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ORIGINAL RESEARCH

Anti-Adhesion Effect of Intraperitoneal Quercetin Associated with Caspase-3 Concentration in a Rat Model of Postoperative Peritoneal Adhesion

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ABSTRACT

Introduction: Postoperative peritoneal adhesion is a common complication that can lead to severe morbidity, including chronic pain and intestinal obstruction. Adhesion formation involves an imbalance between proliferation and apoptosis of mesothelial cells, with suppression of caspase-3 activity playing a key role. Quercetin has been suggested to possess anti-adhesion potential through caspase-3 activation; however, experimental evidence supporting this mechanism remains limited. This study aims to evaluate the effect of intraperitoneal quercetin administration on caspase-3 levels and peritoneal adhesion formation in a Wistar rat (Rattus norvegicus) model following laparotomy.

Methods: This true experimental study employed a posttest-only control design involving 36 healthy male Wistar rats aged 3–4 months and weighing 200–250 g. The animals were randomly allocated into two groups: control (normal saline irrigation) and treatment (intraperitoneal quercetin at 50 mg/kg body weight). Peritoneal adhesion was induced by standardized peritoneal abrasion. On post-operative day 7, evaluations included caspase-3 level measurement using ELISA and macroscopic adhesion grading with a validated scoring system. Statistical analysis was performed using the Mann–Whitney test.

Results: Caspase-3 concentration in the quercetin-treated group was significantly higher than in the control group (2.968 ± 0.970 vs. 1.277 ± 0.482; p = 0.000), indicating enhanced apoptosis. The mean adhesion score was significantly lower in the quercetin group (1.280 ± 1.127 vs. 2.220 ± 1.047; p = 0.012), demonstrating a protective effect of quercetin against pathological adhesion formation.

Conclusion: Intraperitoneal quercetin significantly reduces peritoneal adhesion formation and is associated with increased caspase-3 levels, suggesting a potential apoptosis-related mechanism. These findings support further investigation into its clinical applicability, including optimization of dosing, safety evaluation, and integration into perioperative pharmacotherapy protocols. If validated in human studies, quercetin may contribute to reducing surgical complications and associated healthcare burdens.

Keywords: Peritoneal Adhesion; Apoptosis; Caspase-3; Quercetin; Adhesion Prevention

INTRODUCTION

Post-operative peritoneal adhesion remains one of the most significant complications in modern surgical practice and continues to pose an unresolved clinical challenge (1). Peritoneal adhesion is defined as a pathological attachment between intra-peritoneal structures, including the small intestine, colon, omentum, and abdominal wall, ranging from thin connective tissue layers to dense fibrous bridges containing blood vessels and nerve fibers (2). Peritoneal adhesions develop in approximately 79–90% of patients undergoing abdominal or pelvic surgery. Although adhesions arise as part of the normal peritoneal healing process and may be asymptomatic in some cases, their formation represents a pathological condition that leads to substantial long-term morbidity (3,4).

The clinical and socioeconomic consequences of adhesions are extensive, including increased rates of reoperation, a higher risk of intraoperative complications such as inadvertent enterotomy and massive bleeding, prolonged hospitalization, and the development of chronic abdominal or pelvic pain (3,5). Most notably, adhesions are the leading cause of adhesive small bowel obstruction (ASBO), accounting for about 65% of small bowel obstruction cases and 12–16% of surgical emergency admissions (6). The risk of recurrent ASBO is also considerable, rising progressively with each surgical episode and reaching up to 63% after four operations (7,8). The high incidence and considerable clinical impact of peritoneal adhesions underscore their importance as a persistent health problem that demands more effective preventive strategies.

Various preventive strategies have been implemented to mitigate adhesion formation, including mechanical barriers, pharmacological agents, and laparoscopic techniques (4,9). However, these approaches present significant limitations. Mechanical barriers typically provide only temporary protection and fail to address the underlying pathophysiological mechanisms of adhesion formation, whereas pharmacological interventions often demonstrate limited efficacy and may be associated with adverse effects or poor bioavailability (10). The persistence of peritoneal adhesions despite the availability of current preventive measures highlights the need for novel therapeutic approaches that target the fundamental biological processes underlying adhesion pathogenesis (9–11). In the absence of a truly effective and safe therapy to prevent adhesion formation at the molecular level, this condition remains a critical unmet need in contemporary surgical practice. It warrants innovative research to develop interventions capable of substantially reducing both the incidence and clinical burden of adhesions.

Peritoneal adhesion formation is a multifactorial process involving inflammatory signaling, fibrinolytic imbalance, mesothelial injury, and extracellular matrix remodeling. While apoptosis dysregulation represents one important mechanism, it interacts with other biological pathways that collectively determine adhesion outcomes (12). Following surgical trauma, the peritoneal cavity is exposed to pro-inflammatory mediators, primarily TNF-α, which trigger a cascade of cellular events. This cascade includes the recruitment of inflammatory cells, abnormal fibrin deposition, impaired fibrinolysis, and the proliferation of fibroblasts that differentiate into myofibroblasts responsible for excessive extracellular matrix production. The remodeling of this pathological tissue results in the formation of fibrotic adhesive structures (12–14). This process is characterized by a paradoxical suppression of apoptosis in mesothelial cells and fibroblasts, which prevents the elimination of damaged cells and permits uncontrolled fibroblast proliferation, predisposing to adhesion formation (12–14).

Within this multifactorial framework, apoptosis dysregulation represents one important contributing mechanism. Caspase-3, as a key executioner enzyme in apoptosis, plays a central role in regulating the removal of damaged mesothelial cells. Impaired caspase-3 activity has been associated with reduced apoptosis and persistent fibroblast activity, thereby contributing to adhesion formation (15). In normal peritoneal healing, adequate apoptosis of damaged mesothelial cells is essential to prevent pathological adhesion formation. However, during adhesion development, increased exposure to TNF-α induces upregulation of anti-apoptotic proteins, notably BCL-2, which ultimately suppresses caspase-3 activity. This suppression of caspase-3, mediated by post-translational modifications such as S-nitrosylation, prevents apoptosis of damaged cells and promotes unrestrained fibroblast proliferation and myofibroblast differentiation, thereby facilitating adhesion formation (15,16). The recognition that apoptosis dysregulation, particularly through caspase-3 inhibition, constitutes a central mechanism in peritoneal adhesion formation underscores the need for therapeutic strategies targeting this molecular pathway specifically. Quercetin has emerged as a promising candidate in this context, given its capacity to modulate apoptotic signaling and restore caspase-3 function, which is suppressed during pathological processes. This naturally occurring bioflavonoid is well characterized for its anti-inflammatory, anticoagulant, antioxidant, and pro-apoptotic properties (17–19). Emerging evidence indicates that quercetin can selectively activate apoptotic pathways in fibroblasts via the intrinsic (mitochondrial) mechanism, involving modulation of BCL-2 family proteins, while simultaneously inhibiting TGF-β signaling to prevent mesothelial–mesenchymal transition (MMT) (20). In addition, its anti–TNF-α activity may enhance fibrinolytic balance and attenuate the pro-inflammatory microenvironment that facilitates adhesion formation (21). Through these multifaceted mechanisms, quercetin appears to be a promising therapeutic agent for preventing peritoneal adhesion, primarily by promoting caspase-3 activation and restoring mesothelial cell apoptosis.

Although previous studies have demonstrated the anti-inflammatory and pro-apoptotic properties of quercetin (19,22), experimental evidence specifically evaluating its association with caspase-3 concentration and macroscopic peritoneal adhesion outcomes in a controlled intraperitoneal model remains limited. Understanding how these pathways interact is essential for developing targeted strategies to prevent pathological adhesion formation. Therefore, this study aims to investigate the association between intraperitoneal quercetin administration, caspase-3 concentration, and peritoneal adhesion formation in a rat model.

METHODS

Study Design

This randomized, controlled experimental study employed a standardized postoperative peritoneal adhesion model in Wistar rats. The study was designed and reported in accordance with the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines 2.0 (23). All experimental procedures were conducted in compliance with institutional and national regulations governing the care and use of laboratory animals, including the Guide for the Care and Use of Laboratory Animals (24). A posttest-only design was selected to evaluate endpoint differences between groups, however, this design does not allow assessment of temporal changes in adhesion formation or apoptosis dynamics.

Animals and Housing Conditions

Thirty-six healthy adults male Wistar rats (Rattus norvegicus), aged 10–12 weeks and weighing 200–250 g at the time of surgery, were obtained from the integrated research laboratory of the Faculty of Medicine, University of Mataram. Male rats were used to avoid potential confounding effects of hormonal fluctuations during the estrous cycle on inflammatory responses, as sex hormones are known to modulate immune function (25). Animals were housed in polycarbonate cages (maximum 3 rats per cage) under controlled environmental conditions: temperature 22 ± 2°C, relative humidity 50–60%, and a 12-hour light/dark cycle (lights on at 07:00). Standard laboratory chow and water were provided ad libitum throughout the study period. All animals underwent a 7-day acclimatization period prior to experimental procedures to minimize stress-related confounders (26). Daily health monitoring included assessment of body weight, food and water intake, general appearance, and behavior.

Sample Size Calculation

The sample size was determined using the Federer formula for experimental animal studies: $(n–1)\ (t–1) \geq 15$, where n represents the number of animals per group and t represents the number of treatment groups (27). With t = 2 groups, a minimum of 16 animals per group was required. To account for potential mortality or exclusion (estimated 10%), 18 animals were allocated to each group (N = 36 total). This sample size was also estimated to provide 80% power to detect a clinically meaningful difference in adhesion scores (effect size d = 1.0) between groups at a two-sided significance level of $\alpha = 0.05$ (28). Although the Federer formula was used as a practical guideline, a formal power calculation based on predefined effect size and primary outcomes was not conducted, which represents a limitation of this study.

Randomization and Blinding

Animals were randomly allocated to two experimental groups using computer-generated random numbers (n = 18 per group): (1) Control group: laparotomy with adhesion induction and intraperitoneal administration of vehicle (normal saline); and (2) Quercetin group: laparotomy with adhesion induction and intraperitoneal quercetin administration. Randomization was performed by an investigator not involved in surgical procedures or outcome assessment. The surgical team was aware of group allocation due to the nature of the intervention; however, all outcome assessors (macroscopic and microscopic adhesion scoring, biomarker analysis) were blinded to treatment allocation throughout the study. Treatment codes were revealed only after all data analyses were completed.

Preparation of Quercetin Solution

Quercetin (≥95% purity, Sigma-Aldrich, St. Louis, MO, USA; Cat. No. Q4951) was dissolved in dimethyl sulfoxide (DMSO; Sigma-Aldrich) and subsequently diluted with normal saline to achieve adequate solubility at a concentration of 50 mg/mL and final DMSO concentration of approximately 10%. This concentration was selected because DMSO is widely used as an effective solvent for poorly water-soluble compounds, while concentrations of 5–10% are generally considered well tolerated for intraperitoneal administration and sufficient to maintain solubility without inducing significant biological effects (29).

To control for potential vehicle effects, the control group received an equivalent volume of normal saline containing the same concentration of DMSO. The quercetin solution was prepared fresh on the day of surgery and protected from light. In the quercetin group, quercetin was administered intraperitoneally at a dose of 50 mg/kg body weight, evenly distributed throughout the peritoneal cavity immediately prior to abdominal wall closure. This dose was selected based on previous preclinical studies demonstrating anti-inflammatory and antifibrotic efficacy without significant toxicity (30).

Induction of Peritoneal Adhesion Model

The rats were anesthetized with inhaled sevoflurane. After adequate anesthetic depth was achieved for the surgical procedure, the abdominal area was shaved, and the skin was disinfected with a standard antiseptic to minimize the risk of contamination. A midline incision measuring approximately 3 cm was then performed to expose the abdominal cavity. Peritoneal adhesions were induced through controlled abrasion by applying approximately 10 uniform strokes to the peritoneum over a 1.5 × 1.5 cm area on the right abdominal wall and were performed by a single trained operator to minimize variability. The abrasion was carried out using sandpaper in accordance with a standardized protocol (31) until uniform superficial damage was observed without significant bleeding. Following abrasion, the intervention group received an intraperitoneal administration of 1 mL quercetin (50 mg/kg body weight), while the control group received 1 mL of normal saline. The abdominal incision was subsequently closed using 4-0 polypropylene sutures to ensure secure closure and prevent leakage.

Outcome Assessment

On day 7 after the adhesion induction procedure, euthanasia was performed using high-dose chloroform inhalation in accordance with the institutional protocol at the time of the study. However, chloroform inhalation may influence tissue integrity and biochemical measurements, therefore, this method should be considered a limitation of the study. After confirming the absence of vital signs, re-laparotomy was performed via a midline abdominal incision to reopen the peritoneal cavity. Peritoneal fluid was then carefully collected using a sterile syringe to prevent contamination from blood or other tissues. The collected fluid samples were stored in microcentrifuge tubes and processed according to the ELISA protocol. The caspase-3 concentration was measured using a Rat CASP3 ELISA Kit (ELK Biotechnology, Catalog ELK1528), following the manufacturer's instructions, including sample preparation, incubation with specific antibodies, and absorbance reading via microplate reader.

After the peritoneal fluid was collected, the degree of peritoneal adhesion was assessed macroscopically. The assessment was conducted using a standardized peritoneal adhesion scoring system (32). The grading system utilized for evaluating adhesion severity is presented in Table 1.

Table 1. Peritoneal adhesion grading

Grade	Descriptions
0	No adhesion
1	Adhesions include fibrin deposits, fine thread-like adhesion strands, or slight organ adhesions that can be lysed with blunt instruments.
2	Also includes adhesion strands that can be lysed with blunt instruments, but partly also ones that are only lysable with sharp instruments, with incipient fragile vascularization.
3	Adhesions include clearly vascularized, strong adhesion strands which are only lysable with sharp instruments.
4	Adhesions are firm, extensive organ adhesions, which are only lysable with sharp instruments and for which surgical treatment for organ damage is almost unavoidable.

Statistical Analysis

Statistical analysis was performed using the Mann-Whitney U test to evaluate differences in means between the treatment and control groups, with a 95% confidence interval. Prior to data analysis, normality was assessed using the Kolmogorov-Smirnov test.

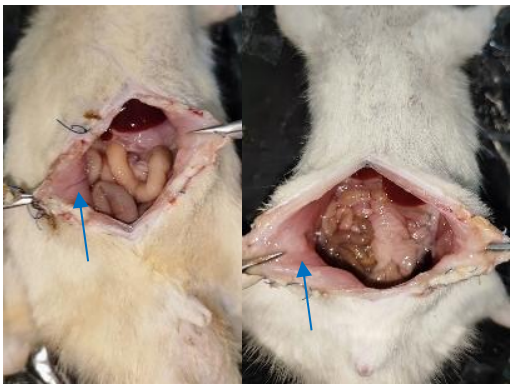
Ethical approval

The study protocol was reviewed and approved by the Health Research Ethics Commission of the Faculty of Medicine and Health Sciences, Universitas Mataram (Reference: 030/UN18.F8/ETIK/2025) prior to the commencement of any experimental procedures. All procedures were conducted in accordance with the approved protocol and institutional standards governing the use of laboratory animals in research.

RESULTS

This study presents an analysis of intraperitoneal quercetin administration on caspase-3 concentration and the degree of peritoneal adhesion formation in a rat model following laparotomy. Enhanced caspase-3 levels serve as a critical indicator of apoptotic activity, which is postulated to play a protective role in preventing the development of pathological adhesive tissue following peritoneal injury. Consequently, this parameter was selected as a molecular marker to evaluate the mechanistic actions of quercetin in apoptotic modulation.

In addition, this study conducted a macroscopic assessment of the degree of peritoneal adhesion to evaluate the anti-adhesive effects of quercetin at the tissue level. A standardized grading system was employed for this assessment, enabling quantitative assessment of adhesion severity. Representative visual depictions of the post-treatment peritoneal tissue are presented in Figure 1.



A. Quercetin-treated group (50 mg/kg)

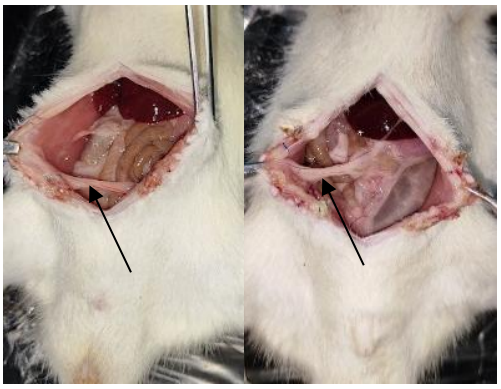

B. Control group (normal saline)

Figure 1. Representative macroscopic appearance of peritoneal adhesion in Wistar rats seven days after laparotomy. **(A)** Quercetin-treated group (50 mg/kg), showing minimal or no adhesion formation (indicated by blue arrow), corresponding to lower adhesion grades (Grade 0–1). **(B)** Control group (normal saline), demonstrating visible fibrous adhesion bands between peritoneal surfaces (indicated by black arrow), corresponding to higher adhesion grades (Grade 2–3). These images illustrate representative findings consistent with the standardized adhesion scoring system used in this study.

Figure 1 presents the macroscopic distinctions between the quercetin-treated group and the control group that are consistent with the quantitative adhesion scores observed in both groups. This visualization provides direct evidence of the preventive effect on peritoneal adhesion formation in rat peritoneal tissue observed seven days post-operatively. To provide a more detailed overview of this study's findings, the results of caspase-3 level measurements in both groups are presented below (Table 2).

Table 2. The effect of intraperitoneal administration of quercetin on Caspase-3 levels in white rats (Rattus norvegicus Wistar strain) after laparotomy

Group	Normal Saline (n=18)			Quercetin (n=18)			*p*	Effect size (r)
	Mean	Median (IQR)	Std. Deviation	Mean	Median (IQR)	Std. Deviation		
Caspase-3	1.277	1.115 (0.89)	0.482	2.968	3.005 (1.43)	0.970	<0.001	0.73

Table 2 presents the comparison of caspase-3 levels between the rat group administered quercetin and the control group. Caspase-3 concentration was significantly higher in the quercetin group compared to the control group (median [IQR]: 3.005 [1.43] vs 1.115 [0.89]; $p < 0.001$), with a large effect size ($r = 0.73$), indicating a substantial treatment effect.
Furthermore, the data on peritoneal adhesion degree scores from macroscopic assessment, which compare the severity of adhesions between the control and quercetin treatment groups, are presented in Table 3.

Table 3. The effect of intraperitoneal administration of quercetin on the formation of peritoneal adhesions in white rats (Rattus norvegicus Wistar strain) after laparotomy.

Group	Normal Saline (n=18)			Quercetin (n=18)			*p*	Effect size (r)
	Mean	Median (IQR)	Std. Deviation	Mean	Median (IQR)	Std. Deviation		
Grade	2.220	2.00 (1)	1.047	1.280	1.00 (2)	1.127	0.012	0.42

Table 3 demonstrates that the macroscopic adhesion degree ($p = 0.012$) significantly lower in the quercetin group compared to the control group (median [IQR]: 1.00 [2] vs 2.00 [1]; $p = 0.012$), with a moderate effect size ($r = 0.42$). This finding indicates that quercetin is associated with a reduction in peritoneal adhesion formation. This series of data forms the basis for discussing the efficacy and mechanism of quercetin as a preventive agent against peritoneal adhesions via apoptotic molecular pathways.

DISCUSSION

This study demonstrates that intraperitoneal administration of quercetin is associated with increased caspase-3 concentration and reduced peritoneal adhesion formation in a rat laparotomy model. Rats treated with quercetin exhibited markedly higher caspase-3 levels compared to the saline control group, with average caspase-3 concentrations nearly twice as high in the intervention group. Correspondingly, the average adhesion scores in the quercetin group were significantly lower, indicating effective prevention of pathological adhesion formation. However, these findings should be interpreted within the context of the study design and its limitations.
These findings support the conclusion that quercetin exerts anti-adhesive effects primarily by inducing apoptosis of mesothelial cells and fibroblasts, mediated by caspase-3. By restoring the balance between cell proliferation and apoptosis following peritoneal injury, quercetin disrupts the cascade that leads to excessive fibroblast activity and collagen deposition, fundamental processes in adhesion development. Therefore, quercetin is positioned as a promising candidate for

pharmacological prevention of post-operative abdominal adhesions (20). Importantly, caspase-3 concentration measured in peritoneal fluid represents a non-specific biomarker and does not distinguish between active and inactive forms, nor provide cell-specific localization. Therefore, these findings should be interpreted as an indication of the presence of an apoptotic process and not as confirmation of apoptosis.

Post-operative peritoneal adhesion formation is a consequence of disrupted healing following surgical injury, primarily involving an imbalance between cell proliferation and apoptosis. In normal wound healing, timely apoptosis eliminates damaged mesothelial cells and regulates fibroblast proliferation, thereby preventing the development of fibrous adhesions. However, surgical trauma triggers a surge of pro-inflammatory cytokines, especially TNF-α, which upregulates anti-apoptotic proteins (e.g., BCL-2) that suppress caspase-3 activity. This suppression allows persistent survival and activity of fibroblasts and myofibroblasts, resulting in uncontrolled extracellular matrix production and fibrotic bridging between peritoneal surfaces (15,16). By reactivating caspase-3 through quercetin's pro-apoptotic mechanisms, this study successfully restored the normal balance between proliferation and apoptosis, creating an environment unfavorable for pathological adhesion formation.

The findings of this study are consistent with previous research demonstrating that optimally activated apoptosis is a crucial component of normal peritoneal healing mechanisms and in preventing pathological adhesion formation. Data from various peritoneal adhesion model studies reveal an intriguing phenomenon: during the initial adhesion formation phase (days 0–3), anti-apoptotic mechanisms predominate, whereas in later periods, pro-apoptotic mechanisms become dominant. This imbalance, particularly the excessive expression of anti-apoptosis during the early phase, creates a critical time window in which the administration of pro-apoptotic agents such as quercetin holds strong potential to significantly alter the course of adhesion development (33–35).

Recent studies indicate that various cell types within adhesion tissue, including fibroblasts, myofibroblasts, endothelial cells, and immune cells, exhibit different sensitivities to pro-apoptotic agents. Quercetin, across multiple investigations, demonstrates cell-type-specific activation of caspase-dependent apoptotic pathways. In fibroblasts, quercetin induces caspase-3 activation via the mitochondrial pathway; in immune cells, it promotes the transition of pro-inflammatory M1 macrophages to anti-inflammatory M2 macrophages, providing a protective effect against adhesion formation (36,37). Understanding the cell-specific responses to quercetin helps explain its efficacy in preventing adhesions without causing excessive collateral damage to normal peritoneal healing processes.

The pro-apoptotic mechanism of quercetin at the molecular level involves activation of the intrinsic (mitochondrial) pathway through modulation of BCL-2 family proteins. Recent studies show that quercetin induces apoptosis by upregulating pro-apoptotic proteins such as BAX and BID while simultaneously inhibiting anti-apoptotic proteins BCL-2, BCL-W, and MCL-1 (35,38). This is particularly significant in the context of peritoneal adhesion, as adhesive fibroblasts are known to exhibit a higher BCL-2/BAX ratio compared to normal peritoneal fibroblasts. This phenomenon contributes to their resistance to spontaneous apoptosis. Studies on pulmonary fibrosis similarly demonstrate that fibrotic fibroblasts express higher levels of anti-apoptotic BCL-2 family members, creating a phenotype primed for sustained survival and proliferation (35).

By downregulating BCL-2 and upregulating BAX/BID, quercetin effectively shifts the balance of BCL-2 family proteins toward a pro-apoptotic state, facilitating mitochondrial outer membrane permeabilization (MOMP), cytochrome-c release, and apoptosome formation that activates procaspase-9. Activation of caspase-9 (the initiator caspase) subsequently activates procaspase-3 into active caspase-3, the executioner caspase (20). In this study, the observed increase in caspase-3 directly reflects activation of this pathway, resulting in the orderly (apoptotic) elimination of adhesive fibroblasts without leaving pro-inflammatory debris that would trigger secondary inflammatory responses.

Recent studies also show that quercetin binds to the TGF-β receptor type II, blocking the signaling cascade that drives the critical process wherein mesothelial epithelial cells transform into fibroblast-like cells (mesothelial-mesenchymal transition/MMT), accompanied by increased extracellular matrix production (collagen, fibronectin) and decreased epithelial markers (E-cadherin), resulting in adhesion formation. Cells undergoing MMT become resistant to apoptosis through upregulation of anti-apoptotic proteins (BCL-2, MCL-1). Quercetin is known to prevent MMT while maintaining cell responsiveness to pro-apoptotic signals via activation of caspase-3, establishing a mechanism for the eradication of fibrotic cells and the prevention of adhesion formation (35).

Quercetin, through its anti-TNF-α effects, may indirectly enhance fibrinolysis by reducing TNF-α-induced PAI release from endothelial cells and fibroblasts (39). More specific studies are required to evaluate whether the increase in caspase-3 and reduction in adhesion observed in this study are partly mediated by improvements in the fibrinolytic balance versus the direct pro-apoptotic effects of quercetin.

Nevertheless, other literature also identifies potential risks associated with excessive and prolonged activation of apoptosis in the context of peritoneal wound healing. Peritoneal wound healing is a balanced process with staged phases: the acute inflammatory phase (0–3 days), during which inflammatory cells aggregate, debris is cleared, and hemostasis occurs; the proliferative phase (3–21 days), characterized by fibroblast proliferation, angiogenesis, and matrix formation; and the remodeling phase (21 days to 2 years), where the matrix matures and final tissue organization takes place (40,41). Excessive and premature apoptosis activation can disrupt the proliferative phase by killing fibroblasts necessary for tissue reconstruction, leading to impaired wound healing, reduced angiogenesis, and ultimately compromised peritoneal barrier function (33,42).

Another important consideration is that procaspase-3, in its role as an executioner caspase, also performs critical non-apoptotic functions in normal cellular processes. Research by Brentnall et al. in 2014 on mouse embryonic fibroblasts demonstrated that procaspase-3 (not merely activated caspase-3) regulates fibronectin secretion and affects cell morphology, adhesion, and migration, effects that occur independently of caspase-3 catalytic activity (43). Excessive caspase-3 activation

can impair these protective functions, resulting in dysregulation of extracellular matrix formation that paradoxically promotes adhesion formation through alternative mechanisms (37).

Other studies also demonstrate that excessive or dysregulated apoptosis induction, particularly when combined with oxidative stress or in an already activated inflammatory environment, can shift the cell death pathway from orderly apoptosis (immunologically silent) to secondary necrosis or necroptosis (immunologically alarming). Secondary necrosis releases potent danger-associated molecular patterns (DAMPs) that robustly activate innate immune signaling, promoting sustained inflammation and paradoxically increasing adhesion risk (44,45). Quercetin, through its antioxidant properties, likely mitigates this risk by maintaining a redox environment consistent with immunologically silent apoptosis.

Intraperitoneal administration of quercetin at a dose of 50 mg/kg body weight in dimethyl sulfoxide (DMSO) at 10% concentration facilitates achieving adequate local concentrations to activate multiple apoptotic pathways. Bioavailability studies demonstrate that quercetin, despite its low aqueous solubility, can attain therapeutic concentrations (~1–3 μM) in target tissues when administered intraperitoneally (46–48). This route of administration offers significant pharmacological advantages over the oral or intravenous routes, particularly in optimizing local concentration and minimizing first-pass hepatic metabolism (48). It is important to note that DMSO, used as a solvent in this study, is known to have biological activities, including anti-inflammatory and membrane-modulating effects, therefore, even in acceptable concentrations, its potential influence to the observed results cannot be fully ruled out.

Study Limitations

The primary limitation is the use of a rat model, which, although it approximates human peritoneal healing, does not fully reflect the complexity and variability of clinical scenarios. The relatively brief observation period of the study (seven days post-laparotomy) precludes assessment of long-term adhesion recurrence and potential delayed toxicity or adverse effects of quercetin. The cell-type selectivity of quercetin-induced apoptosis in pathological versus physiological fibroblasts was not directly measured, nor was its impact on standard wound-healing parameters such as re-epithelialization, angiogenesis, or peritoneal barrier integrity. Importantly, this study did not include direct apoptosis assays (e.g., TUNEL staining), histopathological evaluation, or pathway-specific molecular analyses. Therefore, the proposed apoptosis-related mechanism remains inferential and should be interpreted with caution.

Additionally, the use of DMSO at a concentration of approximately 10% may introduce potential confounding effects due to its known biological activity. Although a vehicle control was used, the independent effect of DMSO cannot be completely ruled out. Finally, this study did not fully address the potential for pro-inflammatory or necrotic responses, particularly in scenarios of oxidative stress or inflammation.

Clinical Pharmacy, Public Health, and Future Research Implications

This study highlights the potential of quercetin as a promising adjuvant therapy for the prevention of post-operative peritoneal adhesions, with important implications for clinical pharmacy practice and public health strategies. From a clinical pharmacy perspective, quercetin may contribute to improved postoperative pharmacotherapeutic management by reducing adhesion-related complications, decreasing the need for reoperations, and minimizing prolonged hospital stays. Optimization of dosing regimens, formulation development, drug–drug interaction assessment, and pharmacokinetic profiling will be critical to ensure safe and effective integration into perioperative care protocols.

In the context of public health, the prevention of peritoneal adhesions has significant implications for reducing surgical morbidity, healthcare costs, and long-term disability. Adhesion-related complications contribute to recurrent hospital admissions, bowel obstruction, infertility, and chronic pain, thereby increasing the burden on healthcare systems. The incorporation of evidence-based adjunct therapies such as quercetin into surgical prevention strategies may enhance patient outcomes and support cost-effective healthcare delivery.

Future research should prioritize well-designed prospective clinical trials to establish efficacy, safety, optimal dosing, and population-specific responses. Health economic evaluations are also warranted to assess cost-effectiveness and potential impact on healthcare resource utilization. Additionally, exploration of other pharmacological agents with similar preventive potential may expand therapeutic options and strengthen comprehensive postoperative complication prevention programs.

CONCLUSION

Intraperitoneal quercetin is associated with increased caspase-3 concentration and reduced post-operative peritoneal adhesion formation. These findings suggest a potential apoptosis-related mechanism; however, further studies incorporating direct mechanistic and histopathological analyses are required.

DECLARATIONS

Author contributions: ZH, AAF, and MR conceived and designed this study. ZH, and IH collected, organized, and interpreted the data. ZH, AAF, MR, and IH wrote, critically reviewed, and approved the final draft of the manuscript. AAF corresponded with the journal and addressed the editor's and reviewer's comments. All authors have critically reviewed, approved the final draft, and are responsible for the content and similarity index of the manuscript.

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Declaration of Generative AI And AI-Assisted Technologies in the Writing Process: The authors declare that the use of AI (ChatGPT and QuillBot) to support language refinement, increase clarity, or improve the readability and overall structure of the manuscript.

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