doi.org/10.36721/PJPS.2024.37.6.REG.1411-1419.1

Evaluation of the anti-cholelithiasis activity of vinegar-soaked *Ficus carica* fruit in adults: A randomized controlled trial

Malaika Bukhari[1], Imtiaz Gull[1], Mishal Fatima[1], Rameen Malik[1], Mashhud ul Hasan Abid[2], Muhammad Ibrar[1], Muhammad Mukhtar[3], Imran Ahmad Khan[4]* and Maliha Khalid Khan[5,6]

[1]Fatima-Tu-Zahra Department of Life Sciences, Muhammad Institute of Medical and Allied Sciences, Multan, Pakistan
[2]Department of Biochemistry, Bahauddin Zakariya University, Multan, Pakistan
[3]Institute of Zoology, Bahauddin Zakariya University, Multan, Pakistan
[4]Department of Pharmacy, Muhammad Nawaz Sharif University of Agriculture, Multan, Pakistan
[5]Department of Pathobiology and Biomedical Sciences, Muhammad Nawaz Sharif University of Agriculture, Multan, Pakistan
[6]Ali-Ul-Murtaza Department of Rehabilitation Sciences, Muhammad Institute of Medical and Allied Sciences, Multan, Pakistan

Abstract: This study aimed to evaluate the efficacy of vinegar-soaked *Ficus carica* fruits (VSFCF) for managing cholelithiasis in adults. A parallel-group, single-blinded, randomized controlled trial at Noreen Nishat Welfare Hospital, Khanewal, Pakistan, was conducted from December 2023 to February 2024. Fifty adults with ultrasound-confirmed cholelithiasis were randomly assigned to receive either VSFCF (1 g/kg/day) or ursodiol (600 mg/day). Primary outcomes included gallstone size reduction, while secondary outcomes assessed symptom relief (pain, bloating, digestive discomfort) and hepatic markers. Data analysis was performed using SPSS with a significance level set at $P<0.05$. Results indicated a significant reduction in gallstone size in the VSFCF group, with mean sizes decreasing from 15.5± 2.3 mm to 7.3±1.0 mm ($P<0.001$), compared to a decrease from 16.1±2.5 mm to 10.8±1.5 mm ($P<0.01$) in the ursodiol group. Additionally, VSFCF significantly improved symptoms and hepatic markers, with P-values ranging from <0.05 to <0.001. These findings suggest that VSFCF is an effective alternative for reducing gallstone size and improving symptoms and hepatic health in cholelithiasis management.

Keywords: Cholelithiasis, *Ficus carica*, vinegar, gallstones, hepatic enzymes.

Submitted on 07-08-2024 – Revised on 22-10-2024 – Accepted on 08-11- 2024

INTRODUCTION

Cholelithiasis, a condition characterized by hardened digestive juice deposits in the gallbladder, a tiny organ behind the liver, is gallstones. Bile, released into the small intestine, is stored in the gallbladder. Cholelithiasis affects 10-15% of Westerners (Jin *et al*., 2024). Between 10% and 20% of persons have cholelithiasis (Qu *et al*. 2020). Gallstone disease (GD), one of the most frequent gastrointestinal diseases, strains healthcare systems (Schläfer, and Lammert, 2020). Many medical conditions can cause gallstones. A chronic, recurring hepatobiliary condition, GD causes gallstones in the hepatic, common, or gallbladder. The illness is caused by poor bile acid, bilirubin, and cholesterol metabolism (Fleishman and Kumar, 2024).

Many factors can generate cholesterol gallstones, but an imbalance between lecithin, bile acids and cholesterol is the main culprit. Bile saturation and cholesterol crystallization occur when cholesterol levels are high. In the gallbladder, crystals precipitate and form gallstones. There are many causes of gallstones. They run in families and are twice as common as women. Being overweight may contribute. The Gastroenterological Society of Australia estimates that 25-30% of Australians over 50 have gallstones (Weir and Ghassemzadeh, 2024). Gallstones are increasing daily in Pakistan (Ali *et al*., 2021). Pakistan has an estimated 8% gallstone frequency in people over 40 and 20% in those over 60. Women account for 80% of cases, with an average diagnostic age of 35-40. Karachi has a seven-fold higher prevalence than other places, possibly due to high-fat and refined sugar diets (Nasir *et al*., 2021). Gallstones are classified by the amount of cholesterol they contain: pigment stones (90 % or more bilirubin), pure cholesterol stones and mixed composition stones (varying amounts of bilirubin and cholesterol plus calcium carbonate, calcium phosphate, and calcium palmitate). Cholelithiasis can be caused by hemolytic diseases, ceftriaxone use, total parenteral nutrition, obesity, familial predisposition, cystic fibrosis, and hypercholesterolemia (Zdanowicz *et al*., 2022). Most gallstone patients without symptoms never need medical attention, but severe cases do.

Commercially known as fruit and wine vinegar, vinegar is fermented grain and fruit liquid (Giudici and Gullo, 2017). Vinegar quality depends on supply, acidity and age (Chen *et al*., 2023). Growing customer demand for natural, premium food products improves product characterization and quality control (Ousaaid *et al*., 2021).

**Corresponding author:* e-mail: imranahmedkhandurrani@gmail.com

Vinegar may reduce the incidence of cancer and cardiovascular disease due to its antioxidant content (Kandylis *et al*., 2021). Many studies indicated that grape vinegar had more antioxidant activity than other vinegars (Antoniewicz *et al*., 2021). Organic acids including tartaric, formic, lactic and malic acids affect vinegar's flavor, along with acetic acid (Yildiz, 2023).

Ficus carica Linnis, a group of 800 species of the Mulberry family Moraceae growing in warm areas worldwide, was one of the first plants cultivated (Isa *et al*., 2020). *F. carica*, native to Southwest Asia and the Mediterranean, is extensively planted for its fig fruit and has over 135 names, including enjeer in Pakistan. *F. carica* is revered in tropical regions for religious symbolism and practical applications and its biological activities have drawn researchers worldwide. *F. carica* exhibits anticancer, antiviral, antibacterial, antihypertensive, antiparasitic, anticoagulant, anti-inflammatory, antioxidant, anti-angiogenic and hepatoprotective properties (Mohammad and Alzweiri, 2022). Traditional medical traditions like Ayurveda, Unani, and Siddha emphasize its wide variety of uses in treating endocrine, respiratory, digestive, reproductive, and infectious diseases. *F. carica* contains about 100 bioactive chemicals, including -amyrins, -carotenes, -setosterols, arabinose and xanthotoxin, which regulate cholesterol, cardiac and respiratory diseases (Fazel *et al*., 2024). Phenolic chemicals, flavonoids, polyphenols, alkaloids, and saponins are found in *F. carica* preparations (Walia *et al*., 2022). The highest mineral content in the world is in figs, including iron, magnesium and strontium. Thus, dried figs are highly nutritious (Sandhu *et al*., 2023). This study aimed to compare the effectiveness of vinegar-soaked *F. carica* fruits versus standard ursodiol treatment in managing cholelithiasis.

MATERIALS AND METHODS

Study design

A pragmatic, community-based, parallel-group, single-blinded, randomized controlled trial (IRCT20230202057310N7) was conducted at the Outdoor Patients Department of Noreen Nishat Welfare Hospital, Khanewal, Pakistan (Ref No: 1015). The study design received approval from the Review Board and the Ethical Committee of Muhammad Institute of Medical and Allied Sciences, Multan, Pakistan (2023/IRB/2/Biochem/23), in compliance with the Declaration of Helsinki (Shrestha and Dunn 2020) and its subsequent revisions. Written informed consent was obtained from all participants after providing detailed information about the study's objectives, methods, benefits and potential risks. Participants were also informed of their right to withdraw from the study at any point without any repercussions.

Material required

In the research on cholelithiasis, *F. carica* dried figs were utilized for their potential medicinal benefits. Grape vinegar was employed, likely for preparation or administration purposes. Boxes were utilized for storage or transportation of samples and equipment. Additionally, standard medicine (ursodiol), procured from Linux Pharmacy, Lahore,Pakistan was used for its known efficacy in dissolving gallstones, aligning with the focus of the cholelithiasis study. These materials collectively formed the core components supporting the research objectives.

Preparation of vinegar-soaked F. carica fruits

Fresh *F. carica* (fig) fruits, renowned for their traditional medicinal properties, were procured from the local market in Multan. The authenticity of the fruits was confirmed with the aid of a taxonomist from the Department of Botany at Bahauddin Zakariya University, Multan, and a herbarium specimen was submitted for documentation (wfo-0000687690). Dry *F. carica* slices weighing 3.5kg were obtained and soaked in 4 liters of grape vinegar for 7 days at room temperature. The soaking procedure was carried out once every seven days for three months. To maintain the extraction efficiency, fresh grape vinegar was substituted for the vinegar at each interval (Castro and Guerrero, 2021).

Inclusion/ exclusion criteria

Participants in the study were adults aged 18 to 50 years who had been diagnosed with cholelithiasis, confirmed by a radiologist through ultrasound testing. Participants of cholelithiasis presented with symptoms such as severe upper right abdominal pain, worsened by fatty foods, along with nausea, vomiting and jaundice due to bile duct blockage. Moreover, participants of cholelithiasis presented with infections that led to fever and chills, indigestion, back pain and clay-colored stools were also included. The exclusion criteria ensured a focused study on adults with untreated cholelithiasis, optimizing the assessment of VSFCF's effectiveness. Individuals with prior gallbladder surgery, previous gallstone medications, or those who were pregnant or lactating were excluded to avoid confounding variables, as these factors could alter treatment response or pose health risks. This approach clearly evaluated VSFCF as an alternative treatment for cholelithiasis.

Sample size estimation

The initial estimate for the required sample size in each group for the cholelithiasis study was 25 adults, determined using G* power software (version 3.1.9) (Beck 2013). The study had a moderate effect size ($d = 0.6$), a statistical power of 0.80 and a significance level of 0.05. However, to accommodate a 20% dropout rate, the sample size was adjusted to 30 subjects per group. This approach was guided by principles from Beck's work on power analysis.

Randomization and blinding

The study assigned eligible adults to therapeutic groups in a 1:1 ratio using the permuted block randomization

method. This technique ensures that the treatment groups remain balanced in size, which helps reduce selection bias and improve the robustness of the findings. Participants were allocated to either the VSFCF or standard treatment group based on a computer-generated random number list. This independent generation of random numbers enhances the transparency of the assignment process. The researcher responsible for randomization was not involved in other study activities, which minimizes potential bias in assigning participants. The design prevented any crossover between groups, ensuring that each treatment condition was maintained throughout the trial. This rigorous approach to randomization strengthens the validity of the results, allowing for a more precise assessment of the efficacy of the interventions being studied. It was a single-blinded study. The statistical analyzer was blinded so that the result obtained should be unbiased. The statistical analyst was kept blinded by treatment allocations when analyzing the data. This was done by coding the treatment groups (e.g., Group A and Group B) and we ensured that the analyzer did not have access to the key that links the codes to the actual treatments until after the analysis was completed. This approach helped to prevent biases throughout the trial.

Intervention

Participants in the treatment group were given VSFCF orally at a standard dose of 1g/kg/day for 3 months to assess the treatment's effects. Conversely, the control group is administered ursodiol 600mg per day (Safadi and Mahabadi, 2019) as the standard drug, ensuring that any observed results in the treatment group are due to the intervention itself rather than the effects of ursodiol. Both groups adhere to the same schedule and duration of administration for a comparative analysis of outcomes.

Outcome measures

Sociodemographic data of participants regarding their age, sex distribution, mean size of gallstones, scoring of pain and bloating and digestive discomfort were recorded using a sociodemographic questionnaire. The radiologist assessed the primary outcome of the study, which was the changes in the gallstone size during baseline measurement and 3-month intervention measurement, using ultrasound techniques. This has helped assess the efficacy of treatment concerning the reduction in the size of gallstones. The secondary endpoints included assessment of structural changes in gallstones, assessment of symptoms of gallstones like pain, bloating and digestion discomfort using a numerical rating scale (0-10), symptomatic improvement and monitoring of adverse events and hepatic markers before and after the trial. All these outcomes collectively and comprehensively assessed the effect of treatment on the management of cholelithiasis.

Data collection and monitoring

Baseline data was obtained at the beginning and it contained demographic information, prior medical conditions, the characteristics of the gallstone, and the symptom score. Therefore, Follow-up visits would be attained for outcome measures and assessment of adherence to the treatment protocol, whereby participants were contacted or seen at regular intervals to monitor the outcomes and record any adverse events. These follow-up assessments were imperative in monitoring the study's progress, assessing the participants response to intervention and keeping records of measures taken for safety and efficacy during the study period.

STATISTICAL ANALYSIS

The data was analyzed using SPSS software (version 16.0), employing repeated measures ANOVA and t-tests. Additionally, the calculations included mean values and standard deviations. A p-value of less than 0.05 was deemed statistically significant.

RESULTS

The study comprises 50 patients who were randomly assigned into two groups. A flow sheet diagram showing the patients' recruitment has been shown in fig. 1.

Baseline characteristics

At the baseline, the treatment and control groups showed no statistically significant differences in age, sex distribution, mean size of gallstones, and scoring of pain and bloating, as well as digestive discomfort. This indicates that both groups were well-matched demographically and clinically, providing a solid foundation for comparing treatment efficacy (table 1).

Changes in gallstone size

Throughout the study, the treatment group experienced a consistent and significant reduction in gallstone size compared to the control group. This reduction became more pronounced over time, with the treatment group showing a markedly faster decrease in gallstone size at each measured interval than the control group. The difference in size reduction between the two groups was statistically significant from the 15-day mark onwards, with the treatment group consistently outperforming the control group regarding gallstone size reduction (table 2). The ultrasound images were used to compare gallbladder conditions in treatment and control groups before and after treatment. The post-treatment images show more significant improvement in the treatment group than the control group, as shown in fig. 2.

Symptoms improvement

The comparison shows symptom scores for pain, bloating, and digestive discomfort between the treatment and control groups over time. Initially, both groups had similar scores. By 45 and 90 days, the treatment group showed significantly greater improvements in all

Table 1: Baseline characteristics

Parameters	Treatment Group (n = 25)	Control Group(n = 25)	P- value
Age (years) Mean ± SD	45.2±5.6	44.8±6.2	0.68
Gender (M/F) n (%)	7/18 , 28% / 72%	10/15, 40% / 60%	0.52
Gallstone size (mm) Mean ± SD	15.5±2.3	16.1±2.5	0.46
Pain (0-10)	7.3±1.5	7.1±1.4	0.65
Bloating (0-10)	6.9±1.3	7.2±1.2	0.42
Digestive discomfort (0-10)	7.5±1.6	7.0±1.3	0.18

SD: Standard Deviation; M: Male; F: Female

Table 2: Changes in gallstone size and composition over time: treatment versus control group

Time point	Treatment group (n = 25)	Control group (n = 25)	P value
Baseline size (mm)Mean ± SD	15.5±2.3	16.1±2.5	0.462
15 Days size (mm)Mean ± SD	13.2±2.2	15.9±2.4	0.018
30 Days Size (mm)Mean ± SD	11.8±1.9	14.8±2.3	0.015
45 Days Size (mm)Mean ± SD	10.4±1.7	13.5±2.1	0.003
60 Days Size (mm)Mean ± SD	9.0±1.5	12.1±1.9	0.002
75 Days Size (mm)Mean ± SD	8.2±1.1	11.3±1.7	0.001
90 Days Size (mm)Mean ± SD	7.3±1.0	10.8±1.5	< 0.001

SD: Standard deviation

Table 3: Symptom scores over time: treatment and control groups

Symptoms	Time Point	Treatment Group (n = 25)	Control Group(n = 25)	P value
Pain (0-10)	Before the trial	7.3±1.5	7.1±1.4	0.65
	After 45 days	5.3±1.1	6.3±1.0	0.01
	After 90 days	3.1±0.8	4.9±1.1	<0.001
Bloating (0-10)	Before the trial	6.9±1.3	7.2±1.2	0.42
	After 45 days	4.8±0.4	5.9±0.9	0.02
	After 90 days	2.9±0.2	4.3±0.7	<0.001
Digestive discomfort (0-10)	Before the trial	7.5±1.6	7.0±1.3	0.18
	After 45 days	5.4±0.7	6.1±1.0	0.01
	After 90 days	2.8±0.3	4.5±0.9	<0.001

symptoms compared to the control group, with P-values indicating statistical significance: less than 0.05 at 45 days and less than 0.001 at 90 days, as shown in the table 3.

Adverse events and heptometabolic parameters

The table 4 presents a comparison of adverse events and various hepatic and inflammatory markers between a treatment group and a control group over time. The adverse events were lower in the treatment group compared to the control group after treatment, though the difference was not statistically significantly. Hepatic markers, including total bilirubin, ALT, AST and ALP, showed significant improvement in the treatment group over 45 and 90 days compared to the control group. Specifically, total bilirubin and ALT levels decreased significantly in the treatment group, while AST and ALP levels showed an increasing trend in the short term but eventually decreased. Additionally, CRP levels, an indicator of inflammation, significantly decreased in the treatment group over time, suggesting a reduction in inflammation compared to the control group. Overall, the treatment group demonstrated notable improvements in liver function and reduced inflammation compared to the control group.

DISCUSSION

Cholelithiasis is gallstone formation in the gallbladder. Bile cholesterol and bilirubin form gallstones in the gallbladder. Patients may experience cholecystitis, biliary obstruction, and biliary colic (Doherty *et al*., 2022). Gallstones are a serious health issue for 10% to 15% of persons in affluent nations. Estimated prevalence in sub-Saharan Africa is under 5%. Up to 80% of gallstone patients do not develop acute cholecystitis, cholangitis, or pancreatitis (Littlefield and Lenahan, 2019). Women have more gallstones than men, although both get them with age. After 40, men's prevalence raises 1-3% per decade (Zhang *et al*., 2024). According to Wang *et al*. 2024, 10-15% of men over 60 may have gallstones, compared to a lower frequency in younger men. Due to increased availability to and usage of abdominal ultrasonography, the incidence in general pediatric populations ranges from 0.1% to 1.0%.

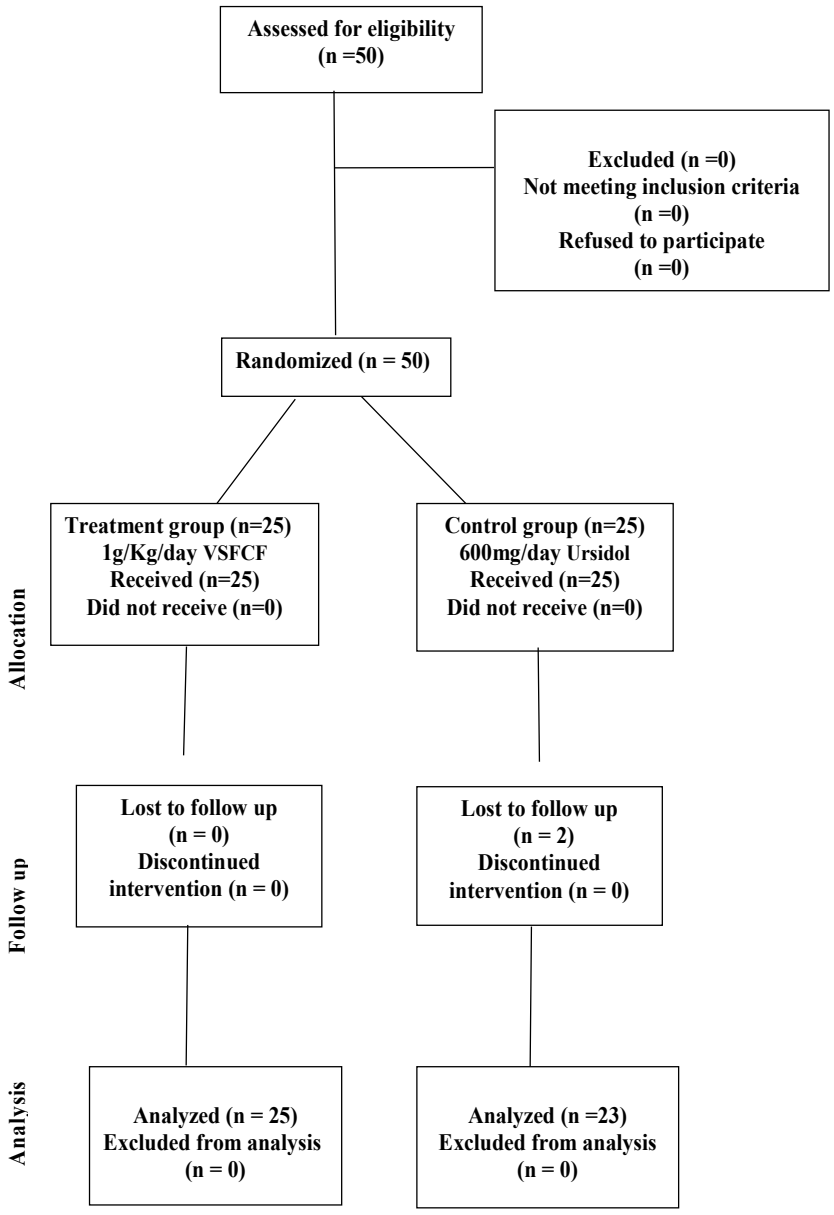

Fig. 1: This figure represents the CONSORT flow diagram of the study

Children have an asymptomatic gallstone rate of 40% to 51%, suggesting they are more prone than adults to have symptoms. Research shows that obesity, biliary tract abnormalities, parenteral nutrition and hemolytic diseases are more common in youngsters. Gallstones were very common in sickle cell disease children in Sudan and Ghana (Mohamed *et al*., 2021).

Antioxidant-rich vinegars may lower the risk of degenerative diseases like cancer and cardiovascular disease (Kandylis *et al*., 2021). Grape vinegar has more antioxidant activity than other vinegars (Antoniewicz *et al*., 2021). Acetic acid, tartaric, formic, lactic and malic acids impart flavor to these vinegars (Yildiz, 2023). Acetic acid dissolves cholesterol gallstones by increasing cholesterol solubility and changing bile composition, preventing new stones. *In vitro* and clinical experiments demonstrate that acetic acid improves lipid metabolism and lowers blood cholesterol, supporting its use in gallstone treatment. *F. carica* (fig) contains about 100 bioactive components, such as amyrins, β-carotenes and β-sitosterols, which enhance cardiovascular health and cholesterol management (Hussain *et al*., 2021). Its extracts contain phenolic components, flavonoids, polyphenols, alkaloids, and minerals such iron, magnesium and strontium (Ghaffari and Fattouch, 2023). Amyrins increase cholesterol solubility in bile and reduce gallstone-related gallbladder and biliary tract inflammation. Modulating enzymes like HMG-CoA reductase and bile acid composition may increase cholesterol solubilization. Amyrins can also combine with cholesterol to make it more soluble and excretable.

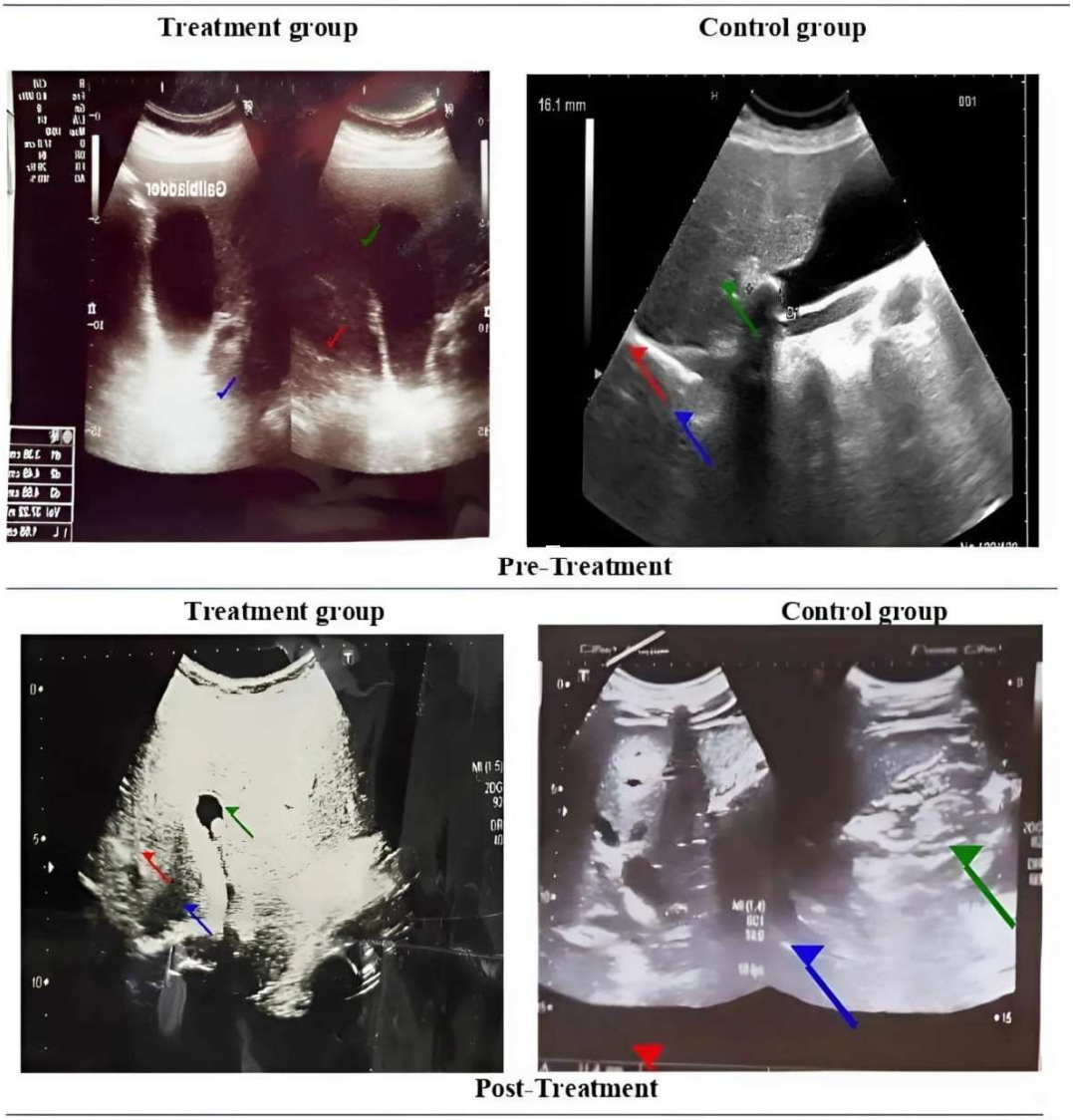

Fig. 2: Pre-treatment ultrasound images showing gallstones (red arrows), thickened gallbladder wall (blue arrows) and dilated biliary duct (green arrows). Post-treatment images demonstrate the resolution of gallstones, normalization of the gallbladder wall thickness and reduction in biliary duct dilation.

Research suggests that magnesium-rich plants like *F. carica* may treat cholelithiasis. This combination dissolves gallstones and prevents new ones (Luo *et al*., 2024). *F. carica*'s anti-cholelithiasis action may be due to its magnesium content (Du *et al*., 2024).

VSFCF was compared to ursodiol for treating cholelithiasis in adults. The intervention group showed a significant reduction in gallstone size over 90 days, with a 52.90 percent decrease ($p<0.001$) compared to a 32.92 percent decline in the control group ($p<0.01$). This shows that VSFCF may be a gallstone treatment option due to its high bioactive chemical content, notably antioxidant-rich phenolic compounds and flavonoids (Wang *et al*., 2023).

The treatment group experienced a 57.5% drop in pain levels, while the control group only saw a 30.9% reduction ($p<0.001$). Significant improvements in bloating and digestive discomfort were seen ($p<0.001$). Neither VSFCF nor ursodiol groups had substantial adverse effects (4% vs. 16%, $p=0.12$). The treatment group experienced significant hepatic marker improvements, including decreased total bilirubin, ALT, AST and ALP levels ($p<0.05$ to $p<0.001$) and reduced C-reactive protein levels ($p<0.01$ at 45 days and $p<0.001$ at 90 days), indicating reduced inflammation. These data imply that VSFCF may dissolve gallstones, improve liver function and reduce inflammation, making it a promising alternative or adjunct therapy for cholelithiasis.

These findings demonstrate VSFCF's benefits compared to other research. Traditional Chinese medicine formulations for cholelithiasis showed only modest gallstone size and symptom relief, suggesting that while

Table 4: Comparison of adverse events and hepatic markers between treatment and control groups over time

Parameters	Time point	Treatment group (n = 25)	Control group (n = 25)	P value
Adverse events (%)	After treatment	1(4%)	4(16%)	0.12
		Hepatic marker		
Total bilirubin (mg/ld.) Mean ± SD	Before the trial	0.8±0.3	0.8±0.3	0.95
	After 45 days	0.28±0.2	0.8±0.3	0.01
	After 90 days	0.18±0.1	0.7±0.3	<0.001
ALT (U/L)Mean ± SD	Before the trial	30±6.0	29.2±5.8	0.92
	After 45 days	21±5.3	29.0±5.7	0.02
	After 90 days	15±4.1	28±5.5	<0.001
AST (U/L)Mean ± SD	Before the trial	26±5.2	26.2±5.4	0.95
	After 45 days	29±4.3	26±5.3	0.02
	After 90 days	22±3.1	25±5.0	0.01
ALP (U/L)Mean ± SD	Before the trial	78.5±12.2	78.0±12.0	0.95
	After 45 days	98.0±10.1	78.0±12.0	0.03
	After 90 days	85.4±9.3	77.0±12.0	0.04
CRP (mg/L)Mean ± SD	Before the trial	10.0±2.5	9.8±2.4	0.88
	After 45 days	5.2±1.8	9.7±2.3	0.01
	After 90 days	3.1±1.2	9.5±2.1	<0.001

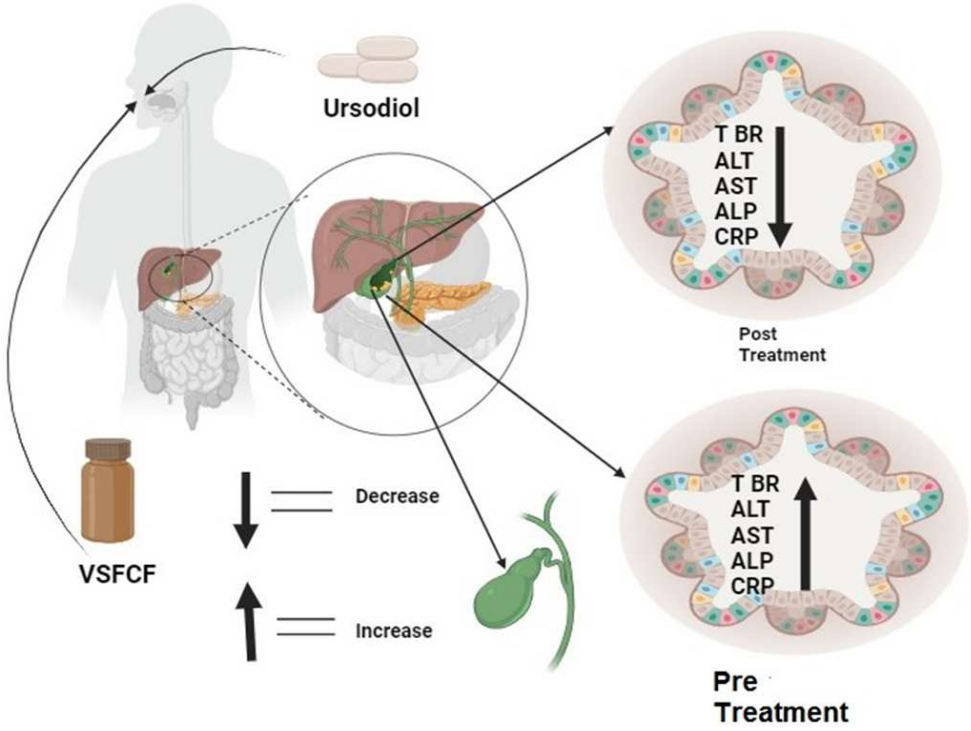

Fig. 3: Proposed mechanism of anti-cholelithiasis of VSFCF in humans

they may be beneficial, VSFCF is more effective (Chen *et al*., 2019). Statins can reduce cholesterol gallstones, but they can also cause liver damage and gastrointestinal issues, unlike VSFCF (Khan *et al*., 2022). Previous studies have shown that *F. carica* can be used as a therapeutic agent due to its high bioactive compound content, including antioxidant and anti-inflammatory phenolic compounds and flavonoids (Gull *et al*., 2024). The improved gallstone size reduction and safety of VSFCF imply it may be a promising alternative to current cholelithiasis treatments. The efficacy and low side effects of herbal therapies like VSFCF warrant further study, especially considering the growing interest in integrative therapy for gallstone disease. The results are promising, but there are some caveats. A larger sample size could have reduced the generalizability of this study's findings. Second, VSFCF's long-term safety and efficacy require longer-term follow-up beyond the 90-day research. This study also needs to precisely analyze bioactive components that could cause the observed effects. Future research will focus on VSFCF's therapeutic mechanisms.

The results are promising, but there are some caveats. A larger sample size could have reduced the generalizability of this study's findings. Second, VSFCF's long-term safety and efficacy require longer-term follow-up beyond the 90-day research. This study also needs to precisely analyze bioactive components that could cause the observed effects. Future research will focus on VSFCF's therapeutic mechanisms.

CONCLUSION

In conclusion, this study provides evidence that VSFCF significantly diminish gallstone size while also changing its composition, implying it may be used to handle cholelithiasis effectively and safely. The positive findings require further research into making VSFCF an effective treatment for this ailment in clinical practice.

ACKNOWLEDGEMENT

We would like to extend our heartfelt appreciation to Noreen Nishat Welfare Hospital for their exceptional support and collaboration during this study.

REFERENCES

Achufusi TG, Safadi AO and Mahabadi N (2019). Ursodeoxycholic acid. 545303.

Ali A, Perveen S, Khan I, Ahmed T, Nawaz A and Rab A (2021). Symptomatic gallstones in young patients under the age of 30 years. *Cureus*, **13**: 19894.

Antoniewicz, Jakubczyk K, Kwiatkowski P, sMaciejewska-Markiewicz D, Kochman J, Rębacz-Maron E and Janda-Milczarek K (2021). Analysis of antioxidant capacity and antimicrobial properties of selected polish grape vinegars obtained by spontaneous fermentation. *Molecules*, **26**: 4727.

Beck and Travis W (2013). The importance of a priori sample size estimation in strength and conditioning research. *J. Strength Cond. Res*., **27**: 2323-37.

Chen GL, Zheng FJ, Lin B, Yang YX, Fang XC, Verma KK and Yang LF (2023). Vinegar: A potential source of healthy and functional food with special reference to sugarcane vinegar. *Front. Nutr*., **10**: 1145862.

Chen Q, Zhang Y, Li S, Chen S, Lin X, Li C and Asakawa T (2019). Mechanisms underlying the prevention and treatment of cholelithiasis using traditional Chinese medicine. *Evid. Based Complement. Alternat. Med*., **2019**: 2536452.

Doherty G, Manktelow M, Skelly B, Gillespie P, Bjourson AJ and Watterson S (2022). The need for standardizing diagnosis, treatment and clinical care of cholecystitis and biliary colic in gallbladder disease. *Medicina*, **58**: 388.

Du W, Yan C, Wang Y, Song C, Li Y, Tian Z, Liu Y and Shen W (2024). Association between dietary magnesium intake and gallstones: The mediating role of atherogenic index of plasma. *Lipids Health Dis*., **23**: 82.

Fazel, MF, Abu IF, Mohamad MH, Mat Daud NA, Ahmad Najib Hasan AN, Aboo Bakkar Z, Md Khir MA, Juliana N, Das S and Mohd Razali MR (2024). Physicochemistry, nutritional and therapeutic potential of *Ficus carica*: A promising nutraceutical. *Drug Des. Devel. Ther*., **18**: 1947-1968.

Fleishman JS and Kumar S (2024). Bile acid metabolism and signaling in health and disease: Molecular mechanisms and therapeutic targets. *Signal Transduct., Target. Ther*., **9**: 97.

Giudici P, DeVero L and Gullo M (2017). Vinegars. *In*: Acetic acid bacteria: Fundamentals and Food Applications; Sengun, IY, Ed. CRC Press Taylor & Francis Group 6000 Broken Sound Parkway NW, Suite 300, pp.54-61.

Grigor'eva IN and Romanova TI (2020). Gallstone disease and microbiome. *Microorganisms*, **8**: 835.

Gull I, Khan IA, Malik A, Bukhari M, Iqbal MO, Usman M, Hussain K, Khan MK and Anwar M (2024). Effect of laxative polyherbal paste for loperamide induced constipation in rats. *Am. J. Transl. Res*.,**16**: 4714.

Gutt C, Schläfer S and Lammert F (2020). The treatment of gallstone disease. *Dtsch. Arztebl. Int*., **117**: 148.

Hussain SZ, Naseer B, Qadri T, Fatima T, and Bhat TA (2021). Fig (*Ficus carica*) Morphology, taxonomy, composition and health benefits. *Fruits Grown Highland Reg. Himalayas Nutr. Health Benefits* Springer, pp.7-90.

Isa MM, Jaafar MN, Kasim KF and Mutalib MFA (2020). Cultivation of fig (*Ficus carica* L.) as an alternative high value crop in Malaysia: A brief review. *IOP Conf. Ser. Mater. Sci. Eng*., **012134**. IOP Publishing.

Jin K, Mi N, He W, Zhong R, Jin B, Liu Z, Dong C, Lin Y, Yue P and Xia B (2024). Dietary patterns, genetic predisposition and risk of cholelithiasis: A large-scale prospective cohort study. *Front. Nutr*., **11**: 1469789.

Jones MW, Weir CB and Ghassemzadeh S (2024). Gallstones (cholelithiasis). In*StatPearls [Internet]* (StatPearls Publishing).

Kandylis P, Bekatorou A, Dimitrellou D, Plioni I and Giannopoulou K (2021). Health promoting properties of cereal vinegars. *Foods*, **10**: 344.

Khan IA, Hussain M, Hussain N, Alqahtani AM and Alqahtani T (2022). Cardioprotective effect of *Rumex vesicarius* Linn. leaf extract against catecholamine-induced cardiotoxicity. *Molecules*, **27**: 3383.

Littlefield A and Lenahan C (2019). Cholelithiasis: Presentation and management. *J. Midwifery Women's Health*., **64**: 289-97.

Luo Z, Zhou W, Xie T, Xu W, Shi C, Xiao Z, Si Y, Ma Y, Ren Q and Di L (2024). The role of botanical triterpenoids and steroids in bile acid metabolism, transport and signaling: Pharmacological and toxicological implications. *Acta Pharm. Sin. B,* **14**: 3385-3415.

Luzón-Quintana LM, Castro R and Duran-Guerrero E (2021). Biotechnological processes in fruit vinegar production. *Foods*, **10**: 945.

Mohamed SO, Ibrahim OA, Mohammad DA and Ali AH (2021). Correlates of gallbladder stones among patients with sickle cell disease: A metaanalysis. *JGH Open*, **5**(9): 997-1003.

Mohammad H and Alzweiri M (2022). Phytochemistry and pharmacological activities of *Ficus carica* latex: A systematic review. *J. Chin. Pharm. Sci*., **31**: 81-96.

Nasir A, Zulfiqar T, Ali A and Zafar H (2021). Prevalence of gallstone disease and its correlation with age among people undergoing abdominal ultrasound in Gujranwala. *EASJ Radiol Imaging Technol*., **3**: 142-45.

Ousaaid D, Mechchate H, Laaroussi H, Hano C, Bakour M, Ghouizi AE, Conte R, Lyoussi B and Arabi IE (2021). Fruits vinegar: Quality characteristics, phytochemistry, and functionality. *Molecules*, **27**: 222.

Portincasa P, Ciaula AD, Bonfrate L, Stella A, Garruti G, and Lamont JT (2023). Metabolic dysfunction-associated gallstone disease: Expecting more from critical care manifestations. *Intern. Emerg. Med*., **18**: 1897-918.

Qu Q, Chen W, Liu X, Wang W, Hong T, Liu W and He X (2020). Role of gallbladder-preserving surgery in the treatment of gallstone diseases in young and middle-aged patients in China: Results of a 10-year prospective study. *Surgery*, **167**: 283-89.

Rasool IF, Aziz A, Khalid W, Koraqi H, Siddiqui SA, Al-Farga A, Lai WF and Ali A (2023). Industrial application and health prospective of fig (*Ficus carica*) by-products. *Molecules*, **28**: 960.

Rebai O, Ghaffari O and Fattouch S (2023). Fig (*Ficus carica*) drying technologies. *In*: Fig *(Ficus carica):* Production, Processing and Properties (Springer), **2023**: 665-688.

Sandhu AK, Islam M, Edirisinghe I, and Burton-Freeman B (2023). Phytochemical composition and health benefits of figs (fresh and dried): A review of literature from 2000 to 2022. *Nutrients*, **15**: 2623.

Shrestha B and Dunn L (2020). The declaration of Helsinki on medical research involving human subjects: A review of seventh revision. *J. Nepal Health Res. Council.*, **17**(4): 548-552.

Walia A, Kumar N, Singh R, Kumar H, Kumar V, Kaushik R and Kumar AP 2022. Bioactive compounds in Ficus fruits, their bioactivities and associated health benefits: A review. *J. Food Qual*., **2022**: 6597092.

Wang J, Gull I, Kousar S and Shahzad R (2023). Evaluation of anti-tyrosinase activity of *Pyrus communis* leaves extract and cosmetic formulation. *Int. J. Pharmacol*., **19**: 834-841.

Wang X, Yu W, Jiang G, Li H, Li S, Xie L, Bai X, Cui P, Chen Q and Lou Y (2024). Global epidemiology of gallstones in the 21st Century: A systematic review and Meta-analysis. *Clin. Gastroenterol. Hepatol.,* **22**: 586-1595.

Yildiz E (2023). Characterization of fruit vinegars via bioactive and organic acid profile using chemometrics. *Foods*, **12**: 3769.

Zdanowicz K, Bobrus-Chcociej A, Pogodzinska K, Blachnio-Zabielska A, Zelazowska-Rutkowska B, Lebensztejn DM and Daniluk U (2022). Analysis of sphingolipids in pediatric patients with cholelithiasisa preliminary study. *J. Clin. Med*., **11**: 5613.

Zhang J, Liang D, Xu L, Liu Y, Jiang S, Han X, Wu H and Jiang Y (2024). Associations between novel anthropometric indices and the prevalence of gallstones among 6,848 adults: A cross-sectional study. *Front. Nutr*., **11**: 1428488.