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# Study the role of GLP-1 receptor agonist (Liraglutide) on experimentally induced diabetic nephropathy in male albino rats

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***Abstract*---**This study is to display glucagon-like peptide 1 (GLP-1) receptor agonist (liraglutide) role in diabetic nephropathy (DN) in rats and to investigate its mechanism of action. 60 male albino rats were divided into: I) control group; II) DN group; III) DN pre-treated group; IV) DN post-treated group. Group II, III & IV revealed a significant reduction in final body weight compared to control. Group II showed significant decreases in e-GFR, GSH and FoxO1 which were significantly elevated in group III & IV near to control. However, Group II showed significant rises in urinary protein, ACR, BUN, serum creatinine, TNFα, insulin, glucose, HOMA IR, MDA, caspase-12 and GRP78 that were significantly reduced in group III & IV near to control. FoxO1 has positive correlations with e-GFR and GSH and has negative correlations with urinary protein, ACR, BUN, serum


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creatinine, TNF-α, insulin, glucose, HOMA IR, MDA, caspase-12 and GRP78 in all groups. The histopathological findings of Group II showed glomeruli distortion and interstitial hemorrhage but group III & IV revealed enhancement in renal architecture. We can conclude that liraglutide has a significant role in the protection and treatment of the kidneys in DN.

***Keywords*---**Caspase-12, Diabetic nephropathy, FoxO1, Glucagon-like peptide 1, GRP78

## 1. Introduction

Diabetic nephropathy (DN) is a main complication of diabetes mellitus (DM) that causes chronic renal failure (Sulaiman, 2019). DN is attributed to renal injury because of prolonged high blood glucose levels, causing proteinuria, a gradual reduction in glomerular filtration rate, and progressive impairment of kidney function (Lim, 2014).

Many factors, such as accumulation of polyols, hemodynamic changes, increased activity of protein kinase C (PKC), enhanced advanced glycation end products (AGEs) formation, oxidative stress and microinflammation are involved in the pathogenesis of DN (Sagoo & Gnudi, 2018; Sinha & Nicholas, 2023).

Liraglutide, a common GLP-1 receptor agonist (GLP-1RA), has a great therapeutic effect in adjusting blood glucose and reducing weight. Liraglutide is likely to play a major renoprotective effect (Mali et al., 2022).

The renoprotective effects of GLP-1RA are reported apart from a decrease of blood glucose, because the receptor of GLP-1 is expressed in the kidney. The renal outcome of patient with type 2 diabetes mellitus (T2DM) can be significantly enhanced by liraglutide, which can delay the progress of DN (Mann et al., 2017; Su et al., 2020).

Theoretically, there is no evidence for whether the incidence of DN in patients with T2DM can be reduced by liraglutide or whether it possesses a curative effect in end-stage renal disease. The aim of this work was to demonstrate the GLP-1RA (liraglutide) effect on experimentally induced DN in male albino rats and to study its mechanism of action.

## 2. Materials and Methods

*2.1. Drugs and diet:*
Liraglutide (Victoza drug) was provided in the form of an injectable pen from Denmark. Streptozotocin (STZ) was obtained from Sigma-Aldrich company, USA as a powder. The standard chow diet is composed of 60% carbohydrates, 26% protein and 14% fat *(Clarke et al., 1977).* HFD consists of 20% carbohydrates, 70% fat, and 10% protein. It is composed of full cream milk, cooked cow fat, green vegetables and bread *(Srinivasan et al., 2005).*

*2.2. Experimental design:*
All experiments of the present study were agreed by the ethical committee of faculty of medicine, (code no: 35374/3/22), Tanta University.
The study used sixty male albino rats weighted 150 to 170 grams. The rats were kept in animal cages (5/cage), with free entry of water and food under controlled environmental conditions, at room temperature.
After 2 weeks of acclimatization, rats were divided into 4 equal groups:
**Group I;(control group)** fed a regular diet, and each received a daily 0.5 ml subcutaneous (SC) injection of normal saline for 4 weeks, then they received 0.5 ml of a single intraperitoneal (ip) injection of citrate buffer. These animals also received SC injections of normal saline daily for 8 weeks.
**Group II;(DN group)** fed a high-fat diet (HFD) with high sugar, received SC injection of normal saline daily for 4 weeks, then they received one ip injection of STZ (40 mg/kg) in citrate buffer (Zhang et al., 2008). They also received SC injection of normal saline daily for 8 weeks (Zhao et al., 2020).
**Group III;( DN pre-treated group):** As in Group II but received SC injection of liraglutide (200 µg/ kg/ day) for 4 weeks before STZ injection (Sonne et al., 2008).
**Group IV;( DN post-treated group):** As in Group II but received SC injection of liraglutide (200 µg/ kg/ day) for 8 weeks after STZ injection (Zhao et al., 2020).

*2.3. Induction of diabetes in rats:*
After four weeks of HFD with high sugar, all animals except the control group fasted for 12 h and received one ip injection of STZ (40 mg/kg) in citrate buffer. Levels of blood glucose were taken from the tail vein after 3 days of STZ injection, and non-diabetic rats were excluded when the level of blood glucose was less than 300 mg/dl (Zhao et al., 2020) and were considered a DN model when the excretion rate of urinay albumin exceeded 20mg/24h and presence of glucose in urine sample after 4 weeks (Xiao et al., 2021).

1 rat from group II, 3 rats from group III and 1 rat from group IV were excluded. In this study, the mortality rate was: 2 rats in group II, 1 rat in group III and 2 rats in group IV. This mortality rate may be due to hyperglycemia and its complications. The final number of rats was 15, 12, 11, 12 rats in the control, DN, DN pre-treated and DN post-treated groups respectively.

2.4. *Urine sample*:
Urine was gathered in a metabolic cage at the end of the experiment. 24-h urinary protein was calculated by the method of *Nishi & Elin, (1985).* Urinary albumin and creatinine concentrations were measured by the method of *Rasanayagam et al. (1973)* & *Bartels et al. (1972)* respectively. After that, the urinary albumin creatinine ratio (ACR) was measured. Then, rats were anaesthized by diethyl ether. Body weight was determined before starting and at the end of this work for all groups.

*2.5. Blood and tissue sampling:*
Blood samples were obtained through cardiac puncture. Then the kidneys were dissected, and each was divided into three parts.
Serum creatinine and BUN were determined by the methods of *Bartels et al. (1972)* & *Deacon, (2009)* respectively.
eGFR was determined by an equation as follows:

If creatinine in plasma was less than 52 mmol/L, eGFR would be calculated by
$(880 \times U^{-0.391} \times C^{-0.660} \times W^{0.695})$.
If creatinine in plasma more than or equal 52 mmol/L, eGFR would be calculated by
$(5862 \times U^{-0.391} \times C^{-1.150} \times W^{0.695})$.
Where eGFR (µL/min), U was urea (mmol/L), C was creatinine concentration (µmol/L), and W was weight (g) by the methods of *Besseling et al. (2021).*
Serum TNF-α and fasting insulin were measured by Shanghai Sunred Biological Technology Co. Ltd and China Calbiotech Inc., USA rat ELISA kits by the methods of *Brouckaert et al. (1993)* & *Temple et al. (1992)* respectively.
Serum fasting glucose and HOMA IR were measured by the methods of *Tietz, (1995)* & *Matthews et al. (1985)* respectively.
Reduced glutathione (GSH) and malondialdehyde (MDA) in the first part of kidney tissue, were determined in renal homogenate usage colorimetric assay kits (Biodiagnostic, Egypt) by *Beutler et al. (1963)* & *Ohkawa et al. (1979*) methods respectively.
Caspase-12, GRP78 and FoxO1 gene expression in the second part of kidney tissue were determined in renal homogenate using real-time PCR (Qiagen, model: Q5plex, Germany) by the method of *Tikellis et al. (2009).*
The third part of the kidney from all rats was fixed in 10% formalin and embedded in paraffin wax. Paraffin-embedded tissues were sectioned into 4µm and stained with hematoxylin-eosin (H&E). The sacrificed rats were collected in a particular package consistent with infection control measures and safety precautions and directed to hospital biohazards.

*2.6. Statistical analysis:*
SPSS software analyzed collected data by version 23.0, using one way ANOVA then the Tukey test. Additionally, an analysis of Pearson's correlation was carried out. If P values were less than or equal to 0.05, they would be statistically significant.

## 3. Results

*3.1. Body weight changes:*
Final body weight (FBW) of all studied groups revealed a significant rise in comparison with initial (In) body weight and FBW of the DN group, DN pre-treated and post-treated groups showed significant decrease in comparison with the FBW of the control group. (Figure 1)

*3.2. Urinary protein level and urinary ACR*
The DN group displayed a significant elevation in urinary protein & urinary ACR levels, which were significantly decreased in DN pre-treated and DN post-treated groups near to the control group. (Table 1)

*3.3. Serum creatinine, BUN, e-GFR, TNF a, fasting insulin, glucose and HOMA IR*
The DN group showed significant increase in serum creatinine, BUN, TNF α, fasting insulin, glucose and HOMA IR levels, which were significantly reduced in DN pre-treated and DN post-treated groups near to the control group while the opposite occurs in e-GFR. (Table 2)

*3.4. Renal GSH, MDA and caspase-12, GRP78 & FoxO1 gene expression*

The DN group showed a significant elevation in renal MDA and caspase-12, GRP78 levels, which were significantly reduced in the DN pre-treated and DN post-treated groups near to the control group, while the opposite occurs in renal GSH and FoxO1. (Table 3)

*3.5. Correlation between the renal FoxO1 gene expression and all other parameters in all studied groups*

-There was significant positive correlation between renal FoxO1 and the final body weight, e-GFR and renal GSH level in all groups. There was significant negative correlation between renal FoxO1 and urinary protein level, ACR, serum creatinine level, BUN, serum TNF-α, serum fasting insulin, serum fasting glucose, HOMA IR, renal MDA level, renal caspase-12 and renal GRP78 in all groups. (Figure 2)

*3.6. Renal histopathology*

3.6.1. Control group:

The rats of this group showed normal kidney architecture regarding normal cortical thickness, normal glomeruli, tubules and normal interstisium. (Figure 3, a)

3.6.2. DN group:

The rats of this group showed tubular cells vacuolation, glomeruli distortion, severe cellular infiltration & interstitial hemorrhage. (Figure 3, b - c and d)

3.6.3. DN pre-treated group:

The rats of this group showed improvement in glomerulus compared with DN group & enhancement of interstitial inflammatory cells infiltration (Figure 3, e)

3.6.4. DN pre-treated group:

The rats of this group also showed enhancement of the glomerulus, lighter tubular edema in comparison with DN group & improvement of interstitial inflammatory cells infiltration. (Figure 3, f)

## 4. Discussion

The results of this work revealed that the final body weight (FBW) of all studied groups showed a significant elevation in comparison with their initial body weight and there was a significant reduction in the FBW of the DN group, DN pre-treated & post-treated groups compared with FBW of control group. The DN group displayed significant decrease in e-GFR, renal GSH, renal FoxO1 gene expression levels and there was significant elevation in urinary protein level, urinary ACR, serum creatinine, serum urea, BUN, serum TNFα, serum fasting insulin, serum fasting glucose, HOMA IR, renal MDA, renal caspase-12 gene expression and renal GRP78 gene expression levels in comparison with the control group, DN pre-treated group and DN post-treated group. However, DN pre-treated and post-treated groups showed significant decrease in e-GFR, renal GSH, renal FoxO1 gene expression levels and there was significant elevation in urinary protein level, urinary ACR, serum creatinine, serum urea, BUN, serum TNFα, serum fasting insulin, serum fasting glucose, HOMA IR, renal MDA, renal caspase-12 gene expression and renal GRP78 gene expression levels as compared to the control group with insignificant changes in DN pre-treated group as compared to DN

post-treated group in all parameters. This agrees with other studies by Chen et al. (2017), Zhao et al. (2020), Xiao et al. (2021).
An imbalance between energy intake and energy output causes weight gain which induces accumulation of fat in white adipose tissue (Seo et al., 2017). Guo et al. (2021) explained that the reduction of body weight that happened after the STZ intake is because of catabolism of fats, protein and dehydration.

Zhou et al. (2014) demonstrated that GLP-1RA would aid fight obesity & its complications by changing white fat to brown fat that burns more calories. Additionally, Tronieri et al. (2020) has reported that liraglutide can suppress appetite, control weight gain and alleviate blood lipid levels in obese patients with T2DM.

DN is the major reason for the worldwide final stage of renal disease (Anders et al., 2018). Proteinuria in DN is mainly because of disruptions in the barrier of glomerular filtration, including the podocyte, the glomerular endothelial cell and the glomerular basement membrane (GBM) (Gil et al., 2020).

Mann et al. (2017) has thought that liraglutide could have beneficial effects on the kidneys. Mali et al. (2022) has also confirmed that treatment with liraglutide has an antiproteinuric effect in patients with T2DM.

Liraglutide could decrease urinary protein levels via antioxidative stress and anti-inflammatory activities, enhance renal function, reduce the incidence of proteinuria in diabetic patients and avoid the presence of DN and acute kidney damage (Mann et al., 2017).

Moreover, liraglutide can improve kidney function and ameliorate extracellular matrix (ECM) accumulation and glomerular injury in DN rats induced by STZ by restoring the signaling of Wnt/β-catenin that has a main effect on renal development, especially the formation of nephron (Huang et al., 2020).

Inflammation has an important role in the pathophysiological mechanisms of DN because TNF-α, the pro-inflammatory cytokine, can result in great production of superoxides and protein hydrolysates, causing increased formation of white blood cells, elevated GBM permeability and provoking kidney damage (Morales et al., 2019).

Ye et al. (2019) described the renoprotective effect of liraglutide by inhibition of TNFα as an inflammatory factor in kidney damage caused by a high fat diet. Additionally, in the STZ diabetic model of Baylan et al. (2022) which used liraglutide as a treatment, it attenuated renal injury by reducing TNF-α and other inflammatory markers which stimulated DN.

Su et al. (2020) illustrated that liraglutide can depress inflammation and delay progression of DN in rat models by decreasing the nuclear factor-kappa B (NF-κB) expression and inhibiting c-Jun N-terminal kinases (JNK) phosphorylation, and extracellular signal-related kinases 1 and 2 (ERK1/2).

Zhang et al. (2020) has reported that HFD feeding rats develop insulin resistance. This might be because HFD can cause an accumulation of fat in the pancreas that induces β-cell stress and hinders production of insulin. This can result in hyperglycaemia (Yi et al., 2020). Simultaneously, the low dose of STZ has impaired insulin secretion that is like the features of the end stage of T2DM (Furman, 2021).

GLP-1 can inhibit the release of glucagon, encourage islet β cell differentiation and proliferation and depress islet β cell apoptosis; GLP-1 also has a hypoglycemic effect in a glucose-dependent manner (Danowitz & De-Leon, 2022).

Wang et al. (2017) indicates that liraglutide can activate the sensitivity of insulin and manage the balance of lipid metabolism. Zhou et al. (2019) showed that liraglutide enhances the sensitivity of insulin in insulin-sensitive tissues via multiple pathways such as increasing oxidation of fatty acids, inhibition of both liver and systemic pro-inflammatory cytokines and reduction of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (Cox-2) expression.

Oxidative stress has a major effect on DN pathogenesis because hyperglycaemia can cause increased reactive oxygen species (ROS) formation via a sequence of pathways, including accumulation of polyols, improved AGEs formation and stimulation of renal NADPH oxidase and PKC (Oguntibeju, 2019). Once DN occurs, ROS increases in the body, resulting in increased glomerular injury, oxidative stress, tubulointerstitial fibrosis and remodeling of the renal matrix (Panigrahy et al., 2017).

Rizzo et al. (2015) displayed in patients with T2DM that liraglutide can decrease oxidative stress not dependent on the hypoglycemic effect. Furthermore, liraglutide ameliorates kidney damage via the reduction of oxidative stress through inhibiting NADPH oxidase activation of the kidney in diabetic rats induced by STZ and depressing the release of macrophage pro-inflammation cytokines (Hendarto et al., 2012).

Hyperglycemia, proteinuria, free fatty acid (FFA) and AGEs have been recognized as main inducers of endoplasmic reticulum stress (ERS) (Su et al., 2023). Frequently, excessive ERS evolves in the intrinsic cells of DM patients in the kidney, which can cause cell damage, apoptosis and finally the formation of DN (Sankrityayan et al., 2019). GRP78 is transferred from the endoplasmic reticulum (ER) to the surface of the cell because of high glucose via an ERS-dependent mechanism (Tsai et al., 2015).

Caspase-12 is located on the ER outer membrane and during ERS, it is activated. The activation of caspase-12 is related to ERS-induced apoptosis (Zhang et al., 2016).

Zhao et al. (2020) illustrated that liraglutide in vivo experiments can enhance oxidative stress and ERS and control apoptosis and cell proliferation via ERS and autotrophy in vitro. Liraglutide also engages protein kinase B signal transducer and transcription 3 signaling activators for helping change nerve cell outcome from apoptosis to survival under chronic ERS (Panagaki et al., 2017).

Reducing the signaling pathway of PI3K/AKT abolishes GLP-1R protective effect by elevating ERS, indicating that this pathway might be affecting GLP-1 on ERS (Chen et al., 2018). Moreover, the PKA pathway might also be associated with GLP-1, guarding cells from apoptosis and reducing the signaling pathway of the ERS (He et al., 2016).

FoxO1, an essential transcription factor of family of the forkhead protein, is a main regulator of the function of pancreatic beta cell and affects beta cell in the developing pancreas of the fetus during their differentiation and under the insulin resistance. FoxO1 also guards beta cells from injury arising from oxidative stress involved in overload of lipid and glucose (Ren et al., 2020).

Additionally, Yadav et al. (2017) has described that FoxO1 reduces expression of insulin-induced plasminogen activator inhibitor-1 (PAI-1) by inhibiting signaling pathway of transforming growth factor-β (TGF-β) / Small mothers against decapentaplegic (SMAD) in hepatocytes. Thus, the reduction of FoxO1 activity might be associated with DN development (Wang and He, 2021).

Du et al. (2016) also showed that liraglutide has a useful influence in kidney damage by reducing oxidative stress and attenuating TGF-β1/ Smad3 pathway activation in diabetic rats. Liraglutide also has a renoprotective role through a FoxO1 upregulated expression of MnSOD in the early DN and improving the activity of FoxO1 through Akt/FoxO1 pathway reduction (Chen et al., 2017).

The histopathological findings of the current work came on the same line and confirm our previously discussed results about the DN group, which showed tubular cell vacuolation, glomeruli distortion, severe cellular infiltration and interstitial hemorrhage. However, DN pre-treated and post-treated groups showed improvement in renal glomerulus, lighter tubular edema and enhancement of the interstitial inflammatory cells' infiltration compared with the DN group. These histopathological findings are agreed with (Zhao et al., 2020; Xiao et al., 2021).

**Conclusion**

GLP-1RA (liraglutide) has a significant role in the protection and treatment of the kidneys in DN, due to reduction in hyperfiltration and albuminuria, renal FoxO1 activation and decreasing the renal endoplasmic reticulum stress by reducing renal caspase-12 and renal GRP78 genes expression levels as assessed by biochemical and histopathological findings.

**Conflict of interest:** nill

**Funding:** nill

Table 1: Urinary protein level and urinary ACR in all studied groups

| | G I (control) | G II (DN) | G III (DN pre-treated) | G IV (DN post-treated) | F | P |
|---|---|---|---|---|---|---|
| Urinary protein (mg/24h) | 3.05 ± 0.44 | 37.27 ± 2.29 [a] | 23.48 ± 1.39 [ab] | 24.54 ± 1.37 [ab] | 1254.8 | <0.05 |
| Urinary ACR (mg albumin/g creatinine) | 15.37± 1.72 | 111.06± 17.61[a] | 34.49±3.22 [ab] | 36.95± 3.45 [ab] | 279.32 | <0.05 |

Data are presented as mean ± SD. [a] displays statistical significance at P≤0.05 in comparison with control group. [b] displays statistical significance at P≤0.05 in comparison with DN group. DN: Diabetic nephropathy, ACR: albumin to creatinine ratio.

Table 2: Serum creatinine, BUN, e-GFR, TNF α, fasting insulin, glucose and HOMA IR in all studied groups

| | G I (control) | G II (DN) | G III (DN pre-treated) | G IV (DN post-treated) | F |
|---|---|---|---|---|---|
| Serum creatinine (mg/dl) | 0.52± 0.09 | 1.77± 0.25 [a] | 0.99± 0.06 [ab] | 1.01± 0.06 [ab] | 184.21 |
| BUN (mg/dl) | 13.39± 0.75 | 34.50±2.92 [a] | 21.22± 1.58 [ab] | 21.72± 1.79 [ab] | 283.77 |
| e-GFR (mL/min) | 1.64± 0.26 | 0.47± 0.08 [a] | 0.88± 0.18 [ab] | 0.82± 0.16 [ab] | 94.88 |
| Serum TNF α (ng/l) | 3.23± 0.35 | 18.13± 1.21 [a] | 9.92± 0.64 [ab] | 10.19± 0.56 [ab] | 901.54 |
| Serum fasting insulin (μIu/ml) | 5.08± 0.45 | 14.79± 0.95 [a] | 6.29± 0.57 [ab] | 6.49 ± 0.55 [ab] | 587.66 |
| Serum fasting glucose (mg/dl) | 81.89± 6.15 | 337.82±43.04 [a] | 189.38±16.88 [ab] | 189.87± 17.56 [ab] | 246.86 |
| HOMA IR | 1.04± 0.13 | 12.36± 1.93 [a] | 2.90± 0.42 [ab] | 3.06± 0.46 [ab] | 328.28 |

Data are presented as mean ± SD. [a] shows statistically significant at P≤0.05 in comparison with control group. [b] shows statistically significant at P≤0.05 in comparison with DN group. eGFR: estimated glomerular filtration rate, BUN: blood urea nitrogen, HOMA IR: homeostatic model assessment of insulin resistance, TNF α: tumor necrosis factor alpha.

Table 3: Renal GSH, MDA and caspase-12, GRP78 & FoxO1 gene expression levels in all studied groups

| | G I (control) | G II (DN) | G III (DN pre-treated) | G IV (DN post-treated) | F |
|---|---|---|---|---|---|
| Renal GSH (mmol /g tissue) | 59.64 ± 2.55 | 23.66 ± 1.92 [a] | 45.54 ± 2.35 [ab] | 43.36 ± 2.74 [ab] | 492.47 |
| Renal MDA (nmol/g tissue) | 11.24± 0.96 | 54.17± 2.68 [a] | 26.38± 1.43 [ab] | 27.42± 1.40 [ab] | 1429.00 |
| Renal caspase-12 gene expression | 1.04 ± 0.04 | 3.74 ± 0.35 [a] | 1.69 ± 0.12 [ab] | 1.75± 0.16 [ab] | 435.76 |
| Renal GRP78 gene expression | 1.02± 0.02 | 3.27 ± 0.42 [a] | 1.49 ± 0.24 [ab] | 1.60 ± 0.32 [ab] | 154.38 |
| Renal FoxO1 gene expression | 1.02± 0.02 | 0.47± 0.08 [a] | 0.83 ± 0.10 [ab] | 0.78 ± 0.11 [ab] | 103.63 |

Data are presented as mean ± SD. [a] displays statistically significant at P≤0.05 in comparison with control group. [b] displays statistically significant at P≤0.05 in comparison with DN group, GSH: glutathione, MDA: malondialdehyde, GRP78: glucose regulated protein 78, FoxO1: forkhead box protein O1.

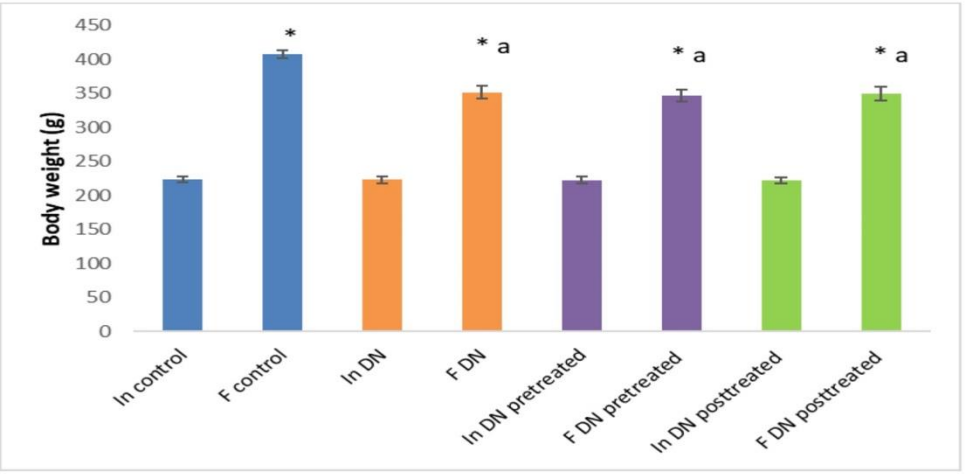
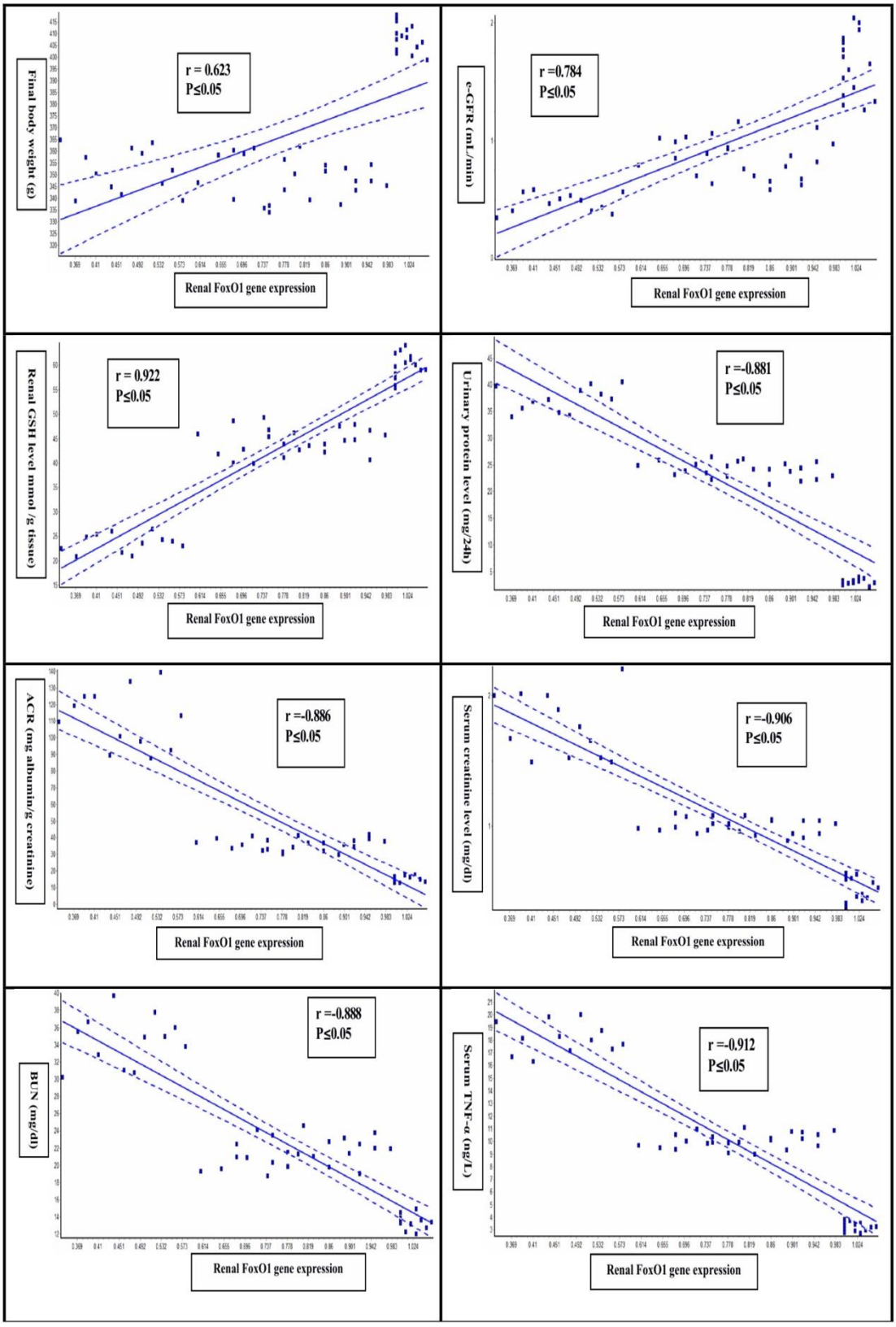



Figure 1: Body weight changes (g) in all studied groups. In, initial body weight; F, final body weight.

*Shows statistically significant at P≤0.05 in comparison with the initial body weight among studied groups.

[a] Shows statistically significant at P≤0.05 in comparison with the FBW of the control group.


Final body weight (g)
r = 0.623
P≤0.05
Renal FoxO1 gene expression
e-GFR (mL/min)
r =0.784
P≤0.05
Renal FoxO1 gene expression
Renal GSH level mmol /g tissue)
r = 0.922
P≤0.05
Renal FoxO1 gene expression
Urinary protein level (mg/24h)
r =-0.881
P≤0.05
Renal FoxO1 gene expression
ACR (mg albumin/g creatinine)
r =-0.886
P≤0.05
Renal FoxO1 gene expression
Serum creatinine level (mg/dl)
r =-0.906
P≤0.05
Renal FoxO1 gene expression
BUN (mg/dl)
r =-0.888
P≤0.05
Renal FoxO1 gene expression
Serum TNF-α (ng/L)
r =-0.912
P≤0.05
Renal FoxO1 gene expression

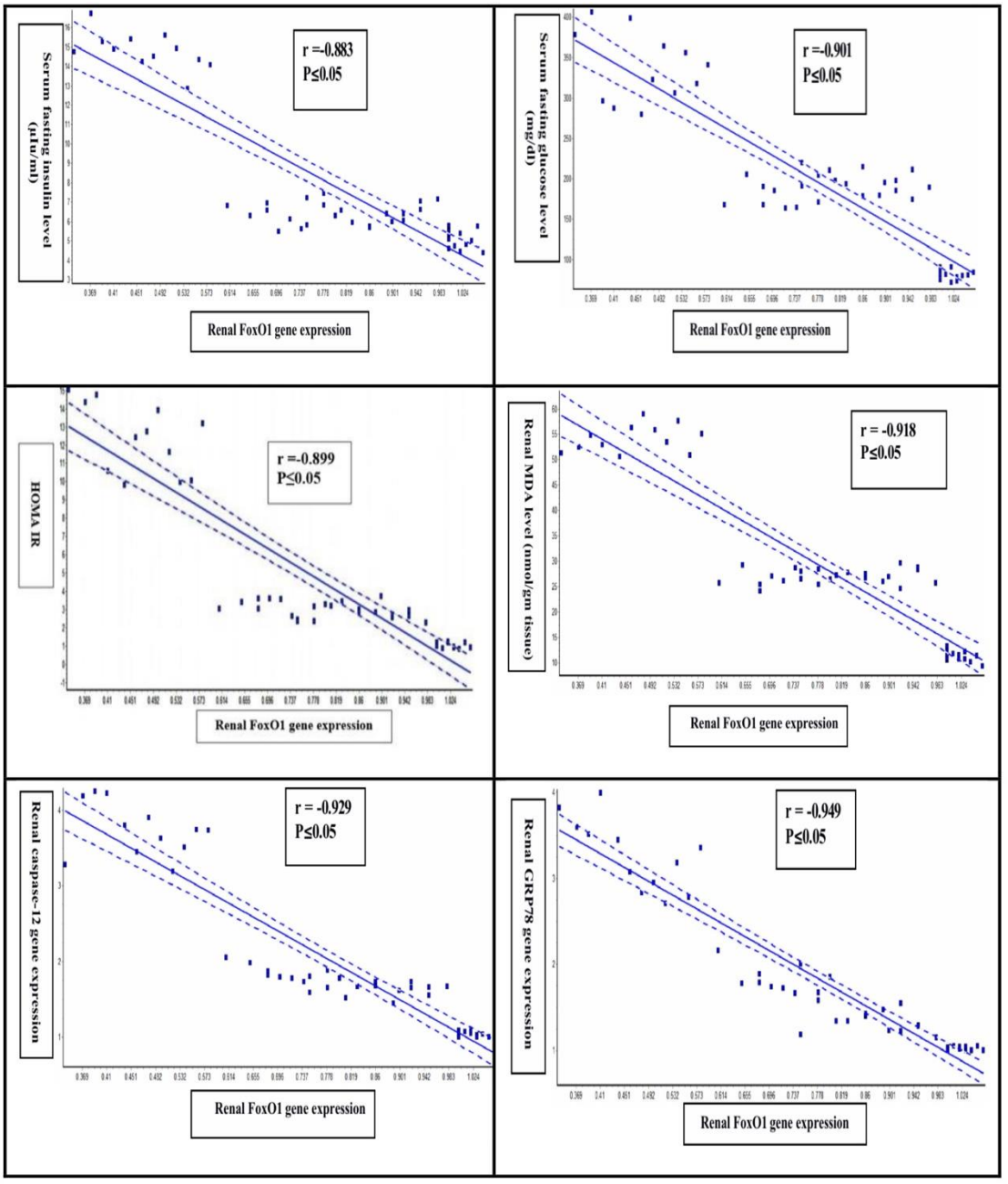



Figure 2: Correlation between FoxO1 & all parameters (final body weight, urinary protein, urinary ACR, serum creatinine, BUN, e-GFR, serum TNF-α, serum fasting insulin, serum fasting glucose, HOMA IR, renal MDA, renal GSH, renal caspase-12 and renal GRP78. r indicates correlation coefficient Vs FoxO1 relative gene expression. P≤0.05 indicates statistically significant.

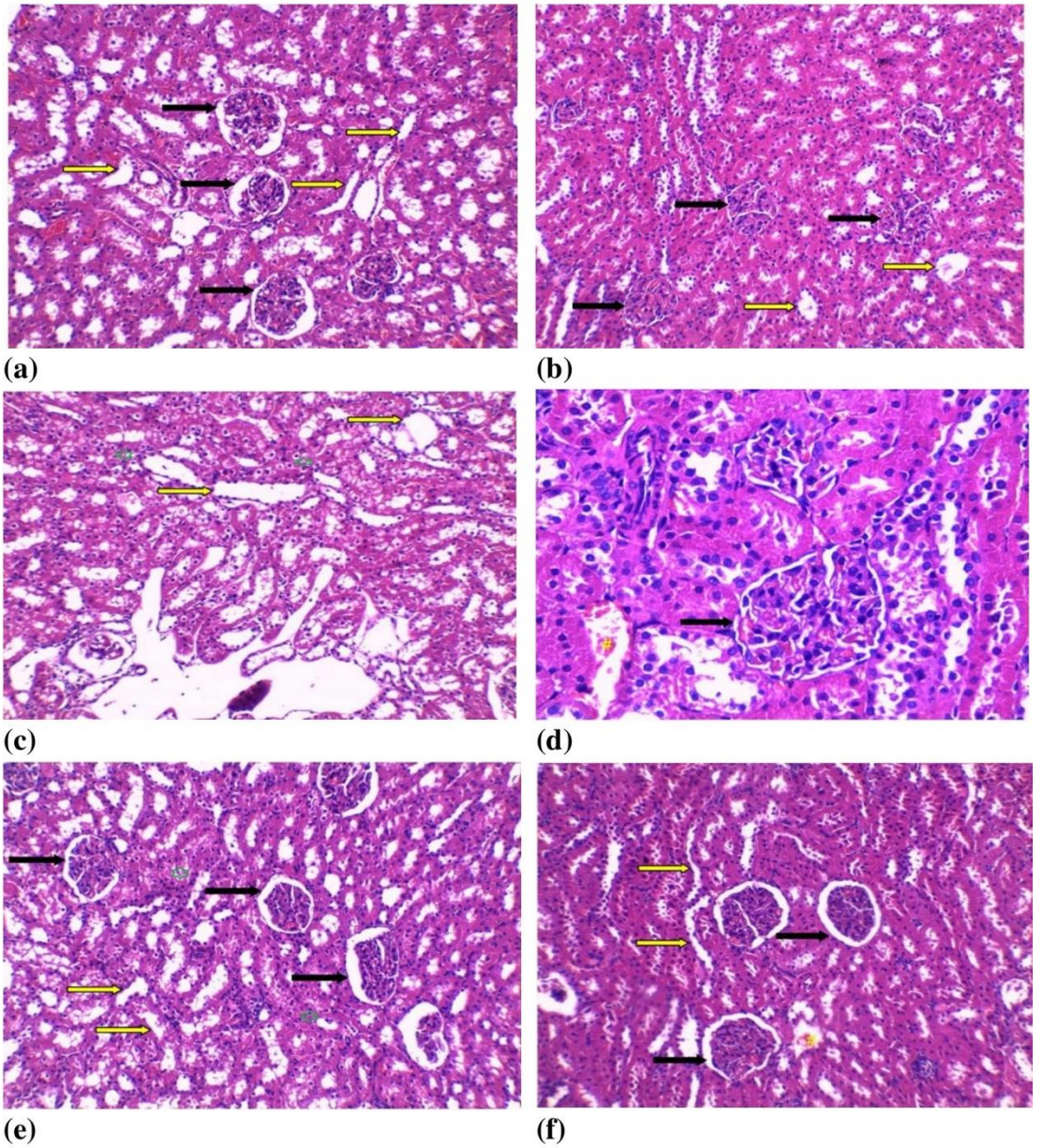



Figure 3: Section from (a) control group showed normal glomeruli (black arrows) and normal tubules (yellow arrows) of the kidney. (H&E x200), (b) DN group showed glomeruli distortion (black arrows) and tubular cells vacuolation (yellow arrows). (H&E x200), (c) DN group showed glomerulus distortion (black arrow), tubular cells vacuolation (yellow arrows) and sever cellular infiltration (✡). (H&E x200), (d) DN group showed glomerulus distortion (black arrow) and interstitial hemorrhage (#). (H&E x400), (e) DN pre-treated group with liraglutide showed a more regular morphology of the glomeruli (black arrows), lighter tubular edema (yellow arrows) and less cellular infiltration (✡). (H&E x200), (f) DN post-treated group with liraglutide showed more normal glomerulus (black arrows), minimal

vacuolated cytoplasm of some renal tubules (yellow arrows) less interstitial hemorrhage (#). (H&E x200).

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