# Synergistic Effects of Bavachinin in Combination With Either Ezetimibe or Atorvastatin on Liver Biomarkers: A Randomized Controlled Trial in Hyperlipidemic Rats With NAFLD

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**Abstract-** Bavachinin, a flavonoid derived from Psoralea corylifolia, exhibits antioxidant and anti-inflammatory properties and functions as a pan-agonist of PPAR nuclear receptors. This study aimed to evaluate the individual and combined effects of bavachinin with either ezetimibe or atorvastatin on liver function markers and hepatocyte apoptosis in a rat model of diet-induced hyperlipidemia. Thirty-five male Wistar rats were randomly assigned to seven groups: normal control (NC), hyperlipidemic control (HC), bavachinin (BAV), atorvastatin (ATV), ezetimibe (EZI), ATV+BAV, and EZI+BAV. Hyperlipidemia was induced in all groups except NC. Serum levels of AST, ALT, ALP, and IL-10 were measured before and after the 4-week intervention period. Liver tissue was assessed using TUNEL staining. Wilcoxon signed-rank tests showed significant within-group reductions in AST in all intervention groups ($P$<0.05). ALT significantly decreased in the BAV and ATV+BAV groups. IL-10 levels significantly increased in the EZI, BAV, ATV+BAV, and EZI+BAV groups. Kruskal-Wallis ANOVA revealed significant between-group differences in AST, ALT, and IL-10 levels across all groups ($P$<0.05). Post hoc Mann-Whitney U tests revealed that both combination groups (ATV+BAV and EZI+BAV) showed significant reductions in AST levels compared with the HC group, and the EZI+BAV group also demonstrated a significant reduction in ALT. IL-10 levels exhibited significant improvements in both combination groups compared with BAV alone. Additionally, TUNEL staining indicated reduced hepatocyte apoptosis in both combination groups as well as in the BAV group relative to the HC group. Bavachinin, in combination with ezetimibe or atorvastatin, demonstrated hepatoprotective and anti-inflammatory effects in a rat model of fatty liver disease. These findings suggest potential therapeutic roles for bavachinin. However, further studies, including complete lipid profiling and oxidative stress markers, are needed.



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receptors (PPAR)

## Introduction

Nonalcoholic fatty liver disease (NAFLD) is a prevalent multisystem disease, the main cause of chronic liver disease and end-stage liver disease globally. NAFLD encompasses a spectrum of hepatic conditions that range from simple steatosis to nonalcoholic steatohepatitis (NASH), which is characterized by hepatocyte ballooning and can progress to cirrhosis and hepatocellular carcinoma (1,2).

The prognosis in NAFLD, including severe complications like cirrhosis and hepatocellular carcinoma (HCC), is closely linked to the degree of fibrosis (3). The exact pathogenesis of NAFLD varies and involves impaired lipid metabolism, increased IL-1β-mediated inflammation, and fibrosis (4). Metabolic syndrome, characterized by central obesity, insulin resistance, dyslipidemia, hypertension, and low-grade inflammation, is associated with NAFLD and NASH and represents a major risk factor for their development; in this context, insulin resistance and low-grade systemic inflammation that accompany metabolic syndrome contribute to hepatic fat accumulation (5-7).

Currently, early NAFLD management emphasizes lifestyle modifications such as weight reduction and increased physical activity, while pharmacological strategies primarily aim to prevent fibrosis; however, no FDA-approved medication specifically targets NAFLD (8). Improving lipid profiles is an important therapeutic target in patients with NAFLD. Lipid-lowering medications, like atorvastatin, are effective in reducing cardiovascular events and improving hepatocyte histology. Statins are commonly used in patients with metabolic syndrome to treat dyslipidemia (9-12). Ezetimibe, a cholesterol-absorption inhibitor acting on NPC1L1, has been shown in recent randomized trials and meta-analyses to improve lipid profiles (notably LDL-C and, in some studies, triglycerides) and to produce modest improvements in hepatic outcomes (e.g., serum transaminases and steatosis) in patients with metabolic syndrome and NAFLD (13,14).

Peroxisome proliferator-activated receptors (PPARs) are nuclear receptors that play central roles in energy balance, lipid metabolism (including cholesterol), and glucose homeostasis, and are therefore attractive targets in NAFLD Management. Activation of the PPAR-α subtype reduces acyl-CoA levels, lowers triglycerides, enhances uptake and β-oxidation of free fatty acids, and upregulates apolipoprotein A-I and A-II expression, leading to increased HDL-C; fibrates (e.g., fenofibrate) act primarily via PPAR-α. Fibrates (e.g., fenofibrate) act primarily via PPAR-α. PPAR-γ promotes adipocyte differentiation, reduces hepatic gluconeogenesis, and improves insulin sensitivity, and is the target of thiazolidinediones (e.g., rosiglitazone). PPAR-β/δ enhances glucose tolerance and stimulates fatty acid oxidation and lipid catabolism (15,16). To date, no FDA-approved PPAR.β/δ agonist exists. Promising substances like cardarine (GW501516) demonstrated carcinogenic effects in rats, and no human dosage has been established (17).

PPARs have a significant role in regulating inflammation by inhibiting multiple inflammatory genes through transrepression. In NAFLD, the pro-inflammatory cytokine IL-1β, produced by liver macrophages, suppresses several PPAR-dependent pathways, leading to lipid accumulation in the liver (18). Conversely, IL-10 is an anti-inflammatory cytokine that can be used as a marker to monitor NAFLD patients. During NAFLD progression, IL-10 levels tend to decrease (19).

Throughout history, nature has been a rich source of inspiration for the discovery of new medications to combat diseases. Bavachinin (BVC), a flavonoid extracted from Psoralea corylifolia seeds, has a long-standing tradition in Chinese medicine for treating type 2 diabetes (20). Interestingly, research indicates that Bavachinin exerts pan-agonistic effects on PPARs in vitro and in animal studies, suggesting its potential therapeutic value. Moreover, when combined with synthetic PPAR agonists, Bavachinin demonstrates synergistic glucose-lowering effects without inducing hepatotoxicity. These findings hold promise for the development of innovative treatments derived from natural compounds (21).

In the present study, we investigated the effects of bavachinin alone and in combination with atorvastatin or ezetimibe on hepatic biomarkers in obese Wistar rats with diet-induced NAFLD. We focused on changes in ALP, AST, ALT, and IL-10 to assess inflammation, while liver injury was assessed by TUNEL staining. We hypothesized that combinations of bavachinin with atorvastatin or ezetimibe would exert distinct effects, and that bavachinin would favorably modulate liver enzymes (reducing ALP, AST, and ALT) and increase IL-10, indicating reduced inflammation. The outcomes aim to clarify the therapeutic potential of these compounds in NAFLD management.

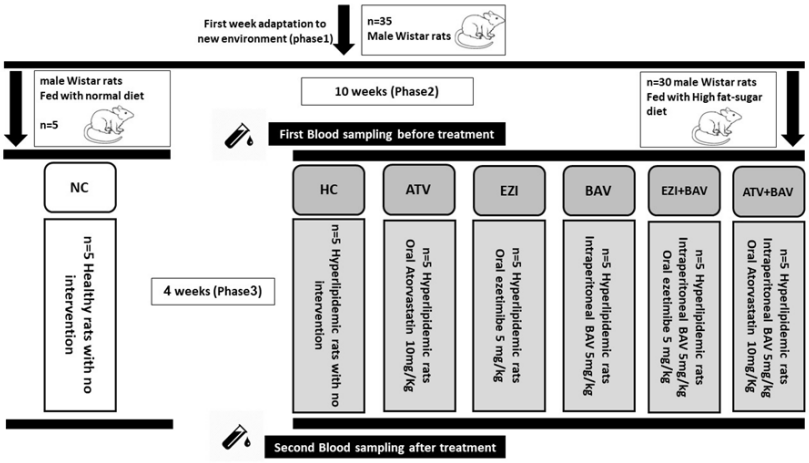


**Figure 1.** Study design. After an adaptation week, 5 subjects from the normal control (NC) group were on a regular diet. The rest are divided into intervention groups of 5: HC (Hyperlipidemic control), ATV (atorvastatin), EZI (ezetimibe), BAV (bavachinin), EZI+BAV (combined ezetimibe and bavachinin), and ATV+BAV (combined atorvastatin and bavachinin)

## Materials and Methods

### Animals

This study was ethically approved by the medical ethics committee of Qazvin University of Medical Sciences with approval code IR.QUMS.REC.1400.300. Thirty-five male Wistar rats, weighing between 200 and 300 grams, were obtained from the University of Tehran, Institute of Biochemistry and Biophysics (IBB) center. The rats were acclimatized to the laboratory conditions for seven days, maintained in standard cages with a controlled environment (25 to 27◦ C degrees, 12-hour light/dark cycle), and provided with ad libitum access to water and food.

### Chemicals and food

Generic ezetimibe and atorvastatin were purchased from a pharmacy (manufactured by Hakim Pharmaceuticals and Sobhan Pharmaceuticals). Bavachinin 99% was purchased from Leap Chem Company, LTD. Dextrose was purchased from Iran Dextrose (Tehran, Iran). The standard food was purchased from Javaneh Khorasan (Mashhad, Iran) and consisted of Protein 14.0%, fiber 4.0%, Fat 4.0%, Calcium 0.6%, Phosphorus 0.4%, Vitamin A 15000 IU, Vitamin $D^3$ 1500 IU, Iron sulfate monohydrate 152 mg, calcium iodate anhydrous 1.5 mg, copper sulfate pentahydrate 20 mg, and manganese. The food to induce hyperlipidemia in the later stage of the study consisted of 40% standard food, with the remainder containing 2% cholesterol, 1% cholic acid, 40% dextrose, and 20% animal fat (Tail fat; all percentages are weight/weight). Tail fat was purchased from Pooya Protein (Tehran, Iran). Cholesterol and cholic acid were purchased from Sigma-Aldrich (USA).

### Preparation

Commercial formulations of atorvastatin (20 mg per tablet) and ezetimibe (10 mg per tablet) were utilized in the study. To prepare the compounds for dosing, the tablets were manually pulverized in a mortar and pestle until a homogeneous powder was obtained, containing both the active substances and the tablet excipients. Precise amounts of the resulting powder were weighed according to each animal's body weight to ensure accurate dosing. The powdered mixture was then suspended in distilled water, which served as a vehicle for oral administration. Due to the limited aqueous solubility of both compounds, the suspension was vigorously stirred immediately prior to loading the syringe to ensure homogeneity and consistent dosing. The prepared suspension was administered orally via gavage at a dosage volume of 1 mL/kg body weight. Dosages were calculated individually to reflect each animal's specific weight. Notably, the tablet formulations used did not contain any added sweeteners such as sucrose or fructose.

### Experimental groups

The animals were randomly divided into seven

experimental groups: 1. Normal control (NC), 2. Hyperlipidemic control (HC), 3. Atorvastatin (ATV), 4. Ezetimibe (EZI), 5. Bavachinin (BAV) 6. Combined atorvastatin and bavachinin (ATV+BAV), 7. Combined ezetimibe and bavachinin (EZI+BAV). The normal control group received a standard rat diet, while the other groups were fed a hyperlipidemic diet designed to induce hyperlipidemia. The feeding phase lasted 10 weeks, and dyslipidemia was confirmed at the end of week 10. All groups, except the normal control group, met the criteria for dyslipidemia (TG> 145 mg/dl) (22). To confirm successful induction of dyslipidemia, triglyceride levels were measured in two randomly selected rats per group at the end of the 10-week feeding phase; however, due to the small sample size, TG data were not included in the statistical analysis. The subsequent 4-week trial stage involved injecting BVC (5 mg/kg/day; i.p.) (23), administering atorvastatin via gavage (10 mg/kg/day) (24), and administering ezetimibe via gavage (5 mg/kg/day) (25). The combined treatment groups received a combination of BVC (5 mg/kg/day; i.p.) and either ezetimibe or atorvastatin at the previously mentioned dosages.

**Sampling**

Blood samples were collected from the tail vein of each subject once at the start of the 4-week trial period, and once at the end (1 ml from each subject). Blood samples were centrifuged for 5 minutes (4000 RPM) to separate the serum, and then the serum was kept at -60 degrees Celsius. The rats were sedated with ketamine before sample collection. Before decapitation, the subjects were put under deep anesthesia; after decapitation, samples from the liver were taken.

**Variables measurement**

The BT 1500 Fully Automatic Biochemistry Analyzer (Biotecnica, Rome, Italy) was used to measure Aspartate Transaminase (AST), Alanine Transaminase (ALT), and Alkaline Phosphatase (ALP) with kits from Pars Peyvand (Tehran, Iran). Tissue sampling was performed during euthanasia under deep anesthesia (10 mg/kg ketamine, Exir Pharma, Tehran, Iran). The Rat IL-10 ELISA kit was purchased from Sigma-Aldrich. The assay's lower limit of detection was 10 pg/mL, with a standard curve range of 8.23-6000 pg/mL. Intra-assay and inter-assay coefficients of variation (CV) were <10% and <12%, respectively. BioTek 800 TS Absorbance Reader (Agilent, USA) was used for ELISA readings.

For histological analysis, after deparaffinization, tissue sections were washed with PBS (Sigma-Aldrich). Endogenous peroxidase activity was blocked by incubating the samples for 10 min in a 1:9 mixture of $H_2O_2$ (Sigma-Aldrich) and methanol, followed by three washes in PBS (5 min each). For partial protein digestion, sections were treated with proteinase K (Sigma-Aldrich) for 30 min at 37 ° C and washed three times with PBS. To increase nuclear permeability, 0.3% Triton X-100 was applied for 10 min, followed by three PBS washes. Samples were then incubated with TdT enzyme for 2 h at 37 ° C and washed again with PBS. Finally, nuclei were counterstained with DAPI, and fluorescent images were captured using an Olympus fluorescence microscope.

TUNEL (Terminal deoxynucleotidyl transferase dUTP nick end labeling) detects apoptosis by labeling the 3'-OH termini of fragmented DNA. During apoptosis, endonuclease activation leads to DNA double-strand breaks. TdT catalyzes the addition of labeled dUTPs to these free 3'-OH ends, allowing apoptotic nuclei to be visualized by fluorescence microscopy (26).

**Statistical analysis**

Non-parametric statistical analysis was used, including Kruskal-Wallis ANOVA, Mann-Whitney's U test, and Wilcoxon's signed-rank test. Bonferroni correction was used for pairwise comparisons. IBM SPSS version 26 software was used to perform the statistical analysis. Statistical significance was indicated as $P<0.05$. All data are presented as median±IQR. Graphs were created using GraphPad Prism version 8.

**Table 1. Changes of Aspartate Transaminase (AST) (IU/L), Alanine Transaminase (ALT) (IU/L), Alkaline Phosphatase (ALP) (IU/L), and IL-10 (pg/ml) levels in the serum, and also TUNEL staining in hepatocytes before and after the intervention in each group**

| Group | AST(IU/L) | | ALT(IU/L) | | ALP (IU/L) | | IL-10 (pg/ml) | | Tunel positive cells (%) |
|---|---|---|---|---|---|---|---|---|---|
| | Before | After | Before | After | Before | After | Before | After | |
| **ATV** | 152±19 | 148±20.5 | 42±14 | 39±11 | 563±223 | 559±77 | 17.79±2.33 | 18.59±5.88 | 39.15±2.26 |
| **EZI** | 148±18.5 | 132±13.5 | 43±12 | 41±8.5 | 539±122.5 | 521±72 | 17.91±3.42 | 18.01±2.25 | 31.23±3.63 |
| **BAV** | 159±22 | 138±23.5 | 46±10 | 37±8.5 | 571±159.5 | 620±154 | 17.47±3.52 | 16.23±2.78 | 39.04±7.25 |
| **ATV+BAV** | 161±29 | 126±20 | 40±11.5 | 34±9 | 568±121 | 596±80 | 16.95±2.86 | 19.41±2.02 | 24.65±6.5 |
| **EZI+BAV** | 151±21 | 128±33 | 44±11 | 35±9 | 557±146 | 550±122 | 18.24±2.63 | 18.59±5.24 | 18.93±3.17 |
| **NC** | 131±19.5 | 132±12 | 29±11 | 31±3.6 | 410±65 | 401±56 | 16.98±2.76 | 16.86±2.19 | 6.35±3.39 |
| **HC** | 142±26.5 | 155±33 | 41±10.5 | 48±11 | 586±133.5 | 659±156 | 17.28±3.23 | 15.59±7.02 | 45.97±7.24 |

## Results

The effect of intervention within each group: AST levels significantly reduced in the ATV group (*P*=0.041), EZI group (*P*=0.043), BAV group (*P*=0.043), ATV+BAV group (*P*=0.043), and EZI+BAV group (*P*=0.042). ALT levels significantly decreased only in the BAV group (*P*=0.041) and EZI+BAV group (*P*=0.043). IL-10 significantly increased in the EZI (*P*=0.043), BAV (*P*=0.043), ATV+BAV (*P*=0.043), and EZI+BAV (*P*=0.043) groups. ALP showed no significant differences before and after the intervention.

The effect of the intervention between groups: Significant differences were observed in IL-10 (H [6]=16.891, *P*=0.01), AST (H [6]=29.443, *P*<0.001), and ALT (H [6]=15.856, *P*=0.015) among all groups. The ATV+BAV group showed a significant decrease in AST compared to the HC group (*P*=0.005). The EZI+BAV group also showed a significant decrease in AST compared to the HC group (*P*=0.001) and the NC group (*P*=0.008). ALT was significantly lower in the EZI+BAV group compared to the HC group (*P*=0.048). IL-10 was significantly increased in the ATV+BAV group compared to the BAV group (*P*=0.007) and the EZI+BAV group compared to the BAV group (*P*=0.018).

The difference in apoptotic cells (TUNEL staining) among all groups was statistically significant (H [6]=32.11, *P*<0.001). The NC group showed significantly fewer apoptotic cells compared to the ATV group (*P*=0.01), the BAV group (*P*=0.009), and the HC group (*P*<0.001). The EZI+BAV group showed significantly fewer apoptotic cells compared to the HC group (*P*=0.004). The ATV+BAV group also showed fewer apoptotic cells than the HC group (*P*=0.043).

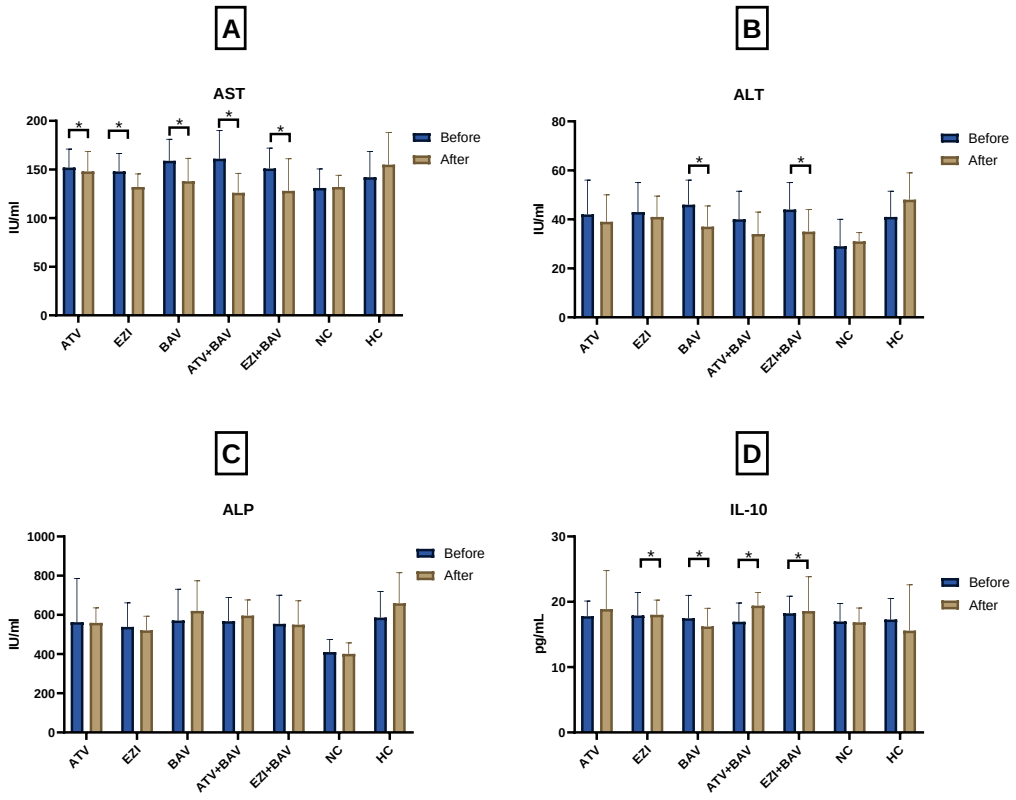


**Figure 2.** Changes in biomarkers before and after intervention within each group. AST = Aspartate transaminase, ALT = Alanine transaminase. ALP=Alkaline phosphatase, IL-10=Interleukin-10. ATV=Atorvastatin group, EZI=Ezetimibe group, BAV=Bavachinin group, ATV+BAV=Combined atorvastatin and bavachinin group, EZI+BAV=Combined ezetimibe and bavachinin group, NC=Normal control group, HC=Hyperlipidemic control group. * indicates statistical significance (*P*<0.05)

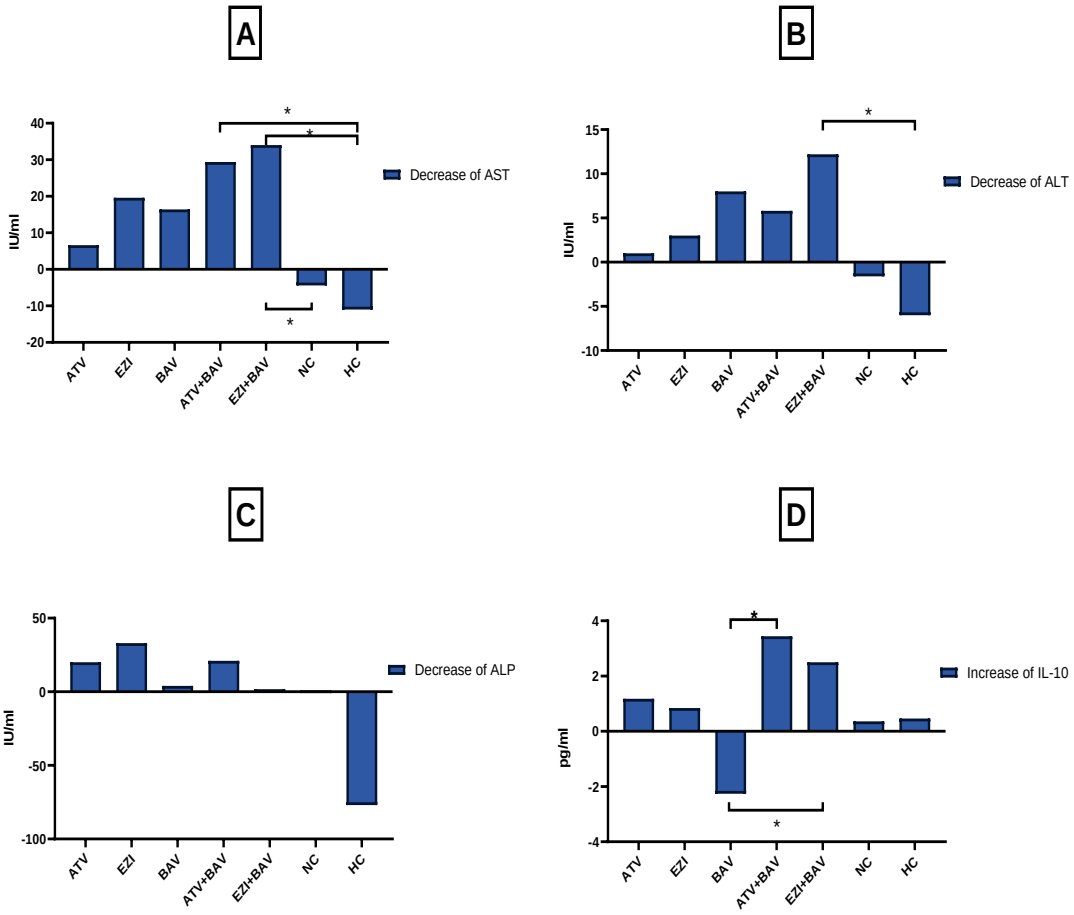


**Figure 3.** Changes in biomarkers between groups after intervention. A=Decrease of AST (Aspartate transaminase), B=Decrease of ALT (Alanine transaminase), C=Decrease of ALP (Alkaline phosphatase), D=Increase of Interleukin-10 (IL-10). ATV=Atorvastatin group, EZI=Ezetimibe group, BAV=Bavachinin group, ATV+BAV=Combined atorvastatin and bavachinin group, EZI+BAV=Combined ezetimibe and bavachinin group, NC=Normal control group, HC=Hyperlipidemic control group. * indicates statistical significance ($P$<0.05).

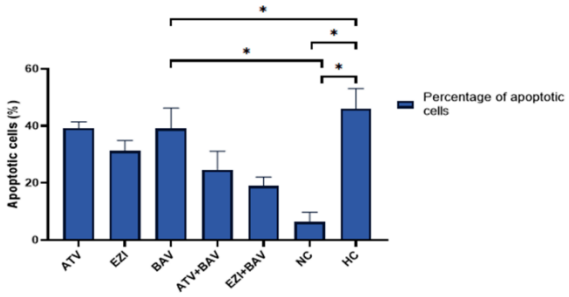


**Figure 4.** Histology results. The percentage of apoptotic cells, as determined by TUNEL staining, after intervention. ATV=Atorvastatin group, EZI=Ezetimibe group, BAV=Bavachinin group, ATV+BAV=Combined atorvastatin and bavachinin group, EZI+BAV=Combined ezetimibe and bavachinin group, NC=Normal control group, HC=Hyperlipidemic control group. * indicates statistical significance ($P$<0.05)

## Discussion

Insulin resistance, diabetes, and obesity are significant comorbidities of NAFLD and NASH, which are closely linked to metabolic syndrome. Presently, there is no specific medication recommended for the treatment of NAFLD and NASH (27). Consequently, the primary approach to managing fatty liver involves

addressing these comorbidities through lifestyle modifications, increased physical activity, and weight control. Various drug classes, such as thiazolidinediones, insulin sensitizers, and statins, have been investigated for their therapeutic potential (28,29). However, the results from these studies have been diverse and often conflicting.

Based on multiple articles highlighting its antioxidant and antidiabetic properties, as well as its pan-agonistic effect on PPAR receptors, bavachinin shows promise as a potential treatment for fatty liver disease and its associated comorbidities (21). However, it's worth noting that some studies have reported sex-related side effects of psoralea corylifolia extract and bavachinin, attributed to their estrogen-like effects (30). To mitigate this concern, the current study employed male Wistar rats as the experimental subjects.

Insulin resistance plays a central role in the progression of fatty liver disease, with various contributing factors, including alterations in the gut microbiome and inflammation, significantly influencing this process. As a result of insulin resistance, there is an accumulation of fats from both de novo synthesis and fats absorbed from the gastrointestinal (GI) tract (31). Elevated levels of free fatty acids surrounding hepatocytes contribute to oxidative stress, leading to cellular damage. Subsequently, this cascade triggers the release of pro-inflammatory cytokines and the onset of inflammation, culminating in histological changes in the liver (32).

Oxidative stress is a significant factor in the development of fatty liver disease. Previous studies on HepG2 and HepaRG cell lines reported that Psoralea corylifolia extract and bavachinin could induce oxidative stress and hepatotoxicity, partially through estrogen-mediated NRF2 deactivation (30,33,34). However, other studies demonstrated antioxidant and anti-inflammatory properties of Psoralea corylifolia, including reductions in ROS, pro-inflammatory cytokines, and oxidative stress induced by NADPH, ascorbate, and CCl4 (35,36). These contrasting findings highlight the importance of experimental context, including dose, sex, and animal model, in interpreting hepatoprotective versus hepatotoxic effects.

Squalene synthase, coded by the FDFT-1 gene, is a critical enzyme in cholesterol biosynthesis. Bavachinin and Psoralea corylifolia also influence lipid metabolism. They can modulate the FDFT-1 gene expression, inhibit SREBP-2, and regulate cholesterol and fat synthesis via the PI3K/Akt/mTOR pathway (37).

Palmitate-induced toxicity occurs when hepatocytes are exposed to high levels of palmitate, resulting in a gradual lipid overload. This exposure causes mitochondrial membrane potential (MMP) collapse, mitochondrial damage, and, eventually, apoptosis. However, in palmitate-induced hepatotoxicity models, bavachinin effectively reduced cholesterol accumulation, improved mitochondrial membrane potential, and decreased apoptosis, demonstrating superiority over atorvastatin in mitigating lipid-induced liver injury (37). These findings highlight the promise of bavachinin as a protective agent against palmitate-induced liver damage and further support its potential clinical applications in the management of fatty liver disease and related conditions.

Our observation that bavachinin, alone or in combination with ezetimibe or atorvastatin, led to significant within-group reductions in AST and ALT levels is consistent with previous studies and strongly supports its hepatoprotective potential. Furthermore, the significant differences in AST and ALT enzyme levels between the HC group and the ATV+BAV and EZI+BAV groups provide additional confirmation, fully reinforcing the evidence for the hepatoprotective effect of bavachinin.

Seo *et al*., similarly reported that Psoralea corylifolia extract reduced HbA1C, insulin resistance, total cholesterol, triglycerides, and downregulated lipogenic and inflammatory genes in diet-induced fatty liver models (38).

These findings suggest that Psoralea corylifolia extract has beneficial effects on metabolic and lipid parameters, indicating its potential as a therapeutic agent for the management of fatty liver disease and associated metabolic disorders. The observed downregulation of lipogenic and inflammatory genes further supports the notion that Psoralea corylifolia extract may mitigate the underlying mechanisms of fatty liver disease development.

Chronic inflammation is a key factor in NAFLD, NASH, and advanced liver disease development. Wong Lee et al. found that Psoralea corylifolia extract reduced IL-6-induced STAT-3 promoter activity, reducing inflammation in the Hep3b cell line (39). Xi Chen *et al*., observed that Psoralea corylifolia extract decreased GFP protein expression, inhibiting GATA-3 and T.bet, and altered the TH1/TH2 ratio by reducing IL-4 levels. Modulating the TH1/TH2 ratio plays a significant role in long-term inflammations, making Psoralea corylifolia extract a promising candidate for managing liver diseases (40).

Bavachinin and Psoralea corylifolia have been

reported in various studies to inhibit Toll-like receptors and reduce the function of iNOS, COX-2, and macrophages (41,42). Nepal *et al*., observed that Bavachinin, in their study on the Human KB cell line, not only decreased the expression of HIF-1 alpha and VEGF but also reduced the level of IL-1 beta. This reduction in IL-1 beta is essential in alleviating chronic inflammation in fatty liver disease (43).

These findings are consistent with our observations that IL-10 levels were elevated in the BAV, EZI+BAV, and ATV+BAV groups. Since a significant between-group difference in IL-10 levels was observed between the BAV group and the EZI+BAV and ATV+BAV groups, the synergistic effect of ezetimibe and atorvastatin with bavachinin was evident.

In cases of fatty liver, progressive hepatocyte injury may ultimately lead to apoptosis, which can be detected by TUNEL staining (28). In the present study, TUNEL staining findings indicated a significant difference in the percentage of apoptotic hepatocytes between the HC group and the EZI+BAV and ATV+BAV groups, providing strong support for our claim regarding the hepatoprotective property of bavachinin. On the other hand, in a study by Jiyang Wang *et al*., investigating the effects of Psoralea corylifolia in Dawley Rats, they observed cholestasis, cholesterol accumulation, and inhibition of pathways involved in bile acid synthesis and excretion. Additionally, female rats exhibited hepatomegaly and a dose-dependent increase in ALT, AST, and GGT levels. Furthermore, necrosis was evident after H & E staining in the mentioned case (44).

In a study by Yu Wu *et al*., on female Dawley rats, both ovariectomized and non-ovariectomized, the investigation of possible estrogen-like side effects of Psoralea corylifolia revealed cholestasis and bile duct dilatation in non-ovariectomized rats. Additionally, Psoralea corylifolia extract reduced the expression of FXR receptors, which control the expression of BSEP, MRP-2, Cyp7a1, and Cyp8b1, crucial factors involved in bile acid synthesis and excretion in non-ovariectomized rats. Furthermore, SULTE 1 and Cyp3A2, which detoxify bile acids, were inhibited by Psoralea corylifolia extract. Molecular docking studies suggested that these effects of Psoralea corylifolia are attributed to its estrogen-like impact on FXR receptors (30).

Numerous studies have highlighted that psoralea corylifolia-induced toxicity may be attributed to the estrogen-like properties of its constituents, such as bakuchiol, psoralen, and isopsoralen. However, in the study by Bojia Liu *et al*., on zebrafish and ovariectomized rats, the Psoralea corylifolia extract used did not contain bakuchiol, psoralen, or isopsoralen. Notably, histological studies of the liver, spleen, and kidneys in ovariectomized rats showed no significant difference or changes compared to the control group. These findings suggest that the absence of these specific constituents might have contributed to the lack of observed toxic effects in the studied tissues (45).

The effect of Several pharmacological interventions on fatty liver disease and its comorbidities has been investigated. PPAR receptors are key regulators of fatty acid oxidation, inflammation, and fatty acid transport. Thus, in various studies, they are known as attractive targets for treating fatty liver disease (46). Several studies have pointed out the damaging effect of intracellular cholesterol accumulation on mitochondria and peroxisomes, which have a vital role in fatty acids degradation; As the level of cholesterol reduces, the function of peroxisomes and mitochondria in fatty acids degradation improves (47).

Several pharmacological interventions have been investigated for their impact on fatty liver disease and related comorbidities. Intracellular cholesterol accumulation is detrimental to mitochondria and peroxisomes, which are essential for fatty acid degradation. Decreasing cholesterol levels can improve the function of these organelles in fatty acid breakdown . (47).

In addition to the anti-inflammatory and hepatoprotective properties of bavachinin reported in previous studies, ezetimibe and atorvastatin have also been widely recognized for their anti-inflammatory and hepatoprotective effects, largely attributed to their influence on cholesterol and other lipid metabolism (13,48). In a study by Park *et al*., involving mice on a low-methionine, low-choline diet, statin administration increased alpha-PPAR receptor function and significantly increased the number of mitochondria and peroxisomes in hepatocytes. This led to enhanced fatty acid oxidation in the liver, resulting in a remarkable reduction in steatosis and inflammation (49).

Paraoxonase-1, a hepatic enzyme, plays a critical role in reducing liver inflammation and oxidative stress. Reduced activity of Paraoxonase 1 (PON1) is considered a reliable marker for Nash progression. Samy *et al*.'s study on patients with NAFLD demonstrated that atorvastatin increased Paraoxonase 1 activity, potentially preventing or delaying the progression of liver steatosis to NASH (50).

Studies have reported elevated levels of AGEs (advanced glycation endproducts) in individuals with

fatty liver disease compared to healthy individuals. AGEs contribute to oxidative stress and activate Stellate Cells, crucial in fibrosis development. Kamara et al.'s study revealed that atorvastatin not only reduced oxidative stress but also slowed the progression of NASH and reduced AGEs levels (51).

NPC1L1 (Niemann-Pick C1-Like 1) is a crucial factor in cholesterol absorption. It is not only expressed in the intestines, where it plays a vital role in cholesterol uptake from the diet, but also in the liver, where it influences the entry of cholesterol into hepatic cells. Several studies have demonstrated that cholesterol exerts detrimental effects on both peroxisomes and mitochondria, leading to reduced fatty acid oxidation and exacerbating the progression of NASH (52).

Yoneda *et al*., conducted a study demonstrating that a 6-month treatment with ezetimibe in individuals with NASH resulted in favorable outcomes. The treatment led to reductions in AST, ALT, and C-Reactive Protein (CRP), indicating reduced liver inflammation. Moreover, they observed a decrease in Type IV collagen in the liver, suggesting a reduction in fibrosis (53).

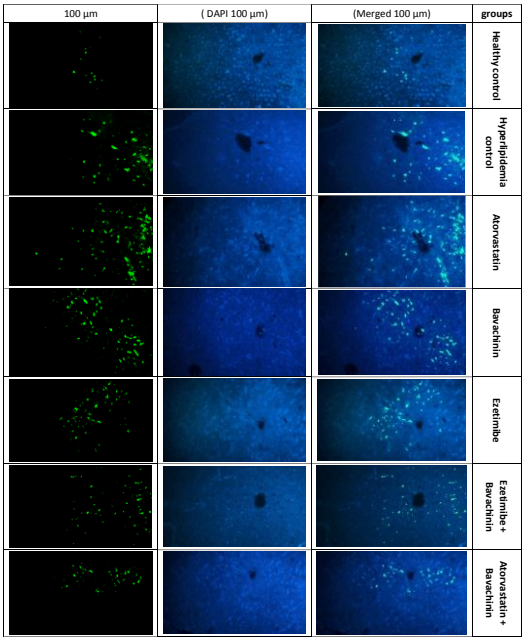


**Figure 5.** TUNEL staining of liver cells

In this study, bavachinin, in combination with ezetimibe or atorvastatin, showed hepatoprotective effects, as evidenced by reductions in serum AST and ALT levels and decreased hepatocyte apoptosis. These findings are consistent with previous studies highlighting the antidiabetic, antioxidant, and pan-PPAR agonistic properties of bavachinin and Psoralea corylifolia, which may contribute to its therapeutic potential in the treatment of metabolic liver disease. Additionally, reports of estrogen-like side effects associated with Psoralea corylifolia raise concerns that require further investigation. Overall, bavachinin appears to be a promising candidate for managing NAFLD and related metabolic disorders, but more comprehensive studies are needed to evaluate its safety and clarify its mechanisms of action.

## Limitations

Future studies are recommended to include the measurement of additional pro- and anti-inflammatory cytokines to provide a more comprehensive understanding of the inflammatory status. Furthermore, assessing oxidative stress biomarkers such as MDA, SOD, CAT, and GSH is suggested to better show the antioxidant effects.

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