Atorvastatin and Hesperidin: A Study into Their Effect on the Liver

Tabarek Falih Chichan*, Inam Sameh Arif, Huda Jaber Waheed

Department of Pharmacology and Toxicology, College of Pharmacy, Mustansiriyah University, Baghdad, Iraq

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Abstract- The liver is a vital organ responsible for metabolism, immune support, and detoxification, making it vulnerable to drug-induced injuries that can lead to severe complications. Among commonly prescribed medications, atorvastatin is effective in lowering cholesterol levels and reducing cardiovascular disease risk but can also cause hepatotoxicity. This study aims to investigate the potential hepatoprotective effects of hesperidin, a flavonoid known for its antioxidant properties, against liver damage caused by atorvastatin. By examining changes in liver enzyme levels, particularly ALT and AST, the research seeks to elucidate hesperidin's role in mitigating statin-induced liver injury. This study utilized thirty healthy male Wistar rats, aged 10 to 12 weeks and weighing approximately 230 grams, housed under standardized conditions at Mustansiriyah University. The rats had unlimited access to tap water and pellet food, with all care methods approved by the university's ethical committee. Atorvastatin and hesperidin were sourced from AstraZeneca (Germany) and a supplier in China, respectively, and dissolved in DMSO for administration. The rats were randomly divided into five groups, with varying treatments over twenty days, including a negative control group receiving saline and groups receiving atorvastatin alone or in combination with different doses of hesperidin. Blood samples were collected for liver enzyme analysis (ALT and AST) following ethical guidelines. Data was analyzed using SPSS software, with significance determined at a *P* less than 0.05. The results indicated significant differences in liver enzyme levels among the experimental groups ($P<0.05$). Group 2, which received 80 mg/kg atorvastatin, exhibited markedly elevated ALT and AST levels compared to the negative control group. In contrast, groups receiving hesperidin in combination with atorvastatin showed significant declines in both ALT and AST levels. Notably, Group 3 (80 mg/kg atorvastatin plus 50 mg/kg hesperidin) demonstrated a significant reduction in ALT compared to Group 2, while Group 5 (200 mg/kg hesperidin plus atorvastatin) exhibited the lowest ALT and AST levels overall. These findings suggest that hesperidin effectively mitigates atorvastatin-induced hepatotoxicity. Thus, incorporating hesperidin may offer a protective strategy against liver damage associated with statin therapy, warranting further investigation into its clinical applications.

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Introduction

The liver is one of the body's most vital organs, playing a critical role in metabolism, immune support, and detoxification. However, these essential functions make the liver susceptible to various complications, including damage from the medications and chemicals it processes (1-3). Drug- induced liver injury is a significant concern, often resulting in severe side effects or even death. These adverse effects can arise from the drug itself or its metabolites, sometimes leading to idiosyncratic reactions (4,5).

Atorvastatin is a commonly prescribed medication that lowers plasma cholesterol levels by inhibiting 3-hydroxy-3-methylglutaryl coenzyme A reductase. While effective in reducing cardiovascular disease (CVD) risks, atorvastatin can also cause side effects such as muscle related issues (6). Elevated lipid levels increase the risk

Corresponding Author: T.F. Chichan
Department of Pharmacology and Toxicology, College of Pharmacy, Mustansiriyah University, Baghdad, Iraq
Tel: +9647718987661, E-mail address: Tabarekbaghdadi246@Gmail.Com

of CVD which remains a leading cause of death globally. The link between high cholesterol levels and CVD is primarily due to the formation of plaques in the coronary arteries which can lead to ischemic heart diseases. Prior to the induction of statins, the primary method to manage this risk is by limiting dietary fat intake (7).

Studies suggest that reducing LDL cholesterol by 1 mmol/L can decrease the rate of major vascular events by 22%. For example, a 40 mg dose of atorvastatin can reduce LDL cholesterol by half. This reduction is independent of patient characteristics, with each doubling of a statin's dose typically reducing LDL by about 6 percentage points (8). In addition to lowering cholesterol, atorvastatin also provides protection against CVD by reducing LDL oxidation, improving endothelial function, and reducing platelet activity (9).

Despite its benefits, atorvastatin can cause several potential side effects, including hemorrhagic strokes, memory impairment, and kidney dysfunction. Generally, statins have been the cause of various side effects with atorvastatin being the most reported agent to cause them. Hepatotoxicity also been noticed with using simvastatin and other statins including atorvastatin (10).

Hesperidin, a flavonoid that has been found in citrus fruits, is a compound with various health related positive effects while being affordable. The deficiency of this agent has been linked to events such as leg cramps, and pain in limbs. It has been found that using hesperidin supplements could be beneficial in situations such as fluid retention in swelled legs. The benefits of hesperidin has been used clinically to produce Daflon tablets that contain 10% hesperidin and proved to be of great use in treating hemorrhoids by elevating symptoms including bleeding and also pain (11). Hesperidin showed its ability to protect the liver against naturally occurring toxicants, in an investigation hesperidin showed improvement in the levels of ALT and AST among others, ALT and AST levels are important indications for measuring liver damage used in many research (12).

Antiviral activities of hesperidin as well as antibacterial and anti-inflammatory effects of hesperidin were all demonstrated in many researches, studies suggested that hesperidin has the ability to alleviate allergic reactions therefor could be used in treating localized edema that is associated with inflammation this theorize that it could affect histamine levels by reducing it and also have antioxidant free radical scavenging capabilities (13). Therefore, the aim of this study is to search whether hesperidin could help protect the liver against damages caused by atorvastatin.

The aim of this study is to investigate the hepatoprotective effects of hesperidin against liver damage induced by atorvastatin. Given the critical role of the liver in metabolism and detoxification, it is essential to understand the potential adverse effects of commonly prescribed medications like atorvastatin, which, while effective in lowering cholesterol and reducing cardiovascular disease risk, can lead to significant liver enzyme elevations and hepatotoxicity. This research will explore whether the addition of hesperidin, a flavonoid known for its antioxidant and anti-inflammatory properties, can mitigate the hepatotoxic effects associated with atorvastatin treatment. By assessing changes in liver enzyme levels, particularly ALT and AST, this study aims to provide insights into the protective mechanisms of hesperidin and its potential therapeutic role in managing statin-induced liver injury.

Materials and Methods

Laboratory animals

A total of thirty apparently healthy male Wister rats between 10 and 12 weeks of age with a weight of approximately 230 grams, were subjects in this experiment. The animal house of Mustansiriyah University was used to house them providing standardized conditions (14, 15), that included standardized humidity as well as temperature, unlimited access to tab water been provided as well as pellet food, all methods used to care for the rats and the process method been viewed and approved by the scientific and ethical committee in the College of Pharmacy, University of Mustansiriyah, the ethical approvals has all been obtained.

Chemicals

Atorvastatin has been purchased from AstraZeneca, Germany. Hesperidin has been purchased from China. Both chemicals were dissolved in DMSO, the amount needed has been calculated depending on the weight of each group.

Study design

The thirty Wister male rats were equally and randomly divided into five groups, each group containing equal number of 6 rats receiving treatment as the following (Figure 1):

- ✓ Group 1 (negative control group): administered normal saline for twenty days.
- ✓ Group 2 (positive control group): administered a toxic dose of 80 mg/kg of atorvastatin to induct hepatotoxicity, the doses were given for twenty

days.

- ✓ Group 3: administered a toxic dose of 80 mg/kg of atorvastatin plus minimal dose of 50 mg/kg of hesperidin, the doses were given for twenty days.
- ✓ Group 4: administered a toxic dose of 80 mg/kg of atorvastatin plus moderate dose of 100 mg/kg of hesperidin, the doses were given for twenty days.
- ✓ Group 5: administered a toxic dose of 80 mg/kg of atorvastatin plus maximum dose of 200 mg /kg of hesperidin, the doses were given for twenty days.

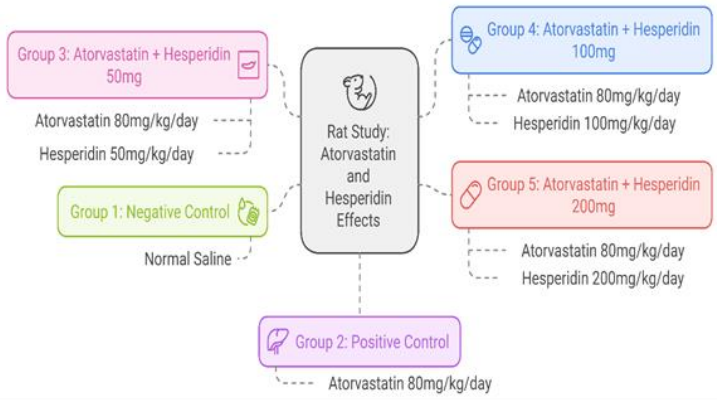

Figure 1. Rat study, atorvastatin and hesperidin effects

All doses were administered by oral gavage. At the end of the experiment blood samples were collected – according to the ethical procedures approved by the facility- for liver profile tests of alanine aminotransferase (ALT) and aspartate aminotransferase (AST), the procedure that was used to measure both ALT and AST was done following the manufacturers' instructions.

Statistical analysis

Statistical Packages for Social Science (SPSS) software was used in this study for analyzing the data collected, it has been reported as mean±standard error of mean (SEM). The T-test was also used to show the significance among groups if found in the study. The differences were considered statistically significantly when *P* was less than 0.05.

Results

ALT and AST

Results showed significant ($P<0.05$) differences between groups. Group 2 that received 80 mg/kg atorvastatin along with group 3 that received 80 mg/kg atorvastatin plus 50 mg/kg hesperidin both showed a significant elevation in ALT levels compared to negative control group, while all other groups showed a significant decline in ALT levels compared to group 2 (Table 1; Figure 2).

Table 1. The effect of atorvastatin and hesperidin on ALT levels of all groups in male rat model. Values expressed as mean±SE

Means±SE	G 1	G 2	G 3	G 4	G 5
ALT IU/L	31±2.6	227±38	133.8±2.2	91.8±10.5	59.5±4.9

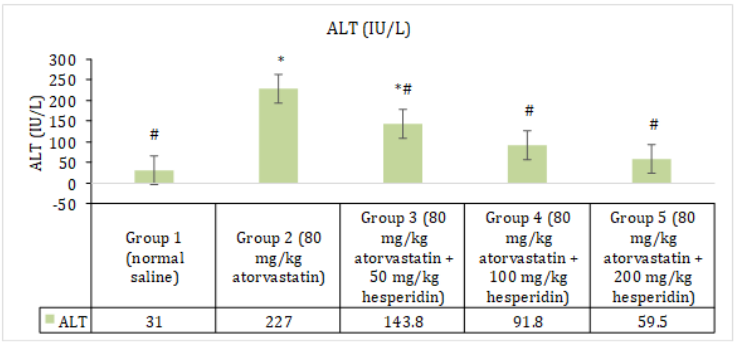

Figure 2. Effects of atorvastatin and hesperidin on ALT levels. Values are means±SD, n=6, when compared to group 1 the use of * indicates a significant (P<0.05) elevation of ALT, while the use of # indicates a significant (P<0.05) decline of ALT compared to group 2

Groups that received hesperidin plus atorvastatin all showed a significant decline in ALT levels compared to other groups, when 50 mg/kg hesperidin was added to 80 mg/kg atorvastatin in group 3 the group showed a significant decline in ALT levels compared to all groups except group 4 that had 100 mg/kg hesperidin plus 80 mg/kg atorvastatin, while group 4 showed a significant decline in ALT levels compared to group 2, and group 5 that received 200 mg/kg hesperidin plus 80 mg/kg atorvastatin showed a significant decline in ALT levels compared to group 2 and group 3 (Table 1; Figure 2).

AST levels reviled similar outcomes with group 2 showing significant [P<0.05] elevation while group 5 showing the decreased levels of AST (Table 2; Figure 3).

Table 2. The effect of atorvastatin and hesperidin on AST levels of all groups in male rat model. Values expressed as mean±SE

Means±SE	G 1	G 2	G 3	G 4	G 5
AST IU/L	29.8±1.4	175.6±10.7	131.8±4.8	70±3.9	64.3±2.3

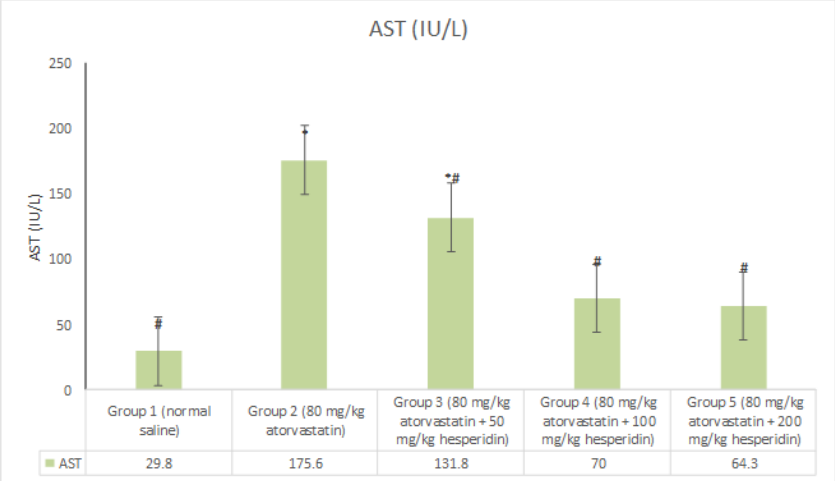

Figure 3. Effects of atorvastatin and hesperidin on AST levels. Values are means±SD, n=6, when compared to group 1 the use of * indicates a significant (P<0.05) elevation of AST, while the use of # indicates a significant (P<0.05) decline of AST compared to group 2

Discussion

The investigation into the effects of atorvastatin and hesperidin on liver enzymes, specifically alanine aminotransferase (ALT) and aspartate aminotransferase (AST), reveals significant findings regarding their hepatotoxicity and potential protective effects. In a study involving male rats, groups treated with atorvastatin alone exhibited elevated ALT and AST levels, particularly the group receiving 80 mg/kg atorvastatin (Group 2), which showed a marked increase in both enzymes compared to the negative control group.

Conversely, the addition of hesperidin at varying doses resulted in a significant decrease in ALT and AST levels, suggesting a hepatoprotective effect of hesperidin when combined with atorvastatin. This pattern indicates that while atorvastatin can induce liver enzyme elevations, hesperidin may mitigate these effects when administered concurrently (16,17).

The results demonstrated that Group 3, which received both 80 mg/kg atorvastatin and 50 mg/kg hesperidin, showed significantly reduced ALT levels compared to Group 2. Additionally, Group 4, receiving 100 mg/kg hesperidin along with atorvastatin, also exhibited lower ALT levels than Group 2. Group 5 further corroborated this trend, as it received a higher dose of hesperidin (200 mg/kg) and showed decreased ALT levels compared to both Groups 2 and 3. This decline in liver enzyme levels with the addition of hesperidin aligns with previous research indicating that flavonoids like hesperidin possess antioxidant properties that can protect against oxidative stress-induced liver damage caused by statins (18,19).

Comparatively, published literature supports these findings. A study reported that atorvastatin can cause acute elevations in hepatic enzymes shortly after initiation of therapy, with elevations often being self-limiting and related to transient hepatocyte membrane alterations rather than direct hepatotoxicity (20). Another study highlighted that while statins like atorvastatin typically lead to mild elevations in ALT and AST, these changes are generally asymptomatic and resolve over time (21). The current findings suggest that the hepatoprotective effects of hesperidin could provide a beneficial adjunct therapy for patients on atorvastatin, potentially reducing the risk of liver enzyme elevation.

Moreover, the mechanisms underlying these observations may involve the oxidative stress induced by atorvastatin. Research indicates that statins can elevate liver enzymes through various pathways that disrupt antioxidant defenses in the liver (22-24). Hesperidin's role as an antioxidant could counteract this oxidative stress, thereby normalizing liver enzyme levels. The current study's results reinforce this notion by demonstrating that groups receiving hesperidin alongside atorvastatin had significantly lower enzyme levels than those receiving atorvastatin alone.

Aminotransferase is an enzyme found in hepatic cells (25), leakage of this enzyme occurs in cases where damage has been done to the cell enough to let the enzyme leak, making it a useful tool in tracking hepatocellular damage caused by multiple causes (26). Induction group showed the most significant elevation in liver enzymes between all groups, this shows the effect of atorvastatin, statins in general proved to cause an increase in liver enzymes in many studies (27), this pushed to an extended observation by the FDA from 2000 to 2009 that came with the conclusion that liver enzymes monitoring was not a mandatory requirement, yet cases were observed of different stages of liver damage but a conclusive decision could not be made for many reasons including that more clinical trials has to take place and the fact that patients requiring statins as part of their healthcare usually were prescribed other medications to control other health issues making it harder to detect which medication is causing the damage (28), however, physicians are yet to agree on statins' safety since reports have showed liver failure in some cases (29).

Other treatment groups although were showing results of significant elevation of liver enzymes but the elevation decreased as hesperidin's doses increased, indicating the protective nature of the medication, this could be attributed to its ability as an antioxidant highlighting the possibility of liver injury done to cells was caused by oxidative stress, this was also found in other studies including a study done on a randomized double blind clinical study done on 2019 (30) and another in vivo study took place on 2022 (31). Group 2 and group 3 both resulted in the most significant increase in liver enzymes supporting the believe of damage caused by atorvastatin, while treatment group 4 and treatment group 5 resulted in the most significant decrease of ALT and AST levels supporting the believe that hesperidin provided protection against the damage and that the damage caused was originated by reactive oxygen species making treatment group 4 and treatment group 5 the healthiest compared to group 2 that has been given 80 mg/kg atorvastatin.

The combination of atorvastatin and hesperidin appears to offer a promising approach to managing the hepatic side effects associated with statin therapy. The significant reductions in ALT and AST levels observed in groups treated with hesperidin suggest their potential as a protective agent against statin-induced liver enzyme elevation. Future clinical studies are warranted to explore the applicability of these findings in human subjects and to further elucidate the mechanisms by which hesperidin exerts its protective effects on liver function during statin therapy.

According to the findings, hesperidin possesses antioxidant abilities that if administered in the right doses could protect liver cells from potential damage caused by atorvastatin. This provides evidence of the protective nature of hesperidin as well as the importance of monitoring liver functions while giving atorvastatin as a

medication, that also includes other statins, this is essential to make sure patients are not in danger of developing liver injuries due to their much needed medications.

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