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Effect of Rosuvastatin on the liver in male albino rats

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Abstract:

Statins are among the most commonly used drugs to lower cholesterol, and rosuvastatin is one of the most important statins, as it inhibits the HMG-CoA enzyme and targets the liver more than other statins. Elderly patients usually receive these drugs to prevent cardiovascular disease. It is known that exposure to drugs for a long time can lead to disorders in some organs, so the study current aimed to find out the effect of rosuvastatin treatment on liver tissues and to what extent it affects biochemical indicators of liver function in albino rats, serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and gamma-glutamyltransferase (GGT) were determined.

The results of the current study indicated that administration of rosuvastatin for 60 days led to a change in serum levels (ALT, GGT and AST), these changes in biochemical tests cause histopathological effects and the possibility of hepatotoxicity.

Keywords: HMG-CoA enzyme, Hepatotoxicity, Rosuvastatin, Statins

1-Introduction

Statins were discovered in the 1970s and are most commonly used worldwide as anti-hyperlipidemia drugs (Lyons and Harbinson, 2009). CoA reductase converts HMG-CoA into mevalonate, which is an early, rate-limiting step in cholesterol production (Downs *et al*.,1998; Shepherd *et al*., 1995).

Rosuvastatin is more efficient than other statins; In the United States, it was the most prescribed brand-name drug with 22.3 million prescriptions (IMS Health, 2014). Rosuvastatin is a synthetic compound consisting of a single nanocomposite formulated and administered as the calcium salt of the

active hydroxycitric acid, which is a white, amorphous substance and a powder that is sparingly soluble in water and methanol and slightly soluble in ethanol, this agent has been shown to be highly effective in lowering LDL-C and improving other components of the lipid profile in atherosclerosis in patients with a variety of dyslipidemias (Olsson *et al*., 2002).

In contrast, some studies have found that it has negative effects, De Dinos *et al*. (2004) reported that patients treated with statins showed increases in serum liver enzymes by 2% to 3%. As (Niza *et al*., 2002) indicated that hepatocytes take up rosuvastatin more selectively, the liver is the primary target organ for rosuvastatin. Famularo *et al*. (2007), which could lead to potential hepatotoxicity (Guthrie and Martin, 2007; Khan and Ibrahim, 2009). Kaplowitz (2004) previously confirmed that the hepatotoxicity of statins is well known. In contrast, other studies have indicated that rosuvastatin may be less likely to cause hepatotoxicity than other statins (Davidson, 2007).

With the limited and contradictory studies presented on rosuvastatin-induced liver injury, the present study aimed to investigate the histopathological changes of the liver with rosuvastatin treatment and to investigate the levels of biochemical indicators of liver function in male albino rats.

2- Methodology of proposed methods

2.1 Animal grouping

In the current study, male albino rats were obtained from the Department of Biology - College of Sciences - University of Thi-Qar. Their ages ranged between (12-14) weeks, and weights (200-250) gm. A veterinarian examined the animals to ensure they were free from diseases, and then the animals were transferred to the animal house in the Department of Biology- College of Education and Pure Sciences, under controlled conditions at a temperature of 20-25 °C. The cabin air was changed continuously using a vacuum ventilation device with a fitted 12 hours light and 12 hours dark cycle.

Thirty-two adult males albino rats were divided randomly into four equal groups; each group included 8 males and gavage for 60 consecutive days according to the method of Mohammed and Hossain (2022) as follows:

1. **Control group:** all rats were given an equivalent volume of normal saline.
2. **Group 10 mg:** all rats were given 10 mg/kg/day of rosuvastatin.
3. **Group 20 mg:** all rats were given 20 mg/kg/day of rosuvastatin.
4. **Group 40 mg:** all rats were given 40 mg/kg/day of rosuvastatin.

All groups were treated with oral gavage for 60 days.

2.2 Preparation of drug

Rosuvastatin is obtained in the form of commercial rosuvastatin (Crestor) in film-coated tablet form and concentrations (10-20-40) mg produced by Pharma Science Incorporated- Montreal/ Canada), approved in the United States since 2003.

Rosuvastatin tablets were mashed with a sterile pestle/mortar and then dissolved in 0.9% sodium chloride solution to obtain rosuvastatin solution as a compound, after which albino rats were orally gavaged according to the animal's body weight (Nair and Jacob, 2016). Then, store the fully dissolved suspension at 4°C until use.

2.3 Sample collection

2.3.1 Blood collection

After the end of the gavage period, animals were anesthetized with chloroform and blood was drawn directly from the heart by cardiac puncture using 5-mL medical syringes, and placed in a gel tube were collected to coagulating, then centrifuged at 3000 rpm for 10 min to get the serum ,and then samples of serum were kept in a freezer at −20 ºC till use (Hassan and Sharhan, 2014).

For liver function enzymes, serum AST and ALT were estimated by using the methodology recommended by the International Federation of Clinical Chemistry (Bergmeyer *et al*., 1986). Measurement of GGT was also estimated using the methodology of Szasz (1976). All calculations were made by using kits provided by (AGAPPE, 2023) Company, India, in compliance with manufacturer's instructions.

2.3.2 Tissue sampling

The animals were dissected for all groups, as the organ required for the study (liver) were removed, and the histological sections were prepared according to the method of Bancroft et al. (1996). starting with the fixation of the tissue organ with formalin 10 %, followed by tissue processing, as follows: it which included several steps, it included dehydration by ethanol at concentrations (70% - 90% - 95% - 100%), then clearing by xylene, and finally infiltration by paraffin wax, then followed by the process of embedding and the process of trimming the wax molds containing the sample, then pasted on the wooden mold for a Rotary microtome, it was cut with a thickness of (4-5) mm. Finally, for histological evaluation of the liver, the sections were placed on plain glass slides and stained with hematoxylin and eosin stains (HE) according to the method of Sharhan *et al*. (2020). HE-stained sections were observed for abnormalities of histopathological features under a light microscope at 40×, 100× and 400×. The histological sections was photographed using Sony a7riv camera attached to Krüss stereomicroscope, then digital images were montaged using software (Helicon Focus™ 8.1.0).

3. Statistical analysis

The statistical analysis of the results was conducted by Analysis of Variance (ANOVA) and significant differences were determined according to Duncans multiple range test and the level of significance ($P \leq 0.05$) by the Statistical Package for Social Sciences (SPSS) program (Bryman and Cramer, 2012).

4. Results

4.1 Effect of rosuvastatin on liver enzymes

The results of the current study show in Table (1) the serum levels of liver enzymes (AST, ALT and GGT). There was a gradual increase in AST levels in the groups treated with rosuvastatin compared with the control group. The statistical results showed insignificant increases (P≤0.05) in the groups (10 mg, 20 mg, and 40 mg) compared with the control group. Also, there were gradual increases (P≤0.05) in ALT levels between the groups treated with rosuvastatin and the control group. The statistical results showed a significant increase (P≤0.05) in the 40 mg group compared with the control and 10 mg groups, At the same time, there was an insignificant increase (P≤0.05) between the 40 and 20 mg groups. The current study showed significant increases (P≤0.05) in GGT levels among the groups treated with rosuvastatin compared to the control group. Statistical results showed a significant increase (P≤0.05) in the 40 mg group compared to the control and 10 mg group. At the same time, there was an insignificant increase (P≤0.05) between the 40 mg group compared to the 20 mg group, and also between the 10 mg group and the control group.

Table (1): Shows the impact of rosuvastatin on the serum levels of AST, ALT and GGT in the study groups (Mean ± SD) at (P≤0.05).

Parameters / Group	AST Mean ± SD	ALT Mean ± SD	GGT Mean ± SD
Control group	71.66±37.56[a]	24.83±12.33[a]	9.43±7.10[a]
Group 10 mg	80.33±38.47[a]	27.00±9.83[a]	13.00±7.80[ab]
Group 20 mg	100.33±35.16[a]	40.66±2.58[ab]	24.66±3.72[bc]
Group 40 mg	114.33±35.62[a]	70.36±48.52[b]	31.63±17.90[c]

*[a-c] Different letters within column indicate significant differences between groups. SD: standard deviation, AST: Aspartate Aminotransferase, ALT: Alanine Aminotransferase, GGT: Gamma-Glutamyl Transferase.

4.2 The histopathological effects of rosuvastatin on liver tissue

The histological sections of the liver in the control group showed the normal architecture of the liver tissue in the arrangement and regularity of the liver cells radially around the central vein. Through histological examination, it was found that the liver is surrounded by connective tissue that extends inward to divide the inner tissue into hepatic lobules that contain hepatocytes inside and have central nuclei. Blood sinusoids are also observed clearly and normally, as in Figure 1.

The results of the liver histopathology study of the 10 mg group showed irregularity of hepatocytes and loss of radial arrangement, dilatation of sinusoids, congestion in hepatocytes and moderate perivascular hemorrhage, as in Figure 2.

As for liver tissue sections in the 20 mg group, the results of the current study showed arrangement of irregular hepatocytes, loss of nuclei, loss of radial arrangement, collection of lymphocytic, dilatation of sinusoids, central venous congestion and necrosis of hepatocytes, as in Figure 3.

While, the results of the liver histopathology study of the 40 mg group showed a loss of radial arrangement of hepatocytes around the hepatic vein, lymphocytic infiltration, congestion in the hepatic vein, as well as the presence of hemorrhage and high degenerative figures in hepatocytes, as in Figure 4.

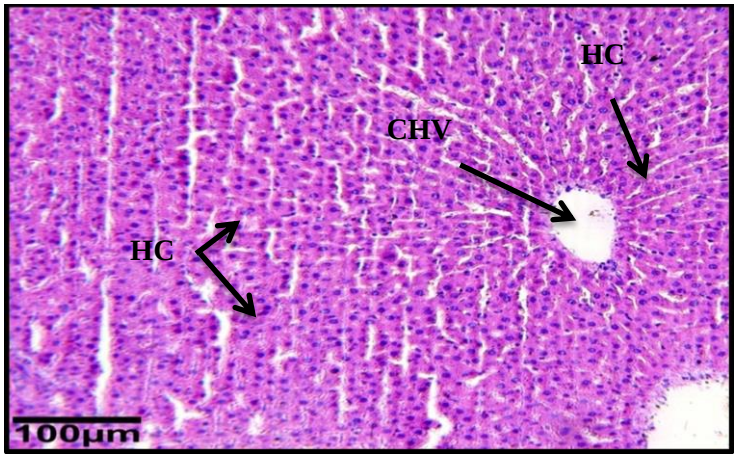

Figure (1): Photomicrograph of rat liver sections of the control group showing normal architecture: central hepatic vein (CHV), hepatocyte (HC), hepatic lobules (HL). H and E, at 40x (scale bar 100 µm).

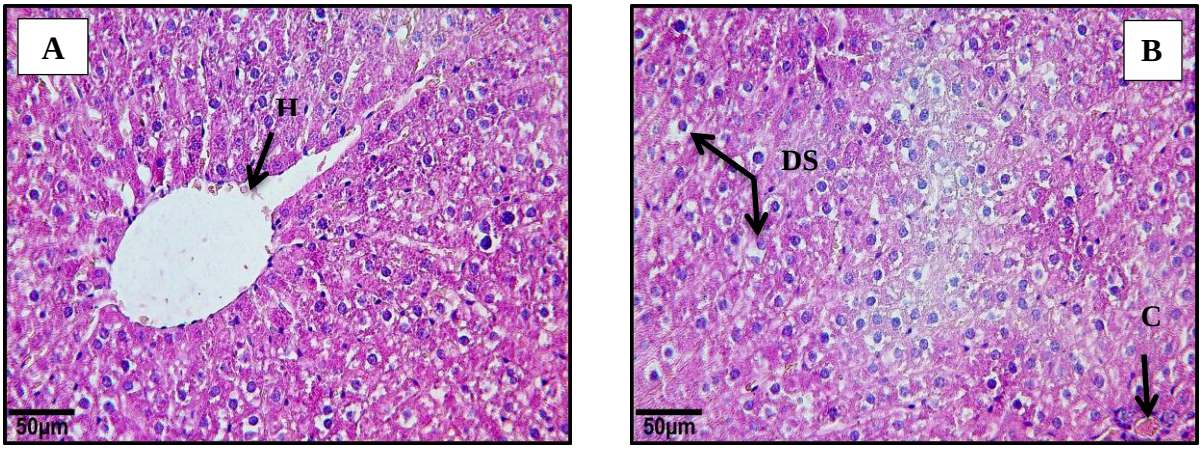

Figure (2 A, B): Photomicrograph of rat liver sections of the rosuvastatin-treated group (10 mg) showing histological effects: dilatation of sinusoids (DS), congestion (C), moderate hemorrhage (H). H and E, at 100x, 400x (scale bar 50 µm).

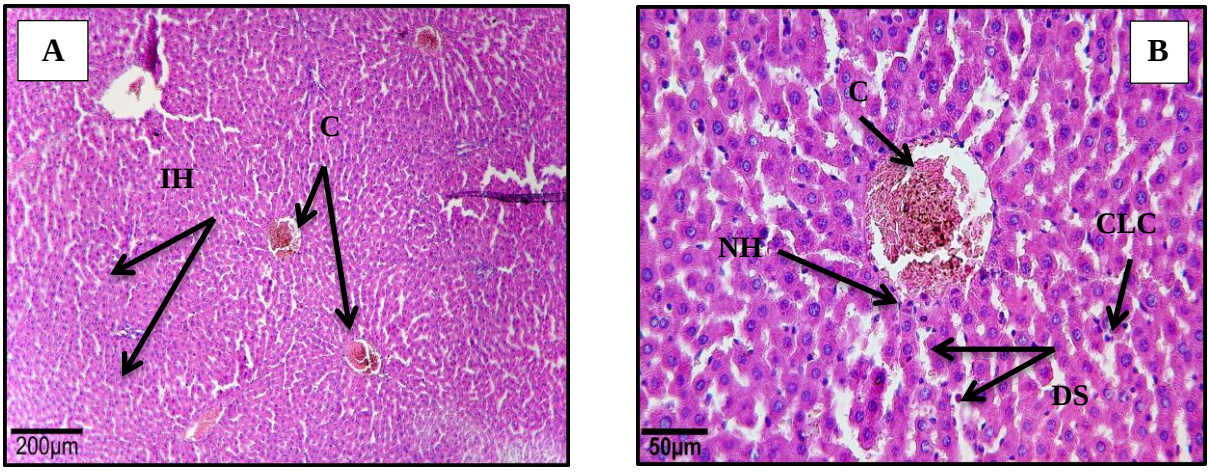

Figure (3 A, B): Photomicrograph of rat liver sections of the rosuvastatin-treated group (20 mg) showing histological effects: congestion in the hepatic vein (C), arrangement of irregular hepatocytes (IH), dilatation of the sinusoids (DS), collection of lymphocytic (CL), necrosis of hepatocytes (NH). H and E, at 40x, 400x (scale bar 200 µm and 50 µm).

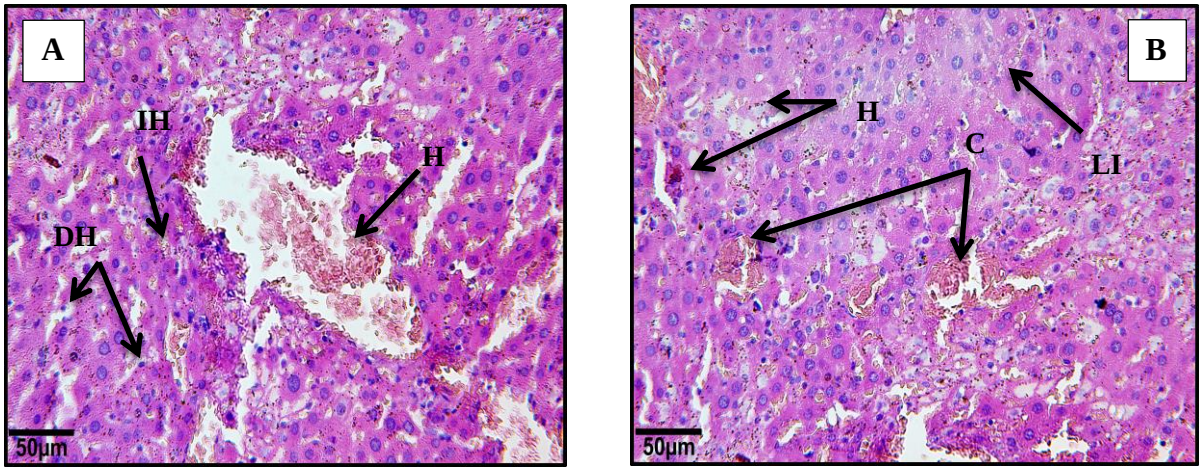

Figure (4 A, B): Photomicrograph of rat liver sections of the rosuvastatin-treated group (40 mg) showing histological effects: arrangement of irregular hepatocytes (IH), lymphocytic infiltration (LI), congestion (C), hemorrhage (H), high degenerative figures in hepatocytes (DH). H and E, at 100x ,400x (scale bar 50 µm).

5. Discussion

The current study showed significant changes in liver function among the study groups, including AST, and the statistical results showed non-significant increases (P≤0.05) in groups (10 mg, 20 mg, 40 mg) compared with the control group, while ALT levels showed results Statistically significant increase (P≤0.05) in the 40 mg group compared to the control and 10 mg group, and this result is consistent with Ashraf *et al*. (2020) and Aziz *et al*. (2020), finding an association of statins with higher levels of ALT compared to AST levels, this is simply consistent with the fact that ALT is a more specific marker of liver injury than AST (Katarey and Verma, 2016). As it was reported (Dujovne, 2002; Tolosa *et al*. 2015) that statins damage mitochondrial membranes, leading to ALT and AST leakage, these findings were also confirmed by Dale *et al*. (2007) and Perdices *et al*. (2014) that statins in increased doses such as atorvastatin, fluvastatin, and rosuvastatin lead to increased ALT levels and a slight increase of AST levels.

This increase in AST and ALT levels may lead to the metabolism of statins, resulting in potential hepatotoxicity, which may be due to an alteration of the cell membrane of hepatocytes without directly injuring the liver (Veillard and Mach, 2002; Clarke and Mills, 2006). A high level of these enzymes in the blood is therefore an indicator of liver damage (Byrne, 2012; Dyson *et al*., 2014).

In addition, the current study showed significant increases (P≤0.05) in GGT levels among the groups treated with rosuvastatin compared to the control group. Statistical results showed a significant increase (P≤0.05) in the 40 mg group compared to the control and 10 mg group, this result is consistent with Xu and Wu (2020) reporting an increase in statin-induced GGT of up to 6 times the normal level after 4 weeks of taking it and a return of serum GGT to normal within 6 weeks of stopping statin administration, and serum GGT levels can be a good indicator of liver injury (Ho *et al*. 2022).

As for the results of the current study on liver tissue showed varying histopathological changes in the study groups, which are consistent with the biochemical results and are associated with increased levels of ALT, AST, and GGT in the blood.

The examination results of the liver sections showed congestion in the central vein, infiltration of lymphocytic concentrated around the central vein and the portal region of the hepatic lobules, and tissue degeneration. These changes increased unevenly with the increase in the concentration of rosuvastatin. The groups most affected by drug treatment were 20 mg and 40 mg, and there is no doubt that these damages resulted from the toxic effect of the drug or one of its metabolites on liver cells, the main organ responsible for drug metabolism. Yeung *et al*. (2003) indicated that rosuvastatin is taken up by hepatocytes selectively and thus associated with potential hepatotoxicity of rosuvastatin. Also, significant hepatic toxicity may occur after a short course of rosuvastatin (Famularo *et al*. 2007).

The results of the current study agreed with the result of Fujimoto *et al*. (2016), which showed that venous congestion increases hepatic hydrostatic pressure and results in edema and hemorrhage, which harms oxygenation and ultimately leads to damage to hepatocytes.

The reason for the concentration of infiltration of lymphocytic in the liver sections of the current study may be the presence of the drug in higher concentrations in the blood serum, and this result agreed with Abdulwahhab (2022).

The results of the current study also agreed with Reyes-Gordillo *et al*. (2017), indicating that exposure of the liver to biotransformed metabolites, alcohol, fats, and viruses lead to liver injury, which can lead to liver degeneration.

6. Conclusion

In the current study, it was concluded that high doses of rosuvastatin lead to liver injury. Moreover, infection was confirmed by AST, ALT and GGT levels, in addition to histopathological changes that revealed liver tissue injury.

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