https://doi.org/10.48047/AFJBS.6.1.2024.742-748

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

Liraglutide alone or in combination with liraglutide ameliorated hyperthyroidism-induced nephropathy via modulating autophagy signaling pathway

Khalifa El-Dawy[1], Saydat Saad[1], Hanaa M. Behairy[1*], and Tarek Khamis[2]

1Biochemistry Department, Faculty of Veterinary Medicine, Zagazig University, 44519 Zagazig, Egypt
2Department of Pharmacology, Faculty of Veterinary Medicine, Zagazig University, 44519 Zagazig, Egypt.
Corresponding author: Hanaa M. Behairy
Email: h.behiry22@vet.zu.edu.eg

Article History

Volume 6, Issue 1, Apr-Aug 2024

Received: 3 March 2024

Accepted: 15 April 2024

Published: 17 April 2024

doi: 10.48047/AFJBS.6.1.2024.742-748

Abstract: Nephropathy is one of the most common complications of the hyperthyroidism with a limited therapeutic strategy. Thus, the current study was designed to investigate the possible ameliorative potency of the glucagon like peptide agonist (liraglutide) alone or in combination with angiotensinogen converting enzyme inhibitors (Captopril). Fifty male adult Sprague Dawley rats were assigned into five equal groups ten rats each, control group, hyperthyroidism induced nephropathy (HTN) group, HTN liraglutide treated group, HTN captopril treated group, and HTN cotreated with captopril and liraglutide group. The renal function test and the gene expression for the renal fibrotic and autophagy markers were assayed. The result revealed that liraglutide alone or combined with the captopril improve renal function tests and alter the expression of the of the renal autophagic markers with outperforming effect for the combination. Taking together the administration of liraglutide alone or combined with captopril modulated the hyperthyroidism-induced nephropathy in rats via altering the expression of renal autophagy signaling pathway.

Keywords: *autophagy; renal fibrosis; hyperthyroidism; liraglutide; captopril; and nephropathy.*

Introduction

It is true that hyperthyroidism induce nephropathy and also hasten CKD.Since the earliest phases of chronic kidney disease (CKD), oxidative stress has been amplified and plays a crucial role in the pathophysiology of renal failure. Podocyte damage, proteinuria, the onset of focal segmental glomerulosclerosis (FSGS), and tubulointerstitial fibrosis have all been directly associated with the induction of renal pro-oxidant enzymes with excessive production of reactive oxygen species (ROS) and accumulation of dityrosine-containing protein products produced during oxidative stress (advanced oxidation protein products, or AOPPs). Vascular oxidative stress is thought to be a major factor in the development of chronic kidney disease (CKD), and ROS

may act as mediators of the disease's compromised renal autoregulation and myogenic responses of afferent renal arterioles. Inflammation and oxidative stress are two characteristics of CKD **(1)**

Glucagon like peptide-1 (GLP-1), a peptide hormone derived from the digestive system, regulates insulin secretion, food intake, and gut motility to effectively maintain postprandial glucose homeostasis. Many of the medications used today to treat type 2 diabetes, obesity, and other conditions are based on GLP-1, as are other novel compounds that are in the development stage **(2).** When nutrients are consumed, intestinal-derived incretin hormones are released. These hormones are secreted at low levels during the basal state and decrease postprandial hyperglycemia by increasing the amount of insulin secreted in response to meals **(3**)

In hyperthyroidism, autophagy is vital in remodeling and conservation of function. A common progression of nearly all chronic kidney illnesses to end-stage kidney disease is renal fibrosis. Autophagy is a highly conserved lysosomal protein degradation system that keeps intracellular homeostasis intact by breaking down protein aggregates, damaged organelles, and invasive infections. Autophagy has a role in the development of renal fibrosis in the glomeruli and tubulointerstitial compartment, according to mounting data. On the other hand, the precise function of autophagy in renal fibrosis remains unclear (**4).** Under stressful circumstances, autophagy activation protects renal cells **(5).**

It has been demonstrated that autophagy, a crucial stress-responsive system, plays a role in the etiology of several kidney disorders, including renal fibrosis (**6).** However, autophagy is not essential for kidney development **(7).** It is vital for adult kidney resident cells and is closely linked to the development of renal fibrosis (**6).**

Furthermore, the following are the main characteristics of renal fibrosis: (1) the infiltration of inflammatory cells, such as dendritic and macrophage cells; (2) the activation and proliferation of myofibroblasts from different sources; (3) the production and long-term accumulation of various ECM components, such as collagens, fibronectin, laminin, glycoproteins, and proteoglycans; (4) and renal tubular atrophy and microvascular rarefaction. And this in agreement with **(8,9).**

Thus, the present study was designed to investigate the renal damage due to hyperthyroidism and the possible effect of GLP-1 agonist and captopril.

2. Material and method.

2.1 Experimental design:

Forty-five adult male albino rats (250-300) gram weight and (6-8) weeks old were purchased from the experimental animal house of the Faculty of Veterinary Medicine, Zagazig University and housed in conditioned room at a temperature of 24 ^{0}C with 12 h dark/ light cycle at a density of 4 rats per cage. After adaptation for 1 week rats were subdivided into five groups, **(G1)** (Control negative): Rats will be serving as anon-treated group and will be given distilled water Dailly by gastric gavage, **(G2):** Rats will be serving as control positive (hyperthyroidism induced nephropathy) which will be given levothyroxine drug (T4 Thyro) orally (0.3ml / kg) daily for 4 weeks **(10). (GP 3):** Rats will be given levothyroxine drug (T4 thyro) orally (0.3ml / kg for 4 weeks daily **(10**). Then they will be given (GLP-1 agonist) liraglutide (Saxenda)150mcg/Kg twice a week for 4 weeks (**GP 4):** Rats will be given levothyroxine drug (T4 thyro) orally (0.3M / kg) daily for 4 weeks (**10**). and then they will be given captopril (100ml/ kg**)** twice a week for 4 weeks **-(12).** (**GP 5):** Rats will be given levothyroxine drug (T4 thyro) orally (0.3M / kg) daily for 4weeks (**10**).and then they will be given combination of saxenda with captopril for twice a week for 4 weeks (150mcg of saxenda -100ml/kg of captopril) (**11,12).**

2.2 Biochemical Analysis:

Blood urea nitrogen, serum uric acid, and serum creatinine were measured according to the manufacturer's instructions (SPINREACT, Gerona, Spain). Additionally, to determine the oxidant/antioxidant activity, the renal tissue was homogenized and centrifuged at 3000 rpm for 10 min; the supernatant was stored at −80 until used. Following manufacturer's instructions, renal malondialdehyde (MDA), reduced glutathione (GSH), catalase (CAT), and superoxide dismutase (SOD) were measured using the sandwich ELISA method (My

BioSource, San Diego, CA, USA) and total antioxidant capacity (TAC) was assayed using a colorimetric assay kit (ABTS, Enzyme method, My BioSource, San Diego, CA, USA).

2.3 Gene expression

Total RNA was isolated from mononuclear cell layer (MNC), Br-MSCs-EXOs, Br-MSCs, and kidney tissue using qiazol (Qiagen, Hilden, Germany). The total extracted RNA concentration and quality was determined using NanoDrop® ND-1000 UV—Vis spectrophotometer (thermos scientific, Waltham, MA, USA). On the other hand, the mRNA was reverse-transcribed with a high-capacity reverse transcriptase kit (Applied Biosystem, Foster City, CS, USA). Finally, the produced cDNA was diluted at 1–5, aliquoted, and stored at −20 °C for the gene expression study. The real-time PCR reaction was conducted in a total reaction volume of 20 µL, 10 µL TOPreal™ qPCR 2X PreMIX, SYBR Green with low ROX, (Enzynomics, Daejeon, Republic of Korea), 1 µL of forward and reverse primer supplied by (Sangon Biotech, Beijing, China) , and nuclease-free water up to 20 µL, as previously reported in **[13,14].** The expression levels of mRNA was normalized to Gapdh. The relative expressions was performed with $2^{-\Delta\Delta CT}$ [**15]**.

2.4 Statical analysis:

The mean ± standard error mean (S.E.M) was used to describe continuous variables. The normality of the data was checked with a Shapiro–Wilk test. In homogeneous data, one-way analysis of variance (ANOVA) was used, followed by Tukey's honest significant difference test for multiple group comparison using SPSS (Version 20; SPSS Inc., Chicago, IL, USA); a *p* value less than 0.05 ($p < 0.05$) was considered statistically significant.

3. Result

3.1 Effect of semaglutide and/or captopril on Uric acid level.

The result of the present work illustrated a significant P<0,05 up regulation in **Uric acid** expression of Nephropathy group than the control group figure (1). However, liraglutide and-or captopril administration show a significant P<0,05 down regulation in **Uric acid** expression compared with Nephropathy group., More over administration of captopril with liraglutide showed more a significant improvement than administration of lira or captopril alone figure (1)

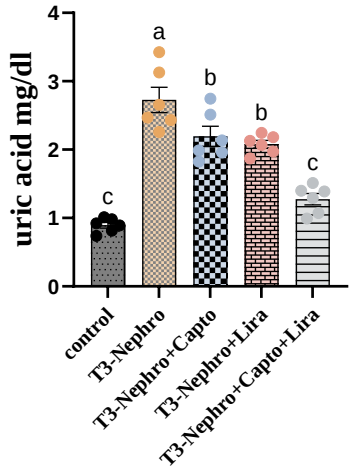

Figure (1) effect of liraglutide and -or captopril administration on Uric acid mg\dl in T4 induced nephropathy.

3.2 Effect of semaglutide and/or captopril on serum creatinine.

The result of the contemporary investigation revealed a significant P<0,05 up regulation in **creatinine** expression of Nephropathy group than the control group figure (2). However, liraglutide and-or captopril administration show a significant P<0,05 down regulation in **creatinine** expression compared with

Nephropathy group., More over administration of captopril with liraglutide showed more a significant improvement than administration of lira or captopril alone figure (2).

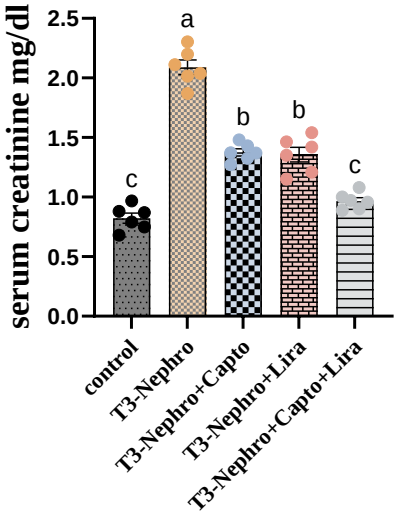

Figure (2) effect of liraglutide and -or captopril administration on creatinine mg\dl in T4 induced nephropathy.

3.3 Effect of liraglutide and/or captopril on serum urea.

The result of the contemporary current work showed a significant P<0,05 up regulation in **Urea** expression of Nephropathy group than the control group figure (3). However, liraglutide and-or captopril administration show a significant P<0,05 down regulation in **Urea** expression compared with Nephropathy group., More over administration of captopril with liraglutide showed more a significant improvement than administration of lira or captopril alone figure (3).

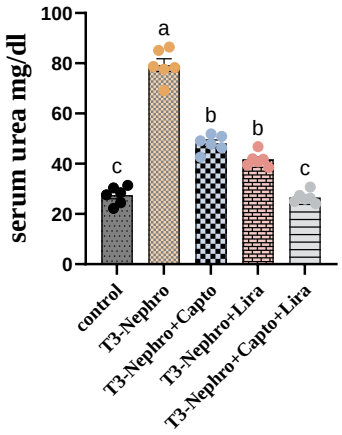

Figure (3) effect of liraglutide and -or captopril administration on Urea mg\dl in T4 induced nephropathy.

3.4 Effect of liraglutide and/or captopril on the expression level of beclin-1.

The result of the contemporary investigation showed a significant P<0,05 down regulation in Beclin-1 expression of Nephropathy group than the control group figure (4). However, liraglutide and-or captopril administration show a significant P<0,05 up regulation in Beclin-1 expression compared with Nephropathy

group., More over administration of captopril with liraglutide showed more a significant improvement than administration of lira or captopril alone figure (4).

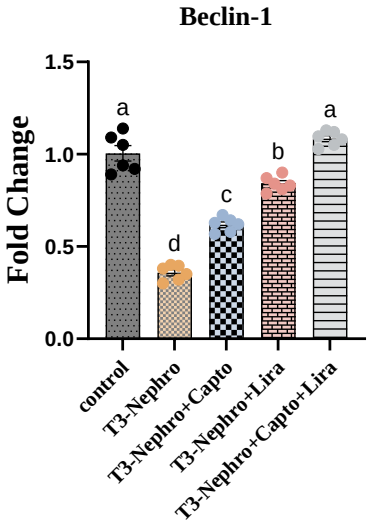

Figure (4) effect of liraglutide and -or captopril administration on Beclin-1 in T4 induced nephropathy.

3.5 Effect of liraglutide and/or captopril on the expression level of LC3-II.

The result of the current study showed a significant P<0,05 down regulation in **LC3-II** expression of Nephropathy group than the control group figure (5). However, liraglutide and-or captopril administration show a significant P<0,05 up regulation in Beclin-1 expression compared with Nephropathy group., More over administration of captopril with liraglutide showed more a significant improvement than administration of lira or captopril alone figure (5).

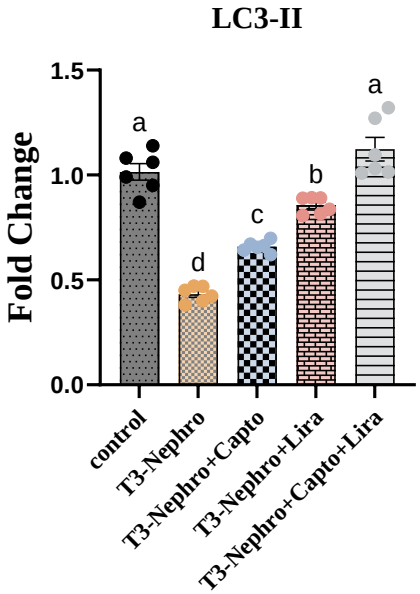

Figure (5) effect of liraglutide and -or captopril administration on LC3-II in T4 induced nephropathy.

3.6 **Effect of liraglutide and/or captopril on the expression level of** P62.

The result of the present work showed a significant P<0,05 up regulation in P62 expression of Nephropathy group than the control group figure (6). However, liraglutide and-or captopril administration show a significant P<0,05 down regulation in P62 expression compared with Nephropathy

group., More over administration of captopril with liraglutide showed more a significant improvement than administration of lira or captopril alone figure (6).

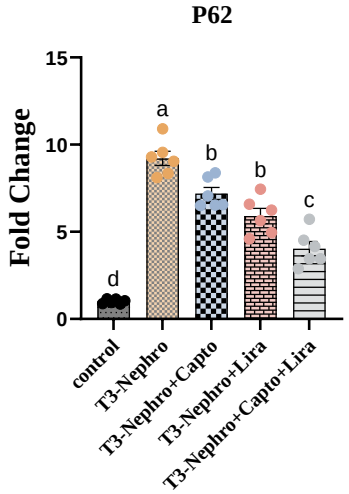

Figure (6) effect of liraglutide and -or captopril administration on P62 in T4 induced nephropathy.

4. Discussion.

The current study aimed to explore nephropathy induced by hyperthyroidism and the benefits of GLP-1agonist (saxenda)and captopril for treating this. Accumulating evidence has implicated that GLP-1 may have a beneficial effect on renal diseases, but the mechanism is not fully understood. Here we show that GLP-1agonist, liraglutide, inhibits oxidative stress. In the proximal tubule, GLP-1 inhibits the sodium-hydrogen ion exchanger isoform 3, which leads to natriuresis. This has been a partial explanation for the antihypertensive effects of GLP-1 receptor agonists. GLP-1 regulates the glomerular filtration rate, though the processes are complex and might be affected by factors such as blood glucose levels. The renal effects mediated by GLP-1 may be signaled through the renin-angiotensin system or atrial natriuretic peptide. GLP-1 therapy has been shown to be reno protective, independent of metabolic benefits, in many rodent studies in models of acute kidney injury and diabetic nephropathy. Probably the mechanism behind this protection is the inhibition of oxidative stress and renal inflammation. Although rare and limited, clinical trials suggestive of GLP-1-mediated renal protection do exist. But acute and chronic kidney disorders are serious global health issues that require action (**16).**

Moreover GLP-1agonist administration showed significantly improved in renal oxidative stress that in line with (**16,17).** The favorable actions of captopril were not a consequence of an overall reduction in serum albumin concentration or glomerular filtration rate because the relative fall in fractional albumin clearance was quantitatively consistent with the reduction in absolute albumin excretion. Such a possibility that reduced albuminuria might be a consequence of improved metabolic management was excluded further by the fact that blood glucose concentrations and hemoglobin Alc percentages during the renal clearance trials remained quite stable inbothgroups.

Even in patients with overt diabetic nephropathy, a continuous subcutaneous insulin infusion that improves metabolic control does not halt or reverse the accelerating increase in albuminuria. On the same text captopril and-or liraglutide administration showed significantly improved in renal oxidative stress without performing effect than the other treatment and this agrees with (**18,19)** Which could be referred to Liraglutide and captopril reduced renal oxidative stress. Furthermore, GLP-1agonist administration showed significantly improved in autophagy and renal fibrosis and this in line with (**20,21).** Moreover, liraglutide administration showed a significant effect in autophagy and renal fibrosis and this agrees with **(22).** On the same text captopril and-or liraglutide administration significantly improve autophagy and renal fibrosis without performing effect than the other treatment. This agrees with (**23)**. Which could be referred to combination of captopril and liraglutide improve autophagy and renal fibrosis.

5. Conclusion:

Administration of liraglutide and- or captopril showed significant effect in hyperthyroidism induce nephropathy via altering renal autophagy signaling pathway.

Conflict of interest: the authors declare that there is no competing conflict of interest.

References

1) Duni, A., Liakopoulos, V., Roumeliotis, S., Peschos, D., & Dounousi, E. (2019). Oxidative stress in the pathogenesis and evolution of chronic kidney disease: untangling Ariadne's thread. International journal of molecular sciences, 20(15), 3711.

2) Gribble, F. M., & Reimann, F. (2021). Metabolic messengers: glucagon-like peptide 1. Nature metabolism, 3(2), 142-148.

3) Baggio LL, Drucker DJ. Therapeutic approaches to preserve islet mass in type 2 diabetes. Annu Rev Med. 2006;57:265–281. Doi:10.1146/annurev.med.57.110104.115624.

4) Liang, S., Wu, Y. S., Li, D. Y., Tang, J. X., & Liu, H. F. (2022). Autophagy and renal fibrosis. Aging and disease, 13(3), 712.

5) Zhang Y-L, Zhang J, Cui L-Y, Yang S (2015). Autophagy activation attenuates renal ischemia-reperfusion injury in rats. Experimental Biology and Medicine, 240:1590-1598.

6) Tang C, Livingston MJ, Liu Z, Dong Z (2020). Autophagy in kidney homeostasisand disease. Nature Reviews Nephrology, 16:489-50

7) Liu S, Hartleben B, Kretz O, Wiech T, Igarashi P, Mizushima N, et al. (2012). Autophagy plays a critical role in kidney tubule maintenance, aging and ischemia-reperfusion injury. Autophagy, 8:826-837.

8) Eddy AA (2000). Molecular basis of renal fibrosis. Pediatr Nephrol, 15:290-301. [DOI] [PubMed] [Google Scholar].

9) Liu Y (2011). Cellular and molecular mechanisms of renal fibrosis. Nature Reviews Nephrology, 7:684-696. [DOI] [PMC free article] [PubMed] [Google Scholar]

10) Wen-yikang 2019. Therapeutic Effect of Scutellaria baicalensis on L-Thyroxine-Induced Hyperthyroidism Rats

11) Sturis J, Gotfredsen CF, Rømer J, Rolin B, Ribel U, Brand CL, Wilken M, Wassermann K, Deacon CF, Carr RD, Knudsen LB2003. GLP-1 derivative liraglutide in rats with beta-cell deficiencies: influence of metabolic state on beta-cell mass dynamics. Br J Pharmacol. 2003 Sep;140(1):123-32. doi: 10.1038/sj.bjp.0705397. Epub 2003 Jul 29. PMID: 12967942; PMCID: PMC1573996.

12) Salah EM, Bastacky SI, Jackson EK, Tofovic SP2018. Captopril Attenuates Cardiovascular and Renal Disease in a Rat Model of Heart Failure With Preserved Ejection Fraction. J Cardiovasc Pharmacol. 2018 Apr;71(4):205-214. doi: 10.1097/FJC.0000000000000561. PMID: 29620605.

13) Khamis, T.; Abdelalim, A.F.; Saeed, A.A.; Edress, N.M.; Nafea, A.; Ebian, H.F.; Algendy, R.; Hendawy, D.M.; Arisha, A.H.; Abdallah, S.H. Breast milk MSCs upregulated β-cells PDX1, Ngn3, and PCNA expression via remodeling ER stress/inflammatory/apoptotic signaling pathways in type 1 diabetic rats. Eur. J. Pharmacol. 2021, 905, 174188. [Google Scholar] [CrossRef]

14) Khamis, T.; Abdelalim, A.F.; Abdallah, S.H.; Saeed, A.A.; Edress, N.M.; Arisha, A.H. Early intervention with breast milk mesenchymal stem cells attenuates the development of diabetic-induced testicular dysfunction via hypothalamic Kisspeptin/Kiss1r-GnRH/GnIH system in male rats. Biochim. Biophys. Acta (BBA)—Mol. Basis Dis. 2020, 1866, 165577. [Google Scholar] [CrossRef] [PubMed]

15) Livak, K.J.; Schmittgen, T.D. Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the 2−ΔΔCT Method. Methods 2001, 25, 402–408. [Google Scholar] [CrossRef]

16) Skov, J. (2014). Effects of GLP-1 in the kidney. Reviews in Endocrine and Metabolic Disorders, 15, 197-207.

17) Winiarska, A., Knysak, M., Nabrdalik, K., Gumprecht, J., & Stompór, T. (2021). Inflammation and oxidative stress in diabetic kidney disease: the targets for SGLT2 inhibitors and GLP-1 receptor agonists. International Journal of Molecular Sciences, 22(19), 10822.

18) Mazzieri, A., Porcellati, F., Timio, F., & Reboldi, G. (2024). Molecular Targets of Novel Therapeutics for Diabetic Kidney Disease: A New Era of Nephroprotection. International Journal of Molecular Sciences, 25(7), 3969.

19) Filippatos, T. D., & Elisaf, M. S. (2013). Effects of glucagon-like peptide-1 receptor agonists on renal function. World journal of diabetes, 4(5), 190.

20) Peng, W., Zhou, R., Sun, Z. F., Long, J. W., & Gong, Y. Q. (2022). Novel insights into the roles and mechanisms of GLP-1 receptor agonists against aging-related diseases. Aging and Disease, 13(2), 468.

21) Yamada, S., Tanabe, J., Sugaya, T., Ichikawa, D., Kimura, K., Shibagaki, Y., & Ikemori, A. (2020). Renoprotective Effect of a GLP-1 Receptor Agonist, Liraglutide, in an Early Phase of Diabetic Kidney Disease in Spontaneously Diabetic Tori Fatty Rats: PO0913. Journal of the American Society of Nephrology, 31(10S), 317-318.

22) Xue, L., Pan, Z., Yin, Q., Zhang, P., Zhang, J., & Qi, W. (2019). Liraglutide promotes autophagy by regulating the AMPK/mTOR pathway in a rat remnant kidney model of chronic renal failure. International Urology and Nephrology, 51, 2305-2313.

23) Wang, N., & Zhang, C. (2024). Recent Advances in the Management of Diabetic Kidney Disease: Slowing Progression. International Journal of Molecular Sciences, 25(6), 3086.