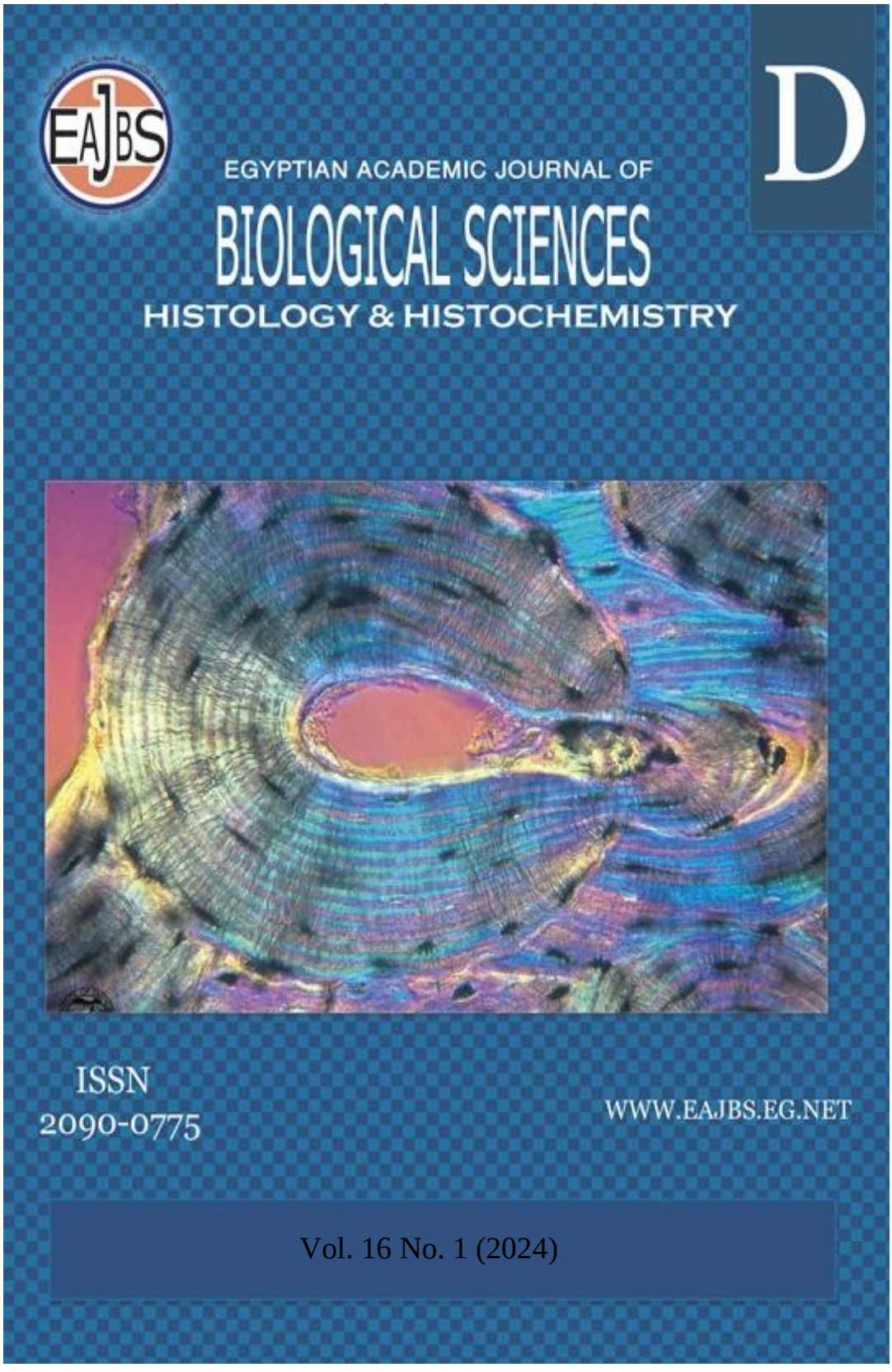

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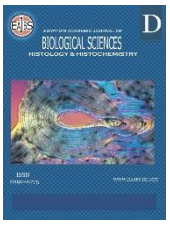

The Renoprotective Effect of Atorvastatin in a Rat Model of High-Fat High-Fructose Diet-Induced Renal Injury

Mamdouh Eldesoqui[1-2], Abdelaty S. Mohamed[2], Ahmed N. A. Nasr[1-3], Sahar K. Ali[4], Mai M. Eldaly[5], Eman M. Embaby[6], Heba S. Ahmed[4], Zeinab M. Saeed[4], Zeinab A. Mohammed[7], Rania H. M. Soliman[8]

[1]Department of Anatomy and Embryology, Faculty of Medicine, Mansoura University, Mansoura 35516, Egypt.
[2]Department of Basic Medical Sciences, College of Medicine, AlMaarefa University, Diriyah, 13713, Riyadh, Saudi Arabia.
[3]Department of Basic Medical Sciences, Faculty of Medicine, Aqaba Medical Science University.
[4]Department of clinical pharmacology, Faculty of Medicine, Zagazig University, Zagazig 44519, Egypt.
[5]Department of Medical Biochemistry and Molecular Biology, Faculty of Medicine, Zagazig University, Zagazig 44519, Egypt.
[6]Department of Physiology, Faculty of Veterinary Medicine, Mansoura University, Mansoura 35516, Egypt.
[7]Department of Forensic medicine and clinical toxicology, Faculty of Medicine, Zagazig University, Zagazig 44519, Egypt.
[8]Department of Human Anatomy and Embryology**,** Faculty of Medicine, Zagazig University, Zagazig 44519, Egypt.

***E-mail:** dr_rania_hassan@yahoo.com

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ABSTRACT

High-fat diets (HFDs) and sedentary lifestyles are associated with obesity, a significant global health issue that affects over 30% of people in industrialized countries. It is associated with metabolic syndrome, type 2 diabetes, high cholesterol levels, and abnormal lipid metabolism. High-fructose diets can lead to type 2 diabetes, insulin resistance, and inflammation in fat tissue. Statins, particularly hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, have been used to treat obesity and diabetes, but their impact on kidney damage in obese rats is limited. This research aimed to investigate the impact of atorvastatin on renal damage resulting from a high-fat, high-fructose diet (HF-HFrD) in rats. This study involved 24 adult male Sprague Dawley rats, divided into four groups: the control group, the atorvastatin (Ator) group, the high-fat-high-fructose diet (HF-HFr) group, and the high-fat-high-fructose diet with atorvastatin (HF-HFr + Ator) group. Rats were anesthetized, weighed, and sacrificed, and blood was collected from the abdominal aorta and kidneys. Biochemical studies were performed to detect serum urea, creatinine, glucose, insulin, lipid profile, malondialdehyde (MDA) levels, and reduced glutathione (GSH) activity. The histopathological evaluation included H&E, Sirius red staining, NF-κB, and caspase-3 immunohistochemical staining. The HF-HFrD group had elevated levels of serum glucose, insulin, HOMA-IR, creatinine, BUN, cholesterol, and triglycerides while showing a reduction in HDL. The renal tissue exhibited increased levels of MDA, decreased levels of GSH, higher collagen accumulation, and increased expression of NF-κB and caspase-3. Atorvastatin therapy effectively improved these alterations in comparison to the HF-HFrD group. In conclusion, atorvastatin improved HF-HFrD-induced renal injury by modulating lipotoxicity, oxidative stress, inflammation, and apoptosis. Atorvastatin may have therapeutic potential for obesity-related kidney damage.

INTRODUCTION

Obesity is a significant global health issue, affecting more than 30% of people in industrialized countries. It is linked to the rise in consumption of high-fat diets (HFDs) and sedentary lifestyles (Kramer and Luke, 2007). Obesity is linked to metabolic syndrome (MS), which involves chronic illnesses including hypertension., cardiovascular diseases, and metabolic disorders (Grundy, 2004, Zaki e*t al.,* 2019). MS is also linked to type 2 diabetes (due to insulin resistance), high cholesterol levels, and abnormal lipid metabolism (Bocarsly *et al.,* 2010). The primary reason for both MS and obesity is the consumption of HFD (Auberval *et al.,* 2014). Consuming foods rich in fat may cause alterations in the way the body processes glucose and lipids, leading to disruptions in metabolism, reduced insulin signaling, and increased deposition of fat. These changes can ultimately culminate in kidney lipotoxicity (Suganami *et al.,* 2012). Furthermore, there are robust correlations among insulin resistance, obesity, and the anomalous proliferation and expansion of adipose tissue (Galic *et al.,* 2010).

Fructose is a crucial ingredient found in many frequently eaten food items. Table sugar, soft drinks, fruit drinks, and jams are the main sources of fructose in our diet (Bantle, 2006). Additionally, the consumption of excessive amounts of carbohydrates, such as in the case of a high fructose diet (HFrD), whether with or without a high-fat diet (HFD), may result in the development of type 2 diabetes (Pereira-Lancha *et al.,* 2012). The rising global prevalence of multiple sclerosis (MS) has stimulated the creation of a laboratory animal model that closely mimics human characteristics. Feeding animal models a diet containing 40-60% high-fat diet (HFD) and/or high-fructose diet (HFrD) may create a model of multiple sclerosis (MS) that closely reflects the human condition (Zaki *et al.,* 2019).

Obesity and insulin resistance can lead to cell death, immune cell infiltration, and localized inflammation in fat tissue, disrupting the production and release of specific signaling molecules known as adipokines, including proinflammatory cytokines such as TNF-α, interleukin 1 (IL-1), and IL-6, which triggers a mild, systemic inflammatory state throughout the body (Suganami *et al.,* 2012, Wanchai *et al.,* 2017), the proinflammatory cytokines can activate a specific tissue nuclear transcription factor, NF-κB leading to local inflammation (Suganami *et al.,* 2012, Wang *et al.,* 2017). This mild inflammation appears to be a key factor in the development of obesity-related insulin resistance, abnormal fat levels, type 2 diabetes, and other health issues (Rao *et al.,* 2015). Moreover, the presence of obesity, together with a modest, widespread inflammatory response, raises the risk of oxidative stress, tissue scarring, organ damage, and cell death. All these conditions are considered vascular disease risk factors that may affect many organs, such as the heart, kidneys, and liver (Rao *et al.,* 2015).

Furthermore, obesity and metabolic syndrome (MS) can cause kidney damage, even in the absence of other clinical indications associated with obesity, such as high blood pressure, high cholesterol, or pre-existing renal diseases. Furthermore, obesity and metabolic syndrome (MS) can cause kidney damage, even in the absence of other clinical indications associated with obesity, such as high blood pressure, high cholesterol, or pre-existing renal diseases (Fujita, 2008, Amaral *et al.,* 2014).

The interest in the beneficial effects of statins, particularly hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, has grown in the context of lipid-lowering treatment.

The 3HMG-CoA reductase enzyme responsible for regulating endogenous cholesterol synthesis and the expression of LDL receptors in the liver. These drugs have been widely used in clinical practice for treating obesity and diabetes (Hebert *et al.,* 1997, Bedi *et al.,* 2016). Statins not only reduce lipid levels but also have other benefits that are not related to cholesterol. These effects include acting as antioxidants, reducing inflammation, and preventing cell death (Bruder-Nascimento *et al.,* 2016). Studies have shown that statins have the ability to safeguard the kidneys of rats against oxidative stress, inflammation, and apoptosis with gentamicin-induced nephrotoxicity and streptozotocin-induced diabetes (Jaikumkao *et al.,* 2016, Thongnak *et al.,* 2017).

However, there hasn't been much research on how statins affect the accumulation of lipids and the resulting kidney damage in obese rats fed a high-fat, high-fructose diet (HF-HFrD). Thus, the purpose of this research was to examine the effect of atorvastatin on renal damage generated by HF-HFrD in rats.

MATERIALS AND METHODS

1-Animals and Grouping:

By using an open epi test for calculation of sample size, 24 adult male Sprague Dawley rats weighing 150-200 g were used in this experiment. after acclimatization for two weeks, the rats were divided into four groups,

I. The Control group was fed on a standard diet with free access to food and tap water.
II. Atorvastatin (Ator) group fed on standard diet with free access to food and tap water and atorvastatin (Ator, Egyptian Pharmaceuticals Industries Co. Egypt) dissolved in saline and will be given in the dose of 10mg/kg/day by oral gavage for 4 weeks (Ben Salem *et al.,* 2019, Pengrattanachot *et al.,* 2020, Thongnak *et al.,* 2020).
III. High Fat-high fructose diet (HF-HFr D) group, for a duration of 8 weeks, a diet consisting of HF-HFrD was administered. The diet included 15% fat, 21% protein, 60% carbohydrate, 3% fiber, and 1% vitamins and minerals. The diet's total caloric content was 5.3 kcal/g. (Attia *et al.,* 2019, Elsisy *et al.,* 2021), with 25% fructose (Sigma, USA) in drinking water (Lozano *et al.,* 2016, Elsisy *et al.,* 2021).
IV. High Fat-high fructose diet (HF-HFr D) and Atorvastatin (HF-HFr D +Ator) group: receive HF-HFr D as in group III for 8 weeks then receive Ator in the same dose as group II for 4 weeks.

2-Estimation of Body Weight and Sampling:

At the end of the experiment, the overnight fasted rats were anesthetized, weighted, and then sacrificed by overdose anesthesia. The abdominal cavity was opened, and blood was collected from the abdominal aorta then kidneys were excised then washed in saline. Subsequently, one kidney was dipped in phosphate buffer and stored in liquid nitrogen for further estimation of oxidative stress parameters and the other kidney was fixed in 10% formalin and embedded in paraffin for histological and histochemical evaluation.

3-Biochemical Studies:

The collected blood was centrifuged, and the separated sera were used for the detection of serum urea, creatinine using commercially available kits (Diamond Diagnostics Company, Egypt), fasting glucose, and insulin using commercially available kits. Fasting blood glucose and serum insulin were used for the calculation of HOMA-IR to determine insulin resistance (Fasting insulin level (μU/ml) x Fasting glucose level (mmol/L)/22.5 (Thongnak *et al.,* 2017). According to Fotschki *et al.,* total cholesterol, triglycerides, low-density lipoprotein cholesterol (LDL), high-density lipoprotein cholesterol (HDL), serum creatinine, and serum blood urea nitrogen (BUN) levels were measured in sera using Pentra C200, Horiba, Tokyo, Japan. biochemical analyzer (Fotschki *et al.,* 2020).

4-Measurement of Oxidative Stress and Antioxidant Activity:

The renal tissue samples from all the rats were weighed and mixed thoroughly in a phosphate buffer saline solution (10% weight/volume) to form tissue homogenate using an automated tissue homogenizer. After that, the mixture was centrifuged at 5000g for 15 minutes at 4 degrees Celsius. The liquid obtained after centrifugation was then used to measure the levels of malondialdehyde (MDA), a product of lipid peroxidation product, and reduced glutathione (GSH) activity as a free radical scavenger using colorimetric methods with commercially available kits from Bio-Diagnostics, Egypt, following the instructions provided by the manufacturer (Eldesoqui *et al.*, 2022).

5-Histopathological Evaluation of The Renal Specimen:

Using a microtome, 3-5µm thickness renal tissue sections were stained with Hematoxylin and Eosin (H&E) for histopathological evaluation and Sirus red staining for detection of collagen fibers and fibrosis. Then the sections will be visualized using a light microscope and photographed by Olympus E-330, Olympus Optical Co. Ltd., Tokyo, Japan. connected to the microscope.

6-Immunohistochemical Staining:

After deparaffinization and rehydration, renal tissue sections were treated with 3% hydrogen peroxide to deactivate any natural peroxidases. The sections were then exposed to 10 mM citrate buffer (pH 6.0) for 30 minutes at 95 degrees Celsius. after that the sections are incubated overnight with the primary polyclonal NFκβ antibody (ABclonal Catalog No. A3108) was diluted to a ratio of 1/100. Dilution of Caspase-3 antibody (Servicebio Catalog No. GB11532) to a ratio of 1/1000 was done. All dilutions at a temperature of 4 degrees Celsius were performed. After rinsing the slides in phosphate-buffered saline, they are treated with a secondary antibody. After staining the slides with diaminobenzidine (DAB) using the mMouse and rabbit HRP/DAB (ABC) detection IHC kit (ab64264, Abcam, UK), the presence of immunoreactivity as a brown color was observed. Hematoxylin was used as a counterstain (Eldesoqui *et al.*, 2023).

7-Morphometric Image Analysis for The Immune-Stained Sections:

For the Sirus red stained sections, the images were transformed into RGB (red, green, blue) stacks, resulting in distinct grayscale images for the red, green, and blue color channels. Then, modification of the threshold for the grayscale picture corresponding to the green channel and quantification of the percentage of the region (Schipke *et al.*, 2017).

The percentage of immunoreactive area in the sections subjected to immunoassay was assessed using Image J software (v 1.53, National Institutes of Health, USA, accessed on 03 October 2023). This analysis involved the utilization of the color deconvolution plugin and the H-DAB vector followed by the measurement of the brown color area percentage using the threshold tool (Elhessy *et al.*, 2023).

8-Statistical Studies:

Analysis of data was done using IBM SPSS Statistics for Windows, Version 22.0 (IBM Corp., Armonk, NY, USA), and GraphPad Prism version 9.0.0 (121). Conversion of the quantitative data into numerical and percentage representations and presentation of the qualitative data using the mean and standard deviation for parametric data was done, after confirming normality with the Shapiro-Wilk test. Several independent groups were evaluated using the one-way ANOVA test and conducted pairwise comparisons using the post hoc Tukey's test. The findings were statistically significant at the 0.05 level.

RESULTS

1-Effect of Atorvastatin on Body Weight, Serum Glucose, Insulin, HOMA-IR, and Renal Function:

A high fat-high fructose diet significantly increased the body weight,

fasting blood glucose, insulin, and HOMA-IR, this increase was significantly ameliorated by atorvastatin in the treated group which showed a non-significant increase compared to control rats except for body weight (Table 1).

Regarding renal functions, the rats fed the HF-HFr diet exhibited a significant rise in serum creatinine and blood urea nitrogen (BUN) levels compared to the control group. Treatment with atorvastatin in the HF-HFr+Ator group resulted in a significant decrease in serum creatinine and BUN levels compared to the untreated HF-HFr group. However, the HF-HFr+Ator group still had a significantly elevated BUN level compared to the control group **(**Table 1**).**

Table 1: Effect of atorvastatin on serum glucose, insulin, and HOMA-IR. The data is provided as the mean value plus or minus the standard error of the mean (SEM). n = 6 rats per group. control - normal diet group; Ator – normal diet with atorvastatin group; HF-HFr - high-fat high-fructose diet group; HF-HFr + Ator - high-fat high-fructose diet with atorvastatin treatment group. a significance versus control group, b significance versus HF-HFr group.

	Control	**Ator**	**HF-HFr**	**HF-HFr+Ator**
Weight (g)	258.8±6.575	253.8±4.836	411.3±9.6a	350±8.45ab
Fasting blood glucose (mg/dl)	114.17±0.5	111.35±6.89	135.24±3.08 a	120.68±2.52 b
Plasma insulin (ng/ml)	3.51±0.38	3.13±0.7	8.21±0.23 a	5.67±0.37 ab
HOMA-IR	17.19±2.67	16.41±6.8	61.29±3.23 a	41.81±3.5 ab
Serum creatinine (mg/dl)	0.58 ± 0.02	0.57 ± 0.01	0.88 ± 0.02 a	0.61 ± 0.02 b
BUN (mg/dl)	0.05 ± 0.01	0.03 ± 0.05	0.22 ± 0.03 a	0.10 ± 0.01 ab

2-Effect of Atorvastatin on Serum Lipid Profile:

Rats in the HF-HFr diet group showed significantly increased plasma levels of cholesterol and triglycerides and decreased levels of HDL when compared to control rats. Administration of atorvastatin significantly reduced cholesterol and triglycerides and increased HDL in the HF-HFr +Ator group compared to the HF-HFr diet group, with a non-significant difference when compared to the control group except for HDL which was still significantly lower in the HF-HFr +Ator group **(**Fig. 1**).**

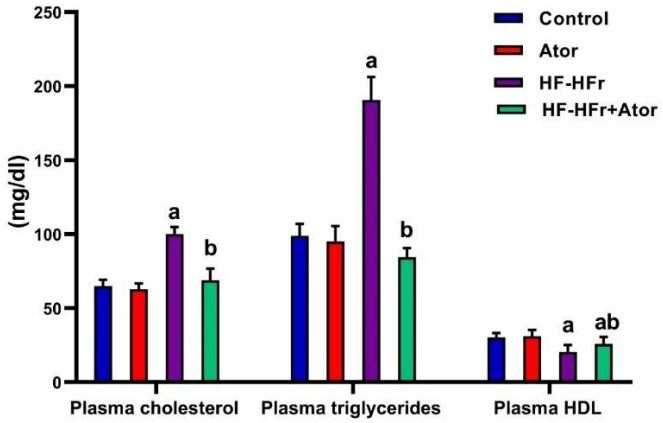

Fig. 1: Plasma levels of cholesterol, triglycerides, and HDL. Data presented as mean ±SEM. n = 6 rats per group. control - normal diet group; Ator – normal diet with atorvastatin group; HF-HFr - high-fat high-fructose diet group; HF-HFr + Ator - high-fat high-fructose diet with atorvastatin treatment group. a significance versus control group, b significance versus HF-HFr group.

3-Effect of Atorvastatin on Renal MDA and GSH:

Significant elevation in renal MDA and reduction in renal GSH were observed in the HF-HFr diet group compared to the control group. Atorvastatin significantly ameliorated these changes in the HF-HFr+Ator group. No significant difference in renal MDA when comparing the HF-HFr+Ator with the control group but renal GSH was still significantly lower than the control group **(**Table 2**).**

Table 2: Effect of atorvastatin on renal MDA and GSH. Data presented as mean ± SEM. n = 6 rats per group. control - normal diet group; Ator – normal diet with atorvastatin group; HF-HFr - high-fat high-fructose diet group; HF-HFr + Ator - high-fat high-fructose diet with atorvastatin treatment group. a significance versus control group, b significance versus HF-HFr group.

	Control	**Ator**	**HF-HFr**	**HF-HFr+Ator**
MDA (nmol/g tissue)	27.18±2.30	27.03±1.7	58.72±3.88 a	29.53±0.01 b
GSH (nmol/g tissue)	1289.12±18.15	1289.12±24.15	517.51±63.48 a	796.95±49.2 ab

4-Effect of Atorvastatin on The Renal Histological Architecture:

The H&E-stained sections from the HF-HFr group showed distorted renal architecture, shrunken glomeruli, widened Bowman space, and widened tubules with tubular cast formation and vacuolation. On the other hand, the HF-HFr diet with atorvastatin showed relative restoration of the renal architecture (Fig. 2).

In Sirus red stained tissue sections, the HF-HFr (high-fat, high-fructose) group had a significant increase in collagen deposition than the control group. However, after administering atorvastatin to the HF-HFr + Ator group, the enhanced collagen deposition was significantly decreased in comparison to the HF-HFr group. Despite the reduction in collagen deposition after atorvastatin therapy, the percentage of collagen in the HF-HFr + Ator group remained considerably higher than those in the control group.

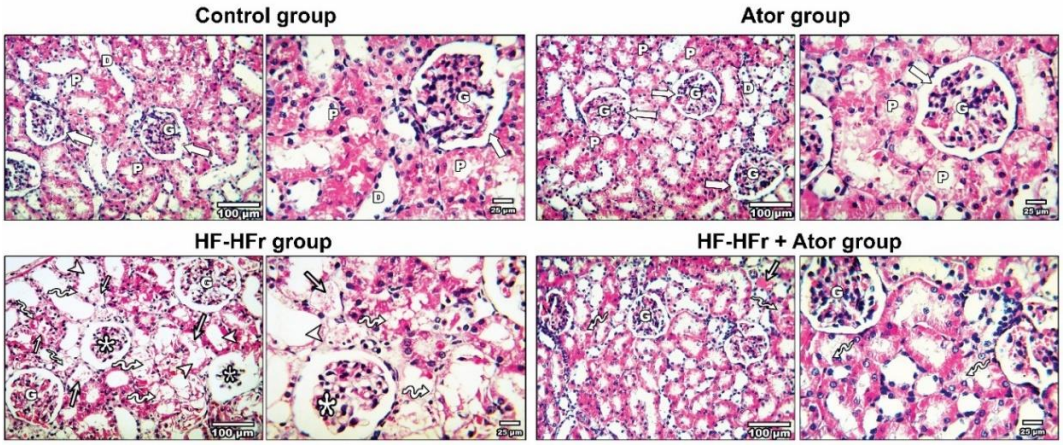

Fig. 2: photomicrographs from the studied groups showing normal renal architecture in the control and Ator groups with normal glomeruli (G), Bowman space (thick arrow), proximal convoluted tubules (P), and distal convoluted tubules (D). HF-HFr group showed shrunken glomeruli and widened Bowman space (star), widened tubules (arrowhead), tubular cast (thin arrow), and tubular vacuolation and degeneration (wavy arrow). HF-HFr+Ator group showed relatively restored renal architecture with some tubular cast (arrow) and vacuolation (wavy arrow).

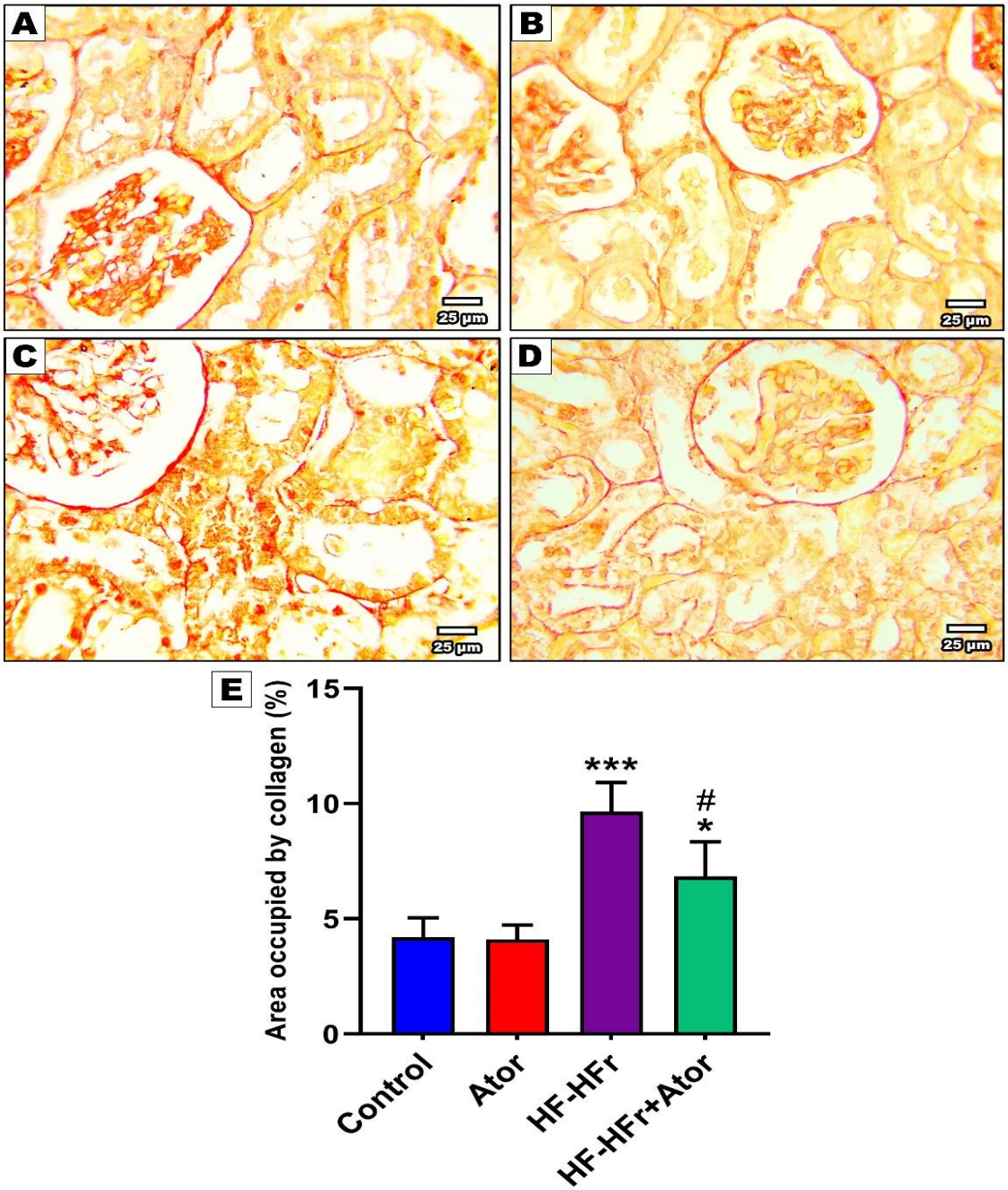

Fig. 3: photomicrographs stained with Sirus red from the control group (A), Ator group (B), HF-HFr group (C), and HF-HFr+Ator group (D). The histogram (E) represents the area occupied by collagen and the data is presented as mean ±SD. * significance against control, # significance against HF-HFr group.

5-Effect of Atorvastatin on Nfκβ and Caspase3 Immunohistochemistry:

The immunohistochemical analysis showed that the expression of NF-κB and caspase-3 was significantly increased in the HF-HFr diet group compared to the control group. However, when the HF-HFr group was treated with atorvastatin, the elevated expression of NF-κB and caspase-3 was significantly reduced. Although atorvastatin lowered the expression of these markers, the levels were still significantly higher in the HF-HFr + Ator group compared to the control group **(**Figs. 4 and 5**).**

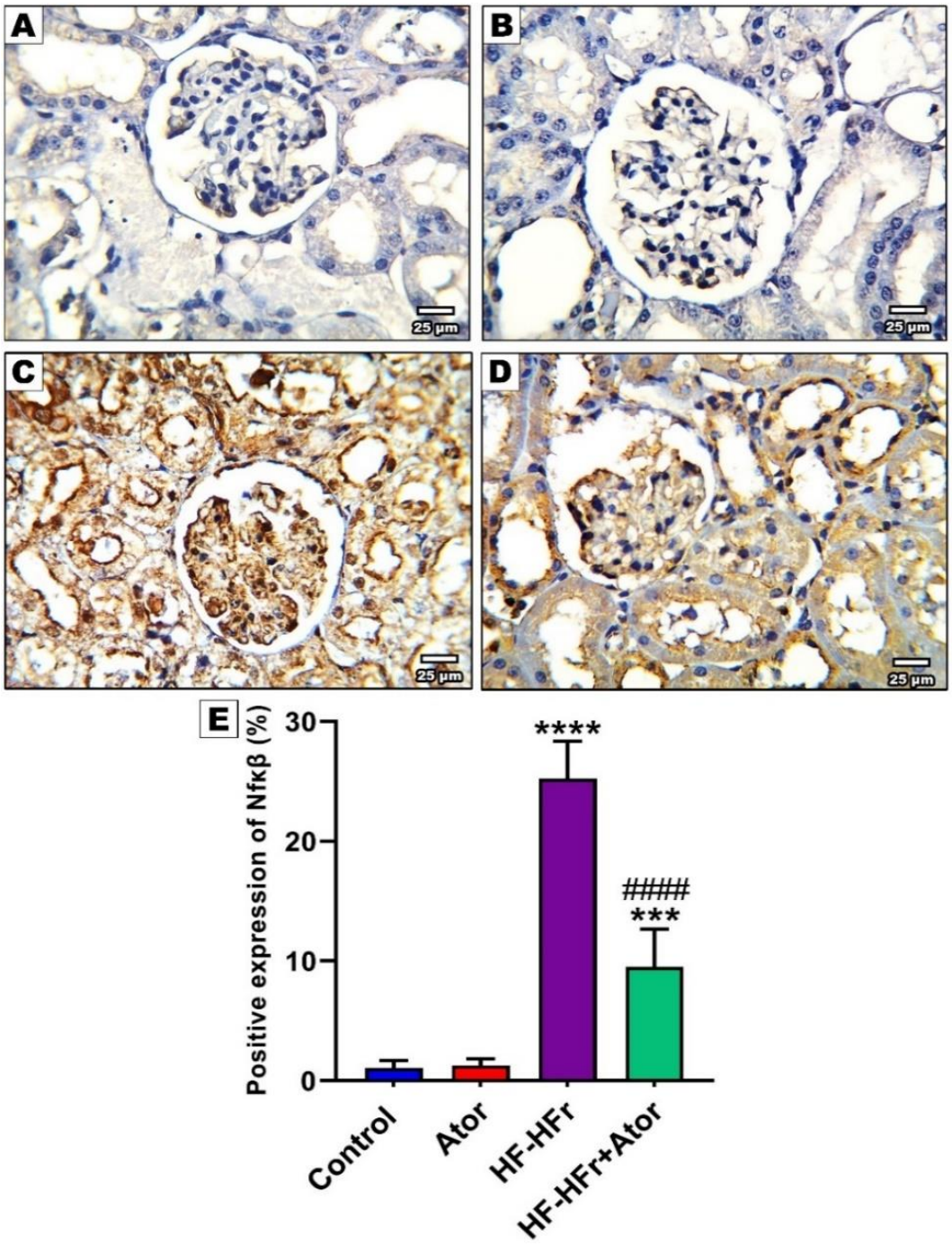

Fig. 4: the immunohistochemistry expression of NF-κβ in renal sections. from the control group (A), Ator group (B), HF-HFr group (C), and HF-HFr+Ator group (D). The histogram (E) represents the percentage of the immunohistochemical positive stained area, the data presented as mean ±SD. * significance against control, # significance against HF-HFr group.

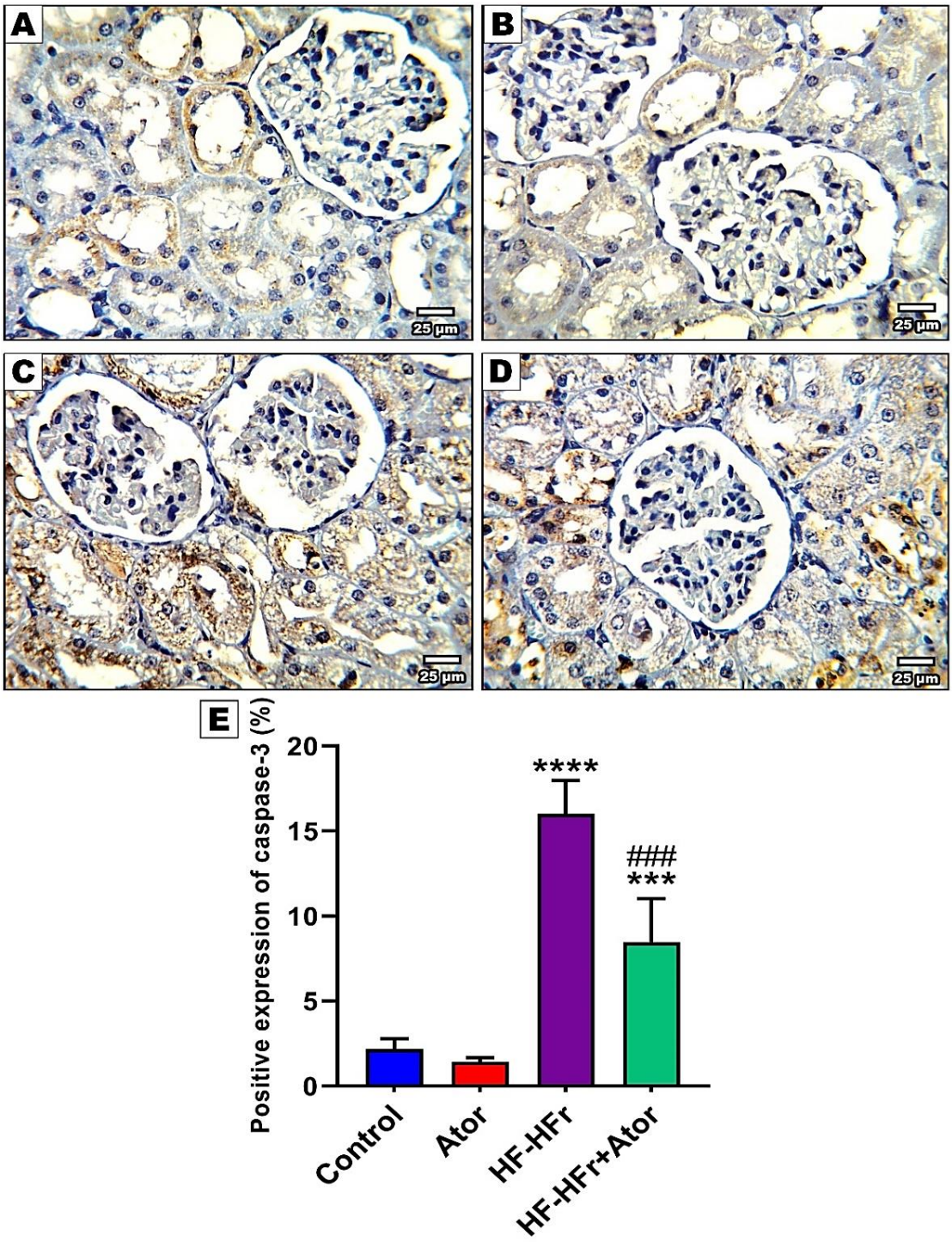

Fig. 5: immunohistochemical expression of caspase-3 in renal sections from the control group (A), Ator group (B), HF-HFr group (C), and HF-HFr+Ator group (D). The histogram (E) represents the percentage of the immunohistochemical positive stained area, the data presented as mean ±SD. * significance against control, # significance against HF-HFr group.

DISCUSSION

Obesity is a major health hazard that is intimately related to increasing dietary fat intake and sedentary lifestyles. Obesity is associated with metabolic syndrome (MS), which includes type 2 diabetes, hypercholesterolemia, and dyslipidemia (Kramer and Luke, 2007, Bocarsly *et al.*, 2010). The consumption of high-fat diets (HFD) is a primary driver of metabolic syndrome and obesity (Auberval *et al.*, 2014).

Fructose is a prevalent component in various commonly

consumed food products (Bantle, 2006), and consuming high-fructose diets (HFrD) has been shown to increase the prevalence of obesity and metabolic syndrome (Bocarsly *et al.*, 2010). Additionally, the consumption of high-carbohydrate diets, including HFrD, with or without HFD, can lead to the development of type 2 diabetes (Pereira-Lancha *et al.*, 2012).

HF-HFr diet model was used as it closely resembles the human metabolic syndrome (Collison *et al.*, 2011, Zaki *et al.*, 2019). The exact mechanisms by which high-fat diets (HFD) and high-fructose diets (HFrD) can lead to renal damage are not yet fully understood (Yang *et al.*, 2014).

Either their hypolipidemic impact, which reduces albuminuria and diabetic kidney disease (Glocker *et al.*, 2011), or their lipid-independent effects on processes that may exacerbate the progression of diabetic nephropathy, provide the renoprotective benefit of statins in diabetes. Statins' pleiotropic mechanisms, which influence cell proliferation, apoptosis, and oxidative stress, may yield positive outcomes unrelated to their lipid-changing properties (Haslinger-Löffler, 2008).

This study demonstrated significant metabolic, histopathological, biochemical, and functional changes in the kidneys that result from consuming a high-fat, high-fructose (HF-HFr) diet. The effects observed were reduced glucose tolerance, higher total cholesterol, an upward trend in plasma triglyceride levels and reduced HDL. Additionally, increased levels of blood creatinine and BUN, serve as indicators of early kidney impairment. This implies a correlation between compromised renal function, obesity, and insulin resistance.

Insulin sensitivity is decreased and normal insulin signaling is disrupted by obesity. Insulin resistance is a disorder that appears in the early stages of type 2 diabetes and is characterized by impaired metabolism of fats and carbohydrates and malfunction of the endothelium. These changes may lead to the development of type 2 diabetes, hyperlipidemia, hyperinsulinemia, and renal impairment (Pengrattanachot *et al.*, 2020).

The kidneys' lipid metabolism is affected by insulin resistance, changes in glucose regulation, and lipid metabolism (Knight and Imig, 2007). Insulin's primary role is to suppress the breakdown of fats in fat cells, The rates of lipolysis in adipocytes were higher in individuals with insulin resistance, leading to the accumulation of lipids in abnormal locations (Morigny *et al.*, 2016). Changes in renal lipid metabolism may significantly contribute to renal damage in the metabolic syndrome. Renal triglyceride and cholesterol levels rose considerably in obese mice produced by HF-HFr diet. Hyperinsulinemia increased the expression of SREBP-1 in the kidneys on diet-induced obesity in mice (Declèves and Sharma, 2015).

The results from this study showed that there is a link between higher levels of triglycerides and cholesterol and kidney inflammation, fibrosis, glomerulosclerosis, lipo-apoptosis, and kidney dysfunction. The hyperlipidemic state led to an accumulation of lipids inside the kidney that led to a decrease in the flow of fluid through the renal tubules and a loss of flexibility in the renal blood vessels. Also, obesity leads to increased density and tension in the glomeruli (Declèves and Sharma, 2015). These changes in structure and function might cause the filtration fraction to go up, as well as renal hyperfiltration, higher glomerular capillary pressure, and damage to the glomeruli (Pengrattanachot *et al.*, 2020). These histopathological alterations were observed in hematoxylin and eosin-stained sections.

Treatment with atorvastatin made the observed increase in Bowman space, smaller glomeruli, tubular degeneration, and broadening better. The excessive filtration rate in the glomeruli and the presence of protein in the urine in individuals were linked with metabolic

syndrome. Furthermore, treatment with atorvastatin reduced the severity of kidney impairment, as measured by the injury score of the H&E stains, in a rat model of obesity-induced kidney injury (Srivastava *et al.,* 2014).

The current study observed a significant increase in oxidative stress in the HF-HFr diet, elevated MDA and reduced GSH. Additionally, increased collagen deposition in Sirus red stained sections, increased expression of NFκβ and subsequent increase in caspase 3 in immunohistochemical stained sections. All these changes were significantly ameliorated after statin treatment.

Rats on a high-fat diet had significantly higher levels of malondialdehyde and lower levels of glutathione in their renal cortex (Pengrattanachot *et al.,* 2020). A high-fat diet induces oxidative stress, which is characterized by impaired mitochondrial activity and increased formation of reactive oxygen species (ROS). This, in turn, triggers molecular responses that contribute to the development of renal cell lesions (Sun *et al.,* 2020). An imbalance in redox led to alterations in the antioxidant response, thereby facilitating a continuous cycle of oxidative stress. This imbalance led to the increased formation of large amounts of MDA and higher levels of MDA were associated with renal damage in high fat-high sugar-fed rats (Rosas-Villegas *et al.,* 2017).

Pengrattanachot *et al.* revealed that statins reduce oxidative stress in HF-HFr-induced kidney damage and regulate it (Pengrattanachot *et al.,* 2020). In the pancreas, atorvastatin showed antioxidant properties and prevented the progression of inflammation and fibrosis, hence improving β-cell function (Wei *et al.,* 2016). Further research has shown that atorvastatin provides additional benefits in sustaining the functions of the kidneys and the pancreas, either in isolation or in conjunction with insulin therapy. These benefits can be achieved in diabetic rats using a gentamicin-induced nephrotoxicity model via regulating endoplasmic reticulum stress, inflammation, and apoptosis (Thongnak *et al.,* 2017, Jaikumkao *et al.,* 2016). As atorvastatin lowers cholesterol and has antioxidant qualities, it may reduce advanced glycation end products (AGEs), which are a source of superoxide-free radical formation (Xu *et al.,* 2014).

A diet high in fat results in fat buildup, elevated levels of inflammatory cytokines, the initiation of glomerular retraction, and impaired kidney function (Muller et al., 2019). Furthermore, because fructose increases the synthesis of uric acid and has metabolic effects on de novo lipogenesis (DNL), it also affects kidney health when consumed in large quantities (Johnson *et al.,* 2010, Fan *et al.,* 2019). Because de novo lipogenesis (DNL) increases the production of inflammatory cytokines, dyslipidemia may result in increased kidney injury. Consequently, the glomerulus and tubules' cell shape and function alter (Hall *et al.,* 2019). NF-κB has the ability to attach itself to the promoter region of genes that induce inflammation. This attachment leads to an increase in the production of proinflammatory cytokines such as TNFα, IL-6, and COX-2 in the renal cortical tissues. Additionally, by upregulating the expression of TGF-β1, the precursor of collagen type IV, NF-κB may ameliorate renal fibrosis (Pengrattanachot *et al.,* 2020).

By inhibiting RhoA, a protein involved in TNF synthesis, NFB activation, and the release of cytokines such as TNF-e, IL-1e, IL-6, and IL-8, statins lessen systemic inflammation (McCarey *et al.,* 2004). The anti-inflammatory impact of atorvastatin was demonstrated by the reduction in the expression of IL-6, TNFαR1, COX2, and NFκB (Pengrattanachot *et al.,* 2020). Regarding statin, atorvastatin has more potent anti-inflammatory effects compared to simvastatin (Ma *et al.,* 2017). Moreover, atorvastatin had a more potent renoprotective effect compared to rosuvastatin.(Takazakura *et al.,* 2015).

Statins improved metabolic irregularities in a study of obese Zucker rats fed a high-fat diet. They also helped to prevent glomerular hypertrophy and mesangial enlargement, as well as reduce renal inflammation (Reisin *et al.,* 2009). Atorvastatin has cholesterol-independent benefits, including antioxidant, anti-inflammatory, and anti-apoptotic properties (Bruder-Nascimento *et al.*, 2016). Atorvastatin, whether administered alone or in conjunction with insulin therapy, has proven additional benefits in maintaining renal and pancreatic functions by controlling oxidative stress, inflammation, and apoptosis in diabetic rats and a model of gentamicin-induced nephrotoxicity (Jaikumkao *et al.,* 2016, Thongnak *et al.*, 2017).

Conclusion:

Atorvastatin improved HF-HFr-induced renal injury through modulation of lipotoxic and oxidative stress-induced inflammation and apoptosis. Atorvastatin decreases triglycerides and cholesterol, increases HDL and modulates renal lipid accumulation with subsequent reduction of insulin resistance, NFκβ, fibrosis and apoptosis.

Declarations:

Ethics Approval: The Ethics Committee for Animal Experimentation guidelines at Zagazig University's College of Medicine approved the research protocol and the animal studies (Approval number: ZU-IACUC/3/F/91/2024). The NIH Guide for the Care and Use of Laboratory Animals was followed throughout the handling and scarification of the animals.

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Author contribution: Dr. Mamdouh Eldesoqui, Sahar k. Ali, Rania H. M. Soliman: Conceptualization, project administration, data analysis, investigation, validation, visualization, writing original draft, and writing-review and editing. Dr. Abdelaty S. Mohamed, Ahmed N. A. Nasr, Mai M. Eldaly, Eman M. Embaby, Heba S. Ahmed, Zeinab M. Saeed, Zeinab A. Mohammed: data curation, formal analysis, tabulation investigation, validation, visualization, writing and editing.

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