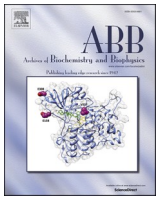
Archives of Biochemistry and Biophysics 751 (2024) 109851

Contents lists available at ScienceDirect

Archives of Biochemistry and Biophysics

journal homepage: www.elsevier.com/locate/yabbi



Effect of angiotensin-converting enzyme inhibitor and angiotensin receptor blocker on oxidative stress and metabolism of elements in kidney of STZ-induced diabetic rats

Nural Pastacı Özsobacı [a,*], Metehan Karataş [b], Matem Tunçdemir [b], Derviş Özcelik [a,c]

[a] Department of Biophysics, Cerrahpasa Medical Faculty, Istanbul University- Cerrahpasa, Fatih, Istanbul, Turkiye
[b] Department of Medical Biology, Cerrahpasa Medical Faculty, Istanbul University- Cerrahpasa, Fatih, Istanbul, Turkiye
[c] Department of Biophysics, Medical Faculty, Istanbul Arel University, Istanbul, Turkiye

ARTICLE INFO

Keywords:
ACE inhibitor
AT1 receptor blocker
Diabetes
Diabetic nephropathy
Oxidative stress
Trace elements

ABSTRACT

In diabetes, increased oxidative stress and impaired trace element metabolism play an important role in the pathogenesis of diabetic nephropathy. The objective of this research was to examine the outcomes of blocking the renin-angiotensin system, using either the angiotensin-converting enzyme inhibitor (ACEI), perindopril, or the angiotensin II type 1 (AT1) receptor blocker, irbesartan, on oxidative stress and trace element levels such as Zn, Mg, Cu, and Fe in the kidneys of diabetic rats that had been induced with streptozotocin.

Thirty-two Wistar albino male rats were equally divided into four groups. The first group was used as a control. The second group of rats developed diabetes after receiving a single intraperitoneal dose of STZ. The third and fourth groups of rats had STZ-induced diabetes and received daily dosages of irbesartan (15 mg/kg b.*w/day*) and perindopril (6 mg/kg b.*w/day*) treatment, respectively.

Biochemical analysis of the kidneys showed a distinct increase in oxidative stress, indicated by heightened levels of malondialdehyde (MDA) and decreased superoxide dismutase (SOD) activities, as well as reduced glutathione (GSH) levels in the kidneys of diabetic rats. In the kidneys of diabetic rats, the mean levels of Fe and Cu were found to be significantly higher than those of the control group. Additionally, the mean levels of Zn and Mg were significantly lower in the diabetic rats compared to the control rats. Both perindopril and irbesartan decreased significantly MDA content and increased SOD activities and GSH levels in the kidneys of rats with diabetes. The Zn and Mg concentrations in the kidneys of diabetic rats treated with perindopril and irbesartan were markedly higher than in untreated STZ-diabetic rats, while the Cu and Fe concentrations were significantly lower. The urinary excretion of rats treated with perindopril and irbesartan showed a pronounced increase in Cu levels, along with a significant reduction in Zn and Mg levels. Although diabetic rats demonstrated degenerative morphological alterations in their kidneys, both therapies also improved diabetes-induced histopathological modifications in the kidneys.

Finally, the present results suggest that manipulating the levels of Zn, Mg, Cu, and Fe - either through ACE inhibition or by blocking AT1 receptors - could be advantageous in reducing lipid peroxidation and increasing antioxidant concentration in the kidneys of diabetic rats.

1. Introduction

Diabetes mellitus is associated with various complications including nephropathy, and it is one of the most life-threatening diseases. Chronic hyperglycemia causes an increase in oxidative stress due to a disparity between the generation and elimination of reactive oxygen species (ROS) [1]. Several mechanisms contribute to the development of oxidative stress in diabetes, but the main factor appears to be hyperglycaemia, commonly termed “glucose toxicity” [2]. The main source of radicals under hyperglycemic conditions are glucose auto-oxidation, activation of the polyol pathway and non-enzymatic glycation of proteins [3–5].

Micronutrients may impact the removal of protectants or pollutants as they are integral components of crucial enzymes in intracellular

* Corresponding author. Department of Biophysics, Cerrahpasa Medical Faculty, Istanbul University- Cerrahpasa, TR-34303, Cerrahpasa, Istanbul, Turkiye.
E-mail address: n.pastaciozsobaci@iuc.edu.tr (N. Pastacı Özsobacı).

https://doi.org/10.1016/j.abb.2023.109851
Received 28 August 2023; Received in revised form 8 October 2023; Accepted 3 December 2023
Available online 6 December 2023

antioxidant defence systems. The imbalance caused by their scarcity or abundance can lead to a pro-oxidant/antioxidant imbalance, thus promoting the growth of secondary problems as the illness advances. Micronutrients are involved in complex processes in the development of secondary complications of diabetes-mellitus in many different areas. These could potentially act as complimentary components of different antioxidant enzymes (such as Cu, Zn, and Mn superoxide dismutase and Se in glutathione dehydrogenase), cofactors in crucial enzymatic processes within glucose and lipid metabolisms (such as Mn and Cu), or potential pro-oxidant catalysers (such as Cu and Fe). There is debate in the literature regarding the role of various factors, including alterations to the homeostasis of certain trace and transition elements, such as Fe and Cu, in the development and prognosis of the condition [6,7].

Recent studies have shown that the kidneys' ROS reactions have a particular inclination towards oxidative stress actions, which are believed to be a contributing factor to the pathogenesis of diabetic nephropathy [1–3]. The increasing lipid peroxidation and reduced antioxidant enzyme activity are found to be associated with the albuminuria prognosis in diabetic rats [8,9]. Patients with diabetes often experience impaired kidney function, resulting in an inability to adequately process water, sodium, and glucose [10].

Activation of the intrarenal renin-angiotensin system (iRAS) has also been implicated as a key player in the pathogenesis of diabetic nephropathy. The angiotensin-converting enzyme (ACE) is a critical regulator of the iRAS and a major contributor to renal damage [11].

Several studies have investigated the beneficial nephroprotective effects of angiotensin-converting enzyme inhibitors (ACEIs). Research has shown that the use of ACEIs or AT1 receptor blockers (ARBs) to block Angiotensin II can reduce protein oxidation and lipid peroxidation while enhancing antioxidant enzyme activity in models of diabetes both in animals and humans. It was also shown that these medicines have antioxidant properties. It is well-established that AT1 receptor antagonists reduce proteinuria and decelerate the advancement of diabetic nephropathy [12–15]. Angiotensin receptor blockers do not prevent the production of angiotensin II. Instead, they occupy the high-affinity type 1 AT1 receptor of this substance, thereby preventing its ability to exert its biological activity [16]. Several studies have demonstrated that using Irbesartan, an AT1 receptor blocker, leads to a notable reduction in the rate at which albumin is excreted [17,18], as well as reduction in renal mass in STZ-diabetic rats [18,19]. The positive impact of various ACEIs, including perindopril, on the progression of nephropathy in experimental diabetes has been investigated. The reduction of kidney weight and microalbuminuria levels, as well as the protective impact on the renal tissue, has been demonstrated in diabetic rats treated with perindopril [20]. Furthermore, there is clinical and experimental evidence indicating that ACEIs and ARBs have renoprotective benefits in addition to their antihypertensive properties, reducing the progression of diabetic nephropathy [19,21,22]. However, the mechanism of the nephroprotective effects could not be fully revealed. Some potential advantages of the use of ACEIs and ARBs in diabetes may be associated with their effects on the content of minerals like basic metals.

Therefore present study designed to examine the potential regulatory impact of perindopril, an ACE inhibitor, and irbesartan, an AT1 receptor blocker, on renal element concentrations in diabetic rats. For this purpose, it was aimed to evaluate element levels and lipid peroxidation status in streptozotocin (STZ)-induced diabetic rats by measuring the ion concentrations of Zn, Cu, Fe, Mg and malondialdehyde (MDA) levels as well as the activity of antioxidant enzymes, superoxide dismutase (SOD) and non-enzymatic antioxidant glutathione (GSH) levels in the kidney tissues of the rats before and after perindopril and irbesartan treatment.

2. Materials and methods

2.1. Animals

Thirty-two male Wistar albino rats weighing between 210 and 240g were obtained from the Animal Research Laboratory (ARL) of the University of Istanbul. The animals were contained at the ARL and allowed to feed ad libitum on a commercially available rat chow (*Eris Chow Industry, Istanbul, Turkey*). The Animal Welfare Act and the Istanbul University Guide for the Care and Use of Laboratory Animals were followed in the maintenance and use of the animals. Starting three days following the injection of STZ, the experiments were conducted over a 4-week period.

2.2. Experimental design

Rats were randomized into four groups ($n = 8$ *in each group*). **The first group** was the non-diabetic control group (C) in which non-diabetic, unsupplemented rats were given 0.9 % w/v saline by gastric gavage.

Diabetes was induced through an intraperitoneal injection of streptozotocin (*STZ; Sigma, St. Louis, MO, USA*) at a dose of 60 mg/kg body weight, dissolved in 0.9 % sodium chloride buffer immediately prior to administration. After injection, animals had free access to food and water. To confirm the diabetic state, after 3 days of STZ injection fasting blood samples were obtained and fasting blood glucose was determined (>300 mg/dl). The rats with blood glucose of 350 mg/dL were considered to be diabetic. Hyperglycemic rats were used for the experiment and classified as follows: **The second group** was the untreated STZ-diabetic group (STZ). **The third group** (STZ + Irb) were STZ-diabetic rats treated with irbesartan (*Sanofi, TURKEY*) dissolved in 0.9 % w/v saline (15 mg/kg b.*w/day; for 30 days*) via gastric gavage. **The fourth group** (STZ-D + Per) were STZ-diabetic rats treated with perindopril (*Servier, TURKEY*) dissolved in 0.9 % w/v saline (6 mg/kg b.*w/day, 30 days*) via gastric gavage. On the 1st and 28th day of the experiment, all rats were kept individually in metabolic cages for the collection of 24 h urine samples and urine volume was measured.

Once the experimental period is finished, the animals were euthanized under anesthesia after that kidney tissue samples were quickly excised and stored at −70 °C for further experiments.

2.3. Evaluation of blood glucose and microalbuminuria

On the first and the 28th day of the experiment, sample collected from the tail vein were used to measure the blood glucose levels with glucose test reagent strips and a glucometer manufactured by Accu-Check Active, Roche, Germany. The microalbuminuria levels were assessed using Micral urine test strips (*Micral test II urine strips, Roche Diagnostics GmbH, Germany*) on the same days that 24-h urine samples were collected.

2.4. Body and kidney weights

The body weight of the rats was recorded at the beginning and end of the experimental period. At the end of the experiment the rats euthanized under anesthesia and dissected to obtain the kidneys. Each rat's left kidney was weighed and noted.

2.5. Histological analysis

At the end of the experiments, rats were anesthetized, and renal tissue samples were obtained for morphological studies before fixation in neutral formalin, followed by embedding in paraffin. Periodic Acid Schiff (PAS) staining were used for histological examinations. Without being aware of the source group, the tissues were examined under a light microscope.

2.6. Preparation of kidney samples for lipid peroxidation and antioxidant analyses

The kidneys used for this procedure were twice bathed in a cold

saline solution, transferred into glass bottles, labelled, and maintained in a deep freezer (−70 °C) without no longer than 10 h. After being weighed, half of the kidney samples were placed on ice, cut into small pieces and homogenized for 2 min at 5000 rpm in 1:5 w/v of ice-cold 50 mM, pH 7.4 Tris–HCl buffer using a glass Teflon homogenizer. The homogenates, which were still on ice, were mixed with 4-L/mL butylhydroxytoluene and utilized to measure the levels of malondialdehyde (MDA), glutathione (GSH), and superoxide dismutase (SOD) immediately.

2.7. Assessment of lipid peroxidation and protein levels

Lipid peroxidation was determined by measuring the MDA levels in tissue homogenates through the thiobarbituric acid reactivity assay using a method previously described using an extinction coefficient $\varepsilon = 01.56 \times 105\ M^{-1}\ cm^{-1}$ at a wavelength $\lambda = 532$ nm [23]. Micromoles were used to express the tissue MDA levels per gram of protein. The Lowry et al. method [24] was used to determine the amount of protein in kidney tissue. Bovine serum albumin used as the standard.

2.8. Superoxide dismutase (SOD) activity

Cu–Zn-superoxide dismutase (Cu–Zn-SOD) activity was determined by the method of Sun et al. [25]. The assay involves inhibition of nitroblue tetrazolium (NBT) and reduction with xanthine-xanthine oxidase (Sigma Co., St.Louise, USA) that was used as a superoxide generator. One unit of SOD is defined as the amount of protein that inhibits the rate of NBT reduction by 50 %. Tissue SOD activities were expressed as units *per* milligram of protein.

2.9. Glutathione (GSH) level

Tissue GSH concentrations were determined according to the method of Beutler et al. [26]. using metaphosphoric acid for protein precipitation and 5′,5′-dithiobis(2-nitrobenzoic acid) for color development. GSH concentration was calculated using $1.36 \times 104\ M^{-1}\ cm^{-1}$ as the molar absorption coefficient. Tissue GSH concentrations were expressed as μmol *per* gram of protein. Biochemical studies were performed using an autoanalyzer (Hitachi 717).

2.10. Measurement of element levels in kidney tissue

From each animal, 0.5–1 g of tissue was extracted for this experiment. After wet-ashing of tissues with nitric and perchloric acid, the liver and kidney tissue Zn, Cu, Fe and Mg levels were studied by atomic absorption spectrophotometry photometry (Shimadzu AA-680, Japan) with an air-acetylene flame. The tissue concentrations of the element are expressed as micrograms *per* gram of wet weight [27].

2.11. Urine collection and metal analysis

Urine samples from control rats and STZ-diabetic rats were collected during experiments. All rats from each group were housed in metal-free metabolic cages in order to collect 24-h urine samples. The urine volumes were measured, and samples were centrifuged at 3000×*g* for 10 min to remove bacteria, cells, casts and other particulate materials. Urine samples were stored at −20 °C. The levels of Zn, Cu and Mg in urine were measured by atomic absorption spectrophotometry (Shimadzu AA-680,Japan) with an air-acetylene flame.

2.12. Statistical analysis

The findings are shown as means standard deviation (SD). The statistical analysis of the data was performed by using SPSS Statistical program (v 10.0, SPSS Inc.Chicago, Illinois, USA). The Mann-Whitney *U* test was applied to analyze the significance of differences between the four groups. The significance level was set at $p < 0.05$.

3. Results

3.1. General metabolic and renal parameters

General metabolic and renal parameters are summarized in Table 1. After three days following STZ injection the rats showed a significant increase ($p < 0.01$) in blood glucose levels, as expected. After four weeks from the STZ injection, STZ-diabetic rats exhibited significantly higher daily urine output levels and microalbuminuria amounts compared to the control group ($p < 0.001$). Kidney weight was significantly reduced in both treated groups (STZ + Irb and STZ + Per) as compared to the untreated-STZ-diabetic group ($p < 0.01$, $p < 0.001$, *respectively*). Significant reduction in the levels of daily urine output ($p < 0.05$) and microalbuminuria ($p < 0.001$) were observed in both STZ + Irb and STZ + Per groups compared to the STZ-diabetic group. The blood glucose values and body weight levels were not significantly different in both treatment groups as compared to the untreated-STZ-diabetic group.

3.2. Renal oxidative stress biomarkers

Renal MDA levels were significantly increased ($p < 0.001$), whereas levels of the nonenzymatic antioxidant GSH($p < 0.01$) and activities of the antioxidant enzymes SOD ($p < 0.01$) were significantly decreased in untreated-STZ-diabetic rats compared with controls (Fig. 1 A,*B,C*). The treatments of both irbesartan and perindopril resulted in a significant decrease of renal MDA ($p < 0.05$, $p < 0.01$, respectively) (Fig. 1A) and significantly elevated renal GSH levels ($p < 0.05$) and significantly increased renal SOD activity ($p < 0.05$) as compared to the STZ-diabetic group (Fig. 1B and C).

3.3. Changes in Zn, mg, Cu and Fe levels

The levels of Fe and Cu in the kidney of the diabetic rats were significantly higher ($p < 0.01$, $p < 0.001$, *respectively*) than in the control rats (Fig. 2B, D). On the other hand, the levels of Zn and Mg were significantly lower ($p < 0.01$) in the kidney of the diabetic rats, in comparison to the control rats (Fig. 2 A,C).

Analysis of the effect of STZ-induced diabetes revealed that the Zn and Mg concentrations significantly increased in the daily urine output of the diabetic rats ($p < 0.01$, $p < 0.001$, *respectively*) (Fig. 2 A,C), while the concentrations of Cu in the diabetics were slightly lower ($p < 0.05$) than in the control rats (Fig. 2C).

Treatments of both irbesartan and perindopril resulted in a significant decrease of renal Fe ($p < 0.05$) and Cu ($p < 0.05$, $p < 0.01$, *respectively*) (Fig. 2 B,D) and significantly elevated renal Zn and Mg levels ($p < 0.01$, $p < 0.05$, *respectively*) as compared to the STZ-diabetic group (Fig. 2 A,C). Both treatments tended to normalize urinary excretion and element levels. There was a significant decrease in urinary Zn and Mg concentration of STZ + Irb and STZ + Per groups as compared to the STZ-diabetic group ($p < 0.001$) (Fig. 2 A,C), while the concentrations of Cu in the STZ + Irb and STZ + Per group were significantly higher ($p < 0.05$) than in the STZ-diabetic group (Fig. 2B).

3.4. Histopathology

The kidneys of nondiabetic rats showed normal histopathological structure (Fig. 3A). In contrast, in untreated-STZ-diabetic rats exhibited glomerular basal membrane thickening, hypertrophic glomeruli, tubular degeneration and dilatations (Fig. 3B). In STZ + Irb (Fig. 3C) and STZ + Per groups (Fig. 3D) glomerular pathology and tubulointerstitial injury were improved, although some cortical and medullary tubules are still degenerated.

Table 1
General metabolic and renal parameters in Control (**C**), untreated-STZ-diabetic (**STZ**), irbesartan-treated (**STZ + Irb**) and perindopril-treated (**STZ + Per**) diabetic rats.

Groups(n = 8)	Blood glucose *(mg/dl)*	Body weight *(g)*	Microalbuminuria *(mg/l/24 h)*		Urine output *(cc)*	Kidney weight *(mg)*	
C	***start***	95.50 ± 3.89	221.01 ± 9,84	1.00 ± 0,00	14.12 ± 2.69		891.25 ± 77.54
	end	95.50 ± 3.85	234.50 ± 5,07	1.00 ± 0,00	15.75 ± 0.70[b]		
STZ	***start***	426.01 ± 39.21[a]	222.12 ± 6.08	1.00 ± 0,00	14.37 ± 2.13		1191.25 ± 73.18[a]
	end	485.12 ± 52.95[a]	199.02 ± 8.71[a]	2.12 ± 0,35[a]	49.00 ± 2.72		
STZ + Irb	***start***	433.25 ± 43.5[a]	223.37 ± 10.36	1.00 ± 0,00	14.37 ± 2.13		1030.12 ± 65.74[a,c]
	end	482.72 ± 63.45[a]	189.75 ± 0.93[a]	1.62 ± 0,51[d]	21.50 ± 3.25[b]		
STZ + Per	***start***	439.12 ± 4.43[a]	228.37 ± 6.09	1.00 ± 0,00	14.50 ± 2.61		975.75 ± 67.85[d]
	end	470.75 ± 45.92[a]	206.00 ± 23.12[a]	1.12 ± 0,35 [d]	24.37 ± 2.92 [b]		

start; *the beginning of the experiment,* ***end;*** *the end of the experiment. Values were given as mean ± SD.*
[a]p < 0.001 vs. non-diabetic control group (C), [b]p < 0.05, [c]p < 0.01, [d]p < 0.001; vs. untreated-diabetic group(STZ).

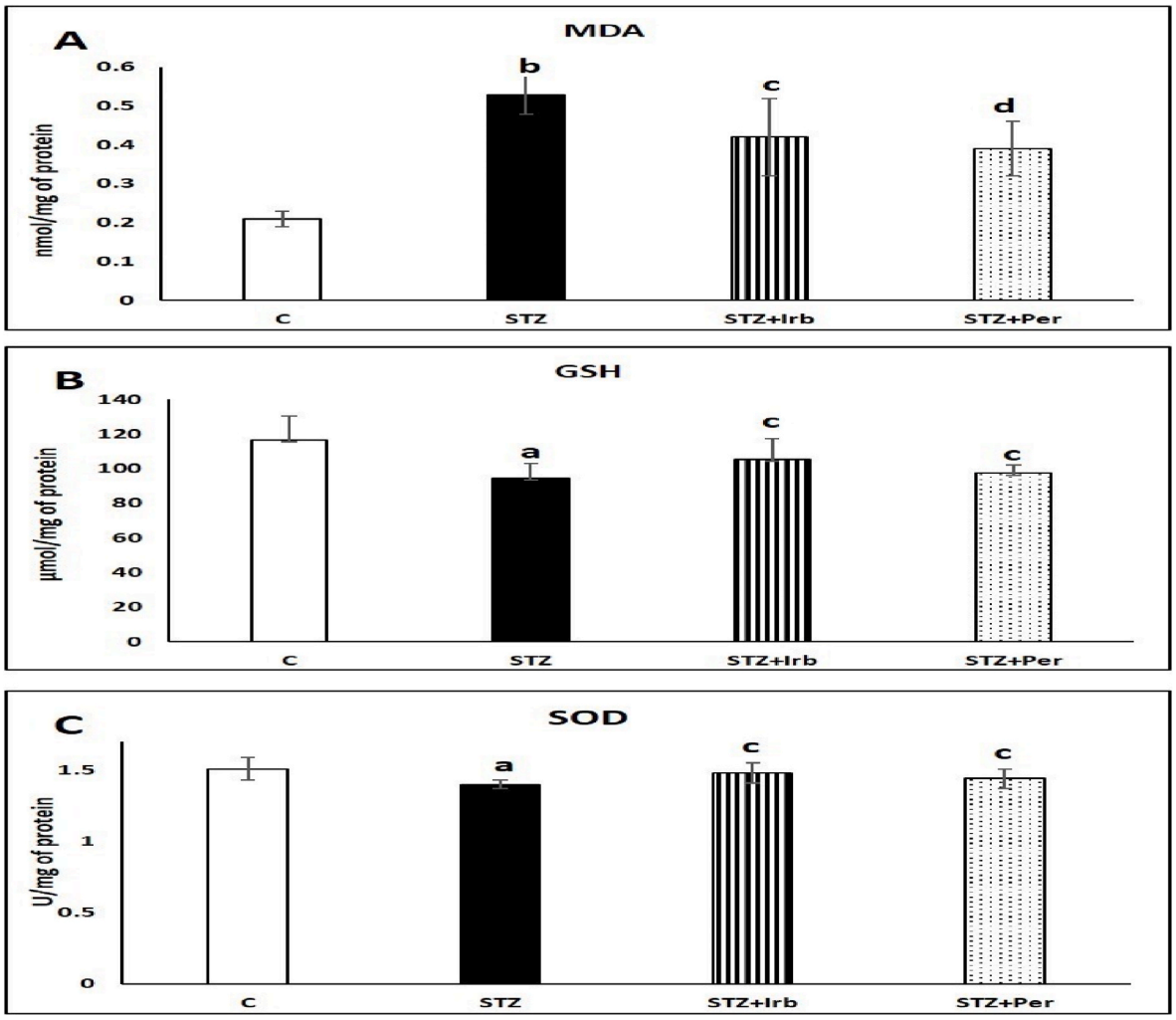


Fig. 1. Changes in renal oxidative stress biomarkers malondialdehyde (MDA) (A), Glutathione (GSH) levels (B) and Superoxide dismutase (SOD) enzymatic activity (C), in Control (C), untreated-STZ-diabetic (STZ), irbesartan-treated (STZ + Irb) and perindopril-treated (STZ + Per) diabetic rats. [a]p<0.01, [b]p < 0.001, vs Control; [c]p < 0.05,[d]p < 0.01 vs. untreated-STZ-diabetic group.

4. Discussion

The current research reveals that rats injected with STZ exhibited common symptoms of diabetes mellitus, including hyperglycemia, polyuria, and developmental delay. Furthermore, it is apparent that there is an increase in urinary albumin excretion in diabetic rats compared to control rats. This is consistent with other reports [28,29] showing a significant increase in oxidative stress, which can increase renal damage in rats and lead to a progressive increase in urinary albumin excretion. The results of this study also showed that the mean levels of Zn and Mg in the renal tissue of diabetic rats were significantly lower than in the control group. The mean levels of Fe and Cu were found significantly higher in the kidneys of the diabetic rats compared to control rats, in agreement with our previous results [30] and those of Greń et al. [31].

In the present study, however, the levels of Zn and Mg were also found to be significantly higher, while the level of Cu was significantly lower in the urinary excretion with STZ-diabetic rats. We were not able to measure the iron values of the rats' urine due to limited amounts of the collected urine. Disturbances in the metabolism of trace elements are

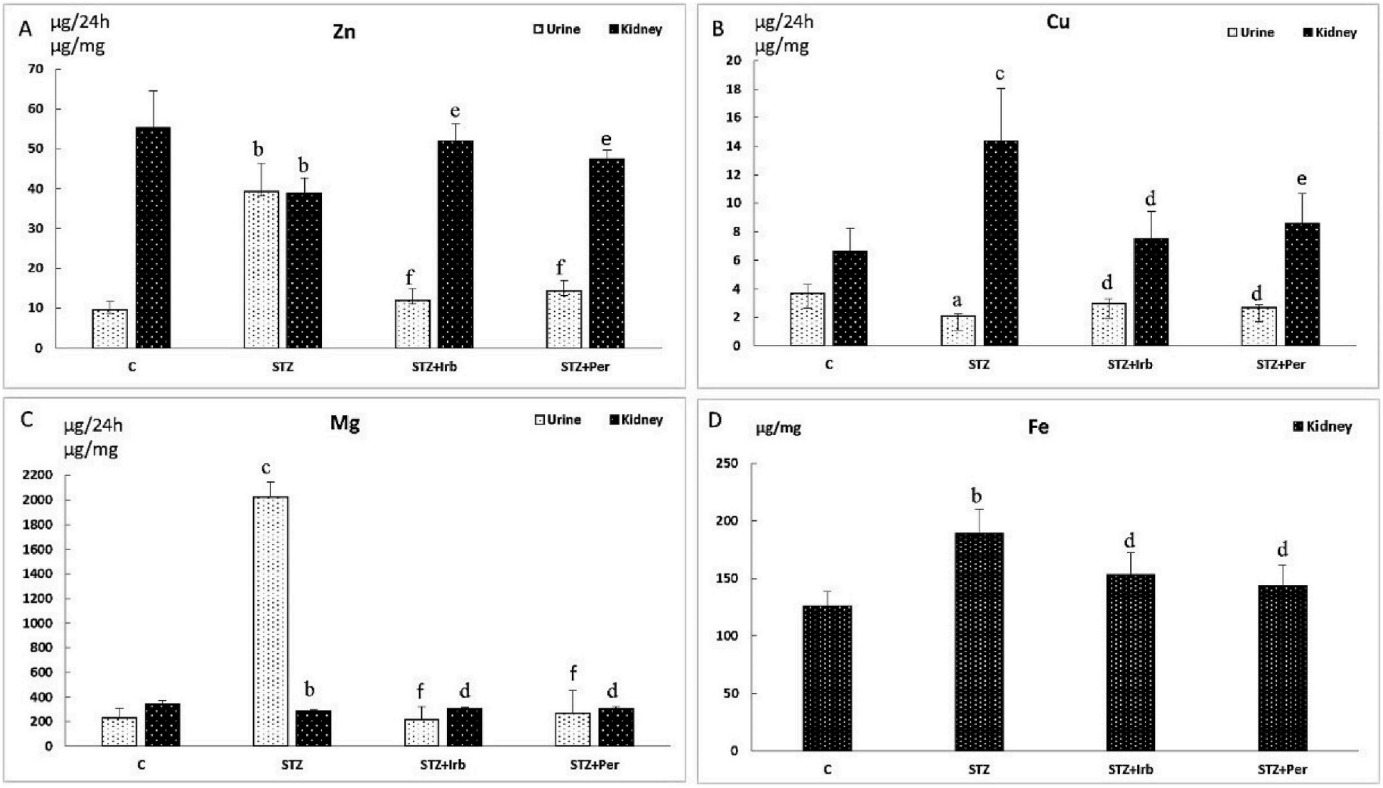


Fig. 2. Changes in (□) urine (µg/24h) and (■) kidney (µg/mg) concentrations of Zinc (Zn) (**A**), Copper (Cu) (**B**), Magnesium (Mg) (**C**) and Iron (Fe) (**D**) in Control (C), untreated-STZ-diabetic (**STZ**), Irbesartan -treated (**STZ + Irb**) and perindopril-treated (**STZ + Per**) diabetic rats. $^{a}p<0.05$, $^{b}p < 0.01$, $^{c}p < 0.001$, vs. control; $^{d}p < 0.05$, $^{e}p < 0.01$, $^{f}p < 0.001$vs.untreated-STZ-diabetic group.

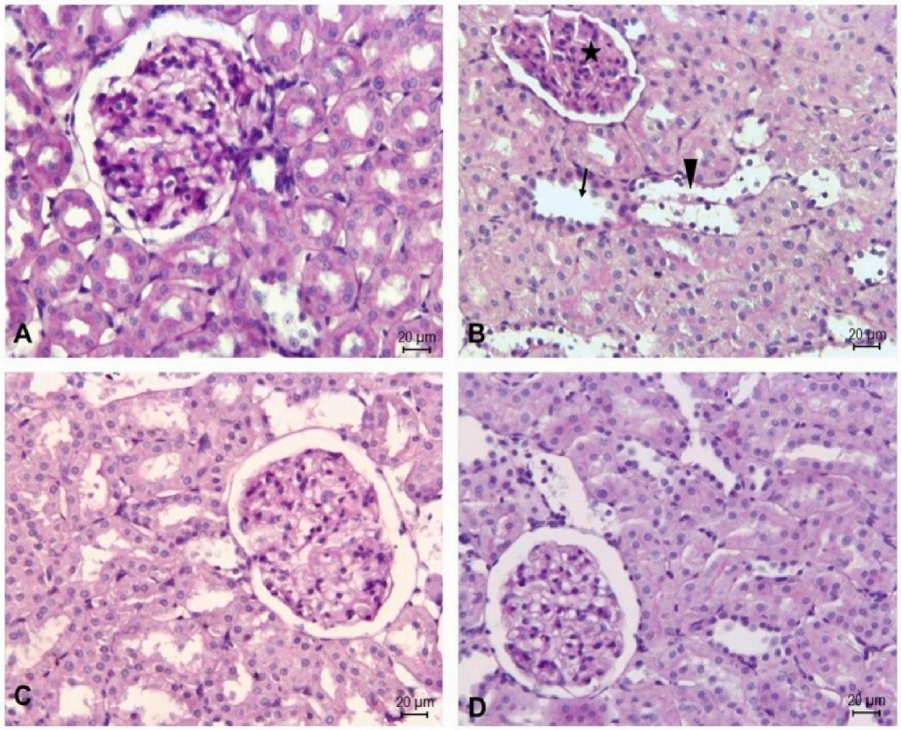


Fig. 3. Light photomicrographs of PAS-stained sections of kidney tissues in experimental groups; non-diabetic control group (A), untreated diabetic (B), Irbesartan treated diabetic group (C) and perindopril-treated diabetic group (D). The asterisk **(*)** indicates glomeruli, arrow **(→)** shows tubular dilatations and arrowhead (▶) tubular degenerations. (Bar: 20 µm).

observed in diabetics. It has been reported that the urinary excretion of calcium, zinc and magnesium is increased in two types of diabetes mellitus, leading to a decrease in blood levels of these elements in these patients [30,32,33].

We also found a significantly increased oxidative stress in the kidneys of STZ-diabetic rats as compared to the control rats. STZ treatment caused many changes in renal antioxidant levels, including an increase in MDA, a decrease in GSH, and antioxidant enzymes such as SOD, which represent the presence of excess peroxides and hydroxyl radicals in diabetic kidneys and are responsible for impaired renal function.

The increased lipid peroxidation and reduced activity of antioxidant enzymes in the kidneys of STZ-diabetic rats revealed in this study are consistent with previous findings and support the hypothesis that free radicals play an important role in the pathophysiology of diabetic nephropathy [3,34]. The decrease of antioxidants may be due to the deficiency of kidneys zinc and magnesium levels in diabetic rats. These observations are supported by the findings that Zn and Mg have antioxidant activities, not only because they form the active sites and/or stabilise the conformation of several antioxidant enzymes, but also because they compete for iron and copper binding sites and can provide protection against transition metal-mediated and free radical-induced damage [35]. Previous researchers have suggested an association between diabetes and various micronutrients, including Mg, Cr, Fe, Zn, Cu, etc. In addition, many studies have shown increased lipid peroxidation in clinical and experimental diabetes [7,36,37]. The results of our previous studies show that impaired ion metabolism of some elements may play a role in the progression of diabetic oxidative complications [30]. Critical elements are essential components of several key enzymes in intracellular antioxidant defence and can have protective or scavenging effects. Their deficiency or excess can result in a shift in the pro-oxidant/antioxidant balance, leading to additional problems as the disease progresses [38].

Hyperzincuria and poor intestinal absorption of zinc in diabetic patients indicate that they are more prone to zinc deficiency, which may be caused by hyperglycaemia, reduced intestinal absorption or increased urinary Zn loss [30,39]. Increased intracellular oxidants and free radicals, as well as deficits in intracellular zinc and zinc-dependent antioxidant enzymes, may be associated with certain diabetic problems [40]. Zinc reduces the severity of diabetes-related problems in a variety of animal models. It also plays a crucial role in controlling the function of the islets of Langerhans [41] and their protective effect against diabetic kidney tissue damage by stimulating metallothionein production and regulating oxidative stress [42].

Magnesium is a cofactor of several enzymes involved in carbohydrate oxidation and plays a vital role in the cell membrane glucose transport mechanism. Magnesium salts have been shown to be effective in the treatment of non-insulin dependent diabetes [43]. Prabodh et al. [44] found that Mg had a significant negative association with cases of diabetic nephropathy.

Changes in metal concentrations in the body occur as a result of the action of reactive oxygen species, but on the other hand, some metals can induce the formation of free radicals, which may be involved in the development of diabetes [45]. The transition metals Fe and Cu are integral components of important enzymes involved in vital biological processes. There is suggestive evidence that Fe plays a pathogenic role in diabetes and its complications such as atherosclerosis. Excess Fe has been implicated in the pathogenesis of diabetes and its complications [46]. The increased levels of Cu, a transition metal that is redox-active and catalyzes lipid peroxidation, may enhance oxidation of low-density lipoproteins, causing increased levels of TBARS in diabetic patients as reported by Heinecke et al. [47].

In the excess of certain metals (particularly Fe or Cu ions), a hydroxyl radical, which is the most powerful ROS, can be produced *via* the Fenton or the metal-catalysed Haber-Weiss reaction [48]. These two chemical reactions appear to account for the majority of the hydroxyl radical production in biological systems and explain, at least in part, why metals such as Fe and Cu induce oxidative stress and ROS-induced damage in cells. Tissue accumulation of transition metal ions is extremely toxic and leads to many pathological conditions associated with oxidative damage to biological membranes and molecules [49].

Angiotensin-converting enzyme inhibitors are a class of drugs that have been shown to protect the kidneys. They may reduce the progression of diabetic nephropathy by regulating haemodynamics in the renal glomerulus, reducing proteinuria and slowing the progression of diabetic nephropathy. Similarly, AT1 receptor antagonists have a similar effect in reducing proteinuria and delaying the progression of diabetic nephropathy, despite the absence of bradykinin effects [19,21,22]. However, the mechanism of their action is not fully understood. As these drugs are commonly used in the treatment of diabetic nephropathy, their effect on Zn, Mg, Cu and Fe concentrations may be additive, resulting in a more pronounced concentration of these elements in diabetic rat tissues.

To the best of our knowledge, no prospective studies have investigated the effect of treatment with the ACEIs and ARBs on renal tissue concentrations of Zn, Mg, Cu and Fe in STZ diabetics.

In this prospective study, we examined the hypothesis that irbesartan, an AT1 receptor blocker, and perindopril, an ACE inhibitor, may have an effect on elemental metabolism by investigating the effects of these drugs on Zn, Mg, Cu and Fe levels in the renal tissue of STZ-diabetic rats. We used the 15 mg/kg dose of irbesartan, an AT1 receptor antagonist, because other studies [17,50] have shown that this dose reduces systemic blood pressure, regulates renal haemodynamic changes and reduces intraglomerular pressure. On the other hand, we have used the 6 mg/kg dose of perindopril, an ACEI, in another group of STZ diabetics. Kelly et al. [51] and Yao et al. [52] showed that in this dose range perindopril reduced systemic blood pressure, decreased urinary albumin excretion rate and protected renal function in normotensive early diabetic nephropathy and diabetic rats. Our data show that there are statistically significant changes in kidney tissue and urinary Zn, Mg, Cu and Fe concentrations.

In the current study, the concentrations of Zn and Mg were found to be significantly higher in the kidneys of diabetic rats treated with perindopril and irbesartan than in the untreated STZ-diabetic rats, whereas the concentrations of Cu and Fe were significantly lower in the treated rats compared to all tissues observed in the untreated STZ-diabetic rats. The levels of Zn and Mg were also significantly lower, while the level of Cu was significantly higher in the urine excreted with perindopril and irbesartan treatment. The loss of these minerals may be attributed to the impaired absorption and/or the excess excretion of these metals in the urine of diabetic patients, which may lead a deficiency or marginal state of these minerals in the blood of diabetic patients [53]. We suppose that the increased kidney tissue levels of Zn and Mg may be due to the decrease in urinary excess by these drugs as well as a possible action of this on the renal cation transporters. Tubek [54] showed that urinary excretion of Zn is normalised in patients with primary arterial hypertension after treatment with perindopril.

In our study, the effect of ACE inhibitor and AT1 receptor blockers on the investigated parameters of oxidative stress in the diabetic kidney tissues were also studied. Our studies revealed that perindopril and irbesartan significantly decreased MDA levels and increased SOD activities and GSH levels after 4 weeks of therapy. These findings are in agreement with the results of Kedziora–Karnatowska [12] who showed that both enalapril and losartan reduced lipid peroxidation in the kidneys of diabetic rats during the early stages of the development of diabetic nephropathy. ACE inhibitors have also been shown to reduce lipid peroxidation in diabetic rat tissues [13] and to increase antioxidant defences in mouse tissues [14]. Irbesartan administration attenuated the increased lipid peroxidation and decreased the antioxidant defence mechanism along with altering renal glomerular filtration rate. This is in line with other observations that chronic treatment with candesartan and losartan, AT1 receptor antagonists, can attenuate oxidative stress and alter the renal function of experimentally induced diabetic animals

[15]. Mastan et al. [55] have reported that Angiotensin-II blockage by the ACEIs (i.e Captopril, Enalapril, Lisinopril) have been shown to increase total antioxidant status (TAS) and reduce total oxidative status (TOS) in the serum during the diabetes, especially in the captopril treated diabetic group. It has also been reported that enalapril, an ACEI, has beneficial effects on the increased oxidative stress in the diabetic rats. To investigate the relationship between the RAS and antioxidant defences, Cavanagh et al. [56] have been administered enalapril to STZ-induced diabetic rats. The results of this study showed that total glutathione and antioxidant enzyme activities were higher in the heart, kidney and liver of enalapril-treated rats than in untreated diabetic rats.

As previously described, the kidneys of STZ rats showed morphological changes such as thickening of the glomerular basal membrane, hypertrophic glomeruli, tubular degeneration and dilatation [57]. The results revealed that diabetic animals treated with irbesartan and perindopril showed a significant improvement in renal function, together with an attenuation of increased renal oxidative stress and morphological changes. It has been reported in another study that treatment with irbesartan can prevent the changed morphology in diabetic kidneys [18, 58]. This finding is consistent with previous research showing that the ACE inhibitor enalapril and the AT1 receptor blocker valsartan can prevent renal impairment and reduce oxidative stress in diabetics [59, 60].

The data from our present study confirm the role of oxidative stress in the development of diabetic nephropathy in the first stage of diabetes development and suggest the possible antioxidant mechanism of the nephroprotective effect of both angiotensin receptor antagonists and angiotensin-converting enzyme inhibitors.

In conclusion, the results of the present study indicate an imbalance in the levels of some elements, including Cu, Fe, Zn and Mg, in the kidney tissue of STZ-diabetic rats. In addition, oxidative stress parameters such as MDA levels are higher in kidney tissue, while the activity of the antioxidant enzyme SOD and the non-enzymatic antioxidant GSH levels are reduced in the tissue. The disturbed oxidative balance in tissues could be affected by the increase in Fe and Cu and the decrease in Zn and Mg levels in the kidneys of STZ-induced diabetic rats. Therefore, all these findings may help to explain the role of impaired ion metabolism of some elements in the progression of diabetic oxidative complications.

In the present study, we have shown in an experimental diabetic animal model that treatment with both irbesartan, an AT1 receptor blocker, and perindopril, an ACE inhibitor, effectively regulate the levels of these elements (Zn, Mg, Cu and Fe) and improve antioxidant levels in the kidney, resulting in a delay in the progression of diabetic nephropathy. Finally, the present results suggest that modulation of the same elements (Zn, Mg, Cu and Fe) by either ACE inhibition or AT1 receptor blockade may be beneficial in reducing lipid peroxidation and increasing antioxidant levels in diabetic rat kidneys.

Preprint of this article

Dervis Ozcelik & Matem Tuncdemir. Effect of Angiotensin-Converting Enzyme Inhibitor and Angiotensin Receptor Blocker on Oxidative Stress and Metabolism of elements in Kidney of STZ-Induced Diabetic Rats. Preprint at https://doi.org/10.21203/rs.3.rs-1746678/v1 (2022).

Author contribution

D.O formulated the present hypothesis. All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by D.Ö, M.T, NPO and M.K. The frst draft of the manuscript was written by D.O, and all authors read and approved the final manuscript.

Funding

The present work was supported by the Istanbul University Scientifc Research Projects Unit (Project No: 283/18062003).

Ethical approval

All experiments were approved by the Istanbul University Cerrahpaşa Faculty of Medicine Ethics Committee for Animal Experiments, and followed the NIH Guide for the Care and Use of Laboratory Animals (1738-23062006).

Declaration of competing interest

No conflicts of interest, financial or otherwise are declared by the author(s). All authors approved the final manuscript.

References

[1] D.V. Ratnam, D.D. Ankola, V. Bhardwaj, D.K. Sahana, M.N. Kumar, Role of antioxidants in prophylaxis and therapy: a pharmaceutical perspective, J. Contr. Release 113 (3) (2006) 89–207.
[2] A. Ceriello, Acute hyperglycemia and active stress generation, Diabet. Med. 4 (Suppl. 3) (1997) S45–S49.
[3] J.W. Baynes, Role of oxidative stress in the development of complications in diabetes, Diabetes 40 (4) (1991) 405–412.
[4] Y. Zheng, X.K. Li, Y. Wang, L. Cai, The role of zinc, copper and iron in the pathogenesis of diabetes and diabetic complications: therapeutic effects by chelators, Hemoglobin 32 (1–2) (2008) 35–45.
[5] W. Wei, Q. Liu, Y. Tan, L. Liu, X. Li, L. Cai, Oxidative stress, diabetes and diabetic complication, Hemoglobin 33 (5) (2009) 370–377.
[6] J.W. Eaton, M. Qian, Interactions of copper with glycated proteins: possible involvement in the etiology of diabetic neuropathy, Mol. Cell. Biochem. 234–235 (1–2) (2002) 135–142.
[7] A. Viktorínová, E. Toserová, M. Krizko, Z. Duracková, Altered metabolism of copper, zinc, and magnesium is associated with increased levels of glycated hemoglobin in patients with diabetes mellitus, Metabolism 58 (1) (2009) 1477–1482.
[8] R. Kakkar, J. Kalra, S.V. Mantha, K. Prasad, Lipid peroxidation and activity of antioxidant enzymes in diabetic rats, Mol. Cell. Biochem. 151 (2) (1995) 113–119.
[9] K.Z. Kedziora-Kornatowska, M. Luciak, J. Blaszczyk, W. Pawlak, Lipid peroxidation and activities of antioxidant enzymes in erythrocytes of patients with non-insulin dependent diabetes with or without diabetic nephropathy, Nephrol. Dial. Transplant. 13 (11) (1998) 2829–2832.
[10] H. Vlassara, M. Torreggiani, J.B. Post, F. Zheng, J. Uribarri, G.E. Striker, Role of oxidants/inflammation in declining renal function in chronic kidney disease and normal aging, Kidney Int. Suppl. 114 (2009) S3–S11.
[11] R. Toto, B.F. Palmer, Rationale for combination angiotensin receptor blocker and angiotensin-converting enzyme inhibitor treatment and end-organ protection in patients with chronic kidney disease, Am. J. Nephrol. 28 (3) (2008) 372–380.
[12] K. Kedziora-Kornatowska, Effect of angiotensin convertase inhibitors and AT1 angiotensin receptor antagonists on the development of oxidative stress in the kidney of diabetic rats, Clin. Chim. Acta 287 (1–2) (1999) 19–27.
[13] K.Z. Kedziora-Kornatowska, M. Luciak, J. Paszkowski, Lipid peroxidation and activities of antioxidant enzymes in the diabetic kidney: effect of treatment with angiotensin convertase inhibitors, IUBMB Life 49 (4) (2000) 303–307.
[14] E.M. de Cavanagh, F. Inserra, L. Ferder, C.G. Fraga, Enalapril and captopril enhance gluthatione-dependent antioxidant defenses in mouse tissues, Am. J. Physiol. Regul. Integr. Comp. Physiol. 278 (3) (2000) R572–R577.
[15] D. Yavuz, B. Küçükkaya, G. Haklar, O. Ersöz, E. Akoğlu, S. Akalin, Effects of captopril and losartan on lipid peroxidation, protein oxidation and nitric oxide release in diabetic rat kidney, Prostaglandins Leukot. Essent. Fatty Acids 69 (4) (2003) 223–227. Oct.
[16] M.R. Weir, V.J. Dzau, The renin-angiotensin-aldosterone system: a specific target for hypertension management, Am. J. Hypertens. 12 (12 Pt 3) (1999) 205S–213S. Dec.
[17] F. Bonnet, M.E. Cooper, H. Kawachi, et al., Irbesartan normalises the deficiency in glomerular nephrin expression in a model of diabetes and hypertension, Diabetologia 44 (2001) 874–877.
[18] B.C. Liu, D.D. Luo, J. Sun, K.L. Ma, X.Z. Ruan, Influence of irbesartan on renal hypertrophy and thickening of glomerular basement-membrane in streptozotocin-induced diabetic rats, Zhonghua Nei Ke Za Zhi 42 (5) (2003) 320–323.
[19] M. Tunçdemir, M. Oztürk, The effects of angiotensin-II receptor blockers on podocyte damage and glomerular apoptosis in a rat model of experimental streptozotocin-induced diabetic nephropathy, Acta Histochem. 113 (8) (2011) 826–832.
[20] S.P. Ertürküner, M. Başar, M. Tunçdemir, İ. Seçkin, The comparative effects of perindopril and catechin on mesangial matrix and podocytes in the streptozotocin induced diabetic rats, Pharmacol. Rep. 66 (2) (2014) 279–287. Apr.

[21] E. Ripley, Complementary effects of angiotensin converting enzyme inhibitors and angiotensin receptor blockers in slowing the progression of chronic kidney disease, Am. Heart J. 157 (Suppl 6) (2009) S7–S16.
[22] C.E. Mogensen, M.E. Cooper, Diabetic renal disease: from recent studies to improved clinical practice, Diabet. Med. 21 (1) (2004) 4–17.
[23] Z.A. Placer, L.L. Cushman, B.C. Johnson, Estimation of products of lipid peroxidation (malonyl dialdehyde) in biological fluids, Anal. Biochem. 16 (2) (1966) 359–364.
[24] O.H. Lowry, N.J. Rosebrough, A.L. Farr, R.J. Randall, Protein measurement with the Folin-Phenol reagent, J. Biol. Chem. 193 (1) (1951) 265–275.
[25] Y. Sun, L.W. Oberley, Y. Li, A simple method for clinical assay of superoxide dismutase, Clin. Chem. 34 (3) (1988) 497–500.
[26] E. Beutler, O. Duran, B.M. Kelly, Improved method for the determination of blood glutathione, J. Lab. Clin. Med. 61 (1963) 882–888.
[27] M.S. Clegg, C.L. Keen, B. Lönnerdal, L.S. Hurley, Influence of ashing techniques in the analyses of trace elements in animal tissue, Biol. Trace Elem. Res. 3 (2) (1981) 107–115.
[28] H. Ha, K.H. Kim, Role of oxidative stress in the development of diabetic nephropathy, Kidney Int. Suppl. 5 (1995) 18–21.
[29] M. Anjaneyulu, K. Chopra, Quercetin, an antioxidant bioflavonoid, attenuates diabetic nephropathy in rats, Clin. Exp. Pharmacol. Physiol. 31 (4) (2004) 244–248.
[30] D. Ozcelik, M. Tuncdemir, M. Ozturk, H. Uzun, Evaluation of trace elements and oxidative stress levels in the liver and kidney of streptozotocin-induced experimental diabetic rat model, Gen. Physiol. Biophys. 30 (4) (2011) 356–363.
[31] A. Greń, G. Formicki, Effects of Iscador and vincristine and 5-fluorouracil on brain, liver, and kidney element levels in alloxan-induced diabetic mice, Biol. Trace Elem. Res. 152 (2) (2013) 219–224.
[32] M.D. Doşa, L.T. Hangan, E. Crauciuc, C. Galeş, M. Nechifor, Influence of therapy with metformin on the concentration of certain divalent cations in patients with non-insulin-dependent diabetes mellitus, Biol. Trace Elem. Res. 142 (1) (2011) 36–46.
[33] J.J. Cunningham, A. Fu, P.L. Mearkle, R.G. Brown, Hyperzin-curea in individuals with insulin-dependent diabetes mellitus: concurrent zinc status and the effect of high dose zinc supplementation, Metabolism 43 (12) (1994) 1558–1562.
[34] S.A. Wohaieb, D.V. Godin, Alterations in free radical tissue defense mechanisms in streptozotocin-induced diabetes in rat, Diabetes 36 (9) (1987) 1014–1018.
[35] N.W. Alcock, Trace elements, in: A.L. Kahn, A.J. Pesce (Eds.), Clinical Chemistry, Theory, Analysis and Correlation, Mosby-Year Book, St.Louis, 1996, pp. 746–759.
[36] L.K. Niskanen, J.T. Salonen, K. Nyyssönen, M.I. Uusitupa, Plasma lipid peroxidation and hyperglycemia: a connection through hyperinsulinaemia? Diabet. Med. 12 (9) (1995) 802–808.
[37] R. Kakkar, S.V. Mantha, J. Radhi, K. Prasad, J. Kalra, Increased oxidative stress in rat liver and pancreas during progression of streptozotocin-induced diabetes, Clin. Sci. 94 (6) (1998) 623–632.
[38] T. Fukada, S. Yamasaki, K. Nishida, M. Murakami, T. Hirano, Zinc homeostasis and signaling in health and diseases: zinc signaling, J. Biol. Inorg. Chem. 16 (7) (2011) 1123–1134.
[39] SalgueiroMJ, N. Krebs, M.B. Zubillaga, R. Weill, E. Postaire, A.E. Lysionek, by, et al., Zinc and diabetes mellitus: is there a need of zinc supplementation in diabetes mellitus patients? Biol. Trace Elem. Res. 81 (3) (2001) 215–228.
[40] S.R. Powell, The antioxidant properties of zinc, J. Nutr. 130 (5S Suppl) (2000) 1447S, 54S.
[41] E. Mocchegiani, R. Giacconi, M. Malavolta, Zinc signalling and subcellular distribution: emerging targets in type 2 diabetes, Trends Mol. Med. 14 (10) (2008) 419–428.
[42] D. Ozcelik, M. Naziroglu, M. Tunçdemir, Ö. Çelik, M. Öztürk, M.F. Flores-Arce, Zinc supplementation attenuates metallothionein and oxidative stress changes in kidney of streptozotocin-induced diabetic rats, Biol. Trace Elem. Res. 150 (1–3) (2012) 342–349.
[43] D.P. Chaudhary, R. Sharma, D.D. Bansal, Implications of magnesium deficiency in type 2 diabetes: a review, Biol. Trace Elem. Res. 134 (2) (2010) 119–129.
[44] S. Prabodh, D.S. Prakash, G. Sudhakar, N.V. Chowdary, V. Desai, R. Shekhar, Status of copper and magnesium levels in diabetic nephropathy cases: a case-control study from South India, Biol. Trace Elem. Res. 142 (1) (2011) 29–35.
[45] M. Naziroğlu, D.M. Dikici, S. Dursun, Role of oxidative stress and Ca(2+) signaling on molecular pathways of neuropathic pain in diabetes: focus on TRP channels, Neurochem. Res. 37 (10) (2012) 2065–2075.
[46] M.C. Thomas, R.J. MacIsaac, C. Tsalamandris, G. Jerums, Elevated iron indices in patients with diabetes, Diabet. Med. 21 (7) (2004) 798–802.
[47] J.W. Heinecke, H. Rosen, A. Chait, Iron and copper promote modification of low density lipoprotein by human arterial smooth muscle cells in culture, J. Clin. Invest. 74 (5) (1994) 1890–1894.
[48] M.G. Repetto, N.F. Ferrarotti, A. Boveris, The involvement of transition metal ions on iron-dependent lipid peroxidation, Arch. Toxicol. 84 (4) (2010) 255–262.
[49] D. Ozcelik, H. Uzun, Copper intoxication; antioxidant defenses and oxidative damage in rat brain, Biol. Trace Elem. Res. 127 (1) (2009) 45–52.
[50] Z. Cao, F. Bonnet, B. Davis, T.J. Allen, M.E. Cooper, Aditive hypotensive and anti-albuminuric effects of angiotensin-converting enzyme inhibition and angiotensin receptor antagonism in diabetic spontaneously hypertensive rats, Clin. Sci. (Lond.) 100 (6) (2001) 591–599. Jun.
[51] D.J. Kelly, A.J. Cox, M. Tolcos, et al., Attenuation of tubular apoptosis by blockade of the renin-angiotensin system in diabetic Ren-2 rats, Kidney Int. 61 (2002) 31–39.
[52] B. Yao, G. Hu, Y. Li, Z. Liao, Y. Zhang, The effect of perindopril in treatment of early diabetic nephropathy with normal blood pressure and microalbuminuria, Zhonghua Nei Ke Za Zhi 40 (12) (2001) 826–828. Dec.
[53] M.A. Abou-Seif, A.A. Youssef, Evaluation of some biochemical changes in diabetic patients, Clin. Chim. Acta 346 (2) (2004) 161–170.
[54] S. Tubek, Differences in selected zinc metabolism parameters in obese and normal-weight hypertensive patients following treatment with spironolactone, Biol. Trace Elem. Res. 114 (1–3) (2006) 135–141.
[55] Y. Mastan Rao, I. Aparna Lakshmi, M. Saroja, Effect of ACE inhibitors on antioxidant status in streptozotocin induced diabetic rats, Asian J. Pharmaceut. Clin. Res. 4 (1) (2011) 134–137.
[56] E.M. de Cavanagh, F. Inserra, J. Toblli, I. Stella, C.G. Fraga, L. Ferder, Enalapril attenuates oxidative stress in diabetic rats, Hypertension 38 (5) (2001) 1130–1136.
[57] T. Sanai, T. Sobka, T. Johnson, M. el-Essawy, E.C. Muchaneta-Kubara, O. Ben Gharbia, by, et al., Expression of cytoskeletal proteins during the course of experimental diabetic nephropathy, Diabetologia 43 (1) (2000) 91–100.
[58] M. Anjaneyulu, K. Chopra, Effect of irbesartan on the antioxidant defence system and nitric oxide release in diabetic rat kidney, Am. J. Nephrol. 24 (5) (2004) 488–496.
[59] B. Kalender, M. Oztürk, M. Tunçdemir, O. Uysal, F.K. Dagistanli, I. Yegenaga, by, et al., Renoprotective effects of valsartan and enalapril in STZ-induced diabetes in rats, Acta Histochem. 104 (2) (2002) 123–130.
[60] T.K. Motawi, S.A. El-Maraghy, M.A. Senousy, Angiotensin-converting enzyme inhibition and angiotensin AT1 receptor blockade downregulate angiotensin-converting enzyme expression and attenuate renal injury in streptozotocin-induced diabetic rats, J. Biochem. Mol. Toxicol. 27 (7) (2013) 378–387. Jul.