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Full-Length Research Article

Comparative Effects of Papain and Fucoidan on Biliary Flow Rate and Electrolyte Concentration in Wistar Rats

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Summary: Papain and Fucoidan are extracted enzyme and a sulfated polysaccharide, respectively, that have gained recognition across the food industry, pharmaceutical and digestive applications, but their comparative effects on Biliary flow rate and electrolytes are not fully elucidated. Hence, this study compared the effects of Papain and Fucoidan on Biliary flow rate and electrolytes. Twenty (20) male albino Wistar rats weighing between 100g and 150g were divided into 4 groups of 5 animals each as follows: Group I (Control group) were administered normal saline and had access to food and water *ad libitum*. Group II (Papain group) was administered 100mg/kg body weight of the stock solution of Papain and were allowed free access to food and water. Group III (Fucoidan group) was given 200mg/kg body weight of the Fucoidan stock solution, with food and water. Group IV (Metoclopramide group) was administered 3mg/kg body weight of Metoclopramide stock solution, with food and water *ad libitum*. The administration lasted 28 days. Bile was collected from the liver (hepatic duct) and bile flow rate and its composition were determined. Papain significantly increased biliary sodium and chloride levels, reduced bicarbonate levels, and had no significant effect on bile flow rate compared with the control group. Fucoidan, on the other hand, has a significant impact on Biliary sodium, chloride, and bile flow rate compared with the control group. These results show that the two compounds have regulatory, but not stimulatory, effects on biliary secretion.

Keywords*:* Inflammation, Fucoidan, Papain, bile flow rate, biliary electrolyte

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INTRODUCTION

Bile is a vital digestive fluid produced by the liver and stored in the gallbladder. It plays a crucial role in the digestion and absorption of fats and fat-soluble vitamins in the small intestine (Moini & Ferdowsi, 2024). It is a complex fluid with several components, such as bile salts (which are synthesized from cholesterol in the liver for enhancing fat digestion and absorption) (Moghimipour *et al.,* 2015; Di Ciaula *et al.,* 2018), bilirubin (which is a by-product of the breakdown of hemoglobin from old red blood cells) (Dzulkifli *et al.*, 2018; Čepelak *et al.,* 2025), cholesterol, and phospholipids (which solubilizes cholesterol and bile salts) (Enright *et al.,* 2020; Wang *et al.,* 2023).

Enzymes are natural catalysts of all biochemical reactions, and they are produced by all living cells (Kellis & Lad, 2016; Bhatia & Bhatia, 2018). For many centuries, the role of plant-derived enzymes has been recognized. Even before the discovery of enzymes, some plant parts and their extracts have also been used in various traditional processes, such as meat softening, using the *Carica papaya* plant (Saeed *et al.,* 2017; Mohd *et al.,* 2023). Some of these plant-derived enzymes are also used as food additives because of consumer preference for clean-label foods and the need for bioactive compounds that improve gastrointestinal and metabolic health (Mohd *et al.,* 2023). These plant-derived enzymes, such as Papain, can be consumed without processing to preserve their full functionality. However, there is still a long way to go in realizing the full potential of plant-derived enzymes and their effects on bile flow and its composition.

Papain is derived from *Carica papaya* (Babalola *et al.,* 2024; Choudhary *et al.,* 2025). It was named in the late 19th century by Wurtz and Boucht, who partially purified the product from papaya sap and recognized it as a proteolitic active constituent of the latex of tropical papaya fruit (Lapuente Salinas, 2021). Papain has been classified as a cysteine protease that processes proteins more broadly than pancreatic proteases (Malek *et al.,* 2016; Verma *et al.,* 2016). Papain has a significant proteolytic action on amino acid

esters, amide bonds, and proteins. In recent years, proteases have arisen as standard biocatalysts in many industrial processes (Naveed *et al.*, 2021; Aruna *et al.*, 2023).

Papain helps in the digestion of proteins, regulates the intestinal function, and has also proven effects in a range of experimental tests (Rahmani *et al.,* 2016; Babalola *et al.,* 2024). In addition to its enzymatic effect on digestion, Papain could modify bile production by enhancing hepatic enzyme activities and altering secretion pathways (Wiszniewsk *et al.*, 2022). However, the exact impact of Papain on biliary flow rate and electrolyte composition has not been thoroughly studied.

On the other hand, Fucoidan, a sulfated polysaccharide found primarily in brown seaweeds (Phaeophyceae), (Ponce & Stortz, 2020; Usov et al., 2022) was first isolated in the early 19th century (Stefaniak–Vidarsson *et al.*, 2017). The term "fucoidan" is derived from the genus name *Fucus*, which includes several species of brown algae (Ponce & Stortz, 2020). Earlier studies have examined its chemical structure and properties (Kopplin *et al.,* 2018; Zayed *et al.,* 2020). In recent years, interest among scientists in Fucoidan has increased significantly due to its diverse biological activities. It has been proven that Fucoidan has antioxidant, anticoagulant, antiviral, antitumor and immunomodulatory activities (Apostolova *et al.,* 2020; Hwang *et al.*, 2022). These bioactivities culminate in Fucoidan being a promising compound in pharmaceutical, nutraceutical and cosmetic uses. In addition, its biocompatibility and biodegradability are significant advantages for its use as a natural therapeutic agent (Barbosa *et al.,* 2019).

Since bile plays a critical role in digestive and metabolic processes (Moini & Ferdowsi, 2024), the effects of natural bioactive compounds on biliary physiology are of significant scientific and therapeutic interest. The paper thus seeks to compare the effects of Papain and Fucoidan on biliary flow rate and electrolyte composition. The rationale for this comparison is to determine whether enzymatic food additives and polysaccharide-based functional ingredients differentially regulate biliary flow and electrolyte composition. The results of this study can shed light on the potential use of these natural compounds to treat biliary and hepatic dysfunction.

MATERIALS AND METHODS

Ethical Approval: Ethical approval was secured from the Research Ethics Committee (REC) of the Faculty of Basic Medical Sciences, University of Calabar, in accordance with the Guide for the Care and Use of Laboratory Animal, with approval number: 484PHY1925.

Animal Care: A total of twenty male Wistar rats, weighing between 100 to 150g, were used for this experiment. The rats were obtained from the animal house of the Physiology department at the University of Calabar. The animals were housed in well-ventilated cages lined with sawdust as bedding, which was changed every day. During their acclimatization process, which lasted for one week at the animal house, the rats were given a commercial rodent diet (pelletized grower feed) and water (Unim *et al.,* 2024).

Animal Grouping: The experimental animals were grouped as follows:

1. Group 1 (Control Group): Rat feed and water.
2. Group 2 (Papain Group): Papain (100mg/kg)
3. Group 3 (Fucoidan Group): Fucoidan (200mg/kg)
4. Group 4 (Metoclopramide Group): Metoclopramide (3mg/kg)

Administration of Experimental Drug: Papain (Batch no: 87398214) and Fucoidan (Batch no: 0809262) extract powder were purchased from Yiwu Maple Craft Co., Ltd., Zhejiang province, China. Both extracts were formulated using normal saline at a dose of 800mg/kg body weight, respectively. Metoclopramide was obtained from a local pharmacy (Betz Pharmacy) at Eta Agbor, Calabar, Cross River State, Nigeria. It was formulated using normal saline into a stock solution and administered once daily at a dose of 30mg/kg body weight. All experimental drugs (Papain, Fucoidan and metoclopramide) were administered orally using an oral gavage tube for a duration of 28 days.

Collection of Biliary Secretion and Calculation of Biliary Flow Rate (BFR): Biliary secretion was collected using the method of Vicker *et al.* (1998). The rats were weighed and anaesthetized by intraperitoneal administration of 23% urethane (0.6ml/100g body weight). They were quickly pinned to a dissecting board for a tracheostomy performed to clear the airways for easy breathing. A pair of scissors was used to reduce the fur around the abdomen. The abdominal region was incised along the *linea alba* to prevent bleeding and expose the contents of the abdomen.

The liver was then located, and the lobes were deflected aside to expose the common bile duct. A piece of white thread was tied around the bile duct to allow bile accumulate in the tiny bile duct to enhance cannulation. The rats were then allowed for 2-3 minutes for bile secreted from the liver to accumulate in the common bile duct. The common bile duct was then cannulated with a Portex cannula (0.5mm in diameter) after a semi-circular incision was made using a pair of scissors. The cannula was ligated in place with two pieces of thread tied around the bile duct. Ligation was a precautionary measure to prevent the cannula from falling off. Plain, simple bottles were now placed where the cannuli discharged. Bile was collected for 3 hours from each rat in each group. The bile secreted by each rat was then measured afterwards and sent to the laboratory for analysis.

The Biliary Flow Rate (BFR), expressed in mL/min, was then calculated for each group using the formula:

$$\textit{Biliary Flow Rate (BFR)} = \frac{\textit{Collected Volume}}{\textit{Collection Time}}$$

Determination of Biliary Electrolytes (Sodium and Potassium): Sodium (Na^+) and potassium (K^+) in bile were determined by flame photometry (model 41°C, Petra Court Ltd., England). The bile was sprayed into a non-luminous gas flame, which changed the colour in response to the metallic ions (cations and anions) present in the bile sample. The non-luminous gas and absorbance read at 598nm and 767nm, respectively, for sodium (Na^+) and potassium (K^+). The light was allowed to fall on a photosensitive detection system (Okpe et al., 2013).

Determination of Biliary Sodium Ion Concentration (Flame Photometry Method): The sodium light filter was inserted, and the galvanometer switched on. The light supply was set to full, the flame ignited, and the air supply at 10 ib/inch was noted. Afterwards, the gas was smoothly adjusted to obtain a discrete flame dome. A few minutes were allowed for warming up. The zero on the galvanometer scale was set with water, and the galvanometer reading was

set to 80 using the standard. The test was read after checking the standards after 2-3 test readings. Biliary sodium was obtained from the following calculation and expressed in mmol/L (Unim et al., 2024).

$$\textit{Calculated sodium (mEq/L)} = \frac{\textit{Reading of Gavanometer}}{X^2}$$

Where X = Dilution factor

Determination of Biliary Potassium Ion Concentration (Flame Photometry Method): The potassium light was inserted, the galvanometer was set with a potassium working standard at 70, and the test was read after checking the standard about 2-3 times. Biliary potassium was obtained from the following calculation and expressed in mmol/L (Obbed et al., 2024).

$$\textit{Potassium (mEq/L)} = \frac{\textit{Reading of Gavanometer}}{X^2}$$

Where X = Dilution factor

Determination of Biliary Chloride Concentration (End point titration method)

Biliary chloride was determined by the principle of the endpoint titration method. 2mL of buffer solution was placed in a conical flask, and 0.2mL of bile was added and mixed. Four drops of Diphenylcarbazone were used as the indicator. This was titrated with mercury nitrate from a 2mL micropipette. The endpoint was indicated by a violet colour. The standard solution (0.2mL) was added to a 2mL buffer solution, and the indicator was added and similarly titrated (Jayant, 1967). Biliary chloride concentration was obtained from the following calculation and expressed in units of mmol/L.

$$\textit{Biliary chloride} = \frac{\textit{Titrated value of test x 100}}{\textit{Titrated value of the standard}}$$

Determination of Biliary Bicarbonate Concentration

Biliary bicarbonate ion was measured by a modified method of Forester *et al.* (1976). Phosphenol pyruvate, carboxylase (PEPC) brings about the catalysis of the reaction between phosphenol pyruvate, carboxylase, pyruvate and carbon dioxide (bicarbonate) to form oxaloacetate and phosphate ions and at the same time, oxidation of an equimolar amount of reduced NADH (Nicotinamide adenine dinucleotide) to NAD^+. The reaction is catalyzed by malate dehydrogenase (MDH), leading to a decrease in absorbance at 340nm that is directly proportional to CO_2 concentration in the sample.

Statistical Analysis

The results were presented as Mean ± SEM. The results were analyzed using one-way analysis of variance (ANOVA), followed by a post hoc test (least square deviation) using computer software, SPSS version 18.0 and Excel. Results were considered significant at $p < 0.05$.

RESULTS

Result of Biliary Flow Rate in the experimental and control groups: Measurement of biliary flow rate showed no statistically significant difference among the test groups. The control group showed a bile flow rate of 0.52 ± 0.07 mmol/L, while the papain- and fucoidan-treated groups displayed slightly higher values of 0.54 ± 0.08 mmol/L and 0.56 ± 0.07 mmol/L, respectively. The metoclopramide-treated group also showed no significant deviation from the control, as shown in Figure 1

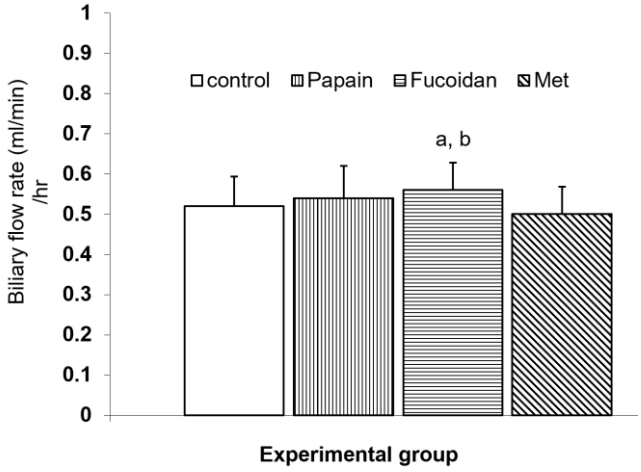

Figure. 1:
Biliary Flow Rate
Values are expressed as mean ± SEM, n = 5
a = p<0.05 vs control; b = p<0.05 vs Papain

Sodium (Na^+) Concentration in the experimental and control groups: The biliary sodium concentration was 131.40 ± 3.16 mmol/L in the control group, 132.20 ± 1.80 mmol/L in the papain-treated group, and 122.40 ± 1.57 mmol/L in the fucoidan-treated group, as displayed in Figure 2. A slight increase was observed in the papain group, whereas a mild reduction was shown in the fucoidan group, which when compared to the papain group showed significant difference ($p<0.05$).

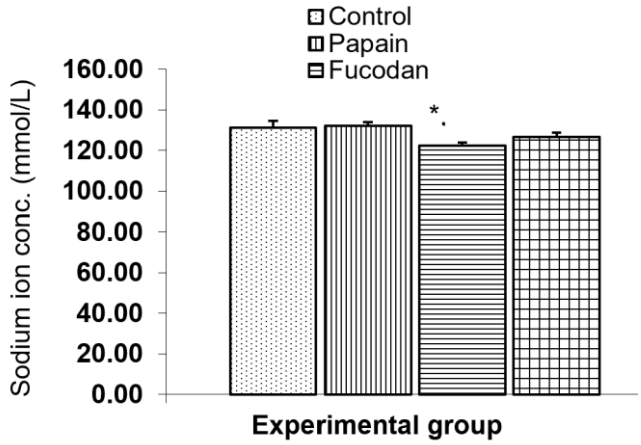

Figure 2:
Comparison of biliary sodium ion concentration between papain and fucoidan experimental groups.
Values are expressed as mean ± SEM, n = 5.
* = p<0.05 vs control; a = p<0.05 vs Papain

Potassium (K^+) Concentration in the experimental and control groups: Potassium levels in bile were 4.38 ± 0.23 mmol/L in the control group, 4.10 ± 0.10 mmol/L in the papain group, 4.44 ± 0.40 mmol/L in the fucoidan group, and 3.78 ± 0.07 mmol/L in the metoclopramide group (Figure 3). Minor variations were observed among the treatment groups compared with the control which were significant.

Chloride (Cl^-) Concentration in the experimental and control groups: The chloride concentrations were 98.40 ± 4.77 mmol/L in the control group, 101.40 ± 2.60 mmol/L in the papain group, 87.60 ± 6.27 mmol/L in the fucoidan group, and 102.00 ± 1.10 mmol/L in the metoclopramide group (Figure 4). A decrease in chloride concentration was

observed in the fucoidan-treated group, while significant increases were recorded in the papain and metoclopramide groups.

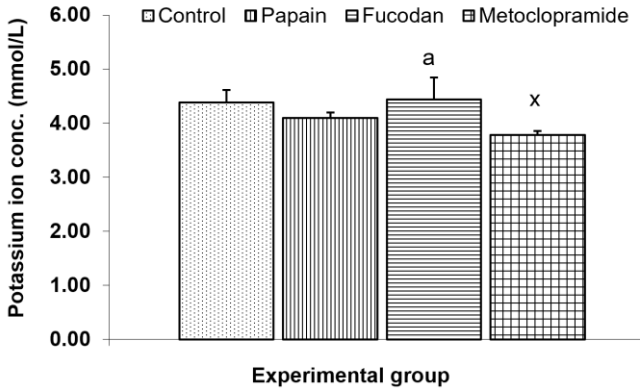

Figure 3:
Comparison of biliary potassium ion concentration between papain and fucoidan experimental groups.
Values are expressed as mean ± SEM, n = 5.
a = p<0.05 vs Papain; x = p<0.05 vs Fucodan

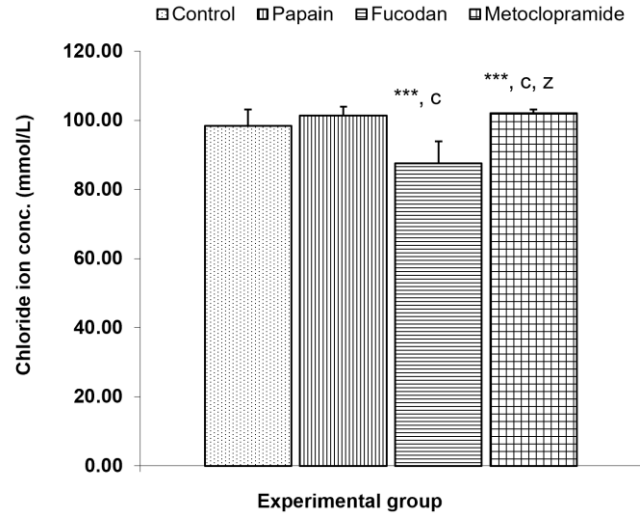

Figure 4:
Comparison of biliary chloride ion concentration in the different experimental groups.
Values are expressed as mean ± SEM, n = 5.
*** = p<0.001 vs control
c = p<0.001 vs Papain; z = p<0.001 vs Phycocyanin

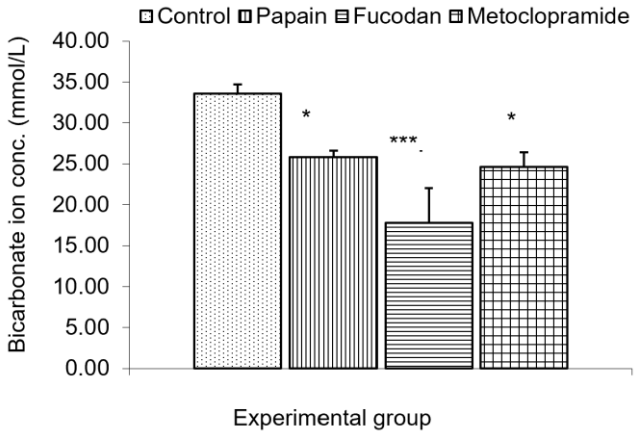

Figure 5:
Comparison of biliary bicarbonate ion concentration in the different experimental groups.
Values are expressed as mean ± SEM, n = 5.
* = p<0.05, *** = p<0.001 vs control; a = p<0.05 vs papain

Bicarbonate (HCO_3^-) Concentration in the experimental and control groups: Bicarbonate levels were 33.60 ± 1.12 mmol/L in the control group, 25.80 ± 0.80 mmol/L in the papain group, 17.80 ± 4.20 mmol/L in the fucoidan group, and 24.60 ± 1.83 mmol/L in the metoclopramide group (Figure 5). Both papain and fucoidan treatments resulted in reduced bicarbonate concentrations compared with the control.

DISCUSSION

The present study evaluated the effects of Papain and Fucoidan on biliary flow and electrolyte composition in experimental animals. The absence of a significant change in the bile flow in the papain and fucoidan groups suggests that neither Papain nor Fucoidan markedly stimulates or suppresses biliary secretion. This observation is consistent with the report of Zhang et al. (2024), who demonstrated that dietary polysaccharides such as Fucoidan exert only mild modulatory effects on bile secretion under physiological conditions. Similarly, although metoclopramide has been reported to enhance bile secretion in some experimental settings, the lack of this effect in the present study may be attributed to variations in dosage, exposure duration, as previously noted by Omar et al. (2024).

The sodium profile displayed a significant increase in papain-treated group when compared to fucoidan-treated group. Sodium plays a fundamental role in bile formation through its involvement in bile salt–dependent flow. The observed increase associated with Papain maybe probably linked to enhanced hepatocellular transport activity, supporting the findings of Wiszniewski et al. (2022), who reported improved hepatic metabolic and enzymatic functions following papain administration. Conversely, the reduction in sodium concentration observed in the fucoidan group is consistent with reports by Dimitrova-Shumkovska et al. (2020) and Zhao et al. (2023), they demonstrated that Fucoidan modulates ionic transport and limits sodium reabsorption, thereby contributing to osmotic balance within the biliary system.

Potassium levels showed only minimal variation across treatment groups, indicating preservation of ionic homeostasis. The significant increase observed in the fucoidan group maybe probably attributed to its sulfated polysaccharide structure, which may have enhanced membrane stability and ion exchange, as reported by Dimitrova-Shumkovska et al. (2020). In contrast, the reduction seen in the papain-treated group when compared to the fucoidan aligns with findings by Pal et al. (2018), who suggested that proteolytic enzymes may transiently alter ionic gradients without inducing pathological disruption. These observations suggest that both agents maintain potassium balance within physiologically acceptable limits.
Chloride concentration was significantly reduced in the fucoidan-treated group when compared with the papain and metoclopramide groups. Chloride plays a key role in bicarbonate exchange and bile alkalinization. The reduction observed with Fucoidan may be attributed to preferential anion exchange involving sulfate groups, consistent with the findings of Kim and Shin (2015), who reported that Fucoidan modulates anion transport mechanisms. The increased observed with Papain supports earlier findings by Ray et al. (2024), who reported enhanced electrolyte transport

following protease exposure, possibly due to increased cholangiocytic activity.

Bicarbonate levels were significantly reduced across all treated groups when compared to the control group, with a more pronounced decrease observed in the fucoidan group, when compared with the papain group. Bicarbonate secretion is essential for maintaining bile alkalinity and protecting the biliary epithelium. The reduction observed may reflect modulation of ductular secretory mechanisms. Fucoidan's known anti-inflammatory and membrane-stabilizing properties, as reported by Vilela et al. (2020), this may have contributed to reduced bicarbonate efflux, while the effect observed with Papain may be related to its influence on oxidative stress and ion transporter regulation, as previously described by Kong et al. (2021).

In conclusion, the findings indicate that Papain and Fucoidan exert a mild and regulatory effects on biliary electrolyte composition without inducing pathological alterations. Their actions appear to support biliary homeostasis rather than stimulate excessive secretion, reinforcing and biochemical safety.

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