Investigating the effect of hesperidin on atorvastatin-related liver toxicity: histopathological investigation

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Abstract:

Background: liver-related side effects can occur with numerous medications, with effects ranging from a mild effect to death. While statins in general, especially atorvastatin, are not usually associated with liver damage, reports recently have been suggesting otherwise.

Objective: this study investigates the effects of various doses of hesperidin against hepatotoxicity caused by atorvastatin in rat model.

Method: Thirty apparently healthy male Wister rats were randomly and equally divided into five groups, each group containing six rats, each group was subjected to certain dose of medication for the entire time of the experiment that lasted for twenty days.

Results: atorvastatin caused numerous liver related toxicity while hesperidin demonstrated dose related hepatoprotective effects.

Conclusion: the characteristic protective features of hesperidin, most likely as an antioxidant, is able to provide protection against atorvastatin hepatotoxicity, however this protective effect is directly affected by the right dose.

Keywords: hepatotoxicity, atorvastatin, hesperidin, histological analysis, rats.

دراسة تأثير الهيسبيريدين على سمية الكبد المرتبطة بأتورفاستاتين: الفحص النسيجي المرضي

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الخلاصة

الخلفية :أن الآثار الجانبية مرتبطة بالكبد من الممكن ان تحدث مع العديد من الأدوية، حيث تتراوح تأثيراتها من تأثيرات خفيفة وقد تصل الى حد الوفاة. الستاتينات بشكل عام، وخاصة أتورفاستاتين، ليست مرتبطة عادةً بسمية الكبد، الا ان التقارير الأخيرة

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تشير إلى خلاف ذلك.

الهدف :تبحث هذه الدراسة في آثار جرعات مختلفة من هيسبيريدين ضد السمية الكبدية التي يسببها أتورفاستاتين في نموذج الفئران.

الطريقة :تم تقسيم ثلاثين من ذكور الفئران الاصحاء من نوع ويستر بشكل عشوائي ومتساوي إلى خمس مجموعات، تحتوي كل مجموعة على ستة فئران، حيث تم تعريض كل مجموعة لجرعة معينة من الدواء طوال فترة التجربة التي استمرت لمدة عشرين يومًا.

النتائج :تسبب أتورفاستاتين في العديد من حالات السمية المتعلقة بالكبد، بينما أظهر هيسبيريدين آثارًا وقائية كبدية مرتبطة بالجرعة.

الخاتمة :تُظهر الخصائص الواقية المميزة لهيسبيريدين، على الأرجح كونه مضادًا للأكسدة، قدرته على توفير الحماية ضد السمية الكبدية الناتجة عن أتورفاستاتين، ومع ذلك فإن هذا التأثير الوقائي يتأثر بشكل مباشر بالجرعة المناسبة.

الكلمات المفتاحية: سمية الكبد، اتورفاستاتين، هسبردين, التحليل الهستولوجي, فئران.

Introduction

The liver is known to be the body's largest organ possessing striking abilities to have many functions including metabolism, supporting immune system, and detoxification. These abilities show the importance of this organ but also the vast issues it might face, one of the major problems would be drug induced toxicity. [1,2]

Drug induced liver injury is a serious problem that could lead to death, this might happen in cases where drugs are given within therapeutic doses as a side effect of the drug itself or its metabolite and it also might happen as an idiosyncratic reaction [3].

Statins show an inhibition effect against 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGCR) [4], Despite the marvelous benefits of the statin patients at risk for CVD , they show namouras potential side effects such as hemorrhagic stroke , potential impairment of memory and enhance formation of cataract with a possibility of compromising kidney function, other side effects have been attributed to statins, including neuropathy , sleep disturbances, suicidal behaviors and diabetes mellitus. [5]

Atorvastatin, as it belongs to the statin group, tends to increase the liver enzymes and serious liver injury, however this causal association is not proven yet, due to the fact that the liver enzymes are none specific to the liver itself, but it may be associated with skeletal muscles [6]. This requires a potential investigation to determine the source of these enzymes by studying other skeletal muscle enzymes such as creatine kinase. Patients with chronic liver diseases such as nonalcoholic fatty liver disease, primary biliary cirrhosis and chronic hepatitis, must undergo liver function test before utilization of any statin. [7]

Hesperidin derived from citrus fruits, having a reasonable price and a variety of medical benefits. 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 also superoxide dismutase (SOD) and TNF- α among others [8,9]

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This study aims to investigate both atorvastatin's ability to cause damage to the liver, and hesperidin's
ability to protect the liver from that damage.

Materials and Methods

Chemicals

Atorvastatin and hesperidin powders were provided, the powders were dissolved in DMSO, the required doses were then calculated to each group carefully depending on the weight and the assigned doses to be given to each group depending on the study design.

Laboratory animals

Thirty apparently healthy male Wister rats between the ages of 10 to 12 weeks old weighting less than 250 grams were used in this experiment, the animals were kept in separate cages to provide enough comfort and as less stress as possible, the cages were made from plastic with 20 x 25 x 35 cm dimensions, the rats were provided with clean tab water and platelets in order to drink and eat freely, the cages were kept clean and well cared for, and the animal house was kept under standard characteristics of 12 hours cycle of day/night and also a comfortable temperature around 25-30 °C, the process was done under the supervision of College of Pharmacy/ Mustansiriyah University and have the ethical approval of the facility.

Study design

Thirty apparently healthy and young rats were divided randomly into 5 groups:

1- Negative control group: (n=6) given normal saline.
2- Induction group: (n=6) given a toxic dose of 80 mg/kg of atorvastatin for hepatotoxicity induction.
3- Group 3: (n=6) given a toxic dose of 80 mg/kg of atorvastatin plus minimal dose of 50 mg/kg of hesperidin.
4- Group 4: (n=6) given a toxic dose of 80 mg/kg of atorvastatin plus moderate dose of 100 mg/kg of hesperidin.
5- Group 5: (n=6) given a toxic dose of 80 mg/kg of atorvastatin plus maximum dose of 200 mg /kg of hesperidin.

All doses were given orally by oral gavage, the gavage been used appropriate to the animals, doses were given approximately at the same time daily to eliminate the effects of other elements, all animals were chosen males to eliminate the possibility of gender effect. At the end of the study, animals were sacrifices and a histological analysis has taken place following known procedures [10,11]

Results

The following was obtained:

1- Negative control group given normal saline:
The histopathological figures of control group were illustrated in figures (1A), (1B), (1C) that revealed normal appearance of hepatic lobules, normal central veins and normally arranged hepatic cords, the magnification of figures revealed normal hepatocytes, sinusoids and kupffer cells.

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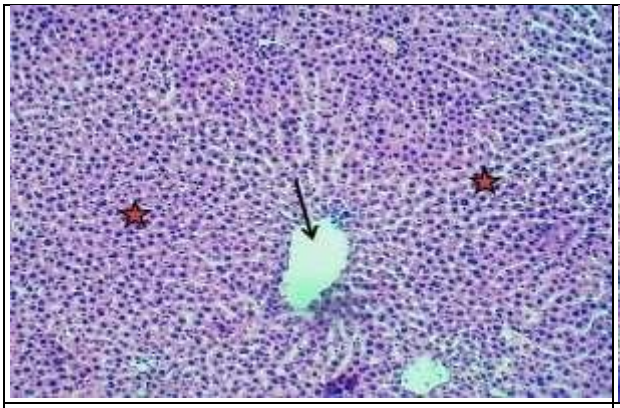

Figure (1A): section of liver shows normal appearance of central vein (Arrow), normally arranged & appearance of hepatocytes (Asterisks). H&E stain.100x.

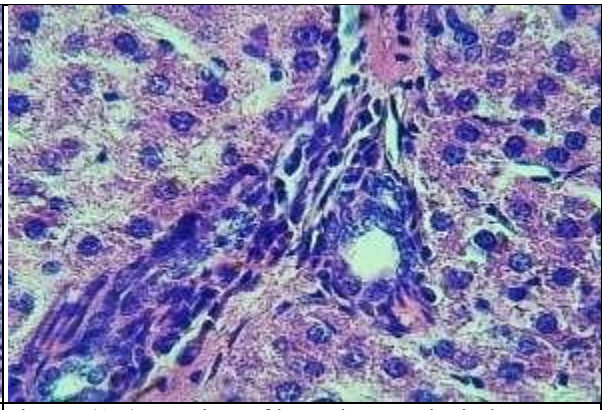

Figure (1B): section of hepatic portal triad shows normal appearance of bile duct & normal appearance of pre portal hepatocytes. H&E stain.400x.

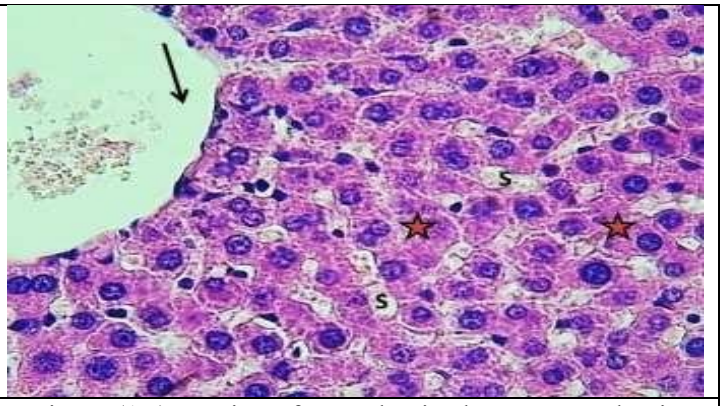

Figure (1C): section of central vein shows: normal vein (Arrow), sinusoids (S) & hepatocytes (asterisk). H&E stain.400x.

2- Induction group given 80 mg/kg atorvastatin:

The histopathological figures of liver showed dilation of central vein that revealed marked irregular outline, marked disarrangement of hepatic cords with zonal degeneration and necrosis (figure 2A). The magnification of the figure showed irregular arranged of hepatocytes with many figures of cellular apoptosis, cellular swelling with granular cytoplasm, cellular necrosis and atrophy (figure 2B). The portal triad revealed marked dilation with congestion of portal vein with portal edema and pre portal necrosis associated with aggregation of mononuclear leukocytes (MNCs) (figure 2C).

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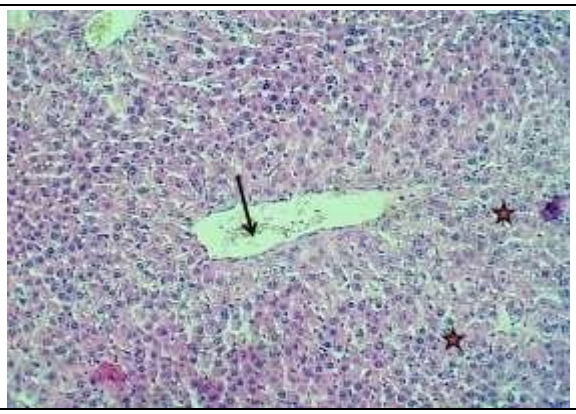

Figure (2A): section of hepatic lobule shows: dilation of central vein with marked irregular outline swelling (arrow), disarrangement of hepatic cords with degeneration and necrosis (asterisk). H&E stain. 100x.

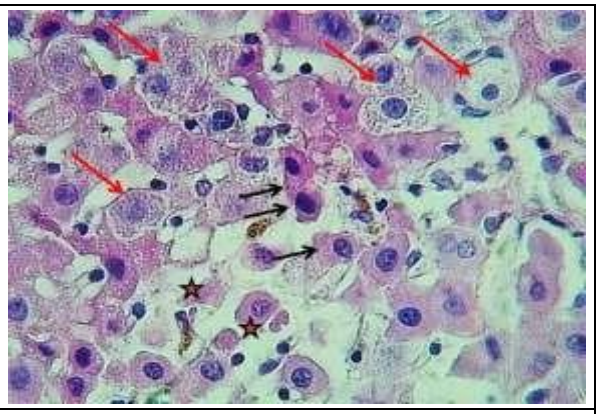

Figure (2B): section of hepatic lobule shows: irregular arranged hepatocytes with many figures of cellular apoptosis (Black arrows), cellular swelling with granular cytoplasm (Red arrows), cellular atrophy and necrosis (asterisks). H&E stain. 400x.

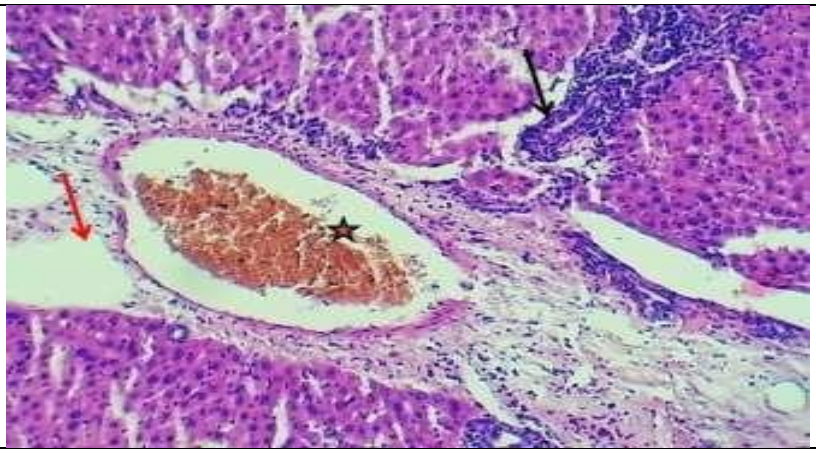

Figure (2C): section of liver shows: sever dilation with congestion of portal vein (asterisk), marked with portal edema (red arrow) & pre portal necrosis with aggregation of MNCs (Black arrows). H&E stain. 100x.

3- Atorvastatin 80 mg/kg + hesperidin 50 mg/kg:

Most histopathological figures of the hepatic lobules showed mild hepatitis with marked portal congestion and pre vascular lymphocytic cuffing (figure 3A), the hepatic parenchyma revealed little sinusoidal infiltration of mononuclear leukocytes (MNCs), with multiple focal necrosis and aggregation of MNCs (figure 3B). Few figures revealed normal appearance of portal and lobular parts of liver with normal appearance of central vein, normally arranged with appearance of hepatocytes (figure 3C).

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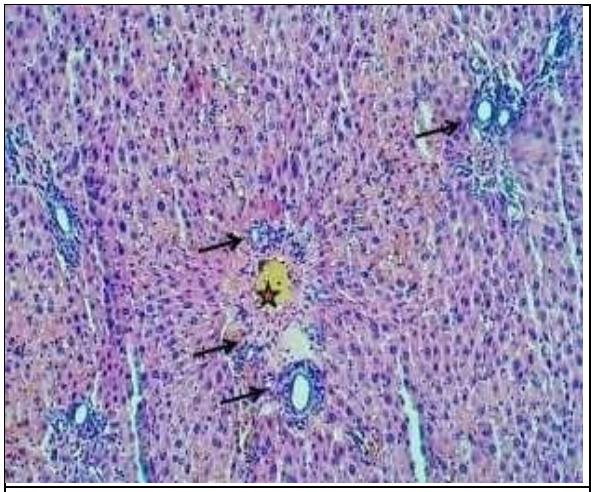

Figure (3A): section of liver shows: moderate hepatitis with marked portal congestion (Asterisk), pre vascular lymphocytic cuffing (arrow). H&E stain.100x.

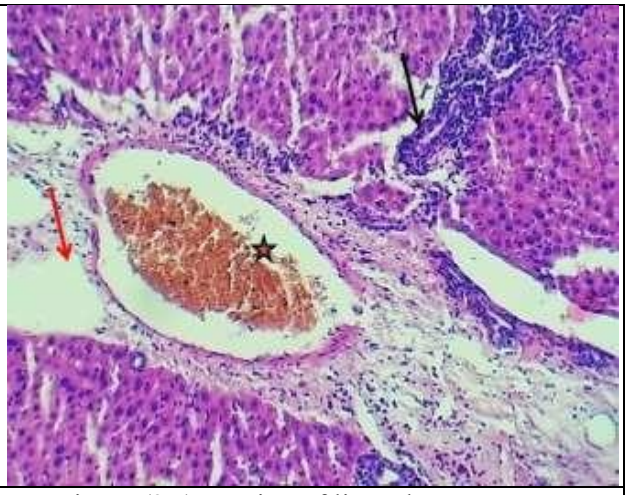

Figure (3B): section of liver shows: sever dilation with congestion of portal vein (asterisk), marked with portal edema (red arrow) & pre portal necrosis with aggregation of MNCs (Black arrows). H&E stain. 100x.

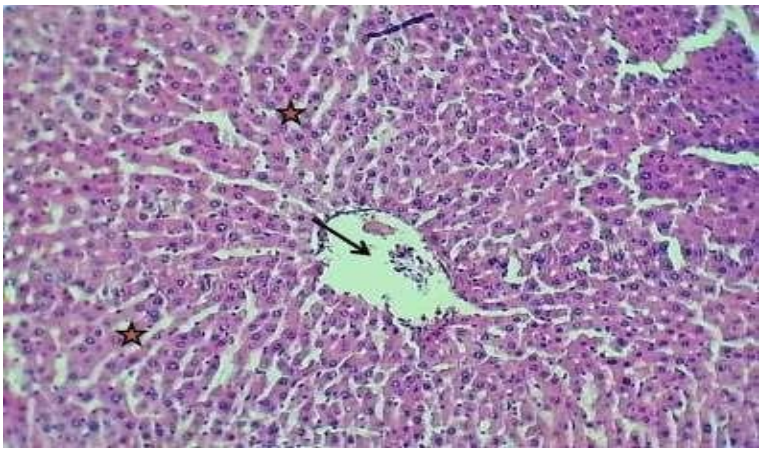

Figure (3C): section of liver shows: normal appearance of central vein (Arrow), normally arranged with appearance of hepatocytes (asterisks). H&E stain.100x.

4- Atorvastatin 80 mg/kg + hesperidin 100 mg/kg:
All histopathological figures of hepatic lobules in showed normal central vein, normally arranged hepatic cords with normal hepatocytes and normal sinusoids (figure 4A, figure 4B). The portal triad showed marked proliferation of cholangiocytes (figure 4C).

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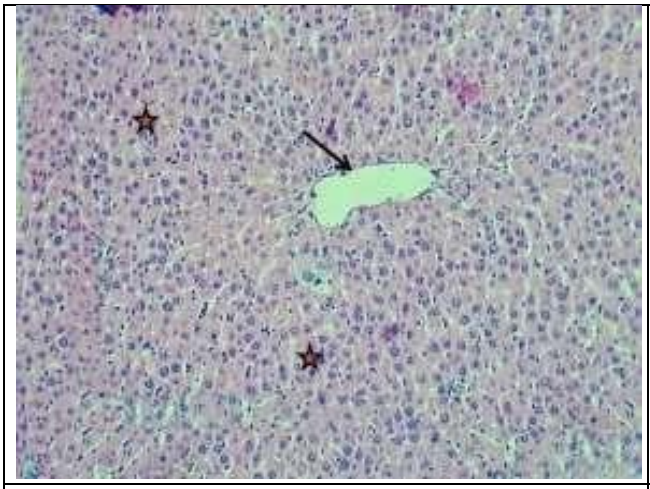

Figure (4A): section of liver shows: normal central vein (arrow) & normal hepatocytes (Asterisk). H&E stain.100x.

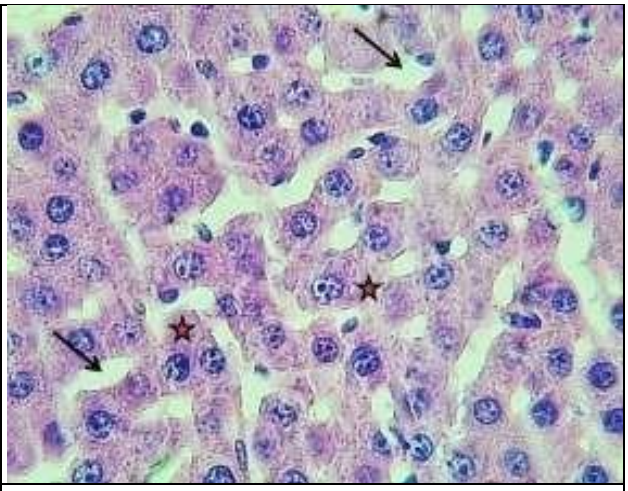

Figure (4B): section of liver shows: normal hepatocytes (Asterisk) with normal sinusoids (arrows) . H&E stain.400x.

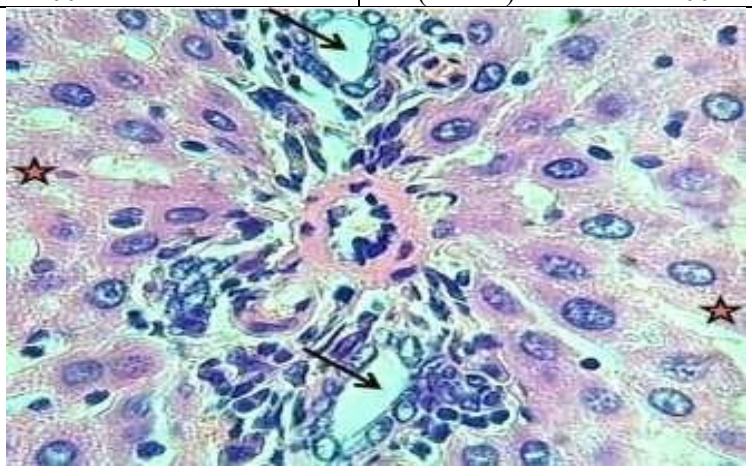

Figure (4C): section of portal triad shows: normal appearance of pre portal hepatocytes (asterisk), with marked proliferation of cholangiocytes (arrows). H&E stain.400x.

5- Atorvastatin 80 mg/kg + hesperidin 200 mg/kg:

The histopathological figures of hepatic lobules showed mild hepatitis with mild congestion of central vein with dilation and congestion of portal vein and mild sinusoidal infiltration of MNCs (figure 5A), the magnification of figures revealed granular degeneration of hepatocytes, with little infiltration of lymphocytes with few figures of apoptotic hepatocytes (figure 5B). Other figures revealed normal lobular and portal liver regions with normal veins, sinusoids and normal hepatocytes (figure 5C).

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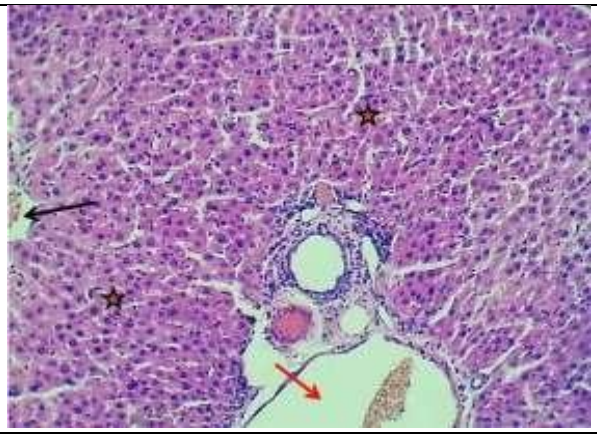

Figure (5A): section of hepatic lobule show mild hepatitis with mild congestion of central vein (Black arrow) with dilation and congestion of portal vein (Red arrow) & mild sinusoidal infiltration of MNCs (Asterisks) .H&E stain.100x.

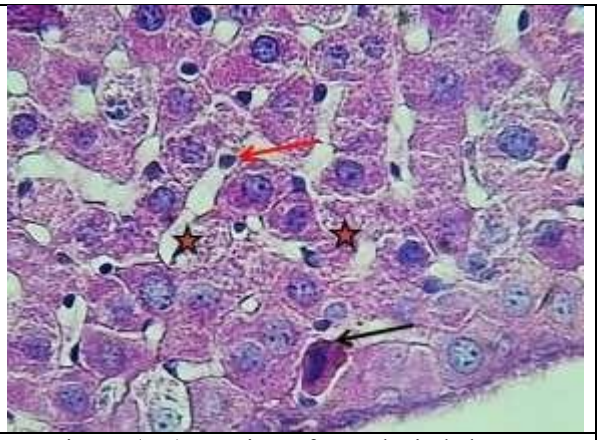

Figure (5B): section of portal triad shows: mild granular degeneration of hepatocytes (asterisk), with little infiltration of lymphocytes (Red arrow), apoptotic hepatocyte (Black arrow). H&E stain.400x.

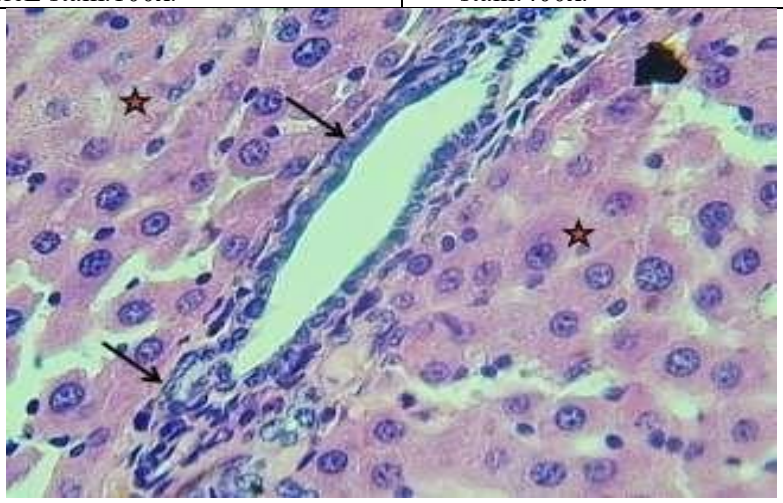

Figure (5C): section of portal triad shows: normal appearance of pre portal hepatocytes (asterisk), with marked proliferation of cholangiocytes (arrows). H&E stain.400x.

Discussion

The notable decline in liver tissue health observed in Group 2, compared to Group1, can be directly attributed to the effects of atorvastatin. Severe necrosis, severe dilation, and congestion of the portal vein among other effects are consistent with those observed in other studies, one study that examined the effect of atorvastatin histologically on liver tissues found out that congestion worsened with the increase of atorvastatin doses, inflammation and necrosis were observed with higher doses [(12)], that study also found similar results of hepatotoxicity when testing for other inflammatory markers, providing a full image of increase risk to the liver connected to increase doses of atorvastatin. A comprehensive clinical review discussing the various effects of statins on healthy liver cells resulted in observation of various hepatocellular injuries. Researchers noted different types of liver damage, such as ballooning degeneration

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that's characterized by liver cells swelling, and also necrosis which is cell death caused by an injury. There were also signs of inflammatory cell infiltration which suggest the possibility of an ongoing inflammatory response within the tissue. Some even more concerning results shared the possibility of autoimmune hepatitis which characterize by the immune system mistakenly attacking liver cells resulting in many complications. The alarm has been raised over the possibility of a potential chronic liver damage as a result of long term using statin [13], this draws the attention over the need to monitor liver functions in patients using statins and specially atorvastatin prescribed to them to manage cholesterol levels.

These changes are not studied closely therefor it cannot be determined which mechanism is exactly responsible for the damage, there could be one mechanism or there could be many involved, studies have provided evidence to suspect it could be explained by mitochondrial dysfunction, oxidative stress affecting apoptosis, enhancing the expression of pro-inflammatory cytokines that could lead to necrosis, and affecting bile flow therefor causing cholestasis. [13]

Groups that had hesperidin in their treatment had healthier outcomes related to different doses,

marked portal congestion with multiple focal necrosis was observed on group of 80 mg/kg atorvastatin + 50 mg/kg hesperidin showing mild hepatitis, 80 mg/kg atorvastatin + 200 mg/kg hesperidin also showed a mild hepatitis with congestion of the portal vein.

In the study, Group 4, which received a combination of 80 mg/kg atorvastatin and 100 mg/kg hesperidin, exhibited the most significant improvement in liver health compared to other groups. This remarkable enhancement underscores not only the positive effects of atorvastatin on liver tissue but also emphasizes the crucial role of dosage in determining the extent of these benefits. The finding suggest this exact combination of atorvastatin and hesperidin might provide a synergic protected effects over liver cells resulting in marinating the atorvastatin effects as well as enhancing the protection of liver cells, making this group the most protected cohort among all participants receiving atorvastatin.

The protective ability of hesperidin was observed in a number of studies and is believed to be directly link to the medication's antioxidant ability. One study investigated the hepatoprotective effects of hesperidin in a rat model of methotrexate-induced liver injury, the results demonstrated that hesperidin possesses significant protective characteristics helping in protecting liver cells from injury, the study found out that hesperidin was able to protect liver cells from damage caused by oxidative stress that happens as a result of an imbalance between free radicals and antioxidant in a living body which can result in cell injury, in addition hesperidin was able to reduce inflammation which could contribute to cell injury as well. [14].

Another study assessed the histopathological effects in rat model with diabetic neuropathy. After administrating hesperidin, researchers observed notable evidence of reduced oxidative stress and significant inflammatory changes. The findings indicated that hesperidin plays a crucial role in alleviating the detrimental effects associated with diabetic neuropathy which is a condition often characterized by heightened inflammation and oxidative damage. [15]

These characteristics helped hesperidin to emerge as a promising candidate for protecting liver cells against drug-induced injury and the results of this study proved these characteristics.

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Conclusion

Atorvastatin do have a damaging effect on liver cells at certain doses resulting in a variety of changes, whoever the liver could be protected against these damages by the presence of hesperidin at the right dose, therefore caution should be takes when using these medications.

Acknowledgment

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Conflict of interest

There is no conflict of interest.

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