RESEARCH PAPER

Therapeutic Effects of Beetroot Extract on Diarrhea and Short-Chain Fatty Acid Concentrations in Post-Cholecystectomy Mice

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ABSTRACT

Background: Post-cholecystectomy may lead to post-cholecystectomy syndrome (PCS), which manifests as various gastrointestinal complaints. Elevated concentrations of secondary bile acids (BAs) and intestinal bacterial dysbiosis—both linked to gastrointestinal dysfunction—are commonly observed in PCS patients. Cholestyramine, a bile acid sequestrant, binds BAs to reduce their ileal absorption and enhance fecal excretion. However, its high cost and limited availability, motivate exploration of alternative agents. In vitro study, beetroot (Beta vulgaris) has demonstrated a bile acid binding capacity of approximately 18%, suggesting potential therapeutic value.

Purpose: This study aimed to evaluate the effect of beetroot extract on diarrhea and fecal short-chain fatty acid (SCFA) levels in mice after cholecystectomy.

Methods: A true experimental post-test only control group design was conducted. Thirty male mice were divided into five groups: healthy controls, PCS model (cholecystectomy-only), cholecystectomy groups treated with three incremental doses of beetroot extract (3.08, 6.16, and 12.32 mg/20 g body weight). Parameters assessed include fecal consistency, stool weight, and fecal SCFA concentrations (acetic, propionic, and butyric acids). Statistical analysis was non parametric, Kruskall Wallis and Mann Whitney, with p-value < 0.05 was considered significant.

Results: Administration of beetroot extract at 12.32 mg/20 g body weight significantly reduced fecal weight compared with cholecystectomy group ($p < 0.05$). This dose also significantly restored SCFA concentrations to levels closest to those of the healthy control group ($p < 0.05$).

Conclusion: Beetroot extract at 12.32 mg/20 g body weight effectively decreased stool weight and normalized SCFA levels comparable to the healthy control. The findings indicate that high-dose beetroot extract exerts the most beneficial therapeutic effect on post-cholecystectomy diarrhea and SCFA modulation.

Keywords: *Beetroot extract, post-cholecystectomy diarrhea, short-chain fatty acids*
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BACKGROUND

Post-cholecystectomy syndrome (PCS) represents a set of gastrointestinal symptoms that occur after gallbladder removal, including diarrhea, bloating, and abdominal discomfort [1,2]. These symptoms have been linked to increased secondary bile acid (BA) concentrations and gut microbiota dysbiosis, leading to impaired intestinal function [3–5]. Bile acid sequestrants, such as cholestyramine, alleviate bile acid diarrhea by binding excess BAs in the intestinal lumen, reducing their reabsorption in the ileum, and increasing fecal excretion [6–8]. However, limitations related to cost and availability have prompted interest in exploring natural alternatives.

Beetroot (*Beta vulgaris L.*) is rich in polyphenols and betalains with bile acid–binding and antioxidative properties [9–12]. Previous *in vitro* studied have shown that beetroot can bind approximately 18% of bile acids and improve gut microbiota composition [13,14], suggesting a

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potential role as a natural bile acid sequestrant. Additionally, beetroot supplementation has been associated with improved intestinal health and short-chain fatty acid (SCFA) production [15–17]. This study aimed to evaluate the therapeutic potential of beetroot extract in reducing diarrhea frequency and improving SCFA profiles in a mice model of PCS induced by cholecystectomy.

METHODS

Study Design

A true experimental post-test only control group design was employed from May 2024 – August 2024. Thirty healthy male mice were divided equally into five groups (n = 6 each) and maintained at the Animal Research Unit, Faculty of Medicine, Gadjah Mada University. Mice were individually caged and kept under controlled laboratory conditions (12-hour light/dark cycle, 22–24 °C, 50–60% humidity) with ad libitum access to food and water [18, 19]. The Ethical Committee of the Faculty of Medicine, University of Diponegoro, Dr. Kariadi Hospital, Semarang, Indonesia approved the experimental protocol. The study has already got ethical clearance with committee number *081/EC-H/KEPK/FK-UNDIP/VIII/2024*. Animals care criteria prepared by the National Academy of Sciences and outlined in the Guide for the Care and Use of Laboratory Animals were applied through-out the experiment. [20].

Dose Determination of Beetroot Extract

To obtain equivalent doses for the mice, conversion was performed using the Body Surface Area (BSA) method as shown in Table 1 [21]. Since the initial dose was expressed as an daily human dose, the calculation was performed as follows, with the body weight of the mice is at 20 grams.

$$\text{Mice dose (in mg)} = \text{Human dose (mg/kg)} \times \left(\frac{K_m \text{ human}}{K_m \text{ animal}}\right)$$

$$\text{Mice dose (mg)} = \text{Human dose (mg)} \times \left(\frac{12.33}{70}\right)$$

K_m is the body surface area correction factor for each species (human $K_m = 37$; mouse $K_m = 3$) [21].

Converted doses for 20 g mice were 3.08 mg, 6.16 mg, and 12.32 mg, equivalent to 250, 500, and 1000 mg in kgBB/day in humans [22], [23], [24], [25], [26].

Beetroot Extract Preparation

The extraction process was carried out using the maceration method. Fresh beetroot was cleaned, sliced, and dried at 40°C until a constant weight was achieved. The dried material was then ground into a fine powder to obtain the simplicia. A total of simplicia powder was macerated with 70% ethanol in a solvent-to-sample ratio of 1:5 (w/v). The mixture was allowed to stand for 48 hours at room temperature and was occasionally stirred. After maceration, the mixture was filtered to obtain the first macerate (M1). The remaining residue (marc) was subjected to a second maceration under the same conditions to obtain the second macerate (M2). Both macerates (M1 and M2) were combined and concentrated under reduced pressure using a rotary evaporator until a thick extract was obtained. The thick extract was stored in an airtight container at 4°C until further analysis [27,28].

Experimental Groups

Mice were randomly divided into 5 groups (n = 6 per group):

1. **Control Group (G1):** No cholecystectomy and no treatment (healthy baseline).
2. **PCS Group (G2):** Cholecystectomy performed, no treatment.
3. **Low-Dose Beetroot Group (G3):** Cholecystectomy performed; mice received beetroot extract (3.08 mg/20 g body weight/day).
4. **Medium-Dose Beetroot Group (G4):** Cholecystectomy performed; mice received beetroot extract (6.16 mg/20 g body weight/day).
5. **High-Dose Beetroot Group (G5):** Cholecystectomy performed; mice received beetroot extract (12.32 mg/20 g body weight/day).

Surgical Procedure

Mice in groups G2 – G5 underwent surgical cholecystectomy to model PCS. Procedure was performed under inhaled isoflurane (2-3% in oxygen) anesthesia. The abdomen was sterilized with povidone iodine solution, and a midline incision was made to access the gallbladder. The cystic duct and artery were ligated, and the gallbladder was excised. The incision was closed with sutures, and mice were monitored for postoperative recovery. [29,30].

Treatment Administration

Treatments were administered orally via gavage once daily for seven days, and output variables were processed on the day 8th.

Outcome Measurements

Fecal Consistency:

Fecal consistency was evaluated using a modified Bristol Stool Chart, in which the original seven-types classification were used three ordinal categories to facilitate data collection [31]. Hard, separate pieces resembling nuts, sausage-shaped with a smooth surface, slightly watery, very soft and watery consistency resembling watery porridge were score 1,2,3,4,5,6 and 7 respectively.

Fecal Weight :

Since each mice was housed individually and clean every morning. Fecal samples were collected twenty four hours, weighed using an analytical balance, and recorded as grams.

SCFA-Analysis
Fecal samples collected on day 8 were analyzed the acetic acid, propionic acid, and butyric acid concentrations levels using gas chromatography (GC) equipped with a flame ionization detector. The methodology involved homogenizing fecal samples in phosphate-buffered saline (PBS), centrifuging samples at 10,000 rpm for 10 minutes, acidifying the supernatant with hydrochloric acid and extracting SCFAs with diethyl ether, injecting the extract into the GC column for analysis [32,33].

Statistical Analysis
All data were analyzed using SPSS (version 25.0, IBM Corp., Armonk, NY). By using Saphiro Wilk we got that all data were non normal distribution, therefore we used Kruskall Wallis and Mann Whitney for comparing among group and between groups. A p-value of < 0.05 was statistically significant [34].

RESULTS
Cholecystectomy induced significant increases in stool weight and softer fecal consistency compared to the healthy control group. It was shown that cholecystectomy only group, the stool weight was higher significantly, in comparison with normal subject and treatment with extract beetroot. However no significantly difference among and between the various dose of beetroot extract as shown in Fig. 1.

Mean rank of stool weight after treatment in group 1,2,3,4 and 5 were respectively 11.17, 27.33, 15.33,10.92 and 12.75. It was shown that in cholecystectomy only group, the fecal weight was significantly higher in comparison with treatment (group 3,4,5) and non cholecystectomy mouse (Group 1) as shown in Fig.2.

Fecal SCFA concentrations were markedly lower in the cholecystectomy-only group, while beetroot extract restored acetic, propionic, and butyric acid levels closer to those of the healthy control. The highest-dose beetroot group exhibited the highest SCFA recovery and lowest diarrhea frequency as shown in Figure 3, Figure 4 and Figure 5.

DISCUSSION
This study demonstrates that beetroot extract ameliorates post-cholecystectomy diarrhea and restores short-chain fatty acid (SCFA) concentrations in a dose-dependent manner [35]. The improvement in stool consistency and normalization of SCFAs indicate a beneficial modulation of intestinal microbiota and bile acid metabolism. Bile acids exert bacteriostatic effects by disrupting bacterial membranes and inhibiting microbial enzyme activity. However, when present in excess, they display detergent-like cytotoxicity that damages the intestinal epithelium, disrupts barrier integrity, and alters microbial homeostasis—ultimately contributing to dysbiosis and diarrhea [36,37]. Accumulation of secondary bile acids such as deoxycholic acid and lithocholic acid suppresses beneficial SCFA-producing bacteria including Faecalibacterium prausnitzii and Roseburia spp., leading to impaired fermentation and reduced butyrat e levels [15,35].

Beetroot extract may counteract these disturbances through several complementary mechanisms. Its polyphenolic and betalain compounds exhibit strong antioxidative and anti-inflammatory effects, limiting mucosal oxidative stress and cytokine activation [6,18,22,25]. Betalains activate the Nrf2/HO-1 pathway and downregulate NF-κB signaling, thereby reducing the expression of proinflammatory mediators such as TNF-α, IL-1β, and IL-6 [26,27]. In addition, beetroot bioactives modulate hepatic lipid and bile acid metabolism by enhancing PPAR-α activity, facilitating cholesterol catabolism, and promoting bile acid synthesis and excretion [14,18,40].

The bile acid–binding potential of beetroot is attributed to its phenolic hydroxyl and carboxyl groups, which can interact electrostatically with bile salts in the intestinal lumen, preventing their reabsorption and enhancing fecal elimination [16,25]. This mechanism resembles that of synthetic sequestrants such as cholestyramine, yet beetroot provides the added benefits of antioxidant protection and improved gut microbial balance [10,27].

Furthermore, beetroot phenolics and fibers act as selective substrates for beneficial microbiota including Lactobacillus and Bifidobacterium spp., thereby supporting the recovery of microbial diversity and SCFA production [13,31,34]. Previous work demonstrated that plant-based polyphenol extracts prevent hypercholesterolemia and modulate gut microbiota composition in high-fat or post-cholecystectomy conditions [5,34,37]. The concurrent restoration of SCFA levels in beetroot-treated groups in this study aligns with these findings, suggesting that beetroot extract improves colonic fermentation by alleviating bile acid toxicity and enhancing microbial cross-feeding among commensal species. In addition to its microbiota-modulating actions, beetroot displays significant antihyperlipidemic and hepatoprotective effects, as documented in hypercholesterolemic and diabetic models [14,18,41]. These metabolic improvements may further contribute to normalized bile acid synthesis and enterohepatic balance.

Collectively, these findings indicate that beetroot exerts multi-targeted benefits—binding excessive bile acids, reducing oxidative and inflammatory damage, restoring microbial equilibrium, and normalizing SCFA production. Compared with cholestyramine, beetroot extract demonstrated comparable or superior outcomes in stool consistency and SCFA recovery, highlighting its promise as a natural, safe, and affordable therapy for bile acid–related diarrhea. Further investigations are warranted to explore the translational potential of beetroot supplementation in human post-cholecystectomy syndromes.

CONCLUSION
Beetroot (*Beta vulgaris)* extract demonstrated a dose-dependent therapeutic effect in mice following

cholecystectomy. The highest dose (12.32 mg/20 g body weight) significantly reduced stool weight while restoring short-chain fatty acid (SCFA) concentrations toward normal levels. These findings indicate that beetroot extract may modulate intestinal bile acid activity and gut microbiota balance, thereby alleviating post-cholecystectomy diarrhea. Further studies are warranted to elucidate the underlying mechanisms comparing with cholestyramine and potential clinical applications of beetroot as a promising natural bile acid–binding agent.

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Conflict of Interest
The authors declare no conflicts of interest.

Ethical Approval
All animal experiments were approved by the Institutional Animal Ethics Committee of Universitas Diponegoro and conducted according to international guidelines for animal research.

Funding Statement
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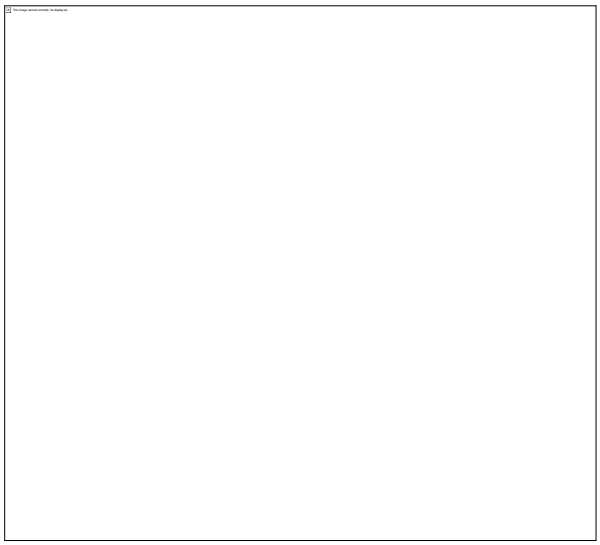
Table and Figures

Table 1. Conversion of human doses to animal equivalent doses (AED) based on body surface area [21].



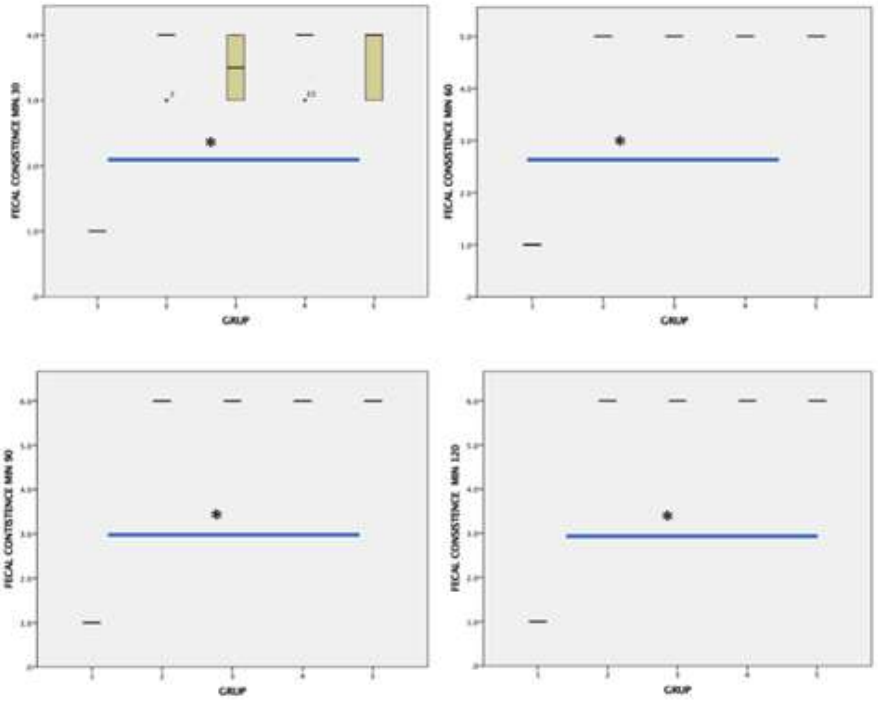


Fig 1. Stool consistence was constant on group 1 (normal healthy mouse) in the beginning of measurement (minute 30th) until minute 120th measurement, there was hard stool (score 1). Meanwhile in the cholecystectomy group feces become more liquid in group without (group 2) and with beetroot extract treatment (group 3,4,5) on the measurement of 60, 90 and 120 minute after the first measurement. (* Kruscal Wallis of group 1 vs group 2,3,4 and 5 on 0, 60,90 and 120 $p<0.05$)

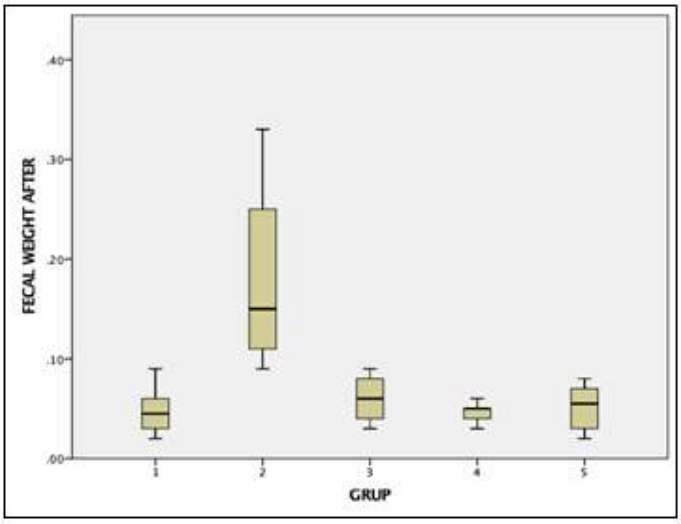


Fig 2. Mean rank of stool weight after treatment in group 1,2,3,4 and 5 were respectively 11.17, 27.33, 15.33,10.92 and 12.75. It was shown that in cholecystectomy only group, the fecal weight was significantly higher in comparison with non Cholecystectomy group (Group 1) and treatment group 3,4,5. Between group treated with beetroot extract (group 3 vs 4 vs 5) there were no significantly difference

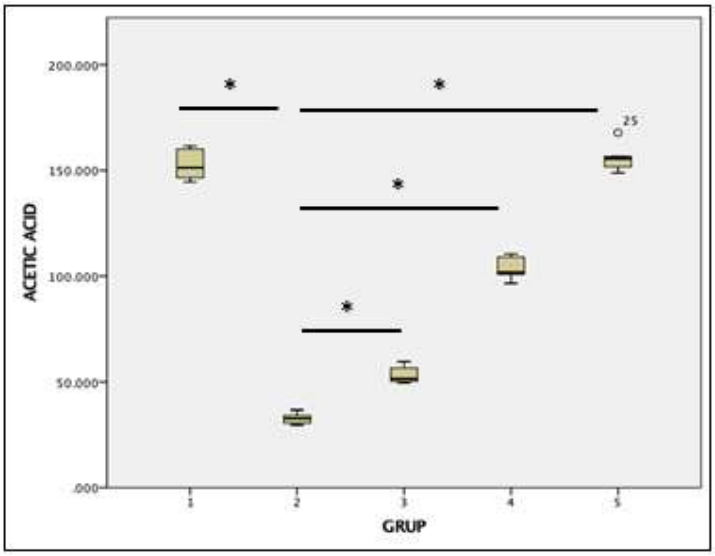


Fig 3. The median and its range of acetic acid in group 1,2,3,4 and 5 were respectively 151.24 (144.46 – 161.61), 32.69 (29.59-36.75), 51.49 (49.52-59.61), 101.73 (96.62-110.55) and 155.63 (148.85-167.77) It was shown that after cholecystectomy only, the acetic acid was decrease significantly in comparison with non cholecystectomy group.. The level of acetic acid were gradually increase significantly in accord with the dose of beetroot extract, (Group 3 vs 2, Group 4 vs 2 and Group 5 vs 2 and reach the normal level in the dose 12,32 mg.

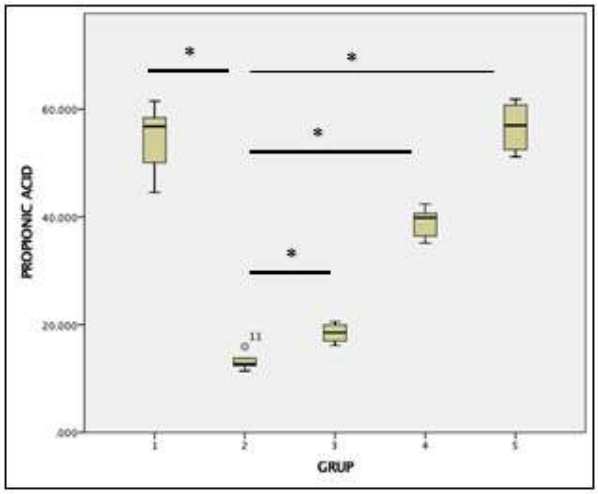


Fig 4. The median and its range of propionic acid in group 1,2,3,4 and 5 were respectively 56,79 (44,53 – 61,50), 12,70 (11,42 – 15,92), 18,53 (16,14 – 20,57), 39,85 (35,15 – 42,40) and 56,99 (51,15 – 61,84).

It was shown that after cholecystectomy only group, the propionic acid was decrease significantly in comparison with non cholecystectomy group. The level of propionic acid gradually increases significantly in accord with the dose of beetroot extract, (Group 3 vs 2, Group 4 vs 2 and Group 5 vs 2 and reach the normal level in the dose 12, 32 mg.

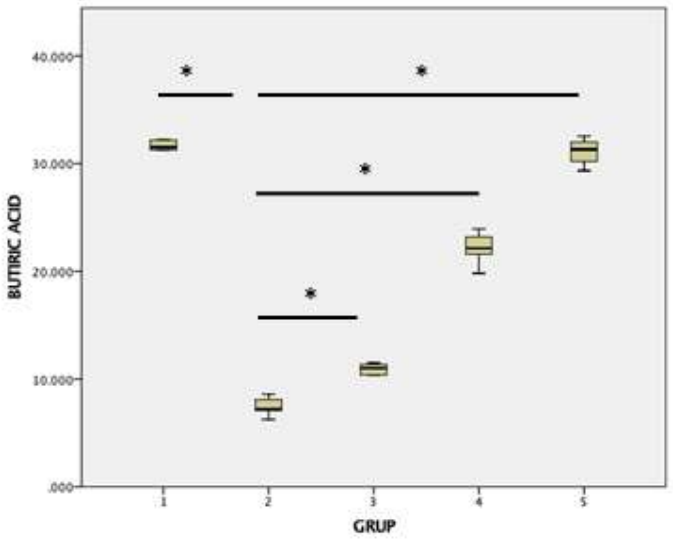


Fig 5. The median and its range of butiric acid in group 1,2,3,4 and 5 were respectively 31.54 (31.22 – 32.24), 7.23 (6.26 – 8.60), 11.00 (10.34 – 11.52), 22.14 (19.81 – 23.94) and 31.32 (29.34 – 32.55). It was shown that after cholecystectomy only, the butiric acid was decrease significantly in comparison with non cholecystectomy group. The level of butiric acid gradually increase significantly in accord with the dose of beetroot extract, (Group 3 vs 2, 4 vs 2 and group 5 vs 2 and reach the normal level in the dose 12,32 mg.