Egyptian Journal of Veterinary Sciences

https://ejvs.journals.ekb.eg/

Evaluation of Renoprotective Effect of Evolocumab and/or Telmisartan on Obesity-Induced Renal Injury in C57BL/6J Mice

Fady Y. Attalla[1], Nabil Abu-Heakal[2], Ahmed I. Ateya[3], Mona M. Elghareep[4], Mahmoud El-Tohamy[5] and Basma H. Osman[6]

[1] *Physiology department, Faculty of Veterinary Medicine, Mansoura University, Egypt.*
[2] *Physiology department, Faculty of Veterinary Medicine, Mansoura University, Egypt.*
[3] *Development of Animal Wealth Department, Faculty of Veterinary Medicine, Mansoura University, Egypt.*
[4] *Physiology department, Faculty of Veterinary Medicine, Mansoura University, Egypt.*
[5] *Physiology department, Faculty of Medicine, Mansoura University. Egypt.*
[6] *Medical Experimental Research Center, Faculty of Medicine, Mansoura University, Mansoura, Egypt.*

Abstract

THIS STUDY evaluated the renoprotective effect of evolocumab and telmisartan on obesity-induced comorbidities at the kidney level. The present investigation was conducted using fifty-six male C57BL/6J black mice divided into eight groups, with seven mice per group. Firstly, the control mice were fed a basal control diet and administered evolocumab and/or telmisartan. Second, mice were fed a high-fat diet [HFD] [TD.88137] to induce obesity for 15 weeks. Thirdly, mice were treated with evolocumab and/or telmisartan. Blood samples and renal tissues were collected to evaluate renal function markers, thyroid hormones levels, oxidative stress and antioxidant markers, gene expressions, and histopathological examination. The results showed that mice fed HFD revealed an impairment of renal function markers, including elevated serum levels of creatinine, angiotensin II, and renin. Hypothyroidism was observed as a reduction in serum levels of triiodothyronine [T3] and tetraiodothyronine [T4]. High renal oxidative stress was noticed as an elevation of malondialdehyde [MDA] and nitric oxide [NO] with a reduction in renal antioxidant enzymes as a decreased levels of the reduced glutathione [GSH], glutathione transferase activity [GST], and total antioxidant capacity [TAC]. Additionally, up-regulation of the *angiotensin II receptor type 1 gene [AT1-R] and nuclear factor kappa B [NF-κB]* gene expression. The histopathological examination represents diffuse tubular degeneration and necrosis. While mice treated with a combination of evolocumab and telmisartan improve renal function markers, as reduction in serum levels of creatinine, angiotensin II, and renin. Thyroid hormones, including serum T3 and T4, were elevated. Alleviation of renal oxidative stress levels and improvement of antioxidant enzyme levels, with down-regulation of *AT1r* and *NF-κB* expressions. Also improving the renal histological structure of obese mice. Conclusion: Evolocumab combined with telmisertan is an effective treatment of obesity-induced renal injury by managing the adverse effects correlated with obesity pathophysiology.

Keywords: Evolocumab; Telmisartan, Obesity, Renal Injury, C57BL/6J Mice.

Introduction

Obesity is a global health issue characterized by excessive fat accumulation. It has worsened over the past 50 years and is expected to increase by 40% in the next decade. This increases the risk of developing diabetes, cardiovascular disease, and chronic kidney disease (CKD). [1]. The link between obesity and CKD is complex, involving decreased kidney function, proteinuria, glomerulomegaly, and progressive glomerulosclerosis. Obesity is also linked to serious factors like insulin resistance, lipotoxicity, adipocytokine dysregulation, hypertension, and elevated glomerular blood pressure. [2].

Thyroid hormone activates renal blood flow and glomerular filtration rate, enhancing the clearance of xenobiotics. It directly affects renal function through changes in GFR, tubular secretory capacity, renal blood flow, electrolyte pumps, and kidney structure.

*Corresponding author: Fady Yousef Attalla, E-mail: Fadyyousef770@mans.edu.eg, Tel.: 01119159205
(Received 22 July 2024, accepted 03 December 2024)
DOI: 10.21608/EJVS.2024.306322.2268

Hypothyroidism and hyperthyroidism deteriorate kidney function, as hypothyroidism raises the risk of impaired renal function by affecting cardiac output, intra-renal hemodynamics, RAAS, and structural changes like impaired glomerular architecture, curtailed tubular mass, and reduced kidney-to-body weight ratio. [3].

In addition, various revolutionary treatment techniques have been developed to address obesity epidemics and related complications, but current medical options are limited and have low efficacy and safety, highlighting the need for further research and development. Meanwhile, using evolocumab as a new lipid-lowering drug is a fully human monoclonal antibody targeting proprotein convertase subtilisin/kexin type 9 [PCSK9], which has the ability to hinder the low-density lipoprotein receptor [LDL-R] disintegration in liver by induction of PCSK9, enhancing more of hepatic LDL-R to eliminate low density lipoprotein [LDL-C] from the circulation [4]. Evolocumab significantly protects endothelium by increasing antioxidant capability and decreasing the level of hydroperoxide which reduces the mortality triggered by H_2O_2, MDA, and levels of lipid peroxide [5].

On the other hand, the liver, small intestine, and kidney are where PCSK9 is mostly expressed, despite being found in the kidney, its impact on kidney disease and function have been shown to be very limited. In vivo models of kidney disease show a considerable increase in serum PCSK9, as demonstrated by introductory investigations. [6].

Telmisartan is a drug with a high binding affinity to angiotensin II receptor type 1[AT1-R] and selectively modulates the receptor of peroxisome proliferator-activated gamma type [PPAR-γ agonist] by 25-30% of full PPAR-γ agonists, which a primary regulator of insulin and glucose metabolism. This dual mechanism of action is believed to offer proactive advantages against obesity-induced vascular and kidney injury. [7].

The objective of this investigation was to evaluate the possible renoprotective impact of evolocumab and/or telmisartan following induction of obesity on C57BL/6J mice. This model can be used for evaluating the biochemical analysis of renal function markers, thyroid hormones levels, renal antioxidant and oxidative stress, renal AT1-R and NF-κB relative gene expressions, and renal histopathological examination.

Material and Methods

Materials

Experimental Animals

Fifty-six male C57BL/6J black mice [aged 6–8 weeks and weighing 25 ± 2 g] were purchased from the animal house of the Medical Experimental Research Center [MERC], Faculty of Medicine, in cooperation with the physiology department of the Faculty of Veterinary Medicine at Mansoura University, Egypt. In an appropriately controlled setting of 12-hour light/dark cycle, 22°C temperature, and 65–70% relative humidity. The mice were housed in a polypropylene enclosure and allowed to acclimate for one week. The mice had unrestricted availability of food and water for the entire duration of the experiment [Seven and a half months]. The ethics committee of the Faculty of Veterinary Medicine at Mansoura University authorized this study, with an assigned registration number, [MU-ACUC; VM.MS.24.04.125].

Experimental diet:

A. Basal Control Diet:

The basal control diet [370 kcal/100G; 12.8% of energy from fat, 18.7% from protein, 68.7% from carbohydrate; TD.05230] was prepared in "MERC" and used for control groups. The diet was produced as a pelleted mouse diet [8].

B. High Fat Diet

The high fat diet [HFD] [450 kcal/100G; 42% of energy from fat, 15.2% from protein, 42.7% from carbohydrate; TD.88137 Harlan Laboratories Inc., Indianapolis, IN, USA, with 2% cholesterol] was also prepared in "MERC. This ration was used to induce obesity model in mice [8].

Drugs and chemicals

Evolocumab

Evolocumab [Repatha®] contained two prefilled syringes [140mg/ml] obtained from Amgen Company, USA. ATC code: C10AX13.

Telmisartan

Telmisartan [Micardis®40 mg] was obtained from Boehringer Ingelheim India Pvt. Ltd. in tablet form.

Methods

Experimental Design

Fifty-six [56] healthy adult male C57BL/6J black mice were divided into eight equal-sized experimental groups at unintentional [seven mice per group] the experimental groups, ration, treatment, and the dose of each treatment for each group are shown as follow:

Group [I] which serve as control group, mice were fed on standard diet, and injected with normal saline I/P, while in group [II] mice fed normal diet and injected S/C with evolocumab [10 mg/kg] every 10 day [9]. In group [III] mice fed normal diet and giving telmisartan orally [5 mg/kg per day] [10]. In group [IV] mice fed normal diet and injected S/C with evolocumab [10 mg/kg] every 10 day with

giving telmisartan orally [5 mg/kg per day]. In group [V] mice fed HFD [TD.88137] and injected with normal saline I/P [8]. In group [VI] mice HFD [TD.88137] then treated with S/C injection of evolocumab [10 mg/kg] every 10 day. In group [VII] mice HFD [TD.88137] then treated with giving telmisartan orally [5 mg/kg per day]. In group [VIII] mice HFD [TD.88137] then treated with combination of S/C injection of evolocumab [10 mg/kg] every 10 day and giving telmisartan orally [5 mg/kg per day]. The mice in group V fed HFD for 15 week, while groups VI, VII, and VIII firstly fed HFD for 15 week then treated for another 15 week with evolocumab and/or telmisartan.

Sampling
Blood samples

The mice were deprived of food for the whole night, and then anesthetized with diethyl ether, blood was drawn from the mouse via heart puncture and placed in plain tubes, which placed in upright position at room temperature, then centrifuged at three-thousand r.p.m for fifteen minutes. A transparent, pale yellow serum sample was drawn up using an automated pipette, moved into fresh, dry, marked vials, and stored in a freezer at -80°C for later use in biochemical testing.

Tissue sampling

The kidneys of the dissected mice were removed, the leftover blood was rinsed with normal physiological saline. Three sections were made from samples:

The first section, 500 mg of renal tissue, was used for tissue homogenization in a phosphate buffer solution using a homogenizer**.** The tissue was homogenized in nine milliliters of ice-cold, pH 7.6 phosphate-buffered saline, a gram of kidney tissue was homogenized. After the completion of the homogenization process, following a twenty-minute centrifugation at four thousand r.p.m., the transparent supernatant was pipetted out and the homogenates were kept at -80 °C until the analytical procedures were performed. The sample was stable for at least one month for subsequent biochemical assay of oxidative activities. The second section was cut into transverse slices to be stored in liquid nitrogen [-176 °C] for gene expression. The third section was preserved in 10% formalin for histolopathological evaluation and staining with hematoxylin and eosin [H&E].

Biochemical assay

Assessment of serum thyroid hormone levels

The determination of T3 using kits [Colorimetric, Calbiotech; Netherland] depends on competitive ELISA for quantitative measurement of T3 in mouse serum. While the determination of T4 using kits [Colorimetric, Calbiotech; Netherland] depends on the mechanism of competitive inhibitor immunoassay using enzymes by adding standards or samples to a pre-coated microtiter plate containing biotin-conjugated T4.

Assessment of serum renal function markers

The creatinine sample content directly correlates with the rate of color formation. Meanwhile, Ang II was measured using an ELISA kit [Colorimetric, Bioassay technology lab; Korain] of Mouse Ang II antibody to detect a sample. In addition, renin was measured using a kit [Colorimetric, Bio Vision; USA] that includes a micro-ELISA plate pre-coated with mouse Renin antibodies.

Assessment of renal oxidative stress and antioxidant enzymes

The oxidative stress markers: the MDA which was estimated by the colorimetric enzymatic method. While the NO was colorimetrically determined، by calculating the wavelength of absorption at 550 nm, which can be determined indirectly. The action of reagents 1 and 2 was to remove the interference of colored matter in the sample.

The antioxidant enzymes: the GSH was determined using pre-made biodiagnostic kits, via spectroscopy by the enzyme colorimetric technique. While the GST triggers the conversion of L-glutathione to CDNB by utilizing the thiol group of the glutathione. The GS-DNB conjugate, formed as a result of the reaction, exhibits absorption at 340 nm. The sample's GST activity was exactly correlated with the rate atwhich absorption increased. In addition, TAC were determined by colorimetric method**.**

Gene expression analysis by quantitative real-time PCR

RNA was extracted from the kidney using the RNeasy Mini kit [Qiagen] according to the instruction manual. The RNA concentration was measured to assess RNA quality and quantity using a NanoDrop spectrophotometer [Implen, USA]. Equivalent to 1µg of total RNA was converted into cDNA using the instructional manual. cDNA was synthesized using a high-capacity reverse transcriptase kit [Thermo Fisher].

The Applied Biosystems software calculated amplification curves and threshold cycles [Ct] values to measure the variance in gene expression on the RNA of the various samples. The Ct values of each sample were compared with those of the control group using the "ΔΔCt" method. Relative gene expression was then calculated by using the following ratio: $[2^{-\Delta\Delta ct}]$.

ΔCT = CT of the gene of interest – CT of the housekeeping gene

ΔΔCt = ΔCT treated – ΔCT control

Fold change due to treatment = $2^{-\Delta\Delta Ct}$

The sequences of target oligonucleotide primers including; mouse *AT1-R* gene, according to Thomas et al., [11]. Mouse *NF-κB* gene, as stated by Li et al., [12]. Mouse *GAPDH* gene, as reported by Bancroft and Gamble. [13].

Histological examination

Kidney specimens [3 from each group] from all groups were collected and immersed in 10% formalin for one day, rinsed with tap water treated with ethanol for dehydration, purified in xylene, and finally embedded in paraffin. From each specimen, a segment with a thickness of five microns was stained by [H &E], for evaluation of the morphometric interstitial changes and fibrosis, and examined microscopically.

The histopathological scoring of renal tissues was conducted according to Sato et al. [14], where a zero [0] score indicates no tubular damage, dilation nor inflammation, or congestion. One [1] indicates minimal tubular damage, dilation with a rare intraluminal cast, a little inflammation, and congestion. Two [2] indicate mild to moderate tubular degeneration, dilation with few intraluminal casts, mild or focal inflammation, and moderate interstitial congestion. Three [3] indicate diffuse tubular necrosis, dilation with severe ectasia, moderate to severe coalescing interstitial inflammatory aggregates, and many interstitial congestions.

Statistical analysis

In the current study, the mean and standard error [SE] [mean ± SE] were used to express the results. Data was analyzed using one-way ANOVA to test for difference among the all the groups employing Turkey as a post hoc test. The means showed a significant difference when [P value < 0.05]. Data was analyzed using SPSS v20 software according to protocol of [15].

Results

Effect of Evolocumab and/or telmisartan on Thyroid hormone levels in obese C57BL/6J mice:

The results of Thyroid hormone levels in serum are dissipated in Figure [1]. In this study, remarkable hypothyroidism was observed in group V which fed HFD [TD.88137], which showed a significant reduction [P<0.05] in serum triiodothyronine [T3] and tetraiodothyronine [T4] levels in comparison with the control groups I, II, III, and IV. In contrast, mice treated with evolocumab and/or telmisertan in groups VI, VII, and VIII induce a significant elevation [P<0.05] in T3 and T4 levels, which was more noticeable in mice treated with a combination of evolocumab and telmisertan in group VIII when compared to the untreated mice receiving a HFD.

Effect of Evolocumab and/or telmisartan on renal function markers in obese C57BL/6J mice:

The results of serum markers of renal function are dissipated in Figure [2]. In the present study, a significant elevation [P<0.05] in the serum levels of creatinine, Ang II , and renin in group V which fed a HFD [TD.88137] comparison with control groups I, II, III, and IV. While mice treated with evolocumab and/or telmisertan in groups VI, VII, and VIII showed a significant reduction [P<0.05] in the serum levels of creatinine, Ang II , and renin, which was more prominent in mice treated with evolocumab combined with telmisertan in group VIII when compared with untreated mice receiving a HFD.

Effect of Evolocumab and/or telmisartan on Oxidative stress and antioxidant enzymes of renal tissue in obese C57BL/6J mice:

The results of renal oxidative stress and antioxidant enzymes are dissipated in Figure [3-4]. In this current study, high renal oxidative damage was observed in group V fed HFD [TD.88137], which was represented by a significant elevation [P<0.05] in the levels of MDA, and NO, meanwhile, the levels of GSH, GST, and TAC were significantly reduced [P<0.05] when compared with control groups I, II, III, and IV. On the other hand, the treatment with evolocumab and/or telmisertan in groups VI, VII, and VIII induces a significant decrease [P<0.05] in the levels of MDA, and NO, besides, the levels of GSH, GST, and TAC were significantly elevated [P<0.05], which was more prominent in mice treated with telmisertan alone in group VII in comparison with those fed a HFD.

Effect of Evolocumab and/or telmisartan on Genes expressions of renal tissues in obese C57BL/6J mice:

The results of renal gene experession are dissipated in Figure [5]. Significant up-regulation [P<0.05] in *AT1-R,* and *NF-κB* genes expressions were noticed in mice receiving a high-fat diet [TD.88137] in group V, in comparison with control groups I, II, III, and IV. Adminsteration of evolocumab and/or telmisertan in groups VI, VII, and VIII induces a significant down-regulation [P<0.05] in *AT1-R*, and *NF-κB* genes expressions, which was more prominent in mice treated with a combination of evolocumab and telmisertan in group VIII when compared with mice received a HFD.

Effect of Evolocumab and/or telmisartan on Histopathological examination of renal tissue in obese C57BL/6J mice:

The results of renal H&E histopathological examination are dissipated in Figure [6-7]. Group I represented in Fig [7; A, B]: showed normal cortical tubules and glomerulus. Group II: upto 90% of renal parenchyma appeared normal except occasional tubular vacuolation with few interstitial congestion, insets, tubular degeneration with vacuolation,

individual tubular cell necrosis. Fig [7; C, D]. Group III Fig [7; E, F]**:** approximately up to 90% of renal parenchyma appeared normal except occasional interstitial inflammation admixed with rare regenerative tubules with basophilic cytoplasm and vesiculated nuclei. Group IV: showed renal tubules approximately are normal with occasional periglomerular inflammation and few interstitial haemorrhage. See Fig [7; G].

Group V dissipated in Fig [7; H]: showed ectatic tubules often lined with attenuated epithelium and rarely contain esinophilic proteinacous materials with interstitial congestion and few inflammation, tubular ectasia lined with attenuated epithelium. Also, in Fig [7; I]: a diffuse tubular degeneration and necrosis characterized by cortical tubules either degenerate [hypertrophied with vacuolated cytoplasm], or necrotic [hypereosinophilic, angular cytoplasm with pyknotic nuclei] epithelial cells, variable amount of intraluminal esinophilic material or sloughed epithelial cells, mild multifocal interstitial infiltrations composed of scattered tiny clusters of neutrophils, macrophages, plasma cells, and lymphocytes, disruption of tubular architecture with either vacuolated epithelial cells or necrotic cell with either pyknotic or karylolytic nuclei.

Group VI Fig [7; J, K]: showed moderate interstitial inflammation or mild interstitial fibrosis surrounded and replaced a necrotic tubules, inset, moderate peritubular aggregation of moderate numbers of lymphocytes, macrophages and fibroblast surrounded a necrotic tubules. Group VII Fig [7; L]: showed focal periglomerular aggregations of inflammatory cells together with minimal interstitial congestion. Meanwhile, in Fig [7; M]: a diffuse tubular vacuolation with rare intratubular sloughed debris and multifocal periglomerular and perivascular minimal to mild aggregation of inflammatory cells. In Fig [7; N]: a minimal periglomerular inflammation and occasional necrotic tubules. In addition, Fig [7; O]: showed minimal interstitail fibrosis admixed with few inflammatory cells and partial tubular regeneration characterized by pile up of 2-3 epithelial cells with vesiculate nucleus. Group VIII: a mild interstitial fibrosis admixed with cellular infiltrates and interstitial congestion surrounded a mild, mild to moderate necrotic tubules. See Fig [7; P].

Discussion

In the present study there was a significant reduction in serum levels of T3 and T4 which indicate a condition of hypothyroidism, in mice fed HFD [TD.88137] group **V**, It is linked to a lower metabolic rate and thermogenesis, as well as attributed to increase the prevalence of obesity, which a low-grade inflammatory condition that persists over time, when adipose tissue is overloaded, it produces more cytokines and other inflammatory markers, such interleukin-6 [IL-6], tumor necrosis factor alpha [TNF-α], and [IL-1] [16], which could suppress the symporter sodium/iodide's mRNA expression, and subsequently affect the thyroid cells' capacity to absorb iodide [16], may conclude in hypothyroidism via depletion of iodine contributing to a reduction in thyroid hormone production. Concurrently, these cytokines can cause the thyroid gland's blood vessels to vasodilate and become more permeable, altering the thyroid's structure and function. [16].

In agreement with our results, Shao et al. [17], noticed that male Sprague-Dawley rats received a HFD containing lard for 24 weeks displayed decreased serum T4 and T4 levels in parallel with elevated serum TSH levels.

On the other hand, the mice treated with evolocumab and/or telmisertan represented a marked elevation in levels of serum T3 and T4. Treating renin-deficient mice with Ang II receptor blocker leads to a notable rise in hypothalamic type 2 deiodinase [DIO2] expression, which responsible for converting T4 to T3. This results in heightened levels of thyroid hormones in the hypothalamus, probably causing inhibition of thyroid-releasing hormone [TRH] via feedback. [18].

According to Chmyr. [19] reported that the treatment with telmisartan promote the activities of hypothalamic-pituitary system result in a noticeable rise in T4 levels and a reduction in thyroid-stimulating hormone [TSH] levels. These findings highlight the role that telmisartan performs in improving metabolic conditions caused by obesity, particularly in relation to symptoms of subclinical hypothyroidism.

While, evolocumab, a novel PCSK9 inhibitor, showed a positive relationship between circulating PCSK9 and serum TSH, but a negative one with T4 and T3. In short-term subclinical and severe hypothyroidism, increased PCSK9 levels correlated with increased total cholesterol, LDL-C, and TSH, while negatively affecting T4 and T3 levels. Previous data showed a reduction in PCSK9 circulation, which negatively correlated with T3 and T4 levels. **[20].** As regard to previous data using evolocumab as treatment lead to reduction in circulation PCSK9 which in-turn negatively correlated with elevation in T3 and T4 levels.

Mice fed HFD [TD.88137] in group V showed marked impairment of renal function markers including; increased serum levels of creatinine, Ang II, and renin. The renal injury triggered by prolonged HFD consumption in lab animals was caused by malfunction of the mitochondria and cellular oxidative stress. It was also shown that excessive fat buildup in the kidney and elevated levels of circulating FFA and pro-inflammatory cytokines were contributing factors to lipotoxicity, which

results in kidney damage including fibrosis of glomerulus, degeneration of podocyte foot process, and tubular cell apoptolysis which attributed to renal dysfunction, evident by reduce GFR, albuminuria, and increased serum creatinine. [21].

Increasingly, HFD attributes to early intrarenal RAAS activation, indicated by elevated urine angiotensinogen concentrations and raised intrarenal Ang II concentrations. In addition, it up-regulates renin expression in adipose tissue by raising the levels of the prorenin receptor [PRR] via elevating PRR expression in adipose tissue, further raising renin levels and hypertension. [22].

In the current study the results agree with Sun et al. [21], illustrated that male C57BL/6 mice fed HFD [TP23520, Trophic Diet, China], which showed a significant raise in creatinine levels. This is also compatible with Gupte et al. [23], clarified that C57BL/6 mice fed an HFD [60% kcal as fat; D12492, Research Diets Inc.; n = 10], exhibiting increased Ang II levels. In harmony with, Dalmasso et al. [24], noted that C57BL/6J mice fed HFD [60% kcal from fat, D12492, respectively; Research Diets, Inc., New Brunswick, NJ] for 20 weeks showed elevated renin activity when compared with control groups.

Treating mice with evolocumab and/or telmisertan improve renal function markers through significant reduction in serum creatinine, Ang II, and renin levels. Telmisartan decreases serum creatinine levels by improving kidney function and renal protective effects in CKD, leading to a decrease in serum creatinine levels, and also ameliorate the renal hypertrophy, reduce glomerular mesangial matrix expansion, and enhance creatinine clearance rate, ultimately lowering serum creatinine levels [25].

Also, telmisertan reduces serum Ang II levels trough it's binding with AT1R, Ang II was inhibited, resulting in blocking the pressor effects on vessels, preventing the kidney's sodium reabsorption ability, and hindering the production and release of aldosterone. Moreover, telmisartan belongs to a class of medications known as renin inhibitors which reduces renin activity [7].

Our finding in agreement with Naguib et al. [26], noticed that male wistar rats fed a HFD for 24 weeks then treated with telmisertan showed a marked decrease in serum creatinine levels when compared with rats fed HFD.

Evolocumab treatment significantly reduced serum creatinine levels, due to a relationship between serum PCSK9 levels and creatinine levels, proposing that chronic renal injury may contribute to the upregulation of PCSK9, increasing its serum concentration. Additionally, evolocumab's effect on serum Ang II levels is due to the correlation between RAAS activity and LDL-C levels, potentially bidirectional, as overfeeding mice attribute to hyperlipidemia, may stimulate RAAS and up-regulate its components including Ang II. [6].

Moreover, evolocumab reduces serum renin levels result from linking the LDL-C and total cholesterol with renin levels, so according to Pizoń et al. [27] who noticed that high-renin hypertensive groups have higher levels of LDL-C and total cholesterol than low-renin hypertensive groups. Briefly, evolocumab is a novel PCSK9 inhibitor and has significant potentiality to lower LDL-C levels [4], which monitor how evolocumab improve renal function markers.

Feeding HFD [TD.88137] to mice induce significant elevation in renal levels of MDA and NO while a significant reduction in renal levels of GSH, GST, and TAC in group V. The animals possess mechanisms that effectively shield them from tissue damage caused by free radical attacks, which including group of natural antioxidant enzymes and proteins, [GST, SOD, CAT, GPX, GRD, and GSH]. Oxidative stress arises when there is an imbalance between the generation of reactive oxygen species (ROS) and antioxidant defense, which contribute to a cascade of alterations that deregulates the performance of cells, resulting in various pathophysiological changes [28]. Several processes, including autoxidation of glyceraldehyde, superoxide generation, and oxidative phosphorylation, can cause oxidative stress in obesity**,** which also associated with lipid peroxidation, led to an increase in MDA levels attributing to obesity-related metabolic syndroms**,** exhibited in kidney as elevated renal MDA levels [29].

Additionally, HFD causes tissue-induced oxidative stress by decreasing renal GSH levels through hypermethylation of a gene promoter associated with glutathione synthesis; neither the decreased expression of glutamine-related enzymes nor accelerated ROS-induced alteration in antioxidant defense system can explain this phenomenon. Moreover, HFD trigger a marked elevation in *IL-6* gene expression, which linked to glutathione deficiency, which may involve heightened cysteine catabolism**,** and stimulate GSH secetion, but not synthesis, by heptic tissue then released into bloodstream. [30]. This finding in agree with Noeman et al. [29], clarified that albino rats fed a HFD containing 46% fat for 16 weeks displayed an elevation in renal MDA levels with significant reduction in GSH levels.

HFD-induced obesity elevate NO level in renal tissue which also increase levels of TNF-α, IFN-γ, IL-1β, and IL-6 in the adipose tissue; these pro-inflammatory mediators trigger iNOS expression which contributes to an increase in the level of iNOS and catalyze the overproduction of NO which is well known to serve in oxidative stress and

inflammatory circumstances. [31]. Compatible with our results, Tsuchiya et al. [32], explained that male C57BL/6J mice fed HFD [82% energy as fat], the results obtained showing induction to production of NO by HFD.

Obesity significantly reduces the activity of GST, GPx, CAT, and PON-1 enzymes in the kidneys of obese mice. A reverse relationship between MDA and antioxidant enzyme actions, demonstrated by elevated lipid peroxidation promoted by obesity, hindering the antioxidant enzymes and proteins, leading to an increase in H2O2, hydroxyl radicals, and superoxide. The antioxidant enzyme's speedy breakdown and storage runout during obesity progression may reduce enzyme levels, explaining why obesity decreases GST levels in renal tissues. [29].

In many different pathophysiological conditions, total antioxidant capacity [TAC] used for the assessment of antioxidant response to free radicals and could be a reliable biomarker for diagnosis of various systemic diseases, including CKD**.** Excessive oxidative stress can cause damage at the molecular, tissue and cellular levels by increasing MDA and decreasing TAC, which are indicative of inadequate antioxidant levels or raised oxidative stress. [33]. This indicate an inverse correlation between TAC and MDA concentration in obesity.

The treated mice, using evolocumab and/or telmisartan, exhibited a significant reduction in the levels of renal MDA and NO while a marked elevation in the levels of renal GSH, GST, and TAC. Obesity leads to systemic inflammation and oxidative stress, so treatment with telmisartan triggers the intrinsic antioxidant defense mechanisms by promoting anti-inflammatory and antioxidant activities via blocking AT1-R. Simultaneously reducing the production of oxidative stress byproducts including the reduction of MDA level through activation of PPAR-γ, which contribute to the diminishment of lipid peroxidation [34].

Furthermore, telmisartan decreases nitric oxide [NO] levels in obese mice by inhibiting NO production through PP2A-mediated eNOS-Ser1179 dephosphorylation [35], reducing both NO formation and activity of iNOS preventing oxidiative/nitrosative stress occurance [35]. Therefore, telmisartan's impact on decreasing MDA and NO levels highlights its potential as a therapeutic agent for combating the oxidative stress associated with obesity.

Our findings concur with Naguib et al. [26], noted that male Wistar rats fed a HFD for 24 consecutive weeks and treated with telmisartan NO and MDA levels were markedly decreased in comparison with the HFD rats.

Moreover, treating rodents with telmisartan induce a marked raise in tissue levels of GSH. Oxidative stress caused by angiotensin can be mitigated by its inhibition, which also induces free radical formation and exhausts GSH. Ang II promotes the production of superoxide radicals, which are cojugated with nitric oxide to produce peroxynitrite, causing oxidative stress associated with an elevation of MDA levels, depletion of SOD activity, and exhaustion of GSH levels in different tissues. [34]. In addition, telmisartan as an angiotensin blocker have a direct impact on elevation of GSH formation. [34]. Telmisartan's capacity to inhibit AT1-R and block the function of Ang II is consequently responsible for it's ability to mitigate oxidative damage and enhance oxidative stress markers in tissues [7].

In the same line with Hamed and Malek. [36], clarified that male albino rats treated with telmisartan lead to normalization in GSH levels and activity of superoxide dismutase in kidney.

As a result of the negative correlation between GST and MDA level [29], telmisartan specifically attaches to GST [ligandin] in the cytosolic region of homogenate from rodent tissue, which exhibited a capacity-limited affinity; however, it was approximately five times more effective for the GST fraction than albumin.**,** so GST was elevated with telmisartan treatment. Telmisartan reduces intracellular ROS levels [37]. Overall, we regarded previous data showing that telmisartan's protective effects against oxidative stress in obese mice involve up-regulation of antioxidant enzymes, reduction of ROS levels, and modulation of oxidative stress, which contribute to increasing TAC.

Obesity is influenced by PCSK9, which activates chemokines and cytokines involved in LDL metabolism, enhancing LDL-oxidation levels and LDLR expression may raising PCSK9 expression, leading to oxidative stress. This stress is further exacerbated by LDL-oxidation, resulting in increased oxidative stress factors like total oxidant status [TOS], NO, and MDA, compared to antioxidant factors like TAC, GSH, SOD, and GPx. [38]. Because of a disparity between oxidants and antioxidants, TAC is markedly decreased than the control group. [33].

Evolocumab is a PCSK9 inhibitor that suppresses hydrogen peroxidation-induced cytotoxicity and markedly decreases the levels of MDA and hydroperoxide. [5]. According to Cammistto et al. [39], PCSK9 inhibitor demonstrated a crucial role in mitigating oxidative stress. As regards previous expressed data, evolocumab has a potent effect on oxidative stress due to its ability as a PCSK9 inhibitor and LDL-C-lowering drug, which in turn elevates the antioxidant protective system.

In the current study HFD [TD.88137] in group V induces a marked up-regulation in expression of renal *AT1-R*. The RAAS plays a critical role in the pathogenesis of obesity. Adipose tissue expresses the components of RAAS, including Ang II receptors, which are involved in managing the metabolism of lipids, adipogenesis, and the pathophysiology of obesity. [22].

The HFD-induced obesity stimulates the RAAS, leading to increased Ang II levels and up-regulation of *AT1-R* expression in kidney, resulting in hypertension by increasing renal sodium reabosorption and regulating natriuresis pressure. In addition, Ang II's ultimate impacts on the target tissue are modulated by the concentration of AT1-R on the cell surface. Drastically, AT1-R stimulation is heightened by elevated Ang II levels. [40]. Our study agrees with Jain et al. [41], illustrated that mice fed HFD [D12079B] for twenty weeks result in significant increased *AT1-R* transcription in renal tissue.

Mice treated with evolocumab and/or telmisertan induce a significant down-regulation in renal *AT1-R* expression. Telmisartan, an Ang II receptor blocker, reduces *AT1-R* expression by modulating gene expression related to the RAAS axis blockade **[7].** It was reported that telmisartan is the only AT1-R antagonist known to be capable of activating PPARγ, which gives telmisartan its uniqueness. **[7].** PPARγ stimulation blocks the expression of *AT1-R*. This blocking is triggered by immediate reactions between proteins, and necessary for this effect, the Sp1 site is presented at the −58/−34 location of the *AT1-R* gene. Which may suppress Sp1 function, leading to a reduction in the expression of *AT1-R* and impairing the response of Ang II on various cells [42].

The results obtained from telmisertan treatment agree with Graus-Nunes et al. [43], who observed that male C57BL/6 mice fed HFD and treated with losartan or telmisartan, which represent that the HFD group also showed higher *AT1-R* gene expression than the Control group. Conversely, both treatments [losartan or telmisartan] restoring the expression of AT1-*R* gene to values lower than the HFD group.

While the impact of evolocumab on the expression of *AT1-R* can be attributed to a direct correlation between the expression of *AT1Rs* in various cells and the levels of LDL**,** the expression of the *AT1-R* gene can be raised by LDL-C, which is associated with hypercholesterolemia. [44]. According to previous data, in conjunction with evolocumab, which is a fast-acting medication that reduces LDL [6], this illustrates the potent effect of evolocumab in downregulating the expression of *AT1-R* subsequence to the induction of obesity.

In this study, the results obtained from feeding HFD [TD.88137] in group V induces a significant up-regulation in renal *NF-κB* expression. HFD induces IL-6 and TNFα elevation, which stimulate the mitogen-activated protein kinase [MAPK] signaling pathway, leading to upregulation of NF-κB expression. This effect is mediated by cognate receptors like tumor necrosis factor receptor 1 [TNFR-1] and tumor necrosis factor receptor 2 [TNFR-2] conclude by upregulation of NF-κB expression. [45].

In addition, the elements of the pathological lipid profile may have the ability to induce non-cognate receptors, which also trigger MAPK signaling. [45]. There is no doubt that *NF-κB* has a vital function in inflammation and insulin resistance caused by obesity. Our findings concur with Gao et al. [45], clarified that feeding a HFD to C57BL/6 mice promote a significant elevation in expression levels of *NF-κB* in comparison with the control group.

Treating mice with evolocumab and/or telmisertan induces a significant down-regulation in renal *NF-κB* expression. TNF-α initiates the expression of proinflammatory cytokines and immune cells, inducing the expression of AGT and Ang II. These cytokines and ROS activate NF-κB, which in turn stimulates nuclear transcription factors. Telmisertan, activating PPAR-γ, blocking NF-κB, COX-2, iNOS, TNF-α, PGE2, and NO, and diminishing IL6 release. Telmisertan also has similar effects on kidneys with oxidative stress, and suppressing Ang II via blocking AT1-R diminsifies *NF-κB* expression and translocation. [46]. As regards previously expressed data, we demonstrate that telmisartan downregulates the expression of renal *NF-κB*.

Our findings coincide with Huang et al. [47] reported that feeding HFD then treating male C57BL/6 mice with telmisertan treatment induced marked reduction of *NF-κB* protein expression than those receive HFD.

In obesity, the LDL serves a provital role in activating NF-κB, which in turn upregulates *PCSK9* via releasing TNF-α, IL-1α, and IL-6. Threrefore, activation of the TLR4/NF-κB signaling pathway [48]. Suppressing the expression of PCSK9 hinders the inflammatory responses induced by oxidized LDL via inhibiting the deterioration of IκB-α and translocation of NF-κB to the nucleus, which is concluded by a significant reduction in inflammatory cytokines such as IL-1α, IL-6, and TNF-α in macrophages. [48]. Consequently, using evolocumab as a PCSK9 inhibitor is able to downregulate NF-κB expression by regulating its pathway. [48].

In unison, the results of group V in oxidative stress markers and histopathological finding; as regard to previous data which revealed a condition of renal oxidative stress which indicate a cellular damage, desregulates the cellular functions and leading to renal injury; demonstrated by renal H&E histopathological finding which showed diffuse

tubular degeneration and necrosis characterized by cortical tubules either degenerate [hypertrophied with vacuolated cytoplasm] and necrotic [hypereosinophilic, angular cytoplasm with pyknotic nuclei], epithelial cells with variable amount of intraluminal esinophilic material or sloughed epithelial cells and mild multifocal interstitial infiltrations composed of scattered tiny clusters of neutrophils, macrophages, plasma cells, and lymphocytes.

Recent research has indicated that HFD is correlated with the potentiation of inflammatory pathways that are involved in triggering tubular damage in the kidney via bluiding up the lipid in tubules, specifically the saturated fatty acids, which have the ability to deteriorate the function and structure of cells within the tubular epithelium by expantion of tubules, detachment of tubular epithelium, and accumulating tubulointerstitial extracellular matrix. [21].

This results in the same line with Rasheed et al. [49], clarify that male albino rats fed HFD expressed multiple lesions in several glomeruli, tubuli, and interstitial matrix. Severe degeneration of the tubular epithelial cells was observed. Observable shedding of tubular cells in the lumina of certain tubules; the tubular cells' nuclei displayed pyknotic and karyolitic alterations; and some tubular epithelial cells were deteriorated, demonstrating the shedding of tubular cells in the lumen.

The treatment using evolocumab and/or telmisertan revealed that renal H&E histopathological examination showing minimal interstitail fibrosis admixed with few inflammatory cells and partial tubular regeneration characterized by pile up of 2-3 epithelial cells with vesiculate nucleus surrounded a mild to moderate necrotic tubules. Telmisartan is possesses nephroprotective efficacy، it ameliorated hyperglycemia, renal expansion, reduced serum levels of urea and creatinine, provide protection against pathological changes in histological structure and also ameliorated oxidative stress, inflammation and apoptosis [25]. Also PPAR-γ agonists significantly reduced glomerulosclerosis and tubulointerstitial fibrosis. Additionally, significant decrease of distened glomerulus, mesangial hypertrophy [7; 25].

In vivo, various models of renal injury demonstrating a marked elevation of PCSK9 level in bloodstream which have been shown in previous studies [29]. PCSK9 is a key factor in the development of dyslipidemia-induced CKD, as demonstrated by the relationship between elevated levels of PCSK9 and cholesterol levels. [6]. A renoprotective impact against obesity-induced kidney injury via suppression of PCSK9 which inturn diminish the HFD-induced ER stress, inflammation, fibrosis, and apoptosis in kidney [6].

Our findings are consistent with Byun et al. [50] who illustrate that HFD feeding for twelve weeks in male mice deificant of PCSK9 of C57BL/6J type treated with evolocumab causing the building up of lipids in relative to cells, inflammation, apoptosis, and fibrosis were significantly decreased protecting against HFD-induced lipotoxicity in kidney.

Conclusions

Obesity is unquestionably one of the biggest health challenges that must be faced in most developed and developing countries because of unhealthy nutritional habits. Which have vast effects, including metabolic dysfunction and chronic inflammation, coming up with a great impact on kidney function. Induction of obesity in C57BL/6J mice by feeding HFD [TD.88137] for 15 week causing renal injury represented by impairment of renal function, marked oxidative stress accompanied by reduction of antioxidant enzymes, upregulation of renal AT1-R and *NF-κB* expression, and finally alteration of histological structrure of kidney. Evolocumab combined with telmisertan is an effective treatment of obesity-induced renal injury by enhancing renal function, diminish the oxidative stress with elevation of antioxidant enzymes, normalization of renal AT1-R and *NF-κB* expression, and improving the histopathological finding of kidney in comparison with mice received HFD.

Acknowledgment

Author and coauthors would like to acknowledge Dr. Basma Hamed Marghani and Dr. Eman Abdel-Wahab Ibrahim for their privotal role for the completion of this research work

Conflicts of interest

There are no conflicts to declare

Funding statement

There is no funding source.

Figures:

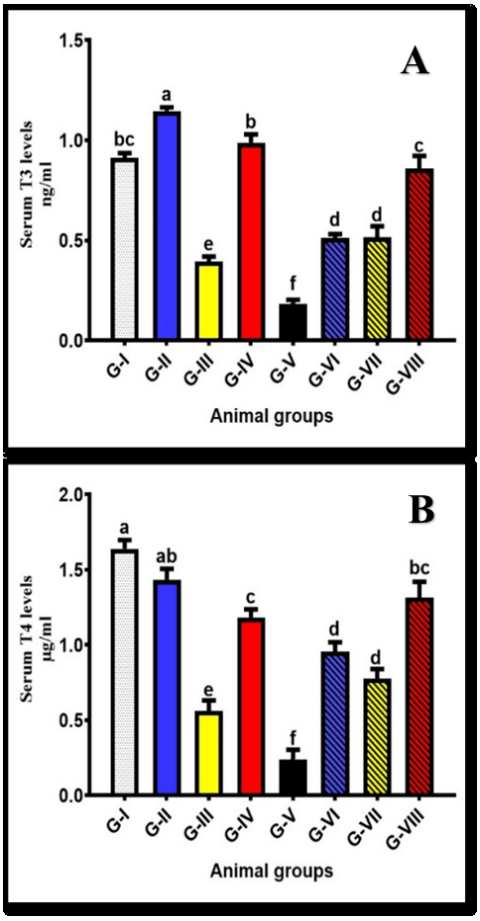

Fig. 1. The impact of Evolocumab and/or Telmisertan on Thyroid hormone levels subsequence to feeding mice a high-fat diet; where [A] indicate serum T3 levels, and [B] indicate serum T4 levels; data expressed as Mean ± S.E where different letter indicates a significant change at [P<0.05]. GI: Control with normal diet; GII: Evolocumab with normal diet; GIII: Telmisartan with normal diet; GIV: Evolocumab & Telmisartan with normal diet ; GV: High fat diet (HFD); GVI: HFD + Evolocumab; GVII: HFD + Telmisartan; GVIII: HFD with Evolocumab & Telmisartan.

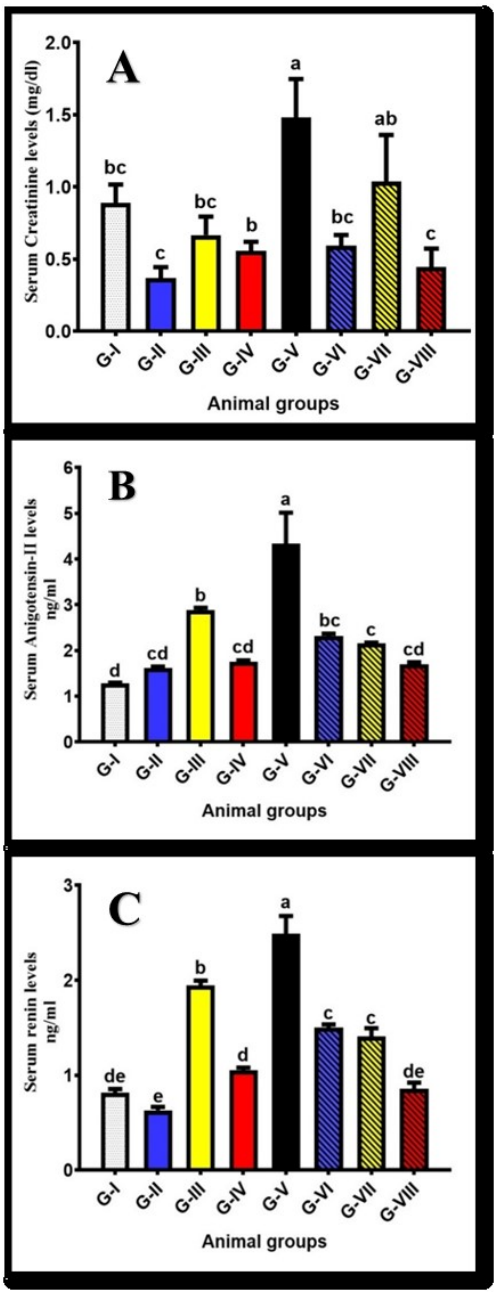

Fig. 2. The impact of Evolocumab and/or Telmisartan on renal function markers subsequence to feeding mice a high-fat diet; where [A] indicate serum creatinine levels, [B] indicate serum Ang II levels and [C] indicate serum renin levels; data expressed as Mean ± S.E where different letter indicates a significant change at [P<0.05]. GI: Control with normal diet; GII: Evolocumab with normal diet; GIII: Telmisartan with normal diet; GIV: Evolocumab & Telmisartan with normal diet ; GV: High fat diet (HFD); GVI: HFD + Evolocumab; GVII: HFD + Telmisartan; GVIII: HFD with Evolocumab & Telmisartan

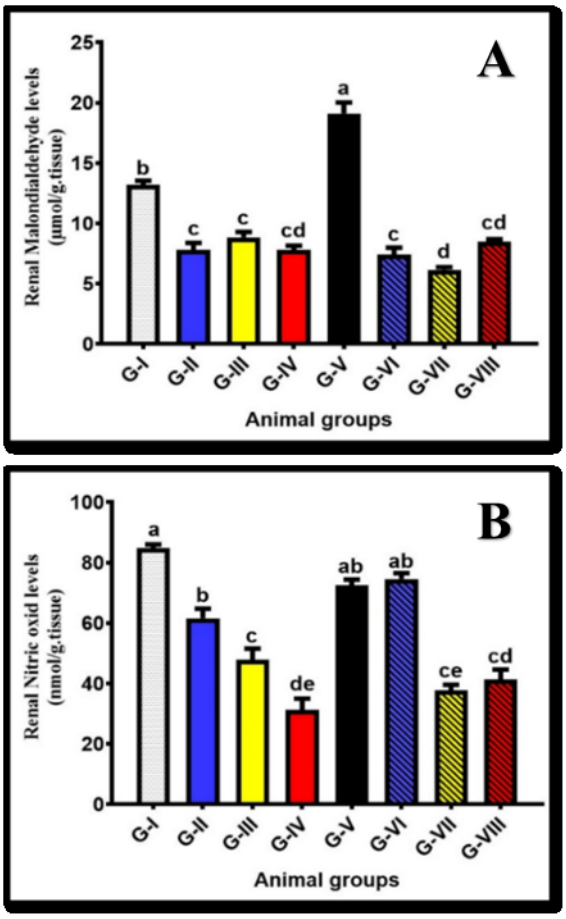

Fig. 3. The impact of Evolocumab and/or Telmisertan on renal Oxidative stress markers subsequence to feeding mice a high-fat diet; were [A] indicate renal MDA level, and [B] indicate renal NO level; data expressed as Mean ± S.E where different letter indicates a significant change at [P<0.05]. GI: Control with normal diet; GII: Evolocumab with normal diet; GIII: Telmisartan with normal diet; GIV: Evolocumab & Telmisartan with normal diet ; GV: High fat diet (HFD); GVI: HFD + Evolocumab; GVII: HFD + Telmisartan; GVIII: HFD with Evolocumab & Telmisartan

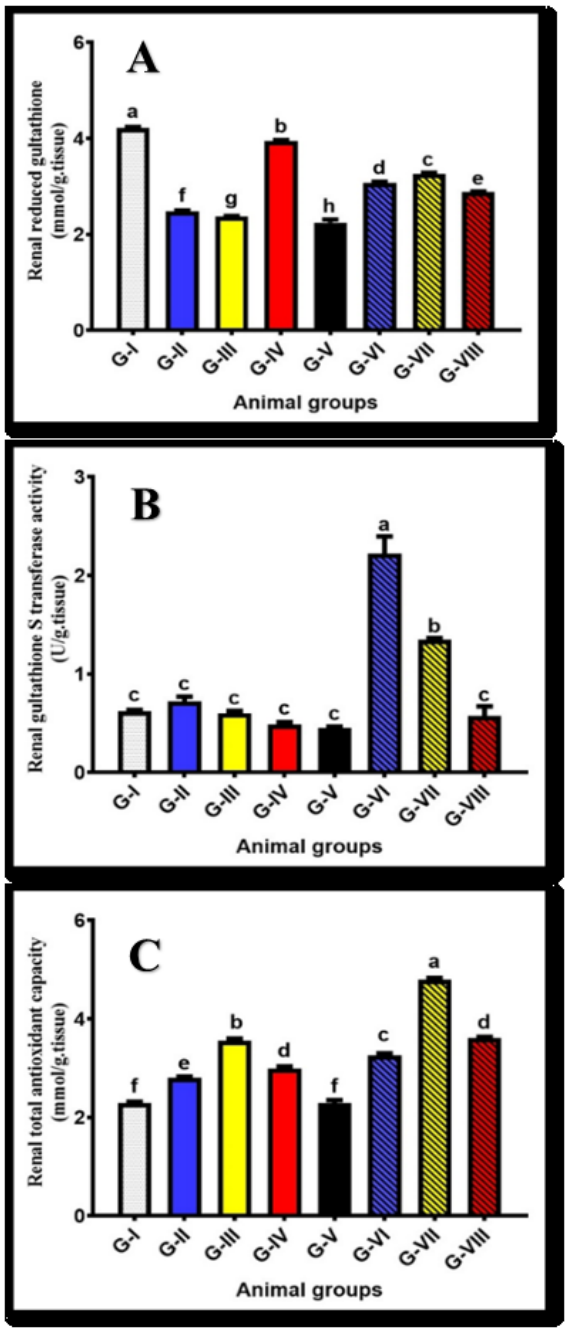

Fig. 4. The impact of Evolocumab and/or Telmisertan on renal antioxidant enzymes subsequence to feeding mice a high-fat diet; where [A] indicate renal GSH level, [B] indicate renal GST levels, and [C] indicate renal TAC levels; data expressed as Mean ± S.E where different letter indicates a significant change at [P<0.05]. GI: Control with normal diet; GII: Evolocumab with normal diet; GIII: Telmisartan with normal diet; GIV: Evolocumab & Telmisartan with normal diet; GV: High fat diet (HFD); GVI: HFD + Evolocumab; GVII: HFD + Telmisartan; GVIII: HFD with Evolocumab & Telmisartan

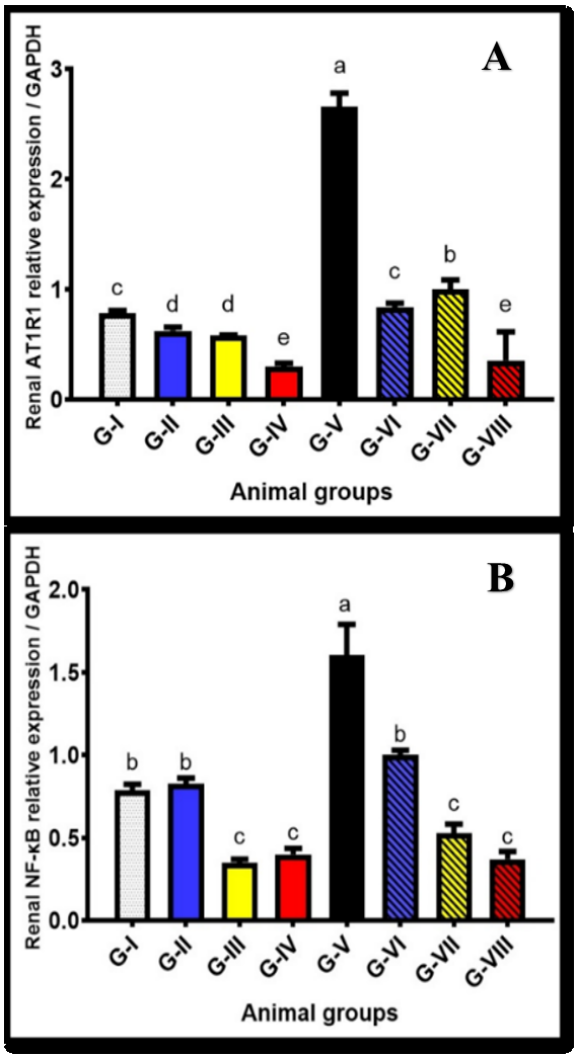

Fig. 5. The impact of Evolocumab and/or Telmisertan on renal *AT1-R* and *NF-κB* gene expression subsequence to feeding mice a high-fat diet; where [A] indicate renal *AT1-R* expression, and [B] indicate renal *NF-κB* expression; data expressed as Mean ± S.E where different letter indicates a significant change at [P<0.05]. GI: Control with normal diet; GII: Evolocumab with normal diet; GIII: Telmisartan with normal diet; GIV: Evolocumab & Telmisartan with normal diet; GV: High fat diet (HFD); GVI: HFD + Evolocumab; GVII: HFD + Telmisartan; GVIII: HFD with Evolocumab & Telmisartan

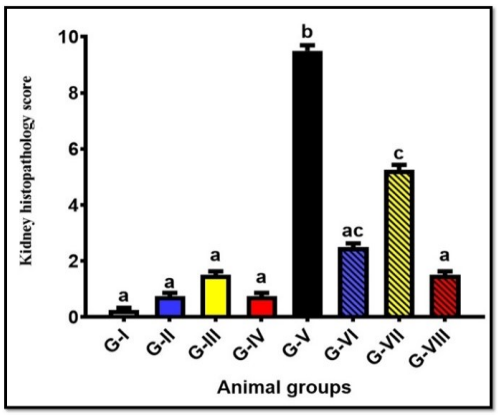

Fig. 6. Scoring for evaluation the impact of Evolocumab and/or Telmisertan on renal histpothological examination subsequence to feeding mice a high-fat diet; data expressed as Mean ± S.E where different letter indicates a significant change at [P<0.05]. GI: Control with normal diet; GII: Evolocumab with normal diet; GIII: Telmisartan with normal diet; GIV: Evolocumab & Telmisartan with normal diet; GV: High fat diet (HFD); GVI: HFD + Evolocumab; GVII: HFD + Telmisartan; GVIII: HFD with Evolocumab & Telmisartan

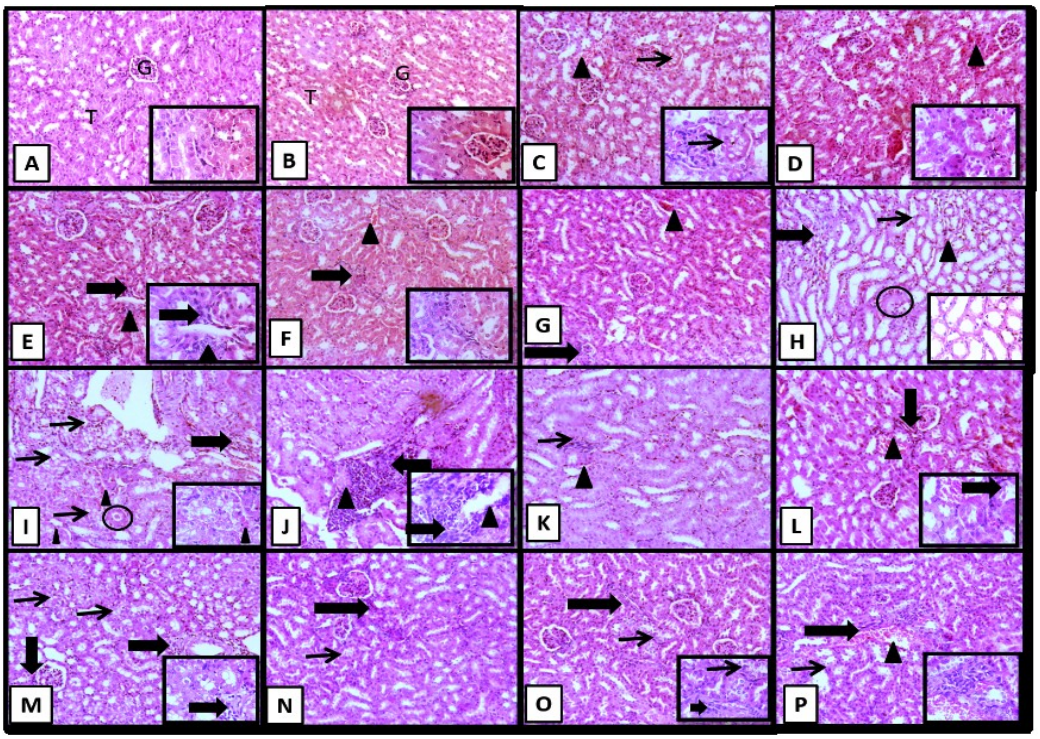

Fig. 7. Representative photomicrograph of Kidney from different groups; A, B] Control group I: showing normal cortical tubules [T] and glomerulus [G], C, D] group II: occasional tubular vacuolation [thin arrow], few interstitial congestion [arrowheads], tubular degeneration with vacuolation [thin arrow]. E, F] group III: occasional interstitial inflammation [thick arrow], and vesiculated nuclei [arrowhead]. G] group IV: occasional periglomerular inflammation [thick arrow] and few interstitial hemorrhage [arrowhead]. H] group V: attenuated epithelium [thin arrow], esinophilic proteinacous materials [circle] with interstitial congestion [arrowhead] and few inflammation [thick arrow]. I] group V: cortical tubules degenerate [hypertrophied with vacuolated cytoplasm] [thin arrow], or necrotic [hypereosinophilic, angular cytoplasm with pyknotic nuclei] epithelial cells [arrowheads], sloughed epithelial cells [circle], scattered tiny clusters of neutrophils [rare], macrophages, plasma cells, and lymphocytes. [thick arrow], necrotic cell with either pyknotic or karylolytic nuclei [arrowhead]. J, K] group VI: moderate interstitial inflammation [thick arrow] or mild interstitial fibrosis [thin arrow], necrotic tubules [arrowheads], peritubular aggregation of moderate numbers of lymphocytes, macrophages and fibroblast [thick arrow] surrounded a necrotic tubules [arrowhead]. L] group VII: focal periglomerular aggregations of inflammatory cells [thick arrow] together with minimal interstitial congestion [arrowhead]. M] group VII: diffuse tubular vacuolation [thin arrows] minimal to mild aggregation of inflammatory cells [thick arrows]. N] group VII: minimal periglomerular inflammation [thick arrow] and occasional necrotic tubules [thin arrow]. O] group VII: few inflammatory cells [thick arrow], epithelial cells with vesiculate nucleus [thin arrow]. P] group VIII: cellular infiltrates [thick arrow] and interstitial congestion [arrowhead] [thin arrow]. Image magnification= 100x, inset= 400x

References

1. Kovesdy, C.P., Furth, S. and Zoccali, C. World Kidney Day Steering Committee. Obesity and kidney disease: Hidden consequences of the epidemic. *Indian J. Nephrol.,* **30** (1), 1-10 (2017).
2. D'Agati, V.D., Chagnac, A., de Vries, A.P., Levi, M. and Porrini E, Herman-Edelstein M, Praga M. Obesity-related glomerulopathy: clinical and pathologic characteristics and pathogenesis. *Nat. Rev. Nephrol.,* **12** (8), 453-471 (2016).
3. Dousdampanis, P., Trigka, K., Vagenakis, G.A. and Fourtounas, C. The thyroid and the kidney: a complex interplay in health and disease. *Int. J. Artif. Organs*, **37** (1), 1-12 (2014).
4. Jakielska, E., Głuszak, P., Walczak, M. and Bryl, W. Effects of PCSK9 inhibitors on metabolic-associated fatty liver disease: a short review. *Prz. Gastroenterol.* **18** (2), 148-153 (2023).
5. Safaeian, L., Mirian, M. and Bahrizadeh, S. Evolocumab, a PCSK9 inhibitor, protects human endothelial cells against H2O2-induced oxidative stress. *Arch. Physiol. Biochem*., **128** (6), 1681-1686. (2022).
6. Feng, Z., Liao, X., Zhang, H., Peng, J., Huang, Z. and Yi, B. Increased serum PCSK9 levels are associated with renal function impairment in patients with type 2 diabetes mellitus. *Ren. Fail*., **45 (**1), 2215880. (2023).
7. Ladino, M. and Hernandez Schulman. I. Renovascular and renoprotective properties of telmisartan: clinical utility. *Int. J. Nephrol. Renovasc. Dis*., **3**,33-38. (2010).
8. Urbizo Uriel , Liceaga Andrea , Reddivari Lavanya , Li Shiyu , Kim Kee-Hong , Cox Abigail and Anderson Joseph. Canary Seed [Phalaris canariensis L.] Peptides Prevent Obesity and Glucose Intolerance in Mice Fed a Western Diet. *Int. J. Mol. Sci.,* **29(**23),14927 (2022).
9. Ason, B., van der Hoorn, J.W., Chan, J., Lee, E., Pieterman, E.J., Nguyen, K.K., Di, M., Shetterly, S., Tang, J., Yeh, W.C., Schwarz, M., Jukema, J.W., Scott, R., Wasserman, S.M., Princen, H.M. and Jackson, S. PCSK9 inhibition fails to alter hepatic LDLR, circulating cholesterol, and atherosclerosis in the absence of ApoE. *JLR*. **55**(11), 2370-2379. (2014).
10. He, H., Yang, D., Ma, L., Luo, Z., Ma, S., Feng, X., Cao, T., Yan, Z., Liu, D., Tepel, M. and Zhu, Z. Telmisartan prevents weight gain and obesity through activation of peroxisome proliferator-activated receptor-delta-dependent pathways. *Hypertension*, **55**(4), 869-879 (2010).
11. Thomas, S.S., Cha, Y.S. and Kim, K.A. Perilla Oil Alleviates High-Fat Diet-Induced Inflammation in the Colon of Mice by Suppressing Nuclear Factor-Kappa B Activation. *J. Med. Food*., **23**(8), 818-826 (2022).
12. Li, K., Yin, H., Duan, Y., Lai, P., Cai, Y. and Wei, Y. Pre-inhalation of hydrogen-rich gases protect against caerulein-induced mouse acute pancreatitis while enhance the pancreatic Hsp60 protein expression. *BMC Gastroenterol*., **21**(1),178 (2021).
13. Bancroft, J.D. and Gamble, M.: The hematoxylins and eosin. In Theory and Practice of Histological Techniques, 5th ed. Edinburgh: Churchill Livingstone. (2002).
14. Sato, N., Takahashi, D., Tsuchiya, R., Mukoyama, T., Yamagata, S. I., Satoh, N. & Kondo, S. Acute nephrotoxicity of aristolochic acids in mice. *JPP.,* **56**(2), 221-229. (2004).
15. Levesque, R., SPSS programming and data management: a guide for SPSS and SAS users. Spss. (2005).
16. Longhi, S., & Radetti, G. Thyroid function and obesity. *J. Clin. Res. Pediatr. Endocrinol.,* **5** (Suppl 1), 40. (2013).
17. Shao, S.S., Zhao, Y.F., Song, Y.F., Xu, C., Yang, J.M., Xuan, S.M., Yan, H.L., Yu, C.X., Zhao, M., Xu, J. and Zhao, J.J. Dietary high-fat lard intake induces thyroid dysfunction and abnormal morphology in rats. *Acta Pharmacol Sin*. **35**(11), 1411-1420. (2014).
18. Wang, C. H., Smithies, O., & Takahashi, N. Inhibition of angiotensin II increases hypothalamic thyroid hormone and stimulates orexigenic NPY/AgRP neurons [LB437]. *The FASEB Journal,* **28**, LB437. (2014).
19. Chmyr, N. V. Dynamics of endocrine and metabolic changes among patients with coronary artery disease, type 2 diabetes mellitus and metabolic syndrome while treating with telmisartan. Int. *J. Endocrinol [Ukraine],* **18**(1), 22-35. (2022).
20. Sadik, N.A., Rashed, L.A. and El-Sawy, S.S. The Relationship of Circulating Proprotein Convertase Subtilisin/Kexin Type 9 With TSH and Lipid Profile in Newly Diagnosed Patients With Subclinical and Overt Hypothyroidism. *Clin. Med. Insights Endocrinol. Diabetes*, **15**, 11795514221093317. (2022).
21. Sun, Y., Ge, X., Li, X., He, J., Wei, X., Du, J., Sun, J., Li, X., Xun, Z., Liu, W., Zhang, H., Wang, Z.Y. and Li, Y.C. High-fat diet promotes renal injury by inducing oxidative stress and mitochondrial dysfunction. *Cell Death Discov.* **11**(10), 914 (2020).
22. Yiannikouris, F., Gupte, M., Putnam, K., Thatcher, S., Charnigo, R., Rateri, D.L., Daugherty, A. and Cassis, L.A. Adipocyte deficiency of angiotensinogen prevents obesity-induced hypertension in male mice. *Hypertension*. **60**(6), 1524-1530. (2012).
23. Gupte, M., Thatcher, S.E., Boustany-Kari, C.M., Shoemaker, R., Yiannikouris, F., Zhang, X., Karounos, M. and Cassis, L.A. Angiotensin converting enzyme 2 contributes to sex differences in the development of obesity hypertension in C57BL/6 mice. *ATVB,* **32**(6), 1392-1399. (2012).

24. Dalmasso, C., Ahmed, N.S., Ghuneim, S., Cincinelli, C., Leachman, J.R., Giani, J.F., Cassis, L. and Loria, A.S. Obese Male Mice Exposed to Early Life Stress Display Sympathetic Activation and Hypertension Independent of Circulating Angiotensin II. *JAHA*. **13**(1), e029511 (2024).

25. Antar, S.A., Abdo, W., Taha, R.S., Farage, A.E., El-Moselhy, L.E., Amer, M.E., Abdel Monsef, A.S., Abdel Hamid, A.M., Kamel, E.M., Ahmeda, A.F. and Mahmoud, A.M. Telmisartan attenuates diabetic nephropathy by mitigating oxidative stress and inflammation, and upregulating Nrf2/HO-1 signaling in diabetic rats. *Life Sci.,* **291**, 120260 (2022).

26. Naguib, Y.M., Samaka, R.M., Rizk, M.S., Ameen, O. and Motawea, S.M. Countering adipose tissue dysfunction could underlie the superiority of telmisartan in the treatment of obesity-related hypertension. *Cardiovasc Diabetol.,* **20,** 1-19**.** (2021).

27. Pizoń, T., Rajzer, M., Wojciechowska, W., Wach-Pizoń, M., Drożdż, T., Wróbel, K., Gruszka, K., Rojek, M., Kameczura, T., Jurczyszyn, A., Kąkol, J. and Czarnecka, D. The relationship between plasma renin activity and serum lipid profiles in patients with primary arterial hypertension. *J Renin Angiotensin Aldosterone Syst*., **19**(4), 1470320318810022. (2018).

28. Blokhina, Olga, Eija Virolainen and Kurt V. Fagerstedt. "Antioxidants, oxidative damage and oxygen deprivation stress: a review." *Ann. Bot.,* **91** (2), 179-194. (2003).

29. Noeman, S.A., Hamooda, H.E. and Baalash, A.A. Biochemical study of oxidative stress markers in the liver, kidney and heart of high fat diet induced obesity in rats. *Diabetol. Metab. Syndr.,* **3**, 1-8(2011).

30. Obrador, E., Benlloch, M., Pellicer, J.A., Asensi, M. and Estrela, J.M. Intertissue flow of glutathione [GSH] as a tumor growth-promoting mechanism: interleukin 6 induces GSH release from hepatocytes in metastatic B16 melanoma-bearing mice. *J. Biol .Chem.,* **286**(18), 15716-15727. (2011).

31. Mount, P. F. and David Anthony Power. "Nitric oxide in the kidney: functions and regulation of synthesis." *Acta Physiol.,* **187**(4), 433-446. (2006).

32. Tsuchiya, K., Sakai, H., Suzuki, N., Iwashima, F., Yoshimoto, T., Shichiri, M. and Hirata, Y. Chronic blockade of nitric oxide synthesis reduces adiposity and improves insulin resistance in high fat-induced obese mice. *Endocrinology*. **148**(10), 4548-4556. (2007).

33. Silvestrini A, Meucci E, Ricerca BM, Mancini A. Total Antioxidant Capacity: Biochemical Aspects and Clinical Significance. *Int. J. Mol. Sci*., **24**(13), 10978 (2023).

34. Rodríguez-Lara, S.Q., Trujillo-Rangel, W.A., Castillo-Romero, A., Totsuka-Sutto, S.E., Garcia-Cobián, T.A., Cardona-Muñoz, E.G., Miranda-Díaz, A.G., Ramírez-Lizardo, E.J. and García-Benavides, L. Effect of Telmisartan in the Oxidative Stress Components Induced by Ischemia Reperfusion in Rats. *Oxid. Med. Cell Longev*., **2019**(1), 1302985**.** (2019).

35. Cho, D.H. Telmisartan Inhibits Nitric Oxide Production and Vessel Relaxation via Protein Phosphatase 2A-mediated Endothelial NO Synthase-Ser1179 Dephosphorylation. *JKMS.* **4, 34**(42), e266. (2019).

36. Hamed, A.A. and Malek, H.A. Effect of telmisartan in experimentally induced diabetetes mellitus in rats. *Int J Health Sci. [Qassim]*. **1**(2), 249-56. (2007).

37. Wienen, W., Entzeroth, M., van Meel, J.C.A., Stangier, J., Busch, U., Ebner, T., Schmid, J., Lehmann, H., Matzek, K., Kempthorne-Rawson, J., Gladigau, V. and Hauel, N.H. A Review on Telmisartan: A Novel, Long-Acting Angiotensin II-Receptor Antagonist. *Cardiovasc. Drug Rev*., **18**(2), 127-154. (2000).

38. Schlüter, K.D., Wolf, A., Weber, M., Schreckenberg, R. and Schulz, R. Oxidized low-density lipoprotein [oxLDL] affects load-free cell shortening of cardiomyocytes in a proprotein convertase subtilisin/kexin 9 [PCSK9]-dependent way. *Basic Res. Cardiol*. ,**112**, 1-11**.** (2017).

39. Cammisotto, V., Baratta, F., Castellani, V., Bartimoccia, S., Nocella, C., D'Erasmo, L., Cocomello, N., Barale, C., Scicali, R., Di Pino, A., Piro, S., Del Ben, M., Arca, M., Russo, I., Purrello, F., Carnevale, R., Violi, F., Pastori, D. and Pignatelli, P. Proprotein Convertase Subtilisin Kexin Type 9 Inhibitors Reduce Platelet Activation Modulating ox-LDL Pathways. *Int. J. Mol. Sci*., **22**(13), 7193. (2021).

40. Ryder, E., Pedreañez, A., Vargas, R., Peña, C., Fernandez, E., Diez-Ewald, M. and Mosquera, J. Increased proinflammatory markers and lipoperoxidation in obese individuals: Inicial inflammatory events? *Diabetes Metab. Syndr.,* **9**(4), 280-286. (2015).

41. Jain S, Puri N, Rana A, Sirianni N, Mopidevi B, Kumar A. Metabolic Syndrome Induces Over Expression of the Human AT1R: A Haplotype-Dependent Effect With Implications on Cardio-Renal Function. *Am J Hypertens*. **31**(4), 495-503. (2018).

42. Sugawara, A., Takeuchi, K., Uruno, A., Ikeda, Y., Arima, S., Kudo, M., Sato, K., Taniyama, Y. and Ito, S. Transcriptional suppression of type 1 angiotensin II receptor gene expression by peroxisome proliferator-activated receptor-gamma in vascular smooth muscle cells. *Endocrinology*, **142**(7), 3125-3134. (2001).

43. Graus-Nunes, F., Santos, F.O., Marinho, T.S., Miranda, C.S., Barbosa-da-Silva, S. and Souza-Mello, V. Beneficial effects of losartan or telmisartan on the local hepatic renin-angiotensin system to counter obesity in an experimental model. *World J. Hepatol.,* **11**(4),359-369. (2019).

44. Li, D., Saldeen, T., Romeo, F. and Mehta, J.L. Oxidized LDL upregulates angiotensin II type 1 receptor expression in cultured human coronary artery endothelial cells: the potential role of transcription factor NF-kappaB. *Circ.,* **102**(16), 1970-1976**.** (2000).

45. Gao, X., Li, Y., Ma, Z., Jing, J., Zhang, Z., Liu, Y. and Ding, Z. Obesity induces morphological and functional changes in female reproductive system through increases in NF-κB and MAPK signaling in mice. *Reprod. Biol. Endocrinol*., **19,** 1-15. (2021).

46. Nagai, N., Izumi-Nagai, K., Oike, Y., Koto, T., Satofuka, S., Ozawa, Y., Yamashiro, K., Inoue, M., Tsubota, K., Umezawa, K. and Ishida, S. Suppression of diabetes-induced retinal inflammation by blocking the angiotensin II type 1 receptor or its downstream nuclear factor-kappaB pathway. *Invest. Ophthalmol. Vis. Sci*., **48**(9), 4342-4350. (2007).

47. Huang, C., Yuan, L. and Cao, S. "Involvement of telmisartan on the protective effects mediated by the peroxisome proliferator- activated receptor- γ pathway in mice." *It J. Diabetes Clin. Res.,* **1,** 15. (2014).

48. Tang, Z., Jiang, L., Peng, J., Ren, Z., Wei, D., Wu, C., Pan, L., Jiang, Z. and Liu, L. PCSK9 siRNA suppresses the inflammatory response induced by oxLDL through inhibition of NF-κB activation in THP-1-derived macrophages. *Int. J. Mol. Med.,* **30**(4), 931-938. (2012).

49. Rasheed, R. A., Elshikh, M. S., Mohamed, M. O., Darweesh, M. F., Hussein, D. S., Almutairi, S. M., & Embaby, A. S. Quercetin mitigates the adverse effects of high fat diet on pancreatic and renal tissues in adult male albino rats. *J. King Saud Univ. Sci.,* **34**(4), 101946. (2022).

50. Byun, J.H., Lebeau, P.F., Platko, K., Carlisle, R.E., Faiyaz, M., Chen, J., MacDonald, M.E., Makda, Y., Yousof, T., Lynn, E.G., Dickhout, J.G., Krepinsky, J.C., Weaver, F., Igdoura, S.A., Seidah, N.G. and Austin, R.C. Inhibitory Antibodies against PCSK9 Reduce Surface CD36 and Mitigate Diet-Induced renal lipotoxicity. *Kidney360.* **3**(8), 1394-1410 (2022).

تقييم التأثير الوقائي لعقار إيفولوكوماب و/أوتيلميسارتان على إصابة الكلى الناجمة عن السمنة في الفئران C57BL/6J

فادي يوسف عطاالله[1] ، نبيل ابو هيكل سيد[2] ، احمد ابراهيم عطيه [3] ، مني مصطفي الغريب [4] ، محمود التهامي [5] وبسمه حامد عثمان [6]

[1] قسم الفسيولوجيا – كليه طب بيطري- جامعه المنصوره – مصر .

[2] قسم الفسيولوجيا – كليه طب بيطري- جامعه المنصوره – مصر .

[3] قسم تنمية الثروة الحيوانية – كليه طب بيطري – جامعه المنصوره – مصر.

[4] قسم الفسيولوجيا – كليه طب بيطري- جامعه المنصوره – مصر .

[5] قسم الفسيولوجيا – كليه الطب - جامعه المنصوره – مصر .

الملخص

في هذه الدراسه تم تقييم التأثير الوقائي للإيفولوكوماب والتيلميسارتان على الأمراض المصاحبة الناجمة عن السمنة على مستوى الكلى. تم إجراء البحث الحالي باستخدام ستة وخمسين فأرًا أسودًا من نوع C57BL/6J ذكرًا مقسمة إلى ثماني مجموعات، بواقع سبعة فئران لكل مجموعة. أولاً، تم تغذية فئران المراقبة بنظام غذائي تحكمي أساسي وتم إعطاؤها إيفولوكوماب و/أو تيلميسارتان. ثانيًا، تم تغذية الفئران بنظام غذائي غني بالدهون [TD.88137] للحث على السمنة لمدة 15 أسبوعًا. ثالثًا، تم علاج الفئران باستخدام عقار إيفولوكوماب و/أو تيلميسارتان. تم جمع عينات الدم وأنسجة الكلى لتقييم وظائف الكلى ومستويات هرمونات الغدة الدرقية والإجهاد التأكسدي وعلامات مضادات الأكسدة والتعبيرات الجينية والفحص النسيجي. وأظهرت النتائج أن الفئران التي تغذت على بنظام غذائي غني بالدهون كشفت عن وجود خلل في وظائف الكلى، بما في ذلك ارتفاع مستويات الكرياتينين والأنجيوتنسين II والرينين في الدم. وقد لوحظ قصور الغدة الدرقية على أنه انخفاض في مستويات مصل ثلاثي يودوثيرونين ورباعي يودوثيرونين. لوحظ ارتفاع الإجهاد التأكسدي الكلوي كارتفاع في المالونديالدهيد وأكسيد النيتريك مع انخفاض في إنزيمات مضادات الأكسدة الكلوية مثل انخفاض مستويات الجلوتاثيون المنخفض ، ونشاط ترانسفيراز الجلوتاثيون ، والقدرة الإجمالية لمضادات الأكسدة. بالإضافة إلى ذلك، تحسين تنظيم التعبير الجيني لمستقبل الأنجيوتنسين II من النوع الأول و العامل النووي-κB. اظهر الفحص النسيجي المرضي تنكسًا أنبوبيًا منتشرًا ونخرًا. في حين أن الفئران المعالجة بمزيج من إيفولوكوماب وتيلميسارتان تحسن مؤشرات وظائف الكلى، مثل انخفاض مستويات الكرياتينين والأنجيوتنسين II والرينين في الدم. ارتفاع هرمونات الغدة الدرقية، بما في ذلك ثلاثي يودوثيرونين ورباعي يودوثيرونين في الدم. التخفيف من مستويات الإجهاد التأكسدي الكلوي وتحسين مستويات الإنزيمات المضادة للأكسدة، مع التنظيم التقليلي لتعبيرات الجيني لمستقبل الأنجيوتنسين II من النوع الأول و العامل النووي-κB. وكذلك تحسين البنية النسيجية الكلوية لدى الفئران السمينة. الاستنتاج: يعتبر إيفولوكوماب مع تيلميسيرتان علاجا فعالا لإصابة الكلى الناجمة عن السمنة من خلال تقليل الآثار الضارة المرتبطة بالفيزيولوجيا المرضية للسمنة.

الكلمات الدالة: الإيفولوكوماب، والتيلميسارتان، السمنه، الخلل الكلوي ، فئران أسودًا نوع C57BL/6J .