacter* spp. can also play a role in the precipitation of calcium through urease activity (73).

Polyphenols undergo metabolism by the gut microbiota, which impacts the composition and function of the microbiome. Additionally, polyphenols have the potential to stimulate the proliferation of beneficial bacteria, such as *Bifidobacterium* and *Lactobacillus*, while restraining the growth of pathogenic bacteria (74). Gut microbiota analysis in Hong et al. study revealed that curcumin (0.1% w/w for 12 weeks in hamsters and 12 μmol/L in cells for 24 hours) can change the gut microbiota, mainly increasing bacteria related to bile acid metabolism and the production of short-chain fatty acids. This subsequently leads to an increase in the expression of hepatic cholesterol 7-alpha hydroxylase and the synthesis of bile acids (5). The Defatted walnut powder extract with its main compound (ellagic acid and glansreginin A) may produce pharmacologically active compounds due to metabolism by intestinal bacteria, which could impact pigment gallstones (43). So, elevated intake of dietary polyphenols is linked to increased diversity in the gut microbiome, which is advantageous for overall gut health.

Improved histopathological changes

It is important to note that GSD induces a wide range of histopathological changes in the gallbladder, including cholesterolosis, acute and chronic inflammation, metaplasia, dysplasia, and gallbladder neoplasms. Inflammation in the mucosa of the gallbladder is typically caused by cholelithiasis, and it is linked to a range of histopathological changes that may precede hyperplasia, metaplasia, and finally, cancer (75,76). The formation of gallstones can manifest as single or multiple entities. Single stones are predominantly comprised of cholesterol and typically exhibit a smooth, yellow-white appearance, sometimes growing to sizes of up to 4 cm (75). Also, GSD is linked to significant changes in the liver's histopathology, and these modifications are more common in people who have experienced symptoms for an extended period (77). Li et al, in a study, revealed that the administration of curcumin at 500 or 1000 mg/kg and/or piperine at 20 mg/kg administered for four weeks could reduce the growth of the mucous membrane and expansion of the connective tissue in the lamina propria, along with a notable reduction in small blood vessels and collagen fibers in gallbladder tissue (39).

Polyphenols antimicrobial properties

The formation of pigment gallstones is significantly linked to bacterial infections in the biliary tract (78). Bacterial enzymes such as beta-glucuronidase alter bile composition, precipitating free bilirubin and calcium bilirubinate. Biliary stasis from conditions like gallstones or reduced gallbladder motility creates an environment for bacterial growth, chronic infection, and inflammation, promoting stone formation. Chronic bacterial infections also lead to inflammation, mucus production, and increased bilirubin load, all of which contribute to the formation of pigment gallstones and mixed stones containing cholesterol, bilirubin, and calcium salts (79).

Moreover, bacteria can create biofilms on the surfaces of the gallbladder and biliary ducts. These biofilms protect the bacteria, making them more resistant to bile flow and immune responses. Furthermore, biofilms can capture bilirubin and other particulate matter, which helps in the formation and growth of stones (80). So, anaerobic bacterial infection (e.g., *Streptococcus faecalis, Clostridium* spp., *Bacteroides* spp.) leads to secondary obstruction and/or stasis (81). The formation of "black" pigment gallstones in the gallbladder is due to excessive bilirubin, which can result from conditions such as hemolysis, ineffective erythropoiesis, and induced enterohepatic circulation of unconjugated bilirubin (UCB). The primary source of free UCB in gallbladder bile is the breakdown of bilirubin conjugates by an enzyme called biliary β-glucuronidase. In the presence of bile salts and Ca^{2+}, UCB forms insoluble salts that precipitate from the bile, forming "black" pigment stones (82).

On the other hand, "brown" stones are a result of bile phase separation and can form in any part of the biliary tree due to chronic stasis and anaerobic bacterial infection. Anaerobic bacteria secrete enzymes that break down biliary lipids and lip pigments into insoluble compounds, leading to the formation of "brown" stones, which can deposit on various obstructions in the biliary system. So, bacterial infections can contribute to pigment gallstone formation (83,84).

Polyphenols have demonstrated antibacterial effects against a broad spectrum of bacteria, including gram-positive and gram-negative bacteria and fungi. The majority of the active polyphenols consist of flavonoids or hydrolyzable tannins. Hydrolyzable tannins include gallotannins and ellagitannins, the latter being the most prevalent in the extracts tested for their antimicrobial potential against clinical samples (85). Moreover, polyphenols can have a synergistic effect when combined with antibiotics and antifungals, offering a promising alternative for combating antibiotic resistance through therapeutic strategies (85). For example, some alkaloids can insert antibiotic effects against *Helicobacter pylori*, *Vibrio cholera*, *Staphylococcus aureus*, and *Escherichia coli* by affecting bacterial cell division, hindering respiration, and inhibiting bacterial enzymes, as well as disrupting bacterial membrane and influencing virulence genes (86). Moreover, most of the polyphenols studied demonstrated the capability to impede bacterial growth and the formation of biofilms. Rutin exhibited the most

encouraging activities in this regard. Further exploration validated rutin's effectiveness in preventing or eliminating *Pseudomonas aeruginosa* and methicillin-resistant *Staphylococcus aureus* urinary catheter biofilms (87). In addition to reducing biofilm biomass, rutin's anti-biofilm mechanisms decreased cell viability, exopolysaccharide, and extracellular DNA levels. Moreover, rutin treatment resulted in a moderate decrease in bacterial adhesion to human keratinocytes. Rutin's antivirulence mechanisms encompassed influencing *P. aeruginosa* protease, pyocyanin, rhamnolipid, and elastase formation and the suppression of the virulence genes (87). Overall, Figure 2 illustrates the main effect of dietary polyphenols in preventing and treating GSD and polyphenol-associated complications.

Complications of polyphenols on gallbladder

The contractile response of smooth muscle in the gallbladder is a critical factor in forming gallstones (88). When the contractile function of the gallbladder's smooth muscle is impaired, it can lead to several issues. These include incomplete emptying, which results in the gallbladder not emptying, leading to residual bile remaining in the gallbladder. Additionally, prolonged retention of bile can lead to increased concentration and supersaturation of bile components, such as cholesterol and bilirubin, known as bile stasis (89,90). The motility of the gallbladder encompasses the complex processes of bile storage, concentration, and delivery. Various factors, including innervation, humoral factors, and neuropeptides, govern the control of gallbladder motor functions. Effective gallbladder emptying relies on the orchestrated contractions of the muscular layers of the gallbladder wall, which are influenced by a diverse array of cell types, including smooth muscle cells, gallbladder neurons, telocytes, and interstitial cells of Cajal (91). The gallbladder motor unit, the SMC-TC-ICC-neuron syncytium, is critical in providing pacemaker activity, establishing propagation pathways for slow waves, transducing inputs from neurons, and exhibiting mechanosensitivity (91). Maintaining proper gallbladder motility is essential in preventing GSD. When motility is impaired, bile supersaturation can occur, leading to the formation of gallstones (54). Moreover, Cholecystokinin (CCK) is a hormone synthesized in the duodenum and jejunum in response to ingesting protein and fat. Its primary role is to modulate various digestive functions, including gallbladder contraction, pancreatic secretion, small intestinal transit, and stomach emptying (92). The CCK-1 receptor (CCK-1R) is responsible for regulating the effects of CCK, and studies in mice have shown that disruption of either the CCK gene or the CCK-1R gene can lead to an increased susceptibility to cholesterol gallstone formation. Notably, anomalies in gallbladder

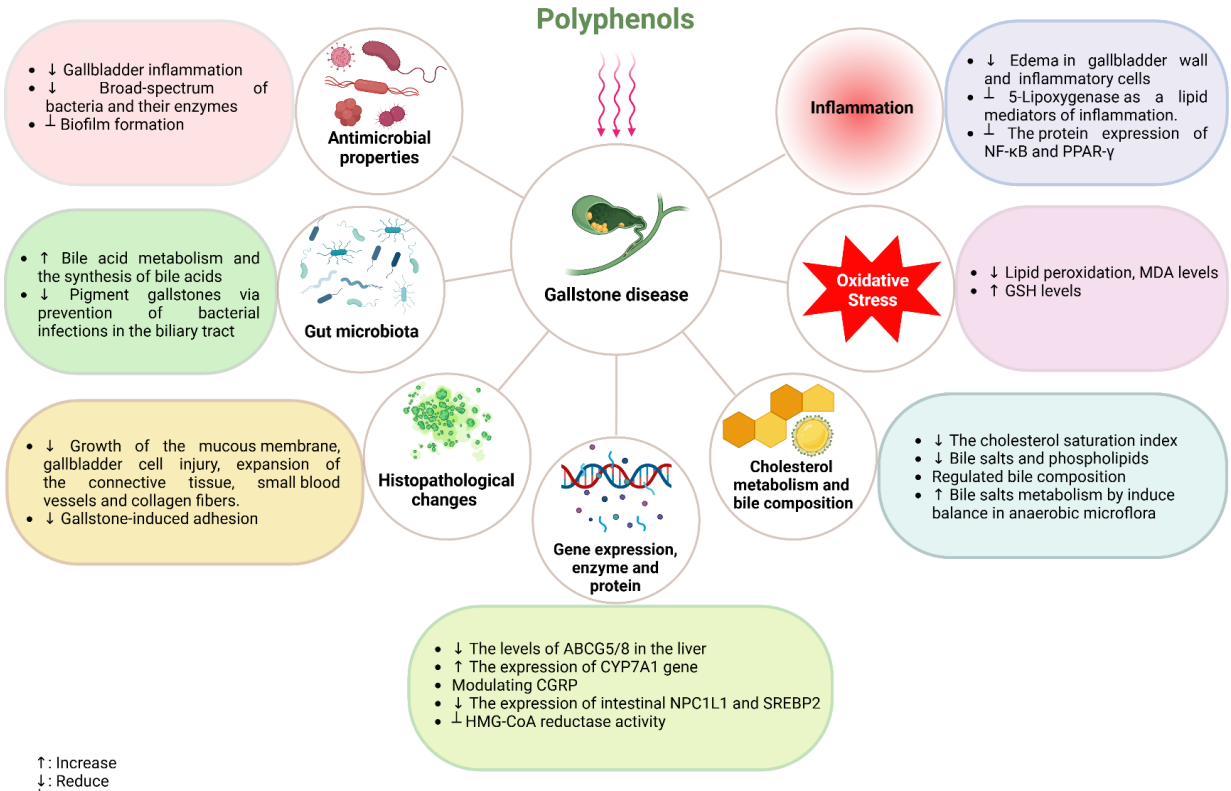

Figure 2. The main biological effects of polyphenols and complications on gallstone disease.

motility in response to CCK are predominantly observed in individuals with cholesterol gallstones (92). So, reduced motility causes biliary stasis, which contributes to the development of cholesterol and pigment stones.

Furthermore, prolonged stasis can result in the formation of biliary sludge, a precursor to gallstones. Inflammation of the gallbladder wall may result from poor motility, further compounding motility issues and increasing the risk of gallstone formation (54,93). However, genistein revealed the relaxation of isolated smooth muscle from the guinea pig gallbladder, which could play a role in the development of GSD. The suppressive effect may be associated with H2 receptors and the discharge of intracellular Ca^{2+} from the sarcoplasmic reticulum (94). Another study also reported that genistein and resveratrol have been discovered to actively inhibit isolated gallbladder muscle's contractile function, whether at rest or when stimulated. These inhibiting impacts are probably a result of the inhibition of tyrosine kinase, the entry of Ca^{2+} through voltage-dependent calcium channels, and the release of Ca^{2+} from the sarcoplasmic reticulum (26). So, polyphenols play a crucial role in relaxing smooth muscle contraction, and this issue can increase the risk of the possibility of GSD in cases where genistein is used instead of hormone therapy in postmenopausal women.

Limitation of the study

It is crucial to acknowledge that most existing research on this subject has relied on preclinical designs involving animal models or in vitro experiments. To effectively apply these findings to clinical practice, conducting well-designed controlled clinical trials involving human subjects is crucial to confirm the findings and establish clinical settings. Moreover, the design, methodology, and patient populations involved in the existing studies are considerably variable. This diversity can complicate drawing consistent conclusions and may restrict the generalizability of the findings. Standardized dosages and treatment durations are crucial for achieving more dependable results.

It is important to highlight substantial variability in the doses of different polyphenols administered and the duration of interventions across the studies, making it difficult to establish optimal therapeutic regimens. Additionally, the relatively low bioavailability and variable absorption and metabolism of polyphenols among individuals may affect the compound's effectiveness in achieving therapeutic concentrations. As a result, further research on these aspects is necessary.

Conclusion

In experimental studies, polyphenols showed promising effects on preventing and attenuating GSD complications through regulation of gene expression, enzyme, and protein, modulation of cholesterol metabolism, reduction of oxidative stress and inflammation, modulation of gut microbiota, and improvement of histopathological changes. However, they cause the relaxation of gallbladder smooth muscles and reduce motility, which are one of the risk factors for GSD occurrence. Future research on polyphenols in GSD should focus on conducting well-designed clinical trials in humans to evaluate their efficacy, safety, and optimal dosages.

Authors' contribution

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Conflict of interests

The authors declared no conflict of interest, financial or otherwise.

Ethical considerations

Nil.

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