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THE EFFECTS OF ROSUVASTATIN EFFECT ON THE KIDNEY IN MALE ALBINO RATS

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Abstract

Background and Aim: Rosuvastatin is known to competitively inhibit HMG-CoA reductase, selectively and reversibly, this enzyme converts HMG-CoA to mevalonic acid in the cholesterol biosynthesis pathway, which is the rate-limiting step in cholesterol synthesis. Rosuvastatin is one of the most important cholesterol-lowering stanins that has been on the market by AstraZeneca since 2003, with unique pharmacokinetic and pharmacodynamic properties. There are some studies that dealt with the effect of rosuvastatin on the kidneys and were contradictory in their results. Therefore, the aim of the current study was to evaluate the effect of rosuvastatin (pharma science Incorporated company - Montreal/ Canada) on the physiological and histopathological parameters in the kidneys of male albino rats.

Material and Methods: Forty adult of albino rats were used in the study. After acclimation, the rats were randomly divided into four groups (8 rats in each group) as follows: The first control group: was given normal saline solution (NS), the second group 10 mg/kg rosuvastatin, the third group was given 20 mg/kg rosuvastatin, while the fourth group was given 40 mg/kg rosuvastatin. The dose was continued for 60 days in a once-daily dosing regimen. After the end of the experiment, chemical analyzes were performed for kidney function, including cystatin C and vitamin D3. Then the rats were dissected, and kidney tissues were taken for histological study.

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Results: Male rats treated with rosuvastatin showed a high level of cystatin C and a low level of D3. While the results of microscopic examination showed significant histopathological changes in the kidneys of rosuvastatin groups compared to the control group.

Conclusion: It was concluded in the current study that the use of rosuvastatin in high doses causes nephrotoxicity.

Keywords: *HMG-CoA, Rosuvastatin Effect, Male Albino Rats*.

Introduction

Statins are among the most widely used drugs in the world. They are effective in reducing cholesterol levels in the blood, of which high levels cause hardening of the arteries and damage to the health of the heart and blood vessels, statins inhibit the 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, the enzyme that catalyzes the rate-limiting step of cholesterol synthesis in the liver and other tissues, thus reducing hypercholesterolemia (Corsini et al. 1995). Statins can reduce the risk of heart attack, stroke, and angina. statins taken alone have shown few side effects, it is possible that patients taking multiple drugs in combination with statins could experience toxic side effects due to drug-drug interactions (DDI). It is also widely used for the treatment of hyperlipidemia as well as for the prevention of atherosclerosis and cardiovascular events (Wainwright, 2005; Bjornsson et al., 2012).

Therapy with high doses of statins can cause nephrotoxicity. Rosuvastatin has several adverse effects on the kidneys, muscles, and liver (Almandoh et al., 2020). There is a direct correlation to renal toxicity with high doses of statins, as well as an indirect correlation by interactions with other factors, and this leads to an increase in the concentration of statins in the blood. Statins cause changes in cell membrane permeability, decreased levels of coenzyme Q10, and reduced isoprenoids due to inhibition of cholesterol production that can lead to nephrotoxicity (Ahmadizar et al. 2023). Hematuria (blood in the urine) and proteinuria (protein in the urine) were diagnosed in 44% of kidney patients who were prescribed high doses of rosuvastatin, so it is recommended that no doses higher than 10 mg per day of rosuvastatin be taken (Shin et al. 2022).

In general, some side effects coincide with the use of drugs, which lead to damage to some organs of the body, even those that were not targeted by the drug. Therefore, the current study came to find out the physiological and histological effects of rosuvastatin drugs in male laboratory rats.

Materials and Methods:

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. The animals were examined by a veterinarian to ensure that they were free from diseases, 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.

Forty adult males Albino rats were divided randomly to the four equal groups, each group included 10 male and gavage for 60 consecutive days as following:

1. Control group: all rats were given an equivalent volume of normal saline via oral gavage for 60 days.

2. Group 10 mg: all rats were given 10 mg/kg/day of rosuvastatin via oral gavage for 60 days.

3. Group 20 mg: all rats were given 20 mg/kg/day of rosuvastatin via oral gavage for 60 days.

4. Group 40 mg: all rats were given 40 mg/kg/day of rosuvastatin via oral gavage for 60 days.

Preparation of drug

Rosuvastatin was obtained in the form of commercial rosuvastatin (Crestor) in the form of film-coated tablets and concentrations (10-20-40) mg produced by the pharma science incorporated company - Montreal/ Canada), which has been approved in the United States since 2003, and was mashed with a sterile pestle / mortar and then dissolved in 0.9% sodium chloride solution to obtain rosuvastatin solution as compound, thereafter white rats were gavaged orally according to the animal's body weight (Nair and Jacob, 2016). The fully dissolved suspension was stored at 4 °C until use.

Sample collection

Blood collection

After the end of the injection period, the body weight for each rat was measured, then the animals were dissected after anesthetizing them with ether and chloroform, then the blood was drawn directly from the heart by cardiac puncture using medical syringes with a capacity of 5 ml, 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°C till use (Al-Alwani, 2021).

For renal function, cystatin C was measured based on the method of Newman (2002) for calculating the concentration of cystatin C in serum, and vitamin D_3 concentration based on the method of Holick (2007) for calculating the concentration of vitamin D_3 in serum.

Tissue sampling

The animals were dissected for all groups, as the organ required for the study (kidneys) were removed, and the histological sections were prepared according to the method of Bancroft and Gamble (2008), 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 kidneys, the sections were placed on plain glass slides and stained with hematoxylin and eosin stains (HE). 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).

Statistical analysis

One way analysis of variance (ANOVA) was used to analyze the data statistically. Significance between the studied criteria was tested by (Duncan multiple ranges) test with a probability level of P≤0.05 by the Statistical Package for Social Sciences (SPSS) program (Bryman and Cramer, 2012).

Results

Effect of rosuvastatin on kidney function

To investigate the effects of rosuvastatin on kidney function, kidney function parameters including cystatin C and vitamin D_3 serum were carried out in experimental groups by biochemical analysis as in table 1.

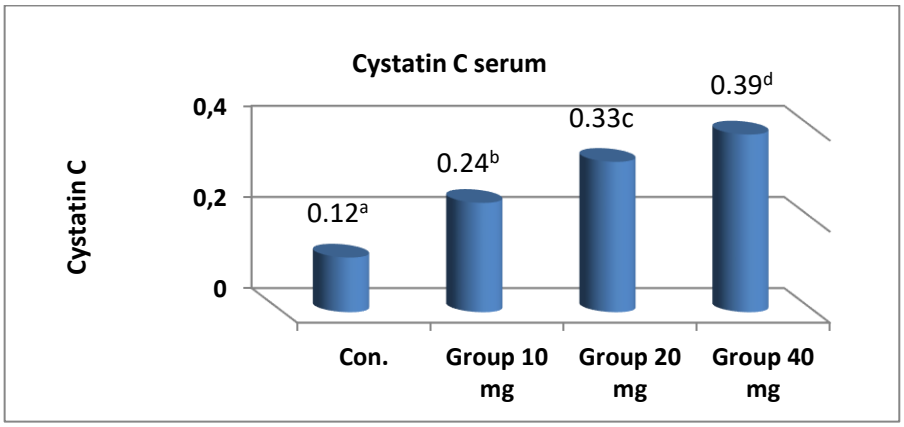
Effect of rosuvastatin on cystatin C levels

Rosuvastatin 10 mg, 20 mg, and 40 mg groups demonstrated a significant (P≤0.05) higher levels of cystatin c as compared with that of control group. Furthermore, rosuvastatin 20 mg, 40 mg groups showed a significant (P≤0.05) higher levels of cystatin c as compared with rosuvastatin 10 mg group. On the other hand, the study showed there is significant elevated in cystatin c level in rosuvastatin 40 mg group when compared with rosuvastatin 20 mg. These results as shown in figure 1.

Table (1): Shows the impact of rosuvastatin on the serum levels of cystatin C and Vitamine D_3 in comparison with control group (mean ± SD).

Parameters / **Group**	**CYS C MG/L** **Mean ± SD**	**D_3 NG/ML** **Mean ± SD**
Control	0.12±0.008[a]	12.30±0.17[a]
Group 10 mg	0.24±0.012[b]	12.46±0.37[a]
Group 20 mg	0.33±0.11[c]	13.06±0.40[a]
Group 40 mg	0.39±0.008[d]	17.20±2.98[b]

***[a-d] Different letters within column indicate significant differences between groups**



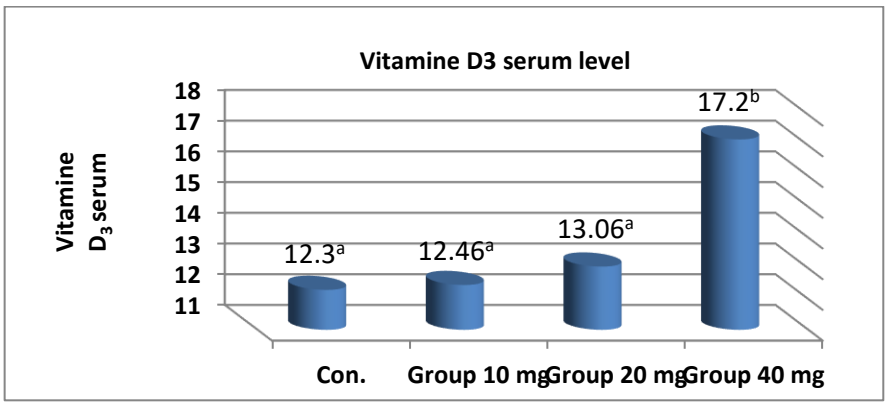
[a-d] **Different letters indicate standard deviation (P≤0.05).**

Figure 1. The mean (n=8) serum cystatin c level (mg/L) in the four experimental groups

Effect of rosuvastatin on vitamin D levels

Rosuvastatin 10 mg, 20 mg, and 40 mg groups showed a significant (P≤0.05) low levels of Vitamin D_3 as compared with Vitamin D_3 level of control group. Additionally, rosuvastatin 20 mg, 40 mg groups exhibited a significant (P≤0.05) low levels of Vitamin D_3 when compared with rosuvastatin 10 mg group.

Also, the current study showed there is a significant (P≤0.05) low levels of Vitamin D_3 rosuvastatin 40 mg group when compared with rosuvastatin 20 mg group. These results were summarized in figure 2.



[a-d] **Different letters indicate standard deviation (P≤0.05).**

Figure 2. The mean (n=8) serum vitamine D_3 level (mg/l) in the four experimental groups

The histopathological effects of rosuvastatin on kidney tissue

The results of the histological study of the kidneys of control group animals showed that the kidney is composed of the cortex region, which contains the renal glomeruli of normal shape and size, the renal tubules (proximal and distal renal tubules, and the collecting tubule), with normal epithelial cells, and the inner region represents medulla region (Fig. 3). The results of the histopathological study of the kidneys of the 10 mg group showed that the tubular structure was

degeneration in the epithelial cells, hemorrhage in the renal tubules, and the beginning of damage to the glomerulus (Fig. 4 A, B). While the histological study of the kidneys of the 20 mg group showed irregularity in the normal form of the renal glomerulus, necrosis and hemorrhage in the blood vessels, congestion in the renal tubules, cysts in the kidney tissue, infiltration of inflammatory cells, and the presence of vacuoles and narrowing of the renal tubules and expansion of some of them (Fig. 4 C-E).

The histological study of the kidneys of the 40 mg group showed severe damage to the tissue structure, severe hemorrhage, glomerular necrosis, hyperplasia in the renal tubule cells, venous congestion, degeneration of the distal renal tubule cells, infiltration of inflammatory cells and loss of the glomerulus to normal form (Fig. 4 F).

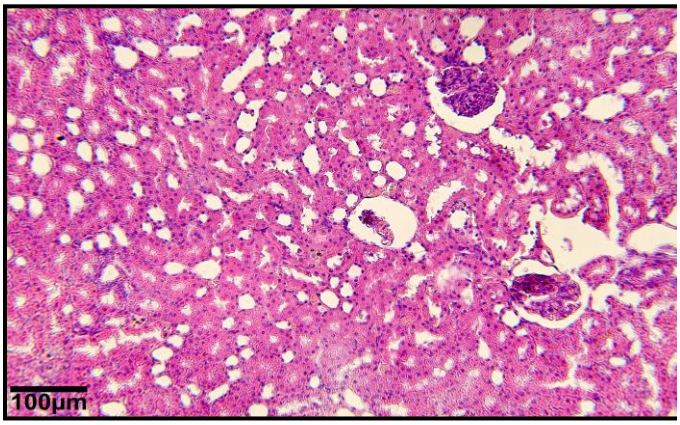


Figure (3): Photomicrograph of rat kidney sections of control group shows kidney normal histology, stained with hematoxylin-eosin, are shown at 10x magnification (scale bar 100 µm).

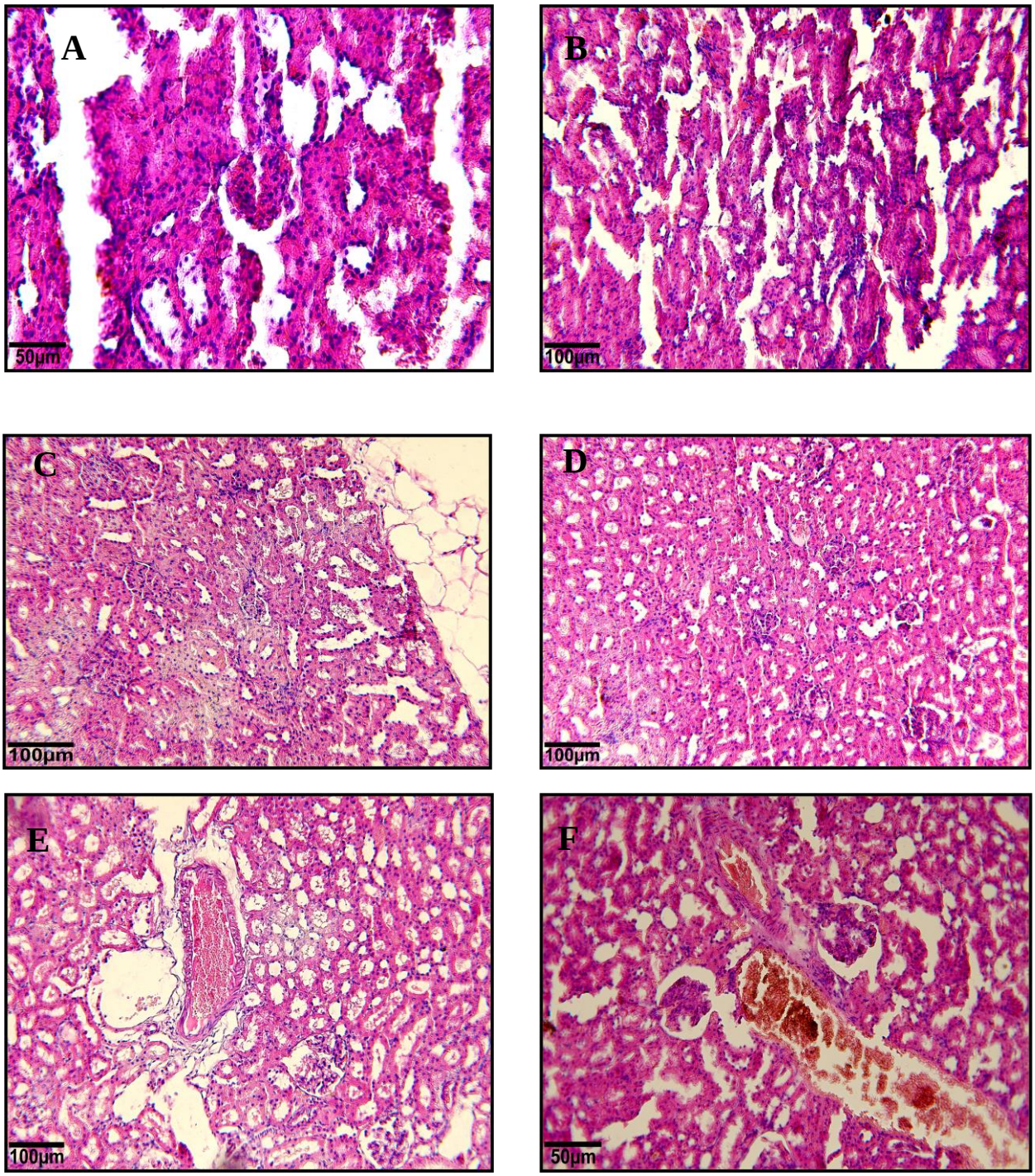


Figure (4 A-G): Photomicrograph of rat kidney sections of rosuvastatin groups (10, 20 and 40 mg/kg) shows histopathological effects, stained with hematoxylin-eosin, are shown at 40x, 100x and 400 magnification (scale bar 100 µm and 50 µm).

Discussion

In the current study, cystatin C was measured, which is filtered by the renal glomeruli and completely reabsorbed by the proximal renal tubule and has a higher molecular size than creatinine (Suzuki et al. 2012), thus, cystatin C is more predictive of events that occur in the kidneys than creatinine or GFR (Vigil et al. 2014). Luaibi et al. (2020) also confirmed that there is a significant correlation between serum cystatin C, urea and creatinine.

The current study showed significant changes in kidney function among the four study groups, including cystatin C, where a significant increase in its level was observed in the three pre-treated groups of rosuvastatin compared with the control group, these results are consistent with Wijesurendra et al (2023), who found an increase in cystatin level while administering rosuvastatin to patients.

As for the relationship between vitamin D_3 levels and statins, Bhattacharyya et al. (2012) indicated that it is still not clear, so studies must be conducted between them. In the current study, the results showed a gradual decrease in the level of vitamin D for all groups treated with rosuvastatin compared with the control group, this result may be due to a defect in renal function, which may result from a reduction in the activity of the enzyme 1-a hydroxylase, which plays a role in converting 25-hydroxyvitamin D into the more effective 1,25-dihydroxyvitamin D, and this was confirmed by Melamed and Thadhani (2012) in that there is a deficiency of vitamin D in diseases Chronic kidney disease in children and adults

As for the histopathological effects in the current study, it was based on the microscopic examination of the kidneys of rats from four experimental groups, and the results showed that the control group had a normal histological structure with undamaged proximal and distal renal tubules. While the groups treated with rosuvastatin had a significant injury to the kidney tissue compared to the control group, cellular degeneration, necrosis and hemorrhage were diagnosed, as well as inflammatory changes. And these results are consistent with Al-Khafaji (2021). Also, the rosuvastatin-treated groups showed that renal corpusclesemia was accompanied by congestion in the glomerular capillaries and increased irregularity of the parietal layer of Bowman's capsule, and this result is consistent with that reported by Ponnuswamy et al. (2009) Rosuvastatin-induced abnormalities in the form of glomerulo-sclerosis and swelling were also seen in proximal and distal renal tubules cells with necrotic nuclei and this is consistent with the findings of Pisoni et al. (2008).

Therefore, the results of the current study showed that there is nephrotoxicity, and these results agreed with other studies as well, indicating the association of acute nephrotoxicity with highly effective statins (Dormuth et al. 2013) , while Ward et al (2016) reported a case of renal tubular toxicity due to initiation of rosuvastatin at a dose of 40 mg in a patient without prior evidence of renal disease. Van Zyl-Smit et al. (2004) also reported a case of direct renal tubular toxicity, which was positively associated with an increase in the patient's dose of rosuvastatin to 80 mg daily and inhibited nephrotoxicity after discontinuation of the drug.

Conclusion

In the current study, it was concluded that high doses of rosuvastatin lead to kidney injury. Moreover, infection was confirmed by high cystatin C level and decreased vitamin D_3 level, in addition to histopathological changes that revealed renal tissue injury.

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