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Valsartan reduces *NOX4* expression and halts diabetic nephropathy in streptozotocin induced diabetic rat model

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Abstract

Background: Diabetic nephropathy is the leading cause of kidney failure worldwide. NADPH oxidase 4 (NOX4) is an obvious cause for reactive oxygen species production in the kidney mainly via renin-angiotensin system. High reactive oxygen species results in kidney damage through direct effect or through activation of other pathways such as the extracellular regulated kinase (ERK) signaling pathway.

Aim: The present study was constructed to investigate the effect of angiotensin II receptor blocker, valsartan, on NOX4, ERK1/2expression, and malondialdehyde level as well as their effects on the progression of diabetic nephropathy in type 1 diabetic rat model.

Methods: The experimental rats were divided into three groups: group I control healthy untreated rats, group II streptozotocin-induced diabetic rats, and group III valsartan treated streptozotocin-induced diabetic rats.

Results: Valsartan showed significantly decreased NOX4 and ERK1/2 mRNA, NOX4 and pERK1/2 protein concentration, and oxidative stress as evidenced by low malondialdehyde concentration in treated diabetic rats. Valsartan attenuates albuminuria, improve overall kidney function parameters, and pathological changes compared to diabetic non-treated rats.

Conclusion: Valsartan treatment impedes renal damage and improve kidney function in treated diabetic nephropathy rats. The beneficial effect of valsartan was mediated through decrease oxidative stress via downregulation of NOX4 and ERK1/2 and decreased reactive oxygen species production.

Keywords: NOX4, Diabetic nephropathy, Valsartan, ERK

Background

Diabetic nephropathy (DN), a major micro-vascular complication of diabetes, is the leading cause of end-stage renal disease worldwide (Collins et al., 2012; Gnudi et al., 2016). Chronic hyperglycemia, the most prominent feature of diabetes, direct the activation of intrarenal renin–angiotensin system (RAS), and increase production of reactive oxygen species (ROS), which leads to renal tissue injury and fibrosis (Urushihara and Kagami 2017; Murphy et al., 2015; Zhang et al., 1999; Singh et al., 1999) .

Angiotensin II (Ang II), a component of renin angiotensin system (RAS), mediate the development of diabetic nephropathy through various mechanisms (Ni et al., 2015;

Onozato et al., 2002; Yadav et al., 2010). Ang II could increase ROS production through activating reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (NOXs) enzyme (Ushio-Fukai 2009), resulting in a state of oxidative stress and altered redox signaling, manifested by damage to macromolecules, eventually lead to inflammation, fibrosis, and vascular dysfunction (Wei et al., 2009; Weidinger and Kozlov 2015). Of the seven NOXs isoform, NOX4 is abundantly expressed in *mesangial and tubular cells* and is considered the major source of ROS production in renal cells in diabetes (Sedeek et al., 2013).

Previous studies had shown that Ang II, ROS, and hyperglycemia could activate the extracellular regulated kinase (ERK) pathway in different types of renal cells, including podocytes and mesangial cells (MCs) (Gorin et al., 2004;

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Lakshmanan et al., 2012; Teng et al., 2014). ERK plays a key role in the intracellular signaling pathway that integrates the transcription of genes involved in numerous cellular responses in DN (Ni et al., 2015; Cheng et al., 2013). It act as an upstream regulator of TGF-B and SMAD pathways which are involved in renal fibrosis, ECM deposition, inflammation, and proliferation, resulting in hypertrophic response in kidney tissue (Ni et al., 2015; Lakshmanan et al., 2012; Cheng et al., 2013; He et al., 2016).

Recent studies pointed out to the role of modulating RAS activity through blocking the generation and action of Ang II using angiotensin-converting enzyme inhibitors (ACEIs) or Ang II receptor blockers (ARBs), in the management of diabetic nephropathy (Seferovic et al., 2017).

Valsartan, a non-peptide triazole derivative antagonist of Ang II, selectively and competitively blocks the binding of angiotensin II to the AT1 subtype receptor in vascular smooth muscle and the adrenal gland (S-i et al., 2011). In type 2 diabetes rat model, administration of high doses of valsartan reduces podocyte injury and renal oxidative stress, which results in not only reduced albuminuria but also arrest the progression of associated glomerulosclerosis (Zhou et al., 2014; Wang et al., 2014).

The role of valsartan in the prognosis of type 1 associated diabetic nephropathy has been less studied. Therefore, the present study was conducted to investigate the effect of valsartan on NOX4-mediated ROS production and ERK signaling pathway in type 1 diabetes rat model.

Methods

Experimental animals and treatment
Sixty adult male white albino rats (weight 200–300 g), obtained from the National research center animal house (Cairo, Egypt), were included in this study. The rats were housed in pathogen-free conditions (five rats/cage), with 12:12 h's light-dark cycles with a stable ambient temperature of 18–22 °C in the animal house of Medicine faculty (Suez Canal University). Animals were provided with normal standard diet of known composition and free access to water. Animals were left to acclimatize for 7 days before the experiment. Animal care before and during the experimental procedures was done in accordance with the guidelines of the Animal Ethics Committee, Faculty of Medicine, Suez Canal University (license number: 2586).

Rats were divided into three groups (20 rats/group): group I (GpI) control healthy untreated rats, group II (GpII) streptozotocin (STZ)-induced diabetic rats as diseased control, and group III (GpIII) valsartan-treated STZ-induced diabetic rats.

Forty rats (comprising GpII and GpIII) were fasted overnight and rendered diabetic by a single intra-peritoneal injection of STZ purchased from Sigma-Aldrich

Chemical Company (St. Louis, MO, USA) in a dose of 65 mg/kg/body weight (BW) (Zhang et al., 2015). Rats had received 5% glucose instead of water for the first 24 h after diabetes induction in order to reduce death due to hypoglycemic shock, resulting from sudden hyperinsulinemia caused by massive beta cell destruction (Saeed et al., 2012). Tail vein blood glucose level were measured 3 days after STZ injection, rats with a fasting blood glucose level ≥ 250 mg/dl for three consecutive determinations were considered as diabetics (Zhang et al., 2015; Hartner et al., 2014). Two weeks later, 20 animals were presented with microalbuminuria, an early sign of DN; GpIII received intragastric valsartan (purchased from the market) at a daily dose of 30 mg/kg/BW for 4 weeks.

Samples collection and preparation
At the end of the experiment, all rats were kept in metabolic cage for 24-h urine collection that is stored at −80 °C after centrifugation. Rats were fasted overnight and sacrificed under anesthesia. Blood samples were collected, kept on ice for 1 h, and centrifuged to separate serum. Serum samples were stored at −80 °C until analysis.

Rats' kidney tissues were dissected, rinsed in ice-cold PBS, and weighted. Left kidneys were fixed in 4% paraformaldehyde for histological studies. The right kidneys were excised into two parts: one part (30 mg) was frozen instantly in −80 °C for RNA extraction, the second part was rinsed in ice-cold phosphate-buffered saline (PBS) to remove excess blood, minced to small pieces, and homogenized in 5–10 mL of cold PBS according to weight with a homogenizer (SR30 model ps 80) on ice. The resulting suspension was subjected to two freeze-thaw cycles, and the homogenates were centrifuged in a cooling centrifuge for 5 min at 5000×g. Supernatant was removed, aliquoted, and stored at −80 °C for biochemical and molecular analysis.

Determination of serum glucose level and kidney function markers
Glucose, creatinine, and urea in serum and urinary creatinine concentration were assayed using automated analyzer (Hitachi, Japan, model7020) with specific commercial kits (Cobas 6000, cobas c501, Roche). Urinary microalbuminuria (UAlb) was assayed using immune-turibidemtric assay, urine albumin/urine creatinine (Ua/Ucr) ratio, and creatinine clearance was calculated.

Renal histopathological examination
Sections (4-μm-thick) cut from 10% formalin-fixed, paraffin-embedded kidney samples were stained with Hematoxylin and Eosin (H&E) and Masson Trichrome staining, for evaluation of renal glomerular damage,

RNA extraction and quantitative RT-PCR detection of renal *NOX4* and *ERK1/2* genes

Thirty milligrams of renal tissues were used for total RNA extraction using RNeasy Mini Kit (cat no 74104, Qiagen, Germany). RNA was quantified using Nanodrop 8000 (Thermo Scientific USA) and cDNA was done using 10 ng RNA by reverse transcription using (QuantiTect Reverse Transcription Kit cat no205311, Qiagen) in a mastercycler gradient thermocycler [Eppendorf, Hamburg, Germany]. Then, 1 μl of cDNA (50 ng/μl) was subjected to PCR amplification performed with an ABI PRISM 7000 Sequence Detector System (Applied Bio system). The assay reactions were performed in 25 μl reaction volume including 12.5 μl SYBR Green PCR mastermix (QuantiTect SYBR Green PCR Kit cat no.204141, Qiagen, Germany) according to the manufacturers' instructions, using specific sequence primers: Primers for *ERK1/2* (QuantiTect primer assay, Qiagen, Cat no: QT00176330), *NOX4* (QuantiTect primer assay, Qiagen, Cat no: QT00186550), and B-actin (QuantiTect primer assay, Qiagen, Cat no: QT00193473) under the following conditions: initial denaturation at 95 °C for 15 min, followed by 45 cycles of denaturation at 95 °C for 15 s, annealing at 56 °C for 30 s, and extension at 72 °C for 40 s. The results were analyzed using the $2^{-\Delta\Delta Ct}$ method of analysis as described by Livak and Schmittgen (2001).

Determination of pERK and NOX4 proteins concentration and malondialdehyde level in kidney tissue homogenate

Quantitative measurement of NOX4 and phosphorylated ERK1/2 (pERK1/2) in kidney tissue homogenate were carried out using NOX4 ELISA kits (cat.no. SEB924Ra cloude-clone corp. USA) and pERK ELISA kits (Instant one™ ELISA, cat.no. 85-86012 eBioSience, Germany) respectively according to manufacturer's instructions. Level of malondialdehyde (marker for lipid per-oxidation, and reflect the level of oxidative stress), was measured using a colorimetric assay with malondialdehyde (MDA) kit (cat no MD 2529) according to manufacturer's instructions.

Statistical analysis

All statistical comparisons were made by means of the one-way ANOVA test followed by Bonferroni post-hoc test. Data were expressed as the mean ± standard error of the mean (SEM) and were analyzed with SPSS statistical software version 20. Chi-square was used to compare histopathological scoring and to find significance between groups. A *p* value of < 0.05 was

considered as statistically significant. Tables and figures were produced using Microsoft Excel 2013 software.

Results

Effect of valsartan treatment on general and metabolic parameters

The effect of valsartan on general and metabolic parameters was shown in Table 1. There was no statistically significant difference in body weight between the three groups at the start point or at the end point of the experiment, although the diabetic group GpII lose weight and treated GpIII group gain weight but the difference was not significant. Fluid and food intake were highly significantly increased in GpII (*p* = 0.001) while fluid and food intake in GpIII was highly significantly reduced (*p* = 0.001).

Rats in GpII showed increased kidney weight that was significantly higher compared to GpI (*p* = 0.001), while kidney weight in GpIII was significantly lowered than GpII (*p* = 0.001). Regarding kidney weight/body weight (KW/BW) ratio, the highest ratio was in GpII, that was significantly higher than GpI (*p* = 0.001). GpIII showed statistically significant lowered KW/BW ratio compared to GpII (*p* = 0.001).

At the end point of the experiment, GpII rats showed significantly elevated blood glucose level (hyperglycemia) (*p* = 0.001) compared to GI and valsartan treated group GpIII which showed significantly reduced blood glucose level (*p* = 0.001) compared to GpII in Fig. 1b.

Effect of valsartan treatment on kidney function

Urine volume/24 h in GpII diabetic rats was significantly increased compared to GpI (*p* = 0.002), while valsartan-treated GpIII showed decreased urine output compared to GpII which was statistically significant (*p* = 0.001), and urine volume of GpIII was slightly elevated than GpI with statistically non-significant difference (*p* = 0.735), Table 2.

Valsartan-treated GpIII rats showed significant improvement of kidney function parameters compared to diabetic non-treated rats GpII, significant low levels of serum creatinine and urea compared to GpII almost approach normal value with a statistically non-significant difference compared to GpI (*p* = 1). Creatinine clearance was significantly elevated in GpIII compared to GPII (*p* = 0.001) and statistically non-significant compared to GI (*p* = 0.140), Fig. 2.

On the other hand, GpII diabetic rats exhibited significant elevated level of kidney function parameters compared to GpI Fig. 2. Urinary creatinine level in GpII displayed marked reduction compared to GpI (*p* = 0.001). GpII creatinine clearance was significantly decreased compared to GpI (*p* = 0.001).

Table 1 General characteristics of the studied groups

Groups variable (mean ± SEM)	GpI	GpII	GpIII
Body weight (g) (start point)	276.4 ± 10.02	266.4 ± 9	261 ± 8
Body weight (g) (end point)	268.35 ± 9.88	248.05 ± 12.21	266.75 ± 10.01
Fluid intake (ml/24 h)	39.66 ± 0.98	214 ± 10.23[**]	64.7 ± 1.18[##]
Food intake (mg/24 h)	29.4 ± 2.4	52.16 ± 1.88[**]	37 ± 1.27[##]
Kidney weight(g)	0.49 ± 0.02	1.03 ± 0.028[**]	0.84 ± 0.038[##]
KW/BW (g/kg)	1.75 ± 0.1	4.3 ± 0.23[**]	3.1 ± 0.17[##]

Values are mean ± SEM, statistically significant difference, **p, ##p ‹ 0.01, (*) compared to GpI: normal group, (#) compared to GpII: DN group. Start point: at the beginning of the experiment before induction of diabetes. End point: at the end of experiment before scarification of rat. *KW* kidney weight, *BW* body weight

Effect of valsartan treatment on histopathological changes in kidney tissues

Histological studies with H&E stains showed normal renal tissues in GpI, with tubular damage score 0, and glomerular damage score 0 (Fig. 3a).

GpII showed glomerular hypertrophy, increased mesangial cells, focal segmental sclerosis in glomerulus, congested blood vessels, and vacuolar degeneration in renal tubules. There were also interstitial inflammatory infiltrate, tubular damage: score 3+, glomerular damage: score 4+; Fig. 3b–d. GpIII showed nearly normal renal tubules, no tubular degeneration, normal Bowman's capsule, and 25%mesangial expansion. There is no interstitial fibrosis, with residual dilatation of few tubules. Tubular damage: score 1+, glomerular damage: score 1+; Fig. 3e, f.

Histological studies (Masson trichrome stained) for assessment of fibrosis showed no evidence of fibrosis, with a score for fibrosis: + 1 in GpI; Fig. 4a. GpII showed pronounced glomerular sclerosis and interstitial fibrosis with a score for fibrosis: + 4; Fig. 4b, c. GpIII showed minimal glomerular sclerosis and no interstitial fibrosis, with a score for fibrosis: + 2; Fig. 4d.

Effect of valsartan treatment on expression level of renal *ERK1/2* and *NOX4* genes

The expression of *NOX4* and *ERK1/2* genes were both statistically significantly decreased in valsartan-treated GpIII compared to GpII ($p = 0.02$, $p = 0.001$) Fig. 5a, b, while their expression were statistically significantly elevated in GpII compared to GpI ($p = 0.032$, $p = 0.003$).
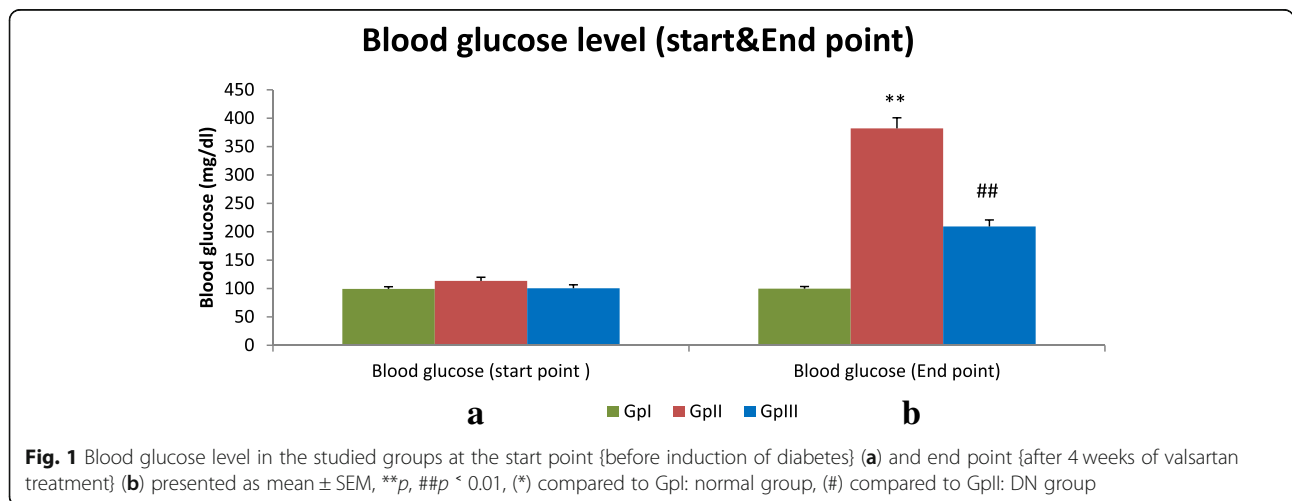
Effect of valsartan treatment on protein concentration of NOX4 and pERK1/2

The highest concentration of NOX4 and pERK1/2 proteins were observed in kidneys of GpII which is statistically significant compared to GpI ($p = 0.001$).

On the other hand, GpIII showed statistically significant decreased NOX4 and pERK1/2 proteins concentration compared to GpII ($p = 0.001$, $p = 0.001$) and non-statistically significant compared to GpI ($p = 1$) Fig. 6a, b.

Effect of valsartan on lipid peroxidation

Malondialdehyde was significantly elevated in GpII compared to GpI ($p = 0.001$), while GpIII showed a significant decrease in MDA concentration compared to

Fig. 1 Blood glucose level in the studied groups at the start point {before induction of diabetes} (**a**) and end point {after 4 weeks of valsartan treatment} (**b**) presented as mean ± SEM, **p, ##p ‹ 0.01, (*) compared to GpI: normal group, (#) compared to GpII: DN group

Table 2 Urine volume/24 h in the studied groups at the endpoint of treatment expressed as (mean ± SEM)

Groups Variable	GI	GII	GIII
Urine volume (ml/24 h)	6.36 ± 0.67	13.93 ± 0.27**	7.10 ± 0.30##

Values are mean ± SEM, Statistically significant difference, **p, ##p ‹ 0.01, (*) compared to GpI: normal group, (#) compared to GpII: DN group

GpII ($p = 0.001$), and statistically non-significant difference from GpI ($p = 0.43$) Fig. 7.

Discussion

Diabetic nephropathy is one of the most common diabetes mellitus complication, with a heterogeneous and complex pathogenesis that involves the interaction between several factors that eventually leads to renal injury. In the present study, we investigated the effect of valsartan on the prognosis of diabetic nephropathy in type 1 diabetes rat model, induced by single STZ
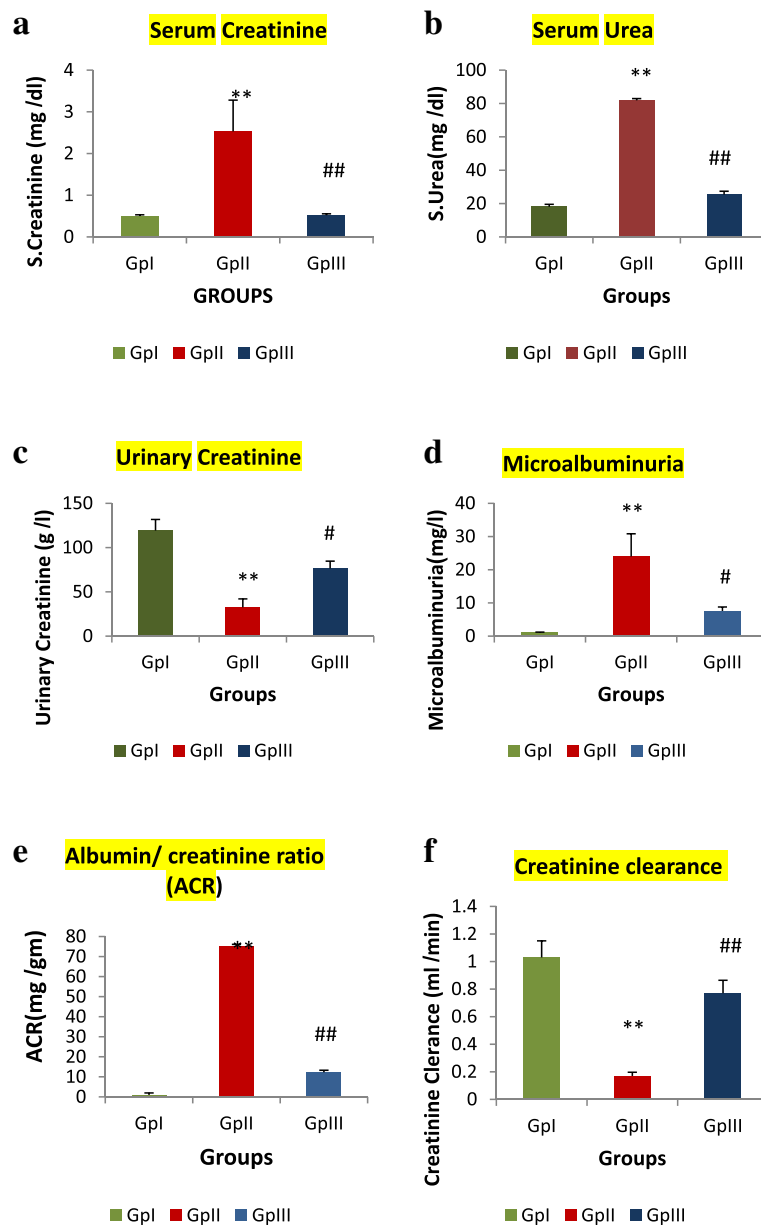
Fig. 2 Mean value of serum creatinine (**a**), serum urea (**b**), urinary creatinine (**c**), microalbuminuria (**d**), albumin creatinine ratio (**e**), and creatinine clearance (**f**), in all studied groups. *p, #p ‹ 0.05, **p, ##p ‹ 0.01, (*) compared to GpI: normal group, (#) compared to GpII: DN group
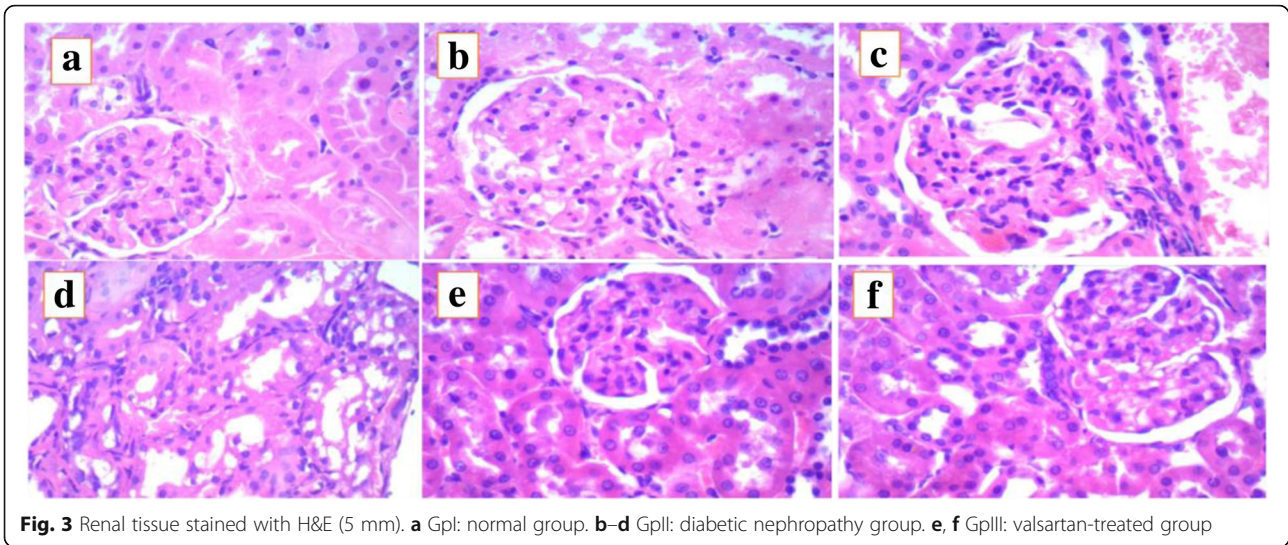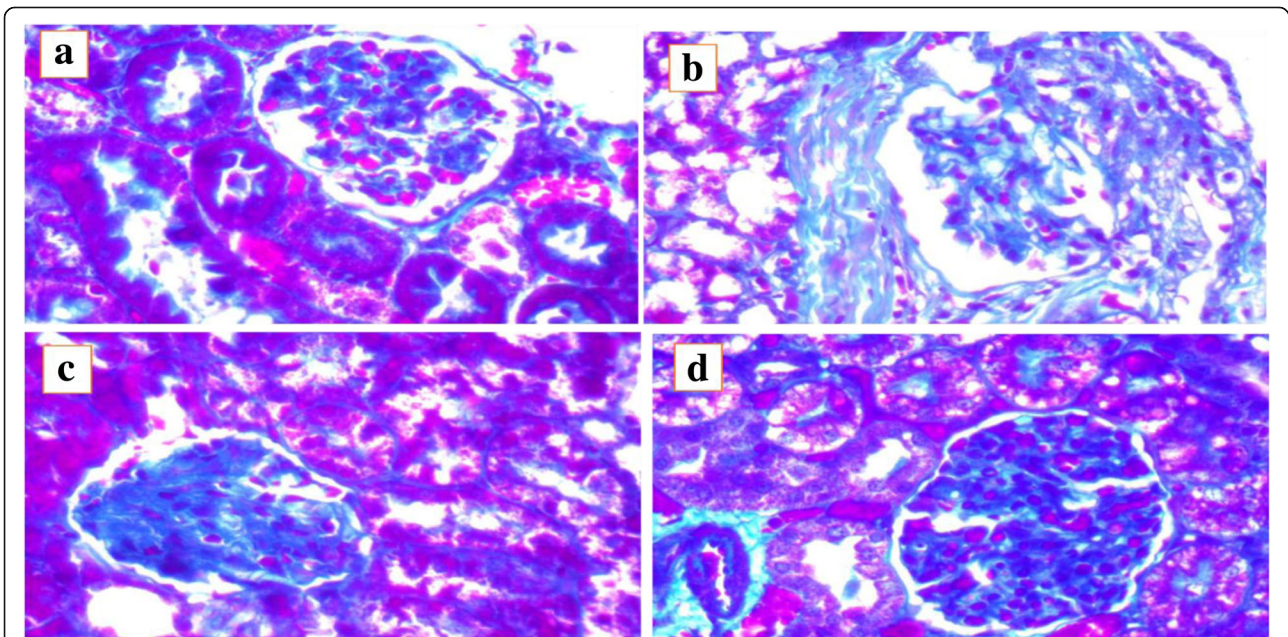
Fig. 3 Renal tissue stained with H&E (5 mm). **a** GpI: normal group. **b–d** GpII: diabetic nephropathy group. **e, f** GpIII: valsartan-treated group
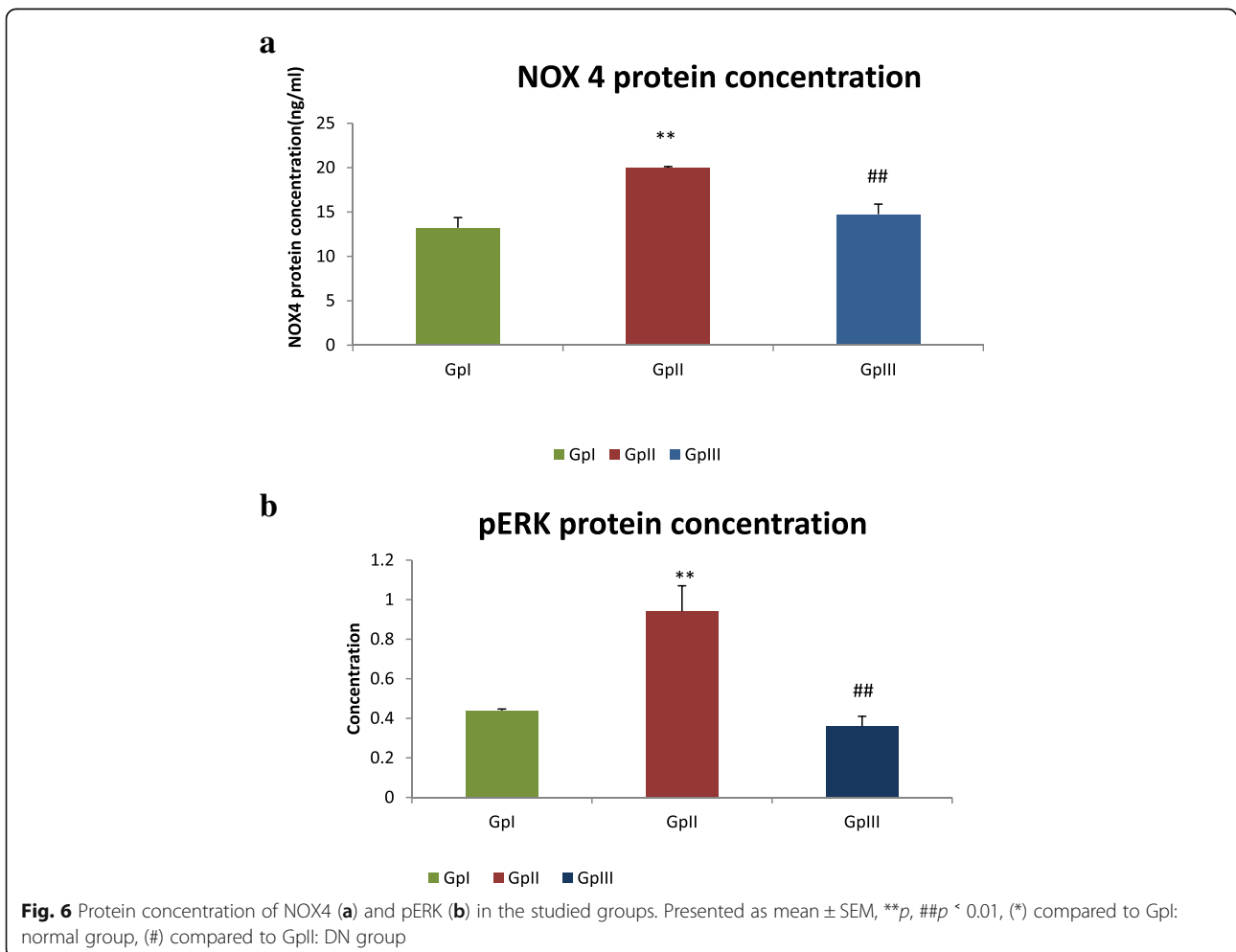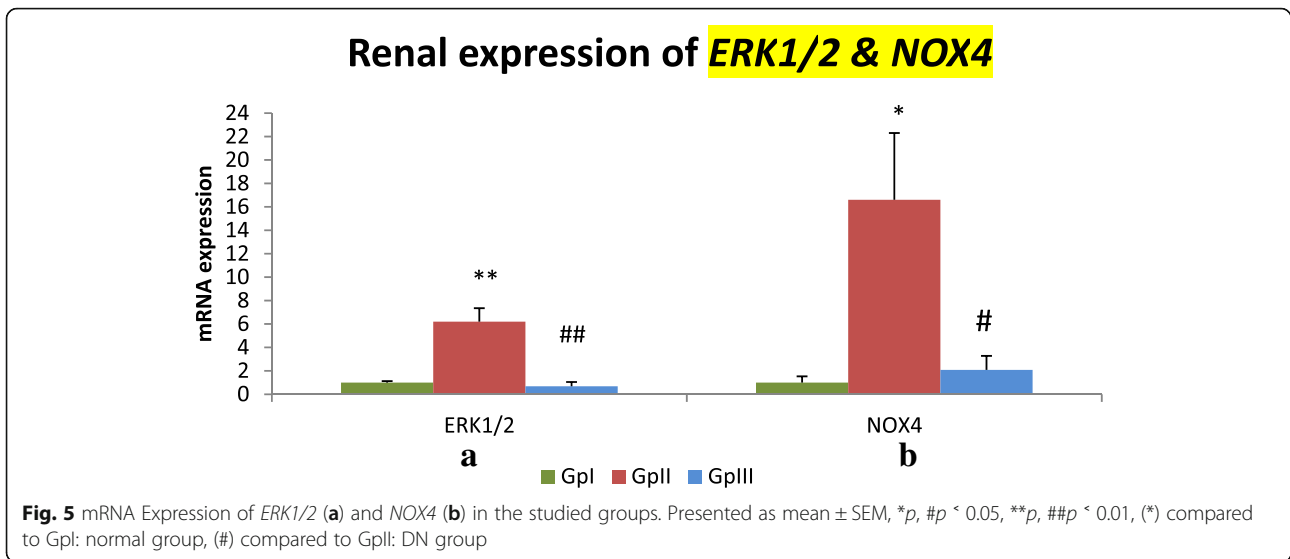
injection (Wu and Yan 2015). The kidneys of diabetic non-treated rats developed advanced pathological changes that were implicated on the kidney function parameters. This diabetic group was characterized by a highly significant increase in proteinuria which considered as a major parameter for monitoring progression of DN, and attributed mainly to glomerular filtration barrier damage (Wang et al., 2014, 2010, 2011).
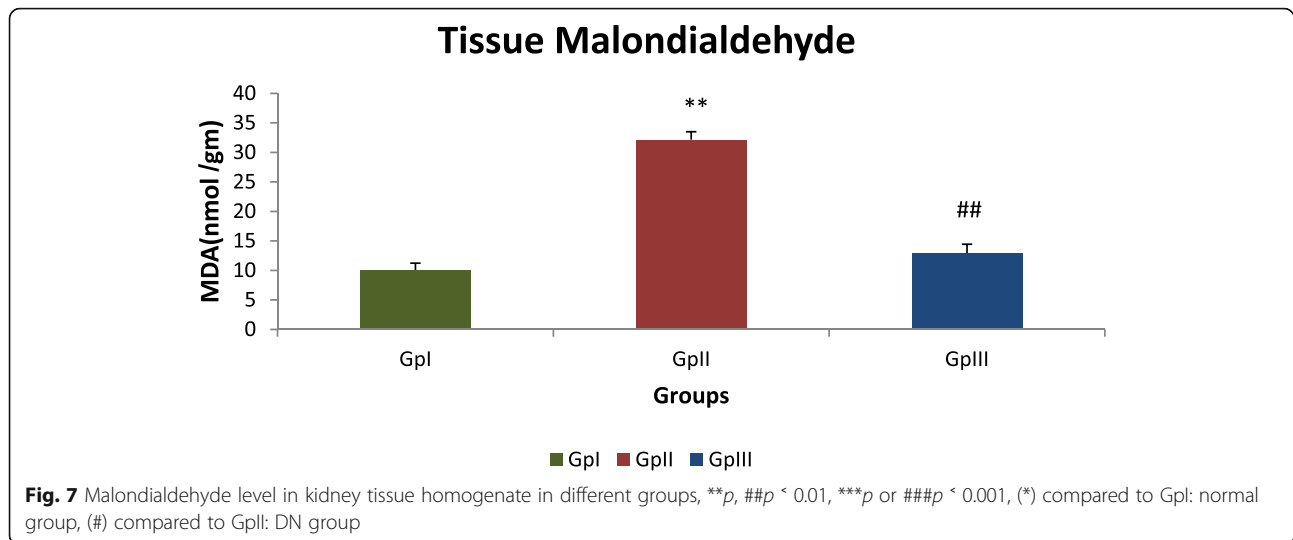
On the other hand, after 4 weeks of valsartan administration, it was evident that it restored the kidney function parameters to nearby normal levels compared to untreated group. In addition, these findings were confirmed by the histopathological study; it was noted that some histopathological changes were comparable to mild DN (class IIA) (Tervaert et al., 2010), manifested by minimal glomerular fibrosis, absence of interstitial fibrosis, nominal sclerosis, and minimal inflammation in treated diabetic rats renal tissues compared to advanced DN (class IV) in non-treated rats. Several studies reported such a beneficial effect of valsartan and other ARBs with different underlying molecular mechanisms. Wang et al., (2014) found that valsartan treatment was

Fig. 4 Renal tissue stained with Masson trichrome (5 mm) from **a** GpI: normal group, **b, c** GpII: diabetic nephropathy group, and **d** GpIII: valsartan-treated group

Fig. 5 mRNA Expression of *ERK1/2* (**a**) and *NOX4* (**b**) in the studied groups. Presented as mean ± SEM, *p, #p ‹ 0.05, **p, ##p ‹ 0.01, (*) compared to GpI: normal group, (#) compared to GpII: DN group

Fig. 6 Protein concentration of NOX4 (**a**) and pERK (**b**) in the studied groups. Presented as mean ± SEM, **p, ##p ‹ 0.01, (*) compared to GpI: normal group, (#) compared to GpII: DN group

Fig. 7 Malondialdehyde level in kidney tissue homogenate in different groups, **p, ##p ‹ 0.01, ***p or ###p ‹ 0.001, (*) compared to GpI: normal group, (#) compared to GpII: DN group

beneficial in protecting kidney injury in diabetes, including improvement of structural and functional changes and inhibition of fibrosis, inflammation, lipid accumulation, and ER stress in the kidney. Gao et al., (2016) reported structural and functional improvement associated with inhibition of Notch pathway and Zhou et al., (2014) related improvement to reduction in podocyte injury and renal NOX-mediated oxidative stress and inflammation. While Chen et al., (2016) relate beneficial effects of irbesartan in DN to its ability to suppress RANKL/RANK and the downstream NF-$_k$B pathway and for olmesartan, Lakshmanan et al., (2012) reveal advantageous effect by modulating MAPK cascade and NOX2 with attenuation of oxidative stress (Lakshmanan et al., 2012; Zhou et al., 2014; Wang et al., 2014; Hartner et al., 2014; Chen et al., 2016; Gao et al., 2016).

In the present study, the molecular mechanisms mediated such effects could be due to the modulation of the activity of NOX4 and pERK in the renal tissues of treated rats; diabetic non-treated rats had significantly elevated NOX4 activity evidenced by a significant increase in the gene expression and the protein concentration, accompanied by high ROS production and high MDA level in renal tissues compared to control group. Surprisingly, valsartan administration maintains NOX4 activity and MDA level within their normal values.

Previous studies confirmed that NOX4 gene expression has been increased in diabetic rodent model animal, along with elevated MDA level and increased H_2O_2 production with subsequent activation of redox-sensitive signaling pathways were previously observed in other studies and such effects were antagonized by NOX4 gene knock down by siRNA and GKT136901 NOX inhibitor (Sedeek et al., 2013; Zhou et al., 2014; Ding et al., 2007; Etoh et al.,

2003; Yaribeygi et al., 2018). In addition, He et al., (2016) found that ROS generation induced by high glucose concentration was mainly derived from NOX4 but not NOX1 or NOX2 in NRK-49f cells and animal model which was reversed by resveratol treatment (He et al., 2016).

Jha et al., (2014) showed that genetic deletion of NOX4 prevent the development of glomerular injury via reduction of renal ROS in diabetic mice (Jha et al., 2014). In addition, in a study on podocyte-specific deletion of NOX4, it was found that NOX4 deletion attenuated albuminuria, glomerulosclerosis, mesangial expansion, and ECM protein deposition and decreased ROS production (Jha et al., 2016), illustrating the role of NOX4 in the development of renal fibrosis.

The ability of valsartan to restrain the expression of NOX4 in renal tissues of type 1 diabetes rodent model was reported by Zhang et al., (2015), in valsartan-treated group, which points to the role of Ang II receptor blockers in inhibiting NOX4 expression in diabetic models. However, Zhou et al., (2014) observed that valsartan treatment had no effect on the elevated NOX4 expression in db/db mice and only reduce NOX2 expression and activity. The reason for this discrepancy may be related to the different animal models used (Zhou et al., 2014; Zhang et al., 2015).

In the present study, valsartan-treated rats develop significant reduction in blood glucose level compared to non-treated DN group. The hypoglycemic effect of valsartan was observed in other studies and could be attributed to increase glucose utilization in peripheral tissues, especially muscles, through upregulation of glucose transporter 4 (GLUT4) (Guan et al., 2015; Chan et al., 2003), along with reduction in both hepatic and renal gluconeogenesis via reduce activity of phosphoenolpyruvate carboxykinase (PEPCK) via the downregulating effect of

valsartan on NOX4-derived ROS and ERK1/2 (Winiarska et al., 2011, 2015). Conversely, several studies reported that valsartan had no effect on blood glucose level in treated compared to non-treated group; one proposed explanation for the controversy regarding valsartan effect on blood glucose may be due to experimental changes to model used (Zhou et al., 2014; Wang et al., 2014; Zhang et al., 2015; Gao et al., 2016; Sun et al., 2015).

Previous data declared that hyperglycemia could increase the expression of *NOX4* mRNA in both direct and indirect pathways. The direct effect is through activation of the NF-kB pathway, mediated by p62/PKζ interaction (Xi et al., 2015). The indirect effect achieved through induction of Ang II, that affect the mitochondrial K_{ATP} channels thereby simulates the expression of mitochondrial Nox4, results in abrupt production of mitochondrial superoxide and H_2O_2 in renal tubular cells (Kim et al., 2012). Similarly, ERK pathway is considered as one of the redox signaling sensitive pathways that could be activated by ROS directly or via hyperglycemia (He et al., 2016; Robinson and Cobb 1997; Fakhruddin et al., 2017; Manda et al., 2015). H_2O_2 mediate ligand-independent phosphorylation and activation of epidermal growth factor receptors, leading to activation of mitogen-activated protein 3 kinase (MAP3K), which results in ERK activation (Meves et al., 2001).

Another possible mechanism for ERK activation by ROS may comprise the inactivation and degradation of the mitogen kinase phosphatase (MKPs) that keep the pathway in an inactive state (Kamata et al., 2005). In addition, Choi et al., showed that hyperglycemia, through glutamate-induced oxidative stress, induces sustained activation of ERK pathway through a mechanism that involves degradation of MKP-1 (Choi et al., 2006).

It was previously reported that ERK1/2 was the main mediator of renal fibrosis, ECM deposition, inflammation, and proliferation in high glucose environment through regulation of TGF-B and SMAD pathways (Ni et al., 2015; Cheng et al., 2013; He et al., 2016; Abe et al., 2002), and has an important role in glomerular-endothelial-mesenchymal transition that characterizes DN (He et al., 2015; Shang et al., 2017).

In the present study, the *ERK1/2* mRNA expression as well as pERK1/2 protein concentration were significantly elevated in diabetic non-treated group compared to control. A result that was consistent with other previous studies (Sedeek et al., 2013; Lakshmanan et al., 2012; Cheng 2013). Moreover, following valsartan administration, *ERK1/2* mRNA expression and pERK1/2 protein concentration were significantly reduced, and such an effect was most likely mediated through Ang II blockage, NOX4-derived oxidative stress reduction, and the hypoglycemic effect.

Conclusions

In conclusion, valsartan could protect the kidney from the deleterious effect of type 1 diabetes-associated nephropathy progression through decreasing oxidative stress, ERK1/2, and NOX4 activity. Early interventions by valsartan to decrease the rate of GFR loss could prolong the time to development of diabetic nephropathy and ultimately end-stage renal disease (ESRD).

Abbreviations
Ang II: Angiotensin II; DN: Diabetic nephropathy; ERK: The extracellular regulated kinase pathway; ESRD: End stage renal disease; MCs: Mesangial cells; MDA: Malondialdehyde; MKPs: Mitogen kinase phosphatase; NADPH: Nicotinamide adenine dinucleotide phosphate; NOX4: NADPH oxidase 4; PEPCK: Phosphoenolpyruvate carboxykinase; RAS: Intrarenal renin–angiotensin system; ROS: Reactive oxygen species; STZ: Streptozotocin

Availability of data and materials
The data used to support the findings of this study are available from the corresponding author upon request.

Authors' contributions
SHAM, LATE, and DIB conceived and designed the experiments. SHAM collected samples and data. SHAM and LATE performed the experiments. DIB analyzed the data. SAM, LATE, and DIB wrote the manuscript. SHAM, LATE, DIB, and TIE were responsible for the final revision and approval of the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate
All the experimental protocols were approved by the institutional research ethics committee at Faculty of Medicine, Suez Canal University.

Consent for publication
Not applicable

Competing interests
The authors declare that they have no competing interests.

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