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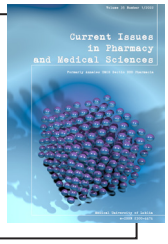

The protective effect of vitamin A on Concor induced structural changes of the liver and kidney in adult rats

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ABSTRACT

Concor is a beta-blocker drug used to treat high blood pressure, acute coronary syndrome, and to control the rapid pulse of the heart such as atrial fibrillation. Some of its adverse effects include hepatitis, increased triglycerides and liver enzymes. Monitoring liver and kidney functions in patients with hepatic or renal impairment who are taking concor is recommended.

The current study was undertaken to define whether vitamin A could improve structural changes in the liver and kidneys. The 24 rats were grouped into the following. The first group was control. The second group was given Vitamin A (5000 IU). Group 3: given concor at a daily dose of 0.9 mg/kg B. wt. Group IV: received concor (0.9 mg/kg B. wt.) and Vitamin A (5000 IU) orally. After 4 weeks, the kidney of the treated group 3 exhibited degenerative alterations in the glomeruli, enlargement of Bowman's space and the epithelium of the proximal kidney tubules showed vacuolar degeneration with necrosis. Liver sections showed degeneration and necrosis of hepatocytes, congestion of the central vein, dilation of sinusoids and inflammatory cell infiltration. Group 4 showed mild degeneration in the glomeruli, expansion of Bowman's space and mild degeneration of tubular epithelium, and normal architecture of the liver with increased Kupffer cells. From this study, we concluded that concor drug induces structural changes in the liver and kidney and these effects were improved by Vitamin A administration.

INTRODUCTION

Bisoprolol is known commercially as concor, bisoprolol, zebeta, and many other trade names. Bisoprolol belongs to the beta blocker group of medication that exert their effects on the cardiovascular system by blocking the activity of several essential chemicals such as epinephrine [1]. This action decreases the cardiac rate, contractility of the heart, and blood pressure [2]. It functions as a cardioprotective agent by blocking beta 1 adrenergic receptors, slowing impulse conduction, and inhibiting the release of renin and angiotensin II by antagonistically binding to beta-1 receptors in the juxtaglomerular cells of the kidney.

Concor is commonly used for the early treatment of hypertension, chronic stable angina and chronic heart failure, and is also used to relieve the symptoms of an overactive thyroid gland [3]. Bisprolol has a high bioavailability with an absorption rate of over 90% and a plasma clearance half-life of 10-12 hours, so in humans it is administered once daily. The clearance of this drug occurs in the liver and kidney, and the inactive metabolites are eliminated predominantly by the kidneys [4]. The main side effects of Concor are headache, fatigue, low blood pressure, hypoglycemia, and slow heart rate [5]. Overdose of this drug can antagonize the β2-adrenergic receptors in the liver and lung, resulting in bronchospasms and low blood sugar [6].

Vitamin A is one of the fat-soluble vitamins that is absorbed in the intestine and stored mainly in hepatic, renal and adipose tissues. The three primary forms of vitamin A are retinal, retinoic acid, and retinaol; the form that the liver stores are retinyl palmitate [7]. The main source of this vitamin is from food, either from animal sources mainly as retinyl esters, or from pigmented vegetables and fruits (carotenoids, especially β-carotene). Usually, vitamin A is absorbed mainly by the intestinal epithelium in the presence of bile salts and intestinal juice [8]. It is assumed that supplements of Vitamin A play a part in the catabolism of carbohydrates, proteins, as well as lipids [9]. During embryonic development, retinoic acid regulates stem cell proliferation,

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differentiation, and division. Also, it is involved in preservation of epithelial cell structure and function and cell apoptosis [10]. Vitamin A helps in the maturation of B-cells and helper lymphocytes (Th) and it is necessary for adaptive immunity. Its deficiency reduces antibody-mediated responses [11]. The relationship of vitamin A with the renal system is well known and retinoid acid is recognized as having a nephroprotective effect and therapeutic benefit in several animal models of kidney disease [12].

The present study is aimed at detecting the effect of Concor drug on the kidney and liver, and on understanding the protective influence of vitamin A upon liver and kidney injury induced by Concor in rats.

MATERIALS AND METHODS

Drug used

Concor was the drug used in this experiment. Each tablet contains 2.5 mg bisoprolol hemifumarate. It is manufactured by Merck Healthcare KgaA, Darmstadt, Germany.

Design of the study

The plan of the study was an interventional non- randomized open experimental study.

Animals used

Twenty-four adult male rats were used, of the same age group (2.5-3) months, weighing (150-200) grams, kept in the animal building of the Veterinary Medicine college, Mosul University. They were housed in clean net cages, 12×20×10 cm in size, in a quiet place, 6 rats in each cage with temperature controlled at 25±2ºC, a 12-hour light-dark period, and access to water and food ad libitum [13]. The animals were left for at least one week to accommodate to the environment of the lab before starting the experiments.

The 24 treated rats were grouped into the following: the first group (G1), considered as a group control, was given normal saline orally for 4 weeks and the second group (G2) was given vitamin A (5000 IU) orally for 4 weeks. The third group (G3) received Concor drug 0.9 mg/kg/day orally for four weeks. Group 4 was given Vitamin A (5000 IU) and Concor drug (0.9 mg/kg B. wt.) orally for 4 weeks. At the end of the 4 week period, the animals were killed humanely, and specimens of the kidney and liver were taken and preserved in 10% formalin solution for one day for fixation, then paraffin blocks formed, then cut by microtome into thin sections (5 microns). Hematoxylin and Eosin stains were used for staining the sections and these were examined by light microscopy and pictures were taken using a color USB 2.0 digital image camera (Omax ToupView 9.0-Megapexil China).

Ethical clearance

The local Ethics Committee of the health team of the school of medicine, University of Mosul, Iraq approved the experimental procedures for this study. It also follows the recommendations of the European Council Directive (2010/63/EU) of September 22, 2010, regarding the standards for protecting animals used for experimental purposes.

RESULTS

Group G1

All the animals in the control group survived and remained active throughout the experiment. They had a good appetite, and reacted very rapidly to stimulus.

Their kidneys were bean shaped, with the outer cortex consisting of normal nephrons and renal tubules and a normal inner medulla. The renal corpuscle of the nephron showed a normal glomerulus, which consisted of glomerular capillary loops that were located within a normal Bowman's space and which were surrounded by a normal Bowman's capsule (Figure 1).

The liver was congested and brown with a firm consistency and a smooth surface. The liver sections from this group showed the lobular architecture of a normal liver, healthy hepatocytes and sinusoids, while the bile ductile appeared normal (Figure 2).

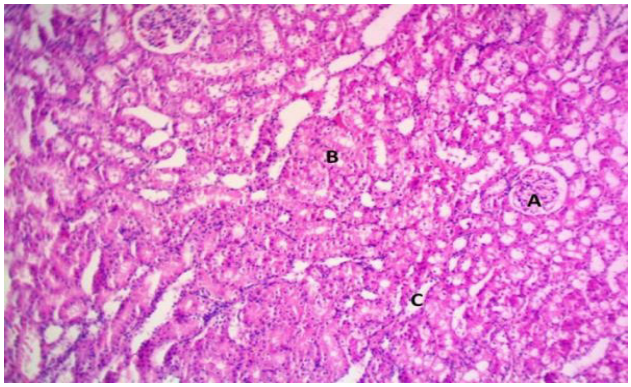

Figure 1. Kidney photomicrograph of the first group displays a typical renal structure showing normal glomeruli (A), proximal convoluted tubules (B), and distal convoluted tubules (C). H and E, 100×

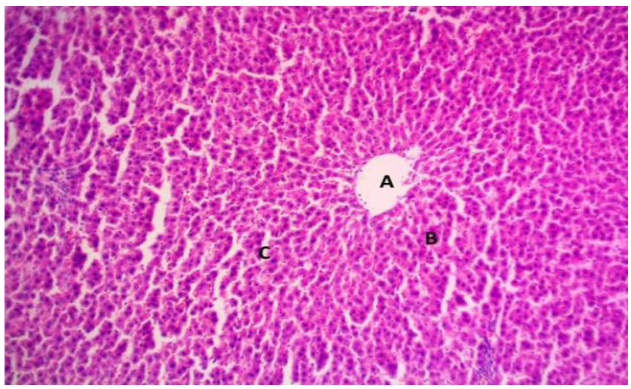

Figure 2. Liver photomicrograph of the first group demonstrates the normal structural organization of the liver in which the central (terminal hepatic venule) vein (A), cords of hepatocytes (B) around, and in between sinusoids (C). H and E, 100×

Group G2

The kidney of the Vitamin A treated group displayed the typical structure expressed by a normal arrangement of the renal tissue. Each glomerulus was composed of a tuft of capillaries encircled by a Bowman's capsule that had normal visceral and parietal layers. The lining epithelium of the proximal and distal renal tubules was cuboidal,

with normal eosinophilic cytoplasm and central nuclei with normal vesicular appearance (Figure 3, 4).

The liver appeared brownish, soft, lobulated with normal porta hepatic, normal hepatocytes around the central vein and mild sinusoidal congestion (Figure 5, 6).

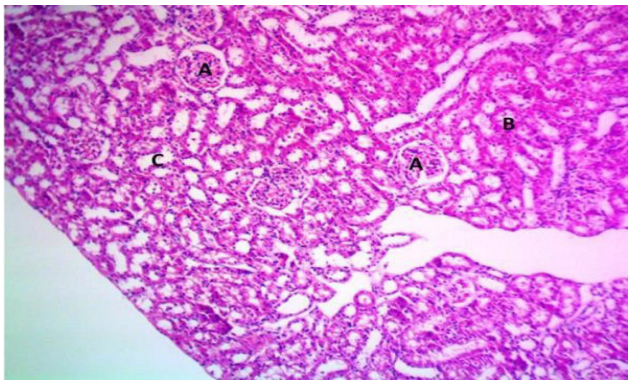

Figure 3. Kidney photomicrograph of the second group (treated with Vitamin A), illustrates renal tissue with a normal architecture showing normal renal glomeruli(A), proximal convoluted tubules (B) and distal convoluted tubules (C). H and E, 100×

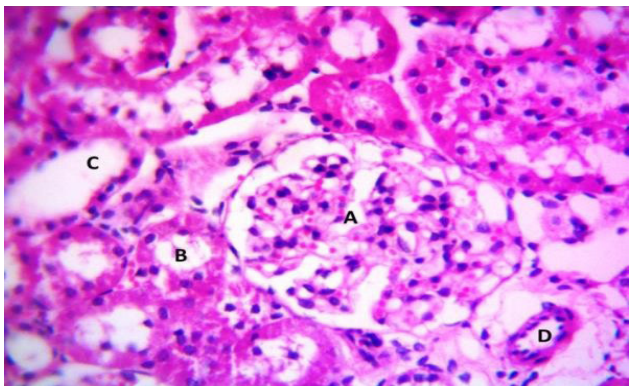

Figure 4: Kidney photomicrograph of the second group showing normal renal tissue, the glomeruli (A), proximal convoluted tubules (B), distal convoluted tubules (C), and blood vessel (D) looks normal. H and E, 400×

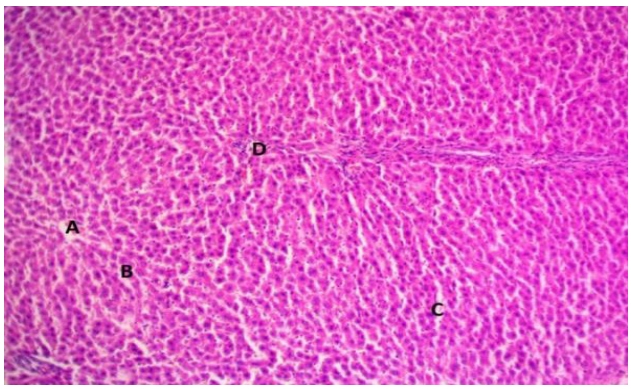

Figure 5. Liver photomicrograph of the second group shows a normal liver structure with central (terminal hepatic venule) vein (A), cords of hepatocytes (B), sinusoids (C), and the portal area (D). H and E, 100×

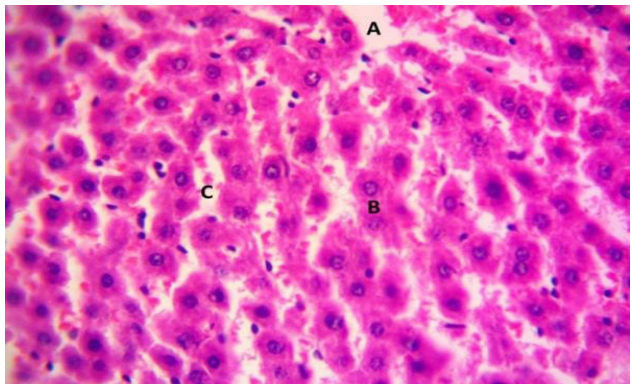

Figure 6. Liver photomicrograph of the second group, illustrates liver tissue with normal architecture, central vein (A), normal hepatocytes (B), and the sinusoids with mild congestion (C). H and E, 400×

Group G3

The kidney of the Concor drug-treated group showed degeneration of some glomeruli with a decrease in the size of the capillary loops of the kidney, an increase in Bowman's space, and hemorrhage in the interstitial space. The lining epithelial of the proximal renal tubules showed vacuolar degeneration and necrosis. Some tubules, particularly in the cortex, were cystically dilated (Figure 7, 8).

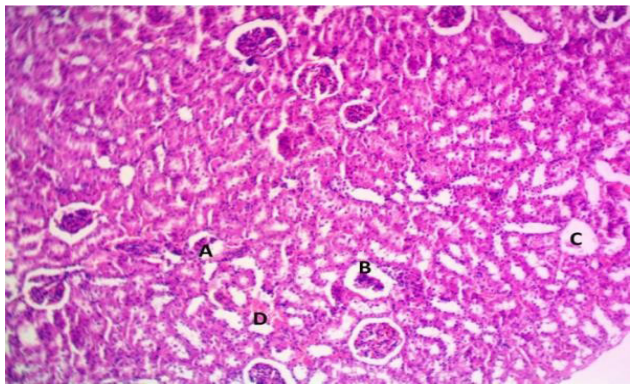

Figure 7. Kidney photomicrograph of concor drug group, shows degeneration of glomeruli(A), expansion of Bowman's space (B), renal cyst (C) and hemorrhage in the interstitial space (D). H and E, 100×

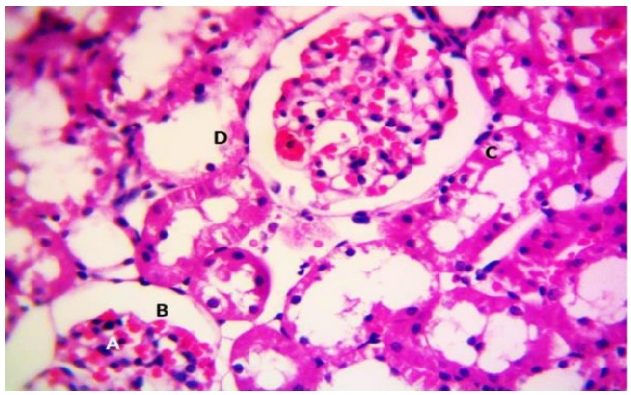

Figure 8. Kidney photomicrograph of concor drug group shows degeneration of glomeruli(A), expansion of Bowman's space (B) and vacuolar degeneration (C) and necrosis (D) of lining epithelial cells of the proximal convoluted tubules. H and E, 400×

The examined liver sections displayed necrosis and degeneration of hepatocytes around the central vein, with sinusoidal dilatation, while the central vein and blood vessels in the portal area were congested. In addition, infiltration of mononuclear inflammatory cells around the periportal area and the central veins was evident (Figure 9-11).

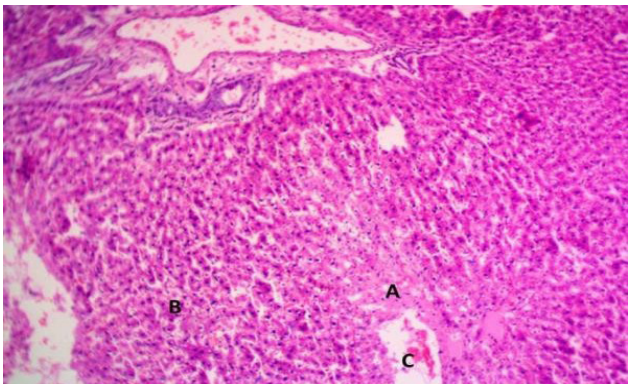

Figure 9. Liver photomicrograph of the concor treated group shows necrosis of hepatocytes around the central vein (A), degeneration of hepatocytes (B), and central vein congestion (C). H and E, 100×

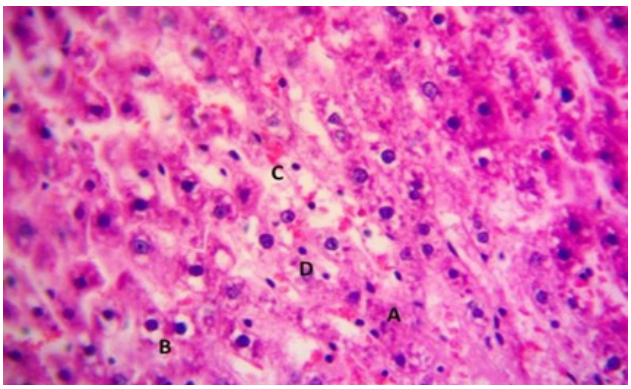

Figure 10. Liver photomicrograph of the concor treated group shows necrosis (A) and degeneration (B) of hepatocytes, congestion and dilation of sinusoids (C) and infiltration by mononuclear inflammatory cells). H and E, 400×

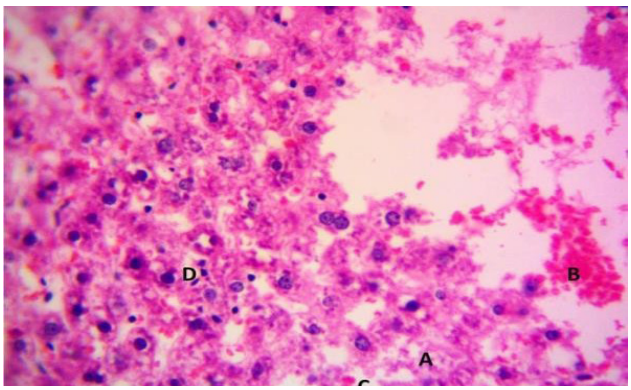

Figure 11. Liver photomicrograph of the concor treated group illustrating necrosis of hepatocytes (A), congestion of central vein(B) and sinusoids with dilation (C) and mononuclear inflammatory cells infiltration (D). H and E, 400×

Group G4

The kidney displayed apparently normal kidney parenchyma, mild degeneration of glomeruli with enlargement of Bowman's space. The lining epithelial cells of some renal tubules showed mild vacuolar degeneration (Figure 12, 13).

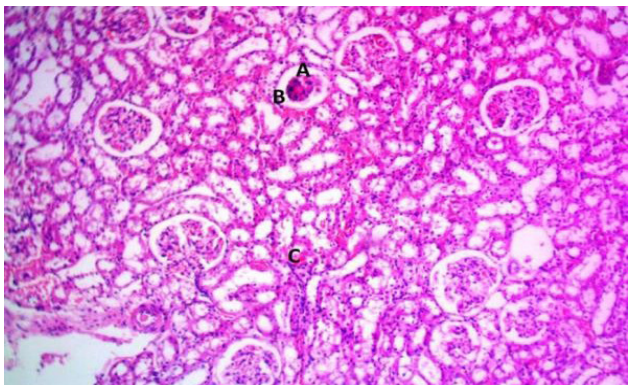

Figure 12. Kidney photomicrograph of concor with vitamin A treated animal shows glomeruli with mild atrophy (A) with the expansion of Bowman's space (B) and congestion of the blood vessels (C). H and E, 100×

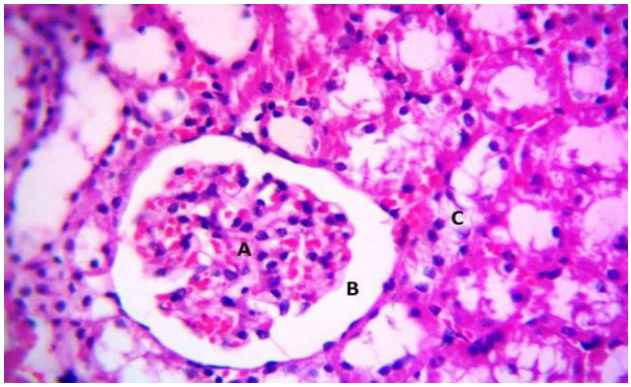

Figure 13. Kidney photomicrograph of concor and vitamin A treated animal illustrates minor atrophy of glomeruli (A) with distention of Bowman's space (B) and the cells of the epithelium lining renal tubules with vacuolar degeneration (C). H and E, 400×

The liver was characterized by normal hepatic lobules with a normal central vein, normal hepatocytes with no degeneration seen, mild congestion of sinusoids with increased numbers of kupffer cells. In addition, congestion of blood vessels in the portal area was less evident than G3 (Figure 14, 15).

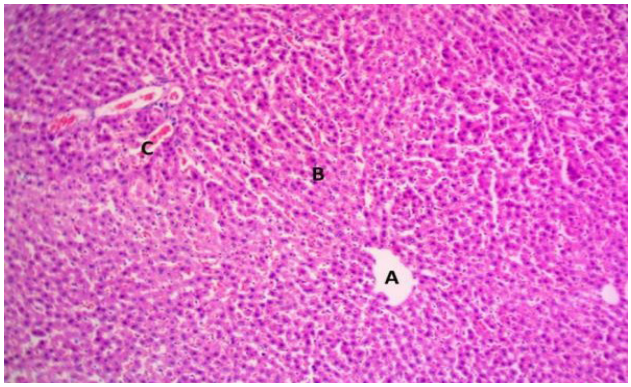

Figure 14. Liver photomicrograph of concor drug with vitamin A treated animal shows a typical structure of liver tissue, normal central vein (A), hepatocytes (B) and congestion (C) of vessels in the portal area. H and E, 100×

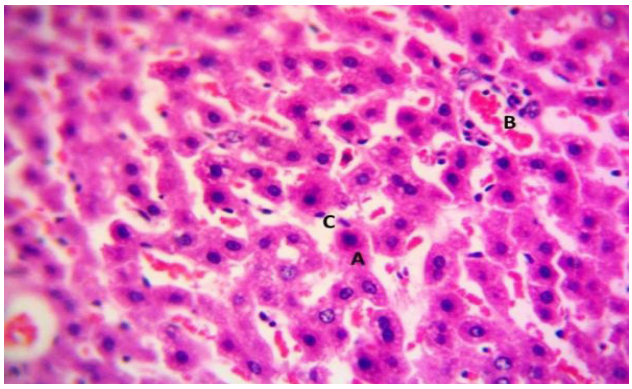

Figure 15. Liver photomicrograph of treated animals with concor and vitamin A shows normal liver lobule architecture with usual hepatocytes (A) and congestion of sinusoids (B) with increased numbers of Kupffer cells (C). H and E, 400×

DISCUSSION

Concor (bisoprolol fumarate) is a synthetic selective β1-blocker medication that is commonly used to treat patients with mild to moderate hypertension. However, nephrotoxicity and hepatotoxicity are unusually conflicting influences of this drug, which physicians should be aware of [14,15]. This study assumed that the β-blockers could affect the liver and kidneys.

The results of this study indicate that Concor administration in a therapeutic dose (0.9 mg/kg b.wt.) once daily orally for 28 consecutive days induced atrophy of some glomeruli of the kidney, and shrinking of the size of the capillary loops that is associated with widening of Bowman's space and interstitial hemorrhage. The lining epithelium cells of the proximal convoluted tubules showed necrosis and vacuolar degeneration, and some tubules, mainly in the cortex, were cystically dilated, which are all signs of tubular kidney degeneration. Our results are similar to the results reported by Alsadek *et al.* [15], who found that bisoprolol (1.8 mg/kg b.wt. once daily for 21 days) induced congested renal blood vessels with perivascular edema and round cell aggregations, as well as degenerative changes in the renal tubules with cystically dilated lumen associated with increased serum uric acid and creatinine levels. The general mechanisms that cause renal disturbance could be explained by the fact that the systolic function of the heart affects kidney function and perfusion. Theoretically, beta-blockers may decrease cardiac output, which causes a drop in kidney perfusion pressure and impairs kidney function [16].

The influence of beta-blockers on oxygenation and hemodynamics of the kidney is not clearly understood. Hall M. *et al.* [17] used magnetic resonance imaging (MR) to calculate blood flow in the renal artery and tissue oxygenation in diabetic and hypertensive patients taking beta-blockers. They found that renal oxygenation in medullary and cortical tissue is not dependent on blood flow in the renal artery, and the improvement in the oxygenation of renal tissue may be caused by a decrease in the kidney's oxygen consumption rather than an increase in renal blood flow [18].

The liver microanatomy in group 3 that received Concor showed congestion of hepatic blood vessels, necrosis and degeneration of hepatocytes around the central vein, with dilatation of sinusoids, and the presence of mononuclear inflammatory cells around and within the central veins and periportal area. This supports the finding of the research done by Alsadek *et al.* [15]. He studied the effects of Zebeta (bisoprolol) and found that the liver showed congestion of blood vessels, portal biliary proliferation, round cell interstitial aggregations, and multifocal necrosis of hepatocytes. These results are consistent with the histological results we found. Dumortier *et al.* [19] described a case of hepatotoxicity caused by atenolol use, which was associated with an increase in aminotransferase enzyme levels. He assumed that the mechanism of hepatotoxicity could be immune-mediated based on the inflammatory cell infiltration that was observed in the liver biopsy samples. The mechanism for hepatotoxicity from beta-blockers is unclear. Rua J *et al.* [20] reported that the Carvedilol drug (non-selective alpha/β-blocker), which is usually given in hypertension and failure of the heart, leads to bile duct injury by toxic substances excreted as metabolites into the bile, and the secondary hepatocyte injury will result in hepatitis, which could progress to cirrhosis and cholestatic syndrome [21]. Mitchell *et al.* [22] noticed that patients with liver injury secondary to the use of β-blockers characteristically develop symptoms of hepatocellular damage and hyperbilirubinemia, and that the laboratory abnormalities will be recovered after stopping using β-blockers. Furthermore, Fisher *et al.* [23] reported that the levels of transaminase enzyme improved rapidly after the gradual withdrawal of both Metoprolol succinate and Carvedilol in two patients with β-blocker-induced liver injury. In conclusion, β-blocker therapy is uncommon to be a cause of severe hepatotoxicity as a side effect, though doctors should be familiar with the aforementioned possible consequences, as these drugs are frequently used and prescribed in patients with advanced liver disease.

In group 2, Vitamin A (5ooo IU) given orally for 4 weeks revealed almost normal renal and liver histology with no evidence of microanatomy changes when compared to control. Rats in group 4, who received Concor medication treatment plus pre-administration of vitamin A, displayed less altered kidney and liver cytoarchitecture than rats in group 3. This could be attributed to the antioxidant role of vitamin A. According to the author's knowledge, no article was found on the protective effect of vitamin A complex on the kidney and liver after Concor therapy in rats, apart from an earlier study on the protective effect of vitamin E complex and the usage of vitamin A against other chemicals like Diclofen drug [24].

As per previous literature, Vitamin A is known to be one of the important antioxidant drugs due to its hepatonephroprotective properties. One such property is that it prevents cadmium [25] and gasoline vapour-induced hepatic injury in rats [26]. The antioxidant action of carotenoids and vitamin A is attributed to the hydrophobic class of polyene groups that lead to a reduction in the singlet oxygen ($^1O_2$), thereby reducing lipid peroxidation, counteracting thiyl radicals, mixing with peroxyl radicals, and stabilizing them. Peroxylic radicals and ($^1O_2$) are regarded as reactive oxygen species (ROS) generated in the liver tissue and are responsible for oxidative stress-related diseases. ROS includes superoxide peroxide and hydroxyl radicals. Their harm is prevented

by antioxidants such as glutathione peroxidase, catalase, superoxide dismutase, and various vitamins such as vitamin E and A, which act as scavengers for this ROS [27].

Vitamin A restores normal levels of catalase, superoxide dismutase and glutathione peroxidase and increases the levels of lipid peroxidation, hydroperoxide and glutathione in the liver. Furthermore, carotenoids, as well as vitamin A, can oxidize during high O_2 tension and are also effective antioxidants even when tissue oxygen tension is low. Moreover, when O_2 tension increases, carotenoids and Vitamin A can be autoxidized. Additionally, carotenoids have a significant role in the preservation of lipoproteins of the cell membranes against peroxyl radical effects [28]. Moreover, some studies have shown that the all-trans retinoic acid (ATRA) which is part of the retained family and is an active metabolite of vitamin A, can decrease inflammatory response in kidney glomeruli and tubules in streptozotocin-induced diabetes [29], and restrict the proliferation of the glomeruli and albuminuria in a settled case of renal damage [30]. It has been shown that treatment with ATRA reserves kidney function and in the initial phases of kidney injury in experimental rats with diabetes, it diminishes kidney hypertrophy by preventing fibrosis through regulation of macrophage infiltration [29]. Finally, from this study, it is evidenced that pre-administration of Vitamin A ameliorates the effect of Concor on liver cells and kidneys; thereby it protects the cellular damage and hepatotoxicity from metabolites produced by Concor free radicals. The mechanism whereby Vitamin A exerts such effects is yet to be fully elucidated.

CONCLUSION

From the results obtained in this study, it could be concluded that Concor in a therapeutic dose has hepatic and renal disturbance effects in rats because these organs are involved in the metabolism of Concor.

In the current work, a protective effect of vitamin A against hepatic and renal adverse effects of bisoprolol is noted. Ultimately, the outcome of the present study showed that vitamin A helps to prevent, improve and accelerate the recovery from the side effects of hepatotoxicity and nephrotoxicity of Concor usage in rats. Therefore, this vitamin might be co-administered at preventive doses in patients who have commonly used this drug.

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