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Article *in* Free Radical Research · July 2016

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To cite this article: Sousou Ibrahim Ali, Naglaa Fathy Alhusseini, Hebatallah Husseini Atteia, Reham Abd El-Satar Idris & Rehab Abdallah Hasan (2016) Renoprotective effect of a combination of garlic and telmisartan against ischemia/reperfusion-induced kidney injury in obese rats, Free Radical Research, 50:9, 966-986, DOI: 10.1080/10715762.2016.1211644

To link to this article: http://dx.doi.org/10.1080/10715762.2016.1211644

Accepted author version posted online: 13 Jul 2016.
Published online: 08 Aug 2016.

FREE RADICAL RESEARCH, 2016
VOL. 50, NO. 9, 966–986
http://dx.doi.org/10.1080/10715762.2016.1211644

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ORIGINAL ARTICLE

Renoprotective effect of a combination of garlic and telmisartan against ischemia/reperfusion-induced kidney injury in obese rats

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ABSTRACT

Obesity enhances the frequency and severity of acute kidney injury (AKI). Telmisartan pre-treatment was used experimentally in the amelioration of ischemia/reperfusion (IR)-induced AKI. However, there is a lack of evidence regarding its beneficial effects on AKI in obese animals. The present study, therefore, aimed to explore the protective effects of garlic and/or telmisartan against renal damage induced by unilateral IR in obese rats. Meloxicam was used as a standard anti-inflammatory agent. Prophylactic oral administration of meloxicam (3 mg kg^{-1}), garlic (500 mg kg^{-1}) and/or telmisartan (5 and 10 mg kg^{-1}) for 4 wk protected against renal function deterioration induced by IR in obese rats. Both doses of telmisartan significantly reduced serum total cholesterol and triacyglycerol levels as well as peri-renal adipocytes size and renal fibrosis. Renal nuclear factor-kappa B immunoreactivity, tumor necrosis factor-alpha content as well as interleukin-10, adiponectin receptor 1 and macrophages (M1, M2) polarization markers (CD11c, CD206) mRNA expressions were down-regulated in ischemic kidney tissues and white adipose tissues around them by all treatments. Moreover, garlic, telmisartan and their combinations significantly suppressed oxidative stress in renal ischemic tissues. Histological picture was also improved by these treatments. Interestingly, the combinations provided a greater protection than their monotherapy in a dose-dependent manner. We suppose that this combination may be a promising prophylactic regimen for managing AKI in case of obesity. Thus, future experimental and clinical large-scale studies are necessary.

ARTICLE HISTORY
Received 15 March 2016
Revised 8 June 2016
Accepted 8 July 2016
Published online 1 August 2016

KEYWORDS
Adiponectin receptor 1; Garlic; telmisartan; renal ischemia/reperfusion injury; Inflammation; macrophages; oxidative stress; peri-renal adipocytes

Introduction

Obesity is closely correlated to metabolic syndromes comprising dyslipidemia, insulin resistance and hypertension in addition to chronic hyper-inflammatory states [1], which enhance the development of diabetes [2] and renal diseases [3,4].

Acute kidney injury (AKI) is a major health problem that can result from renal ischemia/reperfusion (IR) injury in native and transplanted kidneys [5], leading to acute renal failure with increased morbidity and mortality [6]. Reduction of renal blood flow and hence reduction of oxygen supply to renal cells can lead to AKI [7]. Reperfusion of ischemic renal tissues initiates a complex series of cellular events that eventually lead to necrotic and apoptotic renal cell death. It also induces oxidative stress with increased generation of reactive oxygen species (ROS) and inflammation leading to the release of pro-inflammatory mediators in the reperfusion phase. These factors seem to play a crucial role in the pathogenesis of renal IR. Consequently, several anti-oxidants and anti-inflammatory agents were found to be effective in reducing renal injury resulting from IR. They improve the tolerability of the kidney to ischemic injury [8].

Garlic (*Allium sativum* L.) is one of the naturally-occurring medicinal plants. It has several pharmacological activities including antibiotic, anti-oxidant properties, lowering of cholesterol level, inhibition of platelet aggregation and enhancement of the immune system [9–11]. It contains water (65%), fructose, sulfur compounds, protein, fiber, free amino acids, high levels of selenium, vitamins A, C, B complex as well as low levels of calcium, magnesium, sodium, iron and manganese [12]. Organosulfur compounds of garlic, S-allylcysteine,

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and, diallyl sulfide, contribute to its pharmacological activities [13]. Previous studies demonstrated the prophylactic effect of garlic against IR injury [14,15].

The renin-angiotensin system plays an important role in the pathophysiology of renal disorders, including IR [16]. Telmisartan is a long-acting, highly-selective angiotensin II type 1 (AT1)-receptor antagonist approved for treatment of hypertension. Clinical studies have revealed the effectiveness and safety of telmisartan in diabetic nephropathy in patients with type 2 diabetes, with or without hypertension [17,18], as well as in non-diabetic patients with hypertensive nephropathy [19]. The renoprotective effect of telmisartan was also demonstrated in some animal models, such as hypertensive diabetic rats [20], spontaneously hypertensive rats [21], and partially nephrectomized rats [22]. In addition, telmisartan possesses anti-oxidant and anti-inflammatory effects independent of AT1-receptor blocking activity [23,24]. Recent studies have shown that pre-treatment with telmisartan significantly protected against renal dysfunction and tissue damage in rats with ischemic AKI [25–27]. However; no evidence is available concerning its effects in obese animals with AKI.

Due to the increasing epidemic of obesity, the current study aimed to assess a prophylactic approach for AKI using a combination of garlic and telmisartan (at two dose levels), to cross the point between adipocytes biology and kidney diseases in order to manage obese renal ischemic rats.

Materials and methods

Experimental animals

One hundred and seventeen adult male Wistar albino rats (170–185 g) were used in the current study. They were obtained from the Faculty of Veterinary Medicine, Moshtohor University, Kaliobeya, Egypt. Rats were acclimatized for one wk prior to experiments in stainless steel cages (4 rats/cage) in the animal house of the Faculty of Pharmacy, Zagazig University, Zagazig, Egypt. They were kept at controlled temperature (23 ± 2 °C), light/dark (12/12 h) cycle and humidity (60 ± 10%) and supplied with normal chow diet and water *ad libitum*.

Ethical statement

Experimental design and animal handling procedures were approved by the Ethical Committee for Animal Handling (ECAHZU) at Zagazig University, Zagazig, Egypt and were according to the guidelines of the Weatherall report. We tried to reduce the number of utilized animals and their suffering during this study.

Drugs

Garlic (Tomex® tablets 300 mg) was obtained from Atos Pharma for production of medicinal herbs (El Salam City, Cairo, Egypt), while telmisartan (Micardis® tablets 80 mg) was supplied from Boehringer Ingelheim Co. (Ingelheim, am Rheim, Germany). Meloxicam (Anticox II® tablets 15 mg) was obtained from Adwia Co. (Obour City, Kaliobeya, Egypt). All other chemicals were of analytical grade. Drugs were crushed and suspended in distilled water immediately before administration using gum acacia.

Rationale of drug dosing

Obese rats were orally treated with 3 mg kg^{-1} meloxicam 4 wk before exposure to renal ischemia [28]. The dose of garlic was chosen based on previous studies [29,30]. According to a study done by Omotoso et al. [31], 500 mg/kg body weight/day of garlic extract was considered as the optimum dosage; hence it was used in the current study. The standard dose of telmisartan for human is 40 mg/50 kg body weight/day. The corresponding daily dose for rats, calculated from the formula: $d_{rat} = d_{human} \times 0.71/0.11$ [32], is 5.16 mg kg^{-1} body weight. Therefore, in this study, we selected 5 mg kg^{-1} day^{-1} as a low dose (TeL) and 10 mg kg^{-1} day^{-1} as a high dose (TeH) [33]. Additionally, the oral doses (5, 10 mg kg^{-1} day^{-1}) were used in the experimental studies for managing diabetes and IR-induced kidney injury [26,33]. The time points for drugs administration were chosen based on the results of Williams et al. [34].

With reperfusion of tissues following reduced or ceased blood flow, molecular oxygen delivery to the tissue can cause irreversible cell damage. A recent study revealed that peak renal damage occurred 24 h following IR [35]; therefore, this time point would be helpful in monitoring the prophylactic effects of drugs under study. Moreover, many studies [25,34,36] have shown that the maximum histopathological renal damage was observed after the 24 h reperfusion phase indicating that the biochemical alterations preceded the pathological changes. Depending on these findings, the administration of drugs was started 4 wk before the IR procedure to achieve effective prophylaxis against IR-induced kidney injury in obese rats.

Experimental design

Rats were randomly divided into nine experimental groups ($n = 13$ each). Group 1 (sham-operated normal rats, Sham group), group 2 (sham-operated obese rats fed a high fat diet (HFD) for 16 wk, Obese sham group),

group 3 (obese rats subjected to renal IR injury, Obese IR group), group 4 (obese rats treated with meloxicam 3 mg kg^{-1}, p.o. daily for 4 wk before renal IR procedure, Melo group), group 5 (obese rats received garlic 500 mg kg^{-1} day^{-1} orally 4 wk prior to renal ischemic conditioning, G group), group 6 (obese rats pre-treated with telmisartan 5 mg kg^{-1} (low dose), p.o. daily for 4 wk and subjected to renal IR, TeL group), group 7 (obese rats received telmisartan 10 mg kg^{-1} day^{-1} orally (high dose) 4 wk prior to renal ischemic conditioning, TeH group), group 8 (obese rats pre-treated with a combination of garlic 500 mg kg^{-1} plus telmisartan 5 mg kg^{-1}, p.o. daily for 4 wk before IR, G + TeL group), group 9 (obese rats received a combination of garlic 500 mg kg^{-1} day^{-1} plus telmisartan 10 mg kg^{-1} day^{-1} orally 4 wk prior to renal IR conditioning, G + TeH group). Rats of groups 1, 2 and 3 received vehicle only.

Methods

Induction of obesity

Following acclimatization, rats were switched from rodent chow to high-fat diet (HFD) for 16 wk to induce obesity. The components of HFD include 25% total fat containing 11% unsaturated fat, 44% carbohydrate, 18% protein, 13% fiber, ash and other ingredients [37]. The animals which had $\geq$30% increase in body weight were selected for this experimental study [38]. The 16-wk feeding protocol ensured that all rats were in the same nutritional state at the time of blood collection. Food intake and body weight were recorded throughout the experimental period to adjust the doses of used drugs.

Induction of IR injury

At the end of the experimental period rats were fasted overnight, but had free access to water. They were anaesthetized with an intra-peritoneal injection of thiopental sodium (120 mg kg^{-1}). Left kidney was exposed by a flank incision and the renal pedicle was dissected to expose the renal vessels. Ischemia was induced by passing a thick cotton thread over the left renal artery for 45 min under aseptic conditions. Complete ischemia was visually confirmed by blanching of left kidneys. At the end of ischemic period, the thread was cut and pulled out to release the renal artery followed by 24 h reperfusion. Left kidneys were observed for 2–5 min to ensure normal blood reflow, which is manifested by the change of kidney color to red again [26]. The wound was closed with 3-0 silk suture. The rats were placed on a heat pad to maintain body temperature at 37 °C throughout the experimental procedure. Sham-operated rats underwent the same surgical procedures without application of cotton thread over the left renal artery.

Blood sampling and serum preparation

At the end of the reperfusion period (24 h), rats were weighed and blood samples were collected immediately from the retro-orbital plexus of rats using microcapillary tubes. Serum was separated by centrifugation of blood tubes at 4500 rpm for 30 min. An aliquot of serum was used immediately for the determination of creatinine and urea. The remaining serum was stored at −80 °C and used for the determination of other biochemical parameters.

Tissue sampling

Rats were sacrificed by cervical dislocation under anesthesia with thiopental sodium (120 mg kg^{-1}). Left Kidneys and peri-renal adipose tissues surrounding them were removed and rinsed thoroughly with saline and divided into two equal parts. The first part was immersed immediately in liquid nitrogen and kept at −80 °C . Tissue homogenates were prepared in ice-cold phosphate-buffer (pH 7.5). Homogenates were centrifuged at 12000*g* for 10 min, and supernatants were used for further biochemical analysis. The other part of renal tissues was kept in 10% neutral buffered formaldehyde at room temperature for histo-pathological and immuno-histochemical examination.

Assessment of biochemical parameters

Serum creatinine and urea levels were determined by colorimetric methods using kits supplied by Diamond Diagnostics (Cairo, Egypt). Serum levels of total cholesterol (TC) and triacylglycerol (TAG) were measured using commercially available enzymatic kits (Spinreact, Spain). Tumor necrosis factor-alpha (TNF-α) contents in the kidney were determined by enzyme-linked immunosorbent assay (ELISA) technique using Rat TNF-alpha ELISA Kit Cat#: ELR-TNFalpha-001C (RayBio® Inc., Norcross, GA). Lipid peroxidation was assessed by measuring the content of malondialdehyde (MDA), a byproduct of the lipid peroxidation process, in renal tissues. The measurement was performed using Oxiselect™ TBARS assay kit (San Diego, CA). Reduced glutathione (GSH) content in the kidney was determined colorimetrically using a diagnostic kit supplied by Biodiagnostic (Dokki, Giza, Egypt). Renal superoxide dismutase (SOD) activity was assessed using Trevigen® Superoxide Dismutase Assay Kit (Helgerman Court, Gaithersburg, ML). All measurements were done following the manufacturer's instructions.

Relative quantitation of mRNA of adiponectin receptor-1, IL-10 and macrophages (M1, M2) polarization markers genes in renal and peri-renal adipose tissues by quantitative Real Time polymerase chain reaction (qPCR) Using SYBR GREEN

Biopsies of kidney and peri-renal adipose tissues were taken and immediately placed in Cryo tubes and stored in RNA later solution (Qiagen, GmbH, Hilden, Germany) at 10 μL/mg tissue at -80 °C for further processing. From each of the stored biopsies, 25 mg of the tissue were cut and homogenized with 300 μL lysis buffer (2-mercapto ethanol) using rotor tissue rupror (Qiagen, GmbH, Hilden, Germany) in a microcentrifuge tube. Total RNA was extracted using total RNA purification kit (Jena Bioscience, GmbH, Hilden, Germany) following the manufacturer's protocol. 50 μL of the eluted RNA was collected and immediately placed in ice. Concentration of the RNA sample was measured Spectrophotometrically = 44 μg mL^{-1} × A260 × dilution factor using Nanodrop spectrophotometer 2000c (Thermo Scientific, Wilmington, DE). The ratio of the readings at 260 and 280 nm (A260/A280) provides an estimate of the purity of RNA. Pure RNA has an A260/A280 ratio of 1.9–2.3.

Two steps RT-PCR. *1st step, Synthesis of complementary DNA (cDNA).* Template of the extracted RNA 5 μL from each sample and distilled water 15 μL were added to Maxine reverse transcriptase (RT) pre-mix tube. cDNA synthesis (reverse transcription) reaction was performed using G-storm rapid thermalcycler (England) at 45 °C for 60 min followed by RTase inactivation step at 95 °C for 5 min.

2nd step, Amplification of targeted genes using specific primers and real time RT-PCR. In ABI 7900HT fast real-time PCR (Applied Biosystems, Foster City, CA) the prepared reaction components were done in 96 well PCR plate (micro Amp® 90 well optical reaction plate with Barcode, code 128). Multiplex reaction was used, where a two primer pair was present in a well using qPCR Green Master Mix (China, Beijing) and real time cycler conditions of 95 °C and 5 min (initial denaturation) followed by 35 cycles of 95 °C (30 s), 55 °C (1 min), 72 °C (30 s) for denaturation, annealing and extension steps, respectively. The primer sets sequences from 5′–3′ of forward and reverse primers for all genes (Operon, inc. Huntsville, Alabama, Germany) were as following:

- AdipoR1 (Forward primer: 5′-CT TCT ACTG CTCCCCACAGC-3′, Reverse primer: 5′-ACACCACTC AAGCCAAGTCC-3′)
- IL-10 (Forward primer: 5′-CCCTGGGTGAGAAGCTGA AG-3′, Reverse primer: 5′-CAC TGC CTTGCTCTTA TTTTCACA-3′)
- M1 macrophage polarization marker (CD11c) (Forward primer: 5′-AGCCTATCCTGAGACCTT-3′; Reverse primer: 5′-TCTTATCTTCACCACAGTTCT-3′)
- M2 macrophage polarization marker (CD206) (Forward primer: 5′-ATCCACTCTATCCACCTTCA-3′; Reverse primer: 5′-TGCTTGTTCATATCTGTCTTCA-3′)
- Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Forward primer: 5′-ACCACCATGGAGAA GGCCGG-3′; Reverse primer: 5′- CTCAGTGTAGCCCAA GATGC-3′). GAPDH was used as a housekeeping gene (internal control).

Analysis of the amplified products. According to the RQ manager program 1.2 ABI SDS software (ABI 7900HT), the data were produced as sigmoid-shaped amplification plots in which the number of cycle was plotted against fluorescence (when using linear scale). Since the samples of the control group were used as calibrators, the expression levels were set to 1. However, since the gene expression levels were plotted as log10 values (log10 of 1 is 0), the expression level of the calibrator samples appeared as 0 in the graph. Since the relative quantities of IL10, AdipoR1, macrophages polarization markers M1 (CD11c) and M2 (CD206) genes were normalized against the relative quantities of the endogenous control GAPDH gene, the expression level of this gene was zero. Fold changes in genes expressions were calculated using the Cycle threshold (CT) equation: $2^{-\Delta\Delta CT}$.

Renal histopathology

After proper fixation in 10% neutral buffered formaldehyde, left kidneys and peri-renal fat specimens were dehydrated in xylene and ascending grades of ethyl alcohol (70, 90 and 100%), cleared in xylol, impregnated and then embedded in paraffin wax. Sections of 5 μm were cut using rotatory microtome. Kidney sections were stained with hematoxylin and eosin (H&E) stain to study the general histological structure of the kidney, periodic acid Schiff (PAS) reaction technique to study the mucopolysaccharides and polysaccharides (basement membrane), Mallory trichrome stain to study collagen fibers in relation to hepatic fibrosis. All slides were examined under light microscope.

Area percentage of collagen fibers/μm^2 surface area in kidney tissue was measured morphometrically by Leica Qwin 500 image analysis system (England) which is connected to a Leica microscope in six high-power microscopic fields randomly selected from each group.

Immunohistochemical staining of kidney sections was also performed to study the protein expression of NF-κB. The protein expression of NF-κB was determined morphometrically by measuring the optical density of NF-κB immunoexpression in six high-power microscopic fields randomly selected from each group using the aforementioned image analyzer where brown color represents a positive expression.

The peri-renal adipose tissue sections were stained with H&E stain only. All slides were examined under light microscope. Morphometric analysis was done to assess the size of peri-renal adipocytes. The mean adipocyte surface area (μm^2) was measured in six randomly selected high-power microscopic fields within the sections for each group using the same computerized image analysis system.

Statistical analysis

Data were expressed as mean ± standard deviation (SD). They were statistically analyzed using one-way analysis of variance (ANOVA) test followed by Tukey's post hoc test using Graphpad prism software v.5 (GraphPad Software Inc., La Jolla, CA) for comparison between the studied groups. Pearson's correlation analysis was performed to determine the correlations between renal function, fibrosis markers and other measured biomarkers. $p < 0.05$ was considered a significant difference.

Results

Effect on biomarkers of kidney function and fibrosis

High fat diet (HFD) did not have any effect on renal function and structure versus the sham group (0.87 ± 0.02 versus 0.7 ± 0.06 mg/dl, creatinine levels), (52.93 ± 1.14 versus 48.59 ± 1.69 mg/dl, urea levels) and fibrosis (3.7 ± 0.4 versus 3.5 ± 0.58 area percentage of collagen fibers/μm^2). Reperfusion of unilateral ischemic kidney significantly elevated serum creatinine, urea levels and fibrosis area% in obese rats as compared with obese sham ones ($p < 0.001$). However, pre-ischemic treatment with either Melo or G or TeL or TeH for 4 wk significantly reduced serum creatinine ($p < 0.01$) and urea levels ($p < 0.05$) compared to untreated obese rats subjected to renal IR injury (Table 1).

The reduction observed in these renal functional parameters with TeH was significantly higher than that of Melo, G and TeL ($p < 0.001$). Furthermore, the combined administration of G + TeH, not only significantly reduced creatinine and urea levels compared to obese IR, G, TeL, TeH and G + TeL groups ($p < 0.001$), but also restored creatinine values near to normal levels seen with sham-operated rats. TeL, TeH, G + TeL, and G + TeH groups exhibited a marked reduction of fibrotic area% in renal ischemic tissues with respect to obese IR group ($p < 0.001$) (Table 1).

Effect on lipid profile, peri-renal adipocytes size and body weight

High fat diet (HFD) significantly increased serum TC (99.83 ± 16.55 versus 49.58 ± 4.7 mg/dl) and TAG (104.97 ± 7.9 versus 63 ± 8.17 mg/dl) levels as well as peri-renal adipocytes surface area (3010 ± 347.66 versus 1402.6 ± 78.29 μm^2) and body weight (392.83 ± 22.44 versus 199.83 ± 12.89 g) with respect to the sham-operated group ($p < 0.001$). Obese rats that underwent renal IR injury exhibited a non-significant change in these parameters compared to the obese sham-operated group. However, TeL or TeH resulted in a significant decrease in serum levels of TC ($p < 0.001$) and TAG ($p < 0.01$) levels as well as the size of peri-renal adipocytes ($p < 0.001$) compared to untreated obese unilateral renal ischemic rats (Table 1).

The concomitant administration of G along with TeL or TeH successfully reduced hypercholesterolemia, hypertriglyceridemia and the size of enlarged adipocytes around the ischemic kidney in the obese IR group ($p < 0.001$). The combination of G + TeL had a similar hypolipidemic effect to TeH alone. G + TeH group exhibited a greater reduction in TAG levels than G + TeL group. TeH either alone or in combination with G was able to reduce body weight significantly from the obese IR group ($p < 0.05$) (Table 1).

Effect on renal NF-κB immunoreactivity and TNF-α content

As shown in Figure 1, HFD caused a significant inflammation in renal tissues as manifested by elevation in NF-κB immunoreactivity (optical density) and TNF-α content in renal tissues when compared to the sham group ($p < 0.01$). Renal IR injury in obese rats resulted in a further significant increase in NF-κB protein immunoexpression (about 1.21-fold, $p < 0.001$) and the content of TNF-α (about 1.46-fold, $p < 0.001$) in kidney tissues compared to obese sham-operated rats. However, administration of Melo or G or TeL or TeH 4 wk prior to renal IR significantly decreased NF-κB immunoreactivity and TNF-α content in ischemic renal tissues compared to untreated rats in obese IR group ($p < 0.001$).

Both G and TeL or TeH, when administered together, significantly inhibited the elevation in these parameters ($p < 0.05$) that (G + TeH) group TNF-α values in renal

Table 1. Changes in some biochemical, histomorphometic parameters and body weight of 4 wk pre-treated obese renal ischemic rats at 24 h following reperfusion.

Groups Parameters	Obese renal ichemic	Meloxicam (3 mg kg^{-1})	Garlic (500 mg kg^{-1})	Telmisartan (5 mg kg^{-1})	Telmisartan (10 mg kg^{-1})	Garlic + Telmisartan (5 mg kg^{-1})	Garlic + Telmisartan (10 mg kg^{-1})
Serum creatinine (mg/dl)	1.73 ± 0.11	1.44 ± 0.09*	1.5 ± 0.14*	1.34 ± 0.09*	0.95 ± 0.09*^{abc}	1.18 ± 0.11*^{abd}	0.77 ± 0.05*^{abcde}
Serum urea (mg/dl)	94.47 ± 4.57	87.73 ± 1.81*	90.18 ± 0.19	85.89 ± 3.41*	74.49 ± 2.8*^{abc}	79 ± 1.73*^{abc}	63.19 ± 2.53*^{abcde}
Area% of collagen fibers/μm^2	11.3 ± 2.48	10.5 ± 0.62	10.8 ± 2.68	6.7 ± 1.27*^{ab}	6.56 ± 1.23*^{ab}	6.9 ± 0.46*^{ab}	6.8 ± 0.26*^{ab}
Serum TC (mg/dl)	96.57 ± 8.14	89.9 ± 5.31	83.5 ± 4.69	76.47 ± 7.01*	65.46 ± 5.64*^{ab}	73.2 ± 6.19*^{ab}	63.92 ± 5.38*^{ab}
Serum TAG (mg/dl)	99.17 ± 6.74	90.12 ± 4.87	91.45 ± 3.87	89.68 ± 2.97*	83.17 ± 2.1*	85.25 ± 2.32*	73.35 ± 5.29*^{abce}
Peri-renal adipocytes size (μm^2)	2940.33 ± 405	2871 ± 172.88	3009 ± 153.09	2279 ± 274.14*^{ab}	1942 ± 115.38*^{ab}	2123 ± 116.89*^{ab}	1973 ± 76.14*^{ab}
Body weight (g)	389.83 ± 21.47	377.17 ± 11.01	379.83 ± 7.78	371 ± 9.63	360.17 ± 7.47*	368.33 ± 9.44	356.67 ± 7.09*
Renal MDA (nmol/g tissue)	30.66 ± 2.51	7.72 ± 0.5*	12.23 ± 0.49*^{a}	16.02 ± 1.58*^{ab}	8.68 ± 0.47*^{bc}	10.83 ± 1.09*^{ac}	6.73 ± 0.45*^{bcde}
Renal GSH (mmol/g tissue)	1.83 ± 0.15	4 ± 0.21*	2.93 ± 0.19*^{a}	2.8 ± 0.1*^{a}	3.74 ± 0.13*^{bc}	3.2 ± 0.28*^{ad}	4.22 ± 0.23*^{bcde}
Renal SOD (U/g tissue)	3.77 ± 0.33	11.44 ± 0.69*	9.62 ± 0.74*^{a}	8.15 ± 0.73*^{ab}	10.67 ± 0.5*^{c}	10.18 ± 0.72*^{ac}	12.6 ± 0.38*^{bcde}

Values are expressed as mean ± SD ($n = 6$/group). Statistical analysis was done using one-way ANOVA, followed by Tukey's Post hoc-test.
*versus obese IR,
[a]versus Melo,
[b]versus G,
[c]versus TeL,
[d]versus TeH,
[e]versus G + TeL ($P < 0.05$).

tissues approached normal levels of sham-operated animals (Figure 1).

Effect on renal oxidative stress biomarkers

Obese rats showed a significantly-higher level of MDA (20.72 ± 1.8 versus 5.57 ± 0.55 nmol/g tissue) as well as a lower GSH content (2.6 ± 0.15 versus 4.47 ± 0.23 mmol/g tissue) and SOD activity (6.03 ± 0.52 versus 15.02 ± 0.95 U/g tissue) in renal tissues as matched to the sham group ($p < 0.001$). IR resulted in a significant elevation of kidney content of MDA, whereas it resulted in a significant reduction of GSH content and SOD activity in obese rats compared to obese sham-operated ones ($p < 0.001$) (Table 1).

In the same manner as described before, individual pre-treatment of obese renal ischemic rats with Melo, G, TeL and TeH significantly diminished the abnormal alterations in these parameters; however, the effect exerted by Melo and TeH monotherapy was significantly higher than that of G and TeL ($p < 0.05$). In addition, a combination of both G and TeH normalized the levels of MDA and GSH to approximate values to those observed in sham-operated rats (Table 1).

Effect on renal AdiopR1 and IL-10 as well as peri-renal adipose tissue macrophages (M1 and M2) polarization markers genes expressions

Obese rats displayed a marked up-regulation of renal AdiopR1 and IL-10 as well as macrophage polarization markers M1 (CD11c) and M2 (CD206) genes expressions in peri-renal adipose tissues by 31.45, 23, 7.09, 13.86 folds, respectively compared with sham-operated animals ($p < 0.001$). Renal IR injury exaggerated the increase in the transcription of these genes by 92.85, 66, 15.63 and 91.3 folds, respectively 24 h after reperfusion in obese rats ($p < 0.05$) (Figure 2(A,C)).

Individual prophylactic administration of Melo, G, TeL and TeH for 4 wk to obese renal ischemic animals protected against HFD and renal IR-induced elevation in renal and peri-renal adipose tissues genes expressions of the aforementioned markers ($p < 0.01$). Both G and TeL or TeH, when administered together, significantly inhibited the up-regulation in AdiopR1 and IL-10 genes expressions in a dose-dependent manner compared to individual treatments ($p < 0.05$). M2 macrophages (CD206) mRNA expression approached the sham group values in TeH and G + TeL groups (Figure 2(B, D)). Fold changes in the studied genes for all treatment groups are shown in Figure 3.

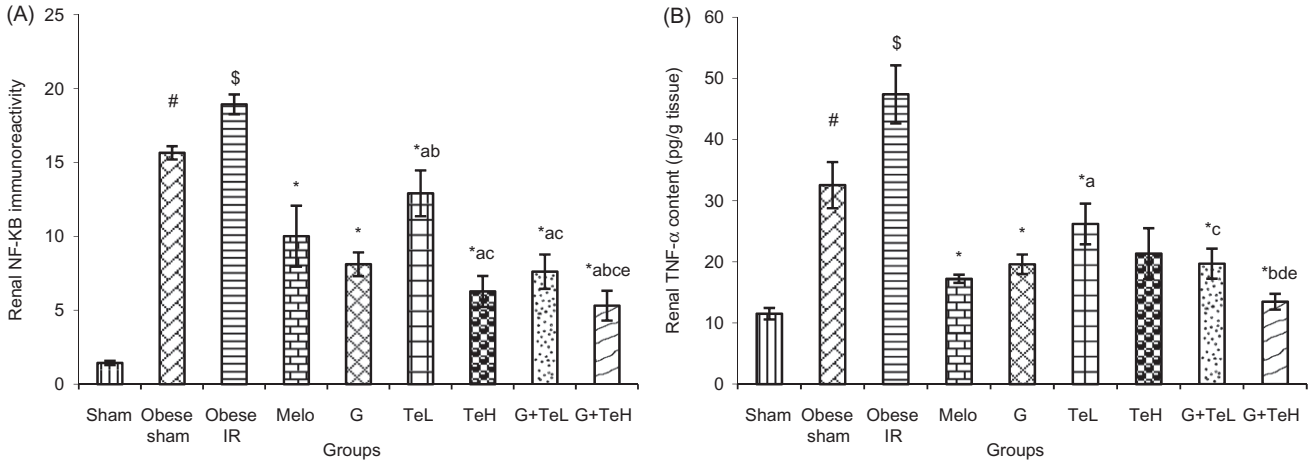

Figure 1. Changes in renal nuclear factor-kappa B (NF-κB) immunoreactivity (A) and content of tumor necrosis factor-alpha (TNF-α) (B) at 24 h following ischemia/reperfusion (IR) injury. Obese rats were pre-treated with Melo, meloxicam (3 mg kg^{-1}); G, garlic (500 mg kg^{-1}); TeL, telmisartan low dose (5 mg kg^{-1}); TeH, telmisartan high dose (10 mg kg^{-1}); G + TeL, G + TeH, a combination of garlic and telmisartan at both doses for 4 wk prior to IR procedure. Results are expressed as mean ± SD ($n = 6$/group). # versus Sham, $ versus Obese sham, * versus Obese IR, [a] versus Melo, [b] versus G, [c] versus TeL, [d] versus TeH, [e] versus G + TeL ($P < 0.05$).

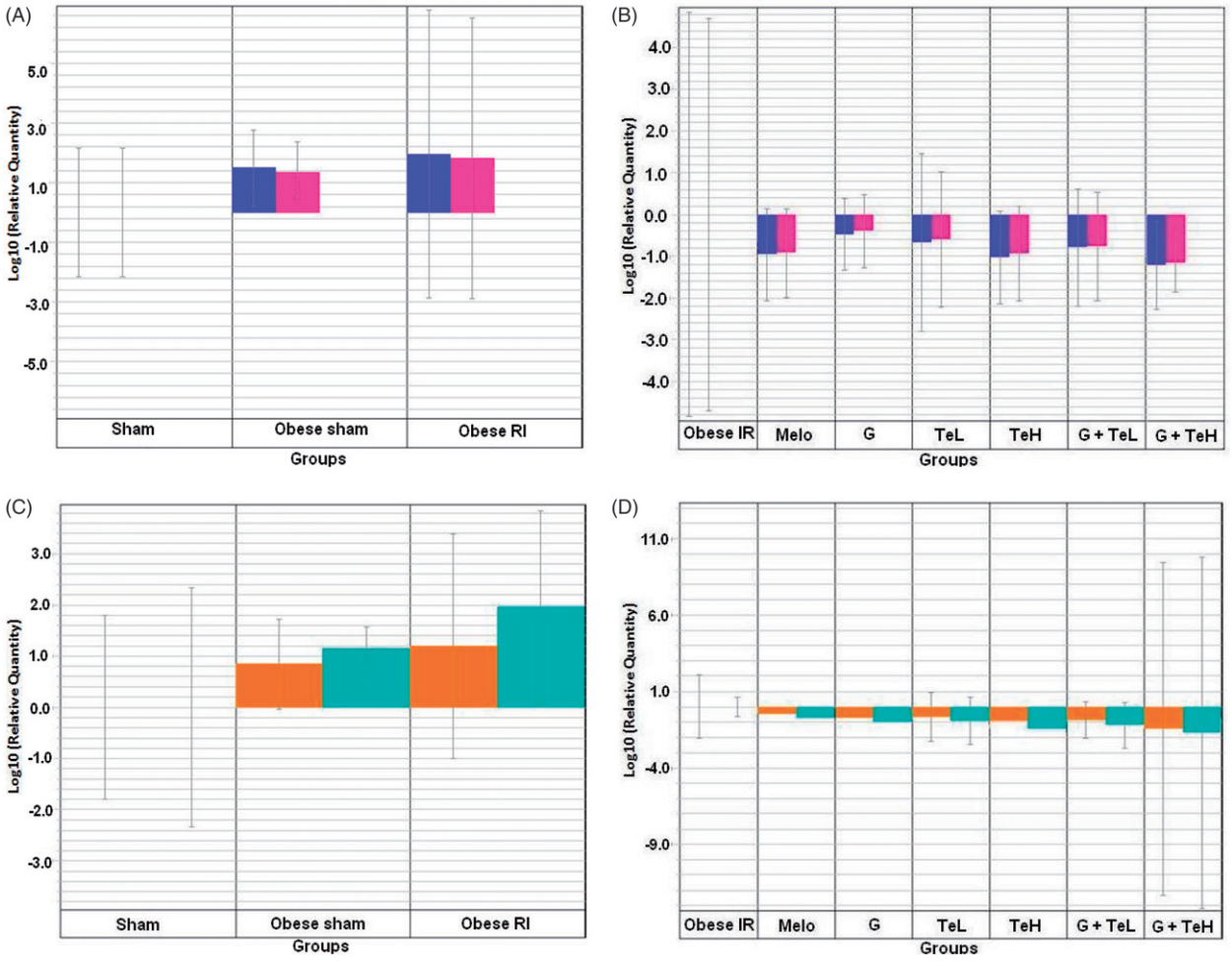

Figure 2. Gene expression (ABI 7900 Fast real time PCR machine) of mRNA for renal AdipoR1 and IL-10 (A,B) & macrophages (M1, M2) polarization markers (CD11c, CD206) in peri-renal adipose tissues (C,D) (target detector genes) indicated by blue, red, orange and green bars (log10 relative quantities) of the studied groups: obese rats were pre-treated with Melo, meloxicam (3 mg kg^{-1}); G, garlic (500 mg kg^{-1}); TeL, telmisartan low dose (5 mg kg^{-1}); TeH, telmisartan high dose (10 mg kg^{-1}); G + TeL, G + TeH, a combination of garlic and telmisartan at both doses for 4 wk and underwent ischemia/reperfusion (IR) procedure.

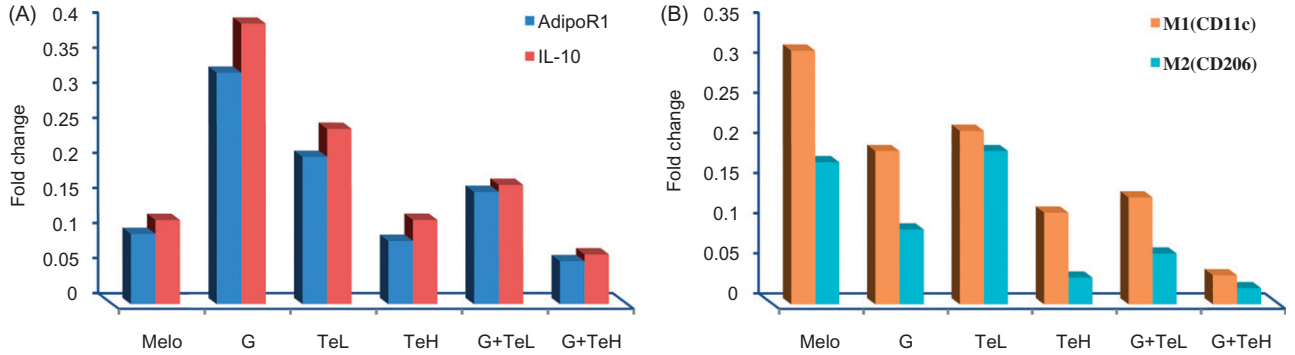

Figure 3. Fold changes of relative genes expressions of (A) AdipoR1 and IL-10 in renal tissues & (B) macrophages polarization markers M1 (CD11c) and M2 (CD206) in peri-renal adipose tissues of obese renal ischemic rats pre-treated with Melo, meloxicam (3 mg kg^{-1}); G, garlic (500 mg kg^{-1}) and/or TeL or TeH, telmisartan (5 or 10 mg/kg^{-1}) for 4 wk relative to obese ischemia/reperfusion (IR) group.

Correlations

Pearson's correlation analysis among the studied groups revealed that there were significant positive correlations between serum kidney functional parameters, fibrosis and each of serum lipids, peri-renal adipocytes size, macrophages (M1 and M2) polarization markers gene expression, renal pro-/anti-inflammatory mediators, MDA and AdipoR1 gene expression. However, these parameters showed negative correlations with renal antioxidants (Table 2).

Histopathological examination of kidney tissues

Sham-operated and obese sham rats showed normal histological structure of the left kidneys (Figure 4(A,B)). They showed also positive reaction of PAS as well as a normal distribution of collagen fibers around the renal glomeruli and renal tubules (Figures 5 and 6(A,B)). Additionally, sham group displayed a very-weak immunoreaction for NF-κB (Figure 7(A)), normal structure of peri-renal adipose tissues (Figure 8(A)). On, the other hand, HFD-fed rats showed a moderate immunoreaction for NF-κB (Figure 7(B)), many enlarged adipocytes along with a marked mononuclear cell infiltration (Figure 8(B)). Similarly, obese rats that underwent IR injury revealed a marked destruction of renal tissues (Figure 4(C)). We also detected a strong positive reaction of PAS in a shrunken glomerulus (Figure 5(C)) as well as a marked increase in collagen fibers deposition around the renal glomeruli and renal tubules (Figure 6(C)). There was a strong immunoreaction for NF-κB (Figure 7(C)). Peri-renal fat tissue specimens of the obese IR group showed increased number of enlarged adipocytes and severe mononuclear cellular infiltration (Figure 8(C)).

Left kidneys of obese IR rats treated with Melo displayed congested glomerular capillaries and vacuolations in some renal tubules (Figure 4(D)). PAS reaction was positive (Figure 5(D)). A marked increase in collagen fibers deposition around the renal glomeruli and renal tubules was also observed (Figure 6(D)). Likewise, we noticed a mild immunoreaction for NF-κB (Figure 7(D)) and some enlarged adipocytes (Figure 8(D)).

Renal tissues obtained from obese renal ischemic rats receiving G prior to IR revealed mild improvement (Figure 4(E)). There was a strong positive reaction of PAS (Figure 5(E)). We noticed a massive increase in collagen fibers deposition around the renal glomeruli and renal tubules (Figure 6(E)). There was a weak immunoreaction for NF-κB, some enlarged adipocytes surrounding renal ischemic tissues and limited mononuclear cellular infiltration (Figures 7 and 8(D,E)).

Telmisartan low dose (TeL) group showed a moderate improvement of the renal tissues structure (Figure 4(F)). TeH resulted also in a moderate improvement of the renal tissues structure (Figure 4(G)). Both doses displayed positive reactions of PAS and a moderate increase in collagen fibers deposition around the renal glomeruli and renal tubules (Figures 5 and 6(F,G). There was a mild (TeL) to weak (TeH) immunoreaction for NF-κB and some enlarged adipocytes around ischemic kidneys (Figures 7 and 8(F,G)).

Administration of a combination of G and TeL or TeH resulted in a marked improvement of the renal and peri-renal adipose tissues structure similar to the picture of the sham-operated group (Figures 4–8(H,I)).

Discussion

The current study showed that feeding of HFD to rats for 16 wk provoked an overt obesity as evidenced by increased body weight gain, fat accumulation around kidneys (enlarged peri-renal adipocytes), hyperlipidemia, inflammation, oxidative stress and up-regulation

Table 2. Correlations between serum kidney function indexes, fibrosis and each of serum lipids, peri-renal adipocytes size, macrophages (M1, M2) polarization markers, renal inflammatory and oxidative stress markers as well as adiponectin receptor1 among the studied groups.

	Serum lipids		Peri-renal adipose tissues			Pro- and anti-inflammatory markers in renal tissues			Oxidative stress markers in renal tissues			
Renal function and fibrosis markers	TC	TAG	Adipocytes size	M1 (CD11c) relative gene expression (log relative units)	M2 (CD206) relative gene expression (log relative units)	NF-KB immuno-reactivity (Optical density)	TNF-α	IL-10 relative gene expression (log relative units)	MDA	GSH	SOD	Renal AdipoR1 relative gene expression (log relative units)
Creatinine	$r = 0.55^{HS}$	$r = 0.54^{HS}$	$r = 0.63^{HS}$	$r = 0.63^{HS}$	$r = 0.81^{HS}$	$r = 0.6^{HS}$	$r = 0.53^{HS}$	$r = 0.7^{HS}$	$r = 0.55^{HS}$	$r = -0.61^{HS}$	$r = -0.58^{HS}$	$r = 0.71^{HS}$
Urea	$r = 0.41^{HS}$	$r = 0.4^{HS}$	$r = 0.48^{HS}$	$r = 0.43^{HS}$	$r = 0.67^{HS}$	$r = 0.44^{HS}$	$r = 0.36^{**}$	$r = 0.63^{HS}$	$r = 0.35^{**}$	$r = -0.48^{HS}$	$r = -0.44^{HS}$	$r = 0.63^{HS}$
Collagen fibers area%	$r = 0.4^{HS}$	$r = 0.3^{*}$	$r = 0.5^{HS}$	$r = 0.36^{**}$	$r = 0.58^{HS}$	$r = 0.34^{*}$	$r = 0.26^{*}$	$r = 0.53^{HS}$	$r = 0.28^{*}$	$r = -0.32^{*}$	$r = -0.28^{*}$	$r = 0.51^{HS}$

*Significant at $p < 0.05$.
**Significant at $p < 0.01$.
^{HS}Significant at $p < 0.0001$.

of AdipoR1 and macrophage polarization markers genes expressions in renal and peri-renal adipose tissues. Despite of these deleterious effects, HFD failed to induce a significant damage in renal functions and histological structure.

High fat diet intake to sham-operated rats here produced a significant increase in serum lipids (TC and TAG) levels as compared to those fed a normal diet. Our results agreed with Goyal et al. [39].

Unlike other organs, the kidney is covered by abundant peri-renal fat tissue containing adipocytes [40]. It has been suggested that a high mass of metabolically-active visceral adipose tissue could also be the primary source of the subclinical inflammatory response in patients with chronic kidney diseases (CKD) [41]. In consistence, we found that increased peri-renal adipocytes size was associated with a severe inflammatory response as manifested by increased macrophages (M1 and M2) polarization markers mRNA expression, mononuclear cell infiltration in peri-renal adipose tissues and elevated immunoexpression of NF-κB protein and levels of the pro-inflammatory cytokine (TNF-α) in renal tissues of obese sham-operated rats.

Adiponectin is significantly elevated in the serum of patients with many diseases, including CKD [42]. Although the adiponectin expression is activated during adipogenesis, the "paradoxical" lowering of plasma levels, a feature of this adipokine, could be due to a negative feedback effect on its production during obesity development [43]. This reduction may be also due to the increase of TNF-α, which inhibits adiponectin expression in adipose tissue [44]. A recent study has shown that the levels of adiponectin in plasma were significantly reduced after feeding HFD [45]. Likewise, up-regulation of AdipoR1 gene expression in the kidney of the obese sham group in this study could be a compensatory response to reduced adiponectin level.

The role of adiponectin in renal failure remains controversial, since it exerts both pro- and anti- inflammatory effects [46,47]. It has been recently observed that short-term exposure to lipopolysaccharide (LPS) in HK-2 cells up-regulates adiponectin protein and AdipoR1 mRNA expression along with its signaling pathway downstream. However, this status was reversed by a knockdown of adiponectin gene. Moreover, adiponectin mediates the nuclear translocation of the transcription factors (NF-κB) and pcFos/pcJun (activator protein 1, AP-1), inducing TNF-α transactivation. These effects in turn could worsen the inflammatory damage in an autocrine-dependent manner [48].

A previous study showed that HFD induced a rise of renal NF-κB activity, TNF-α and monocyte chemo-

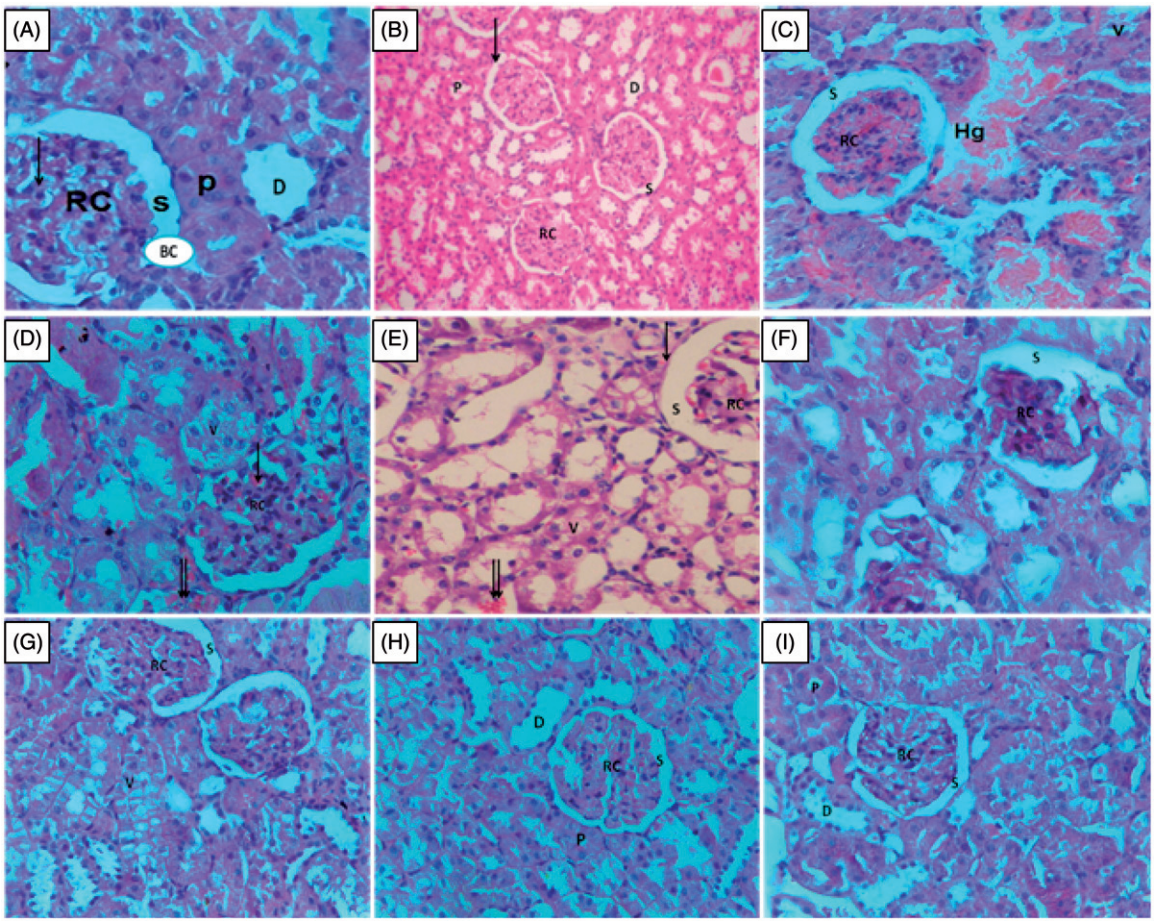

Figure 4. Histological examination of renal tissue specimens at 24 h after ischemia/reperfusion (IR) procedure (H&E dye × 400). Representative light photomicrograph are depicted from (A) sham-operated rats, (B) obese sham-operated rats: showing normal histological structure with renal corpuscles (RC) and glomerular capillaries (arrow) surrounded by Bowman's capsules (BC), subcapsular space (S), proximal convoluted tubule (P) and distal tubule convoluted (D), (C) obese rats underwent IR only: showing marked destruction of the renal tissue with shrunken corpuscle (RC), wide subcapsular space (S) and extensive hemorrhage areas inside it. Several vacuolations (V) in renal tubules with dilatation of their lumen can be detected, (D) obese renal ischemic rats pretreated with meloxicam (3 mg kg^{-1}, p.o.): showing congested glomerular capillaries (arrow). Some glomerular capillaries are ruptured with extravasated blood (double arrows). Vacuolations (V) in some renal tubules can be seen, (E) obese rats underwent IR procedure and pre-conditioned with garlic (500 mg kg^{-1}, p.o.): showing mild improvement of the renal tissue. Renal corpuscles (RC) surrounded by Bowman's capsules (arrow) and subcapsular space (S) are clearly seen. Also, several vacuolations (V) in renal tubules with dilatation of their lumen can be detected. Hemorrhage in between the renal tubules (double arrows) can be observed, (F,G) obese IR group gavaged with telmisartan (5,10 mg kg^{-1}) 4 wk prior to IR procedure: showing moderate improvement of the renal tissue with dilated subcapsular space (S) and vacuolations in some renal tubules and (H,I) obese rats subjected to renal IR and received both garlic (500 mg kg^{-1}) and telmisartan (5,10 mg kg^{-1}) orally 4 wk prior to IR procedure: showing marked improvement of the renal tissue. The histological picture is similar to that of the sham group. The renal corpuscle (RC), subcapsular space (S), proximal convoluted tubule (P) and distal convoluted tubule (D) are noticed.

attractant protein-1 (MCP-1) levels [49]. TNF-α is involved in the genesis of inflammation and contributes to renal damage [50]. Gene expression profiles in glomeruli obtained from renal biopsy samples of patients with obesity-related glomerulopathy (ORG) showed an increase of TNF-α and its receptors, suggesting a role for TNF-α in the development of ORG [51]. TNF-α also has been shown to induce the expression of MCP-1, which is a key regulator in recruiting monocytes to the glomeruli and also contributes to renal damage [52]. Supporting this notion, we reported that HFD-induced enhanced AdipoR1 expression triggered an inflammatory response by increasing NF-κB immunoreactivity and TNF-α content in renal tissues of sham-operated rats.

We found that the obese sham group exhibited a significant increase in renal MDA contents and a decrease in GSH content and SOD activity. In line with this, we reported that HFD induced also a significant increase in the size adipocytes around the kidney. Our findings agree basically with those of Olusi [53] and Vincent and Taylor [54] who reported that, obesity

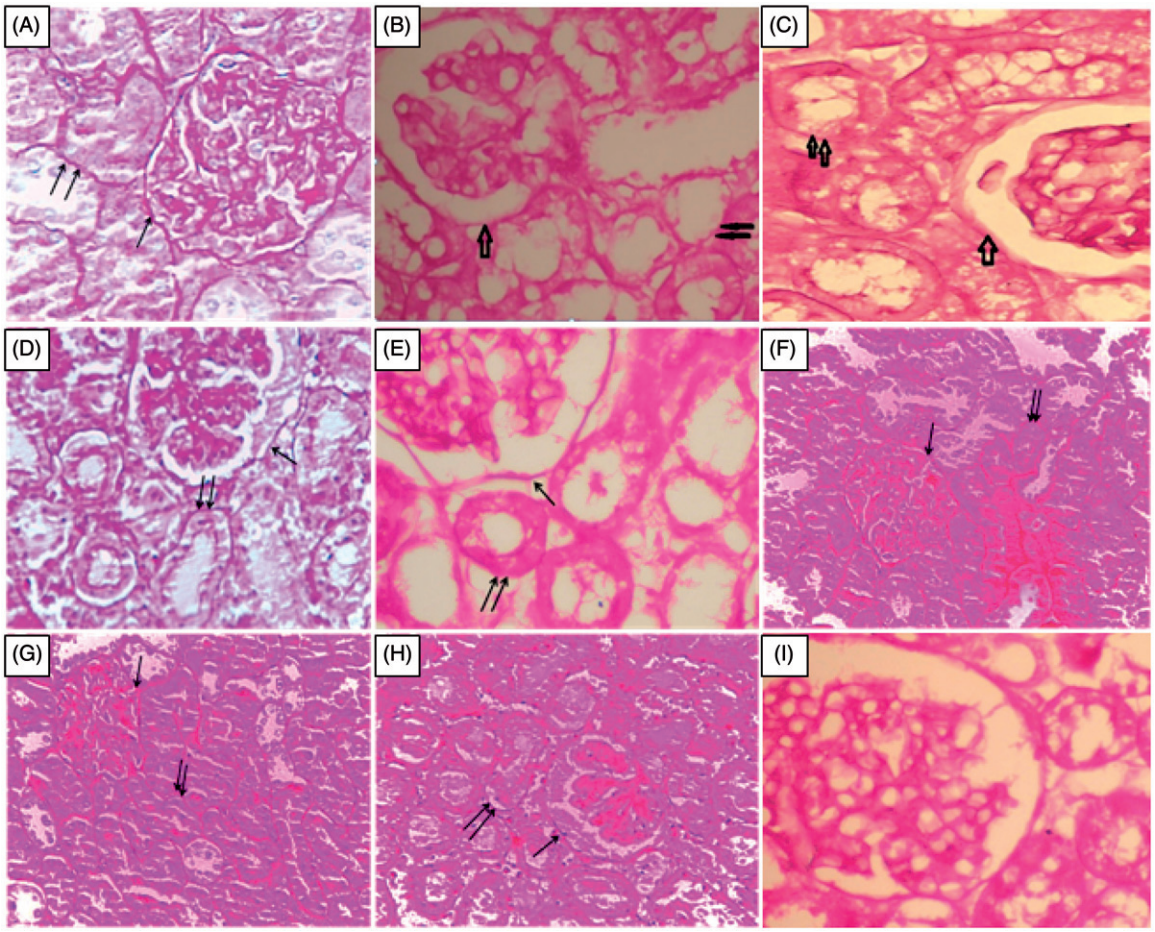

Figure 5. Histological examination of renal tissue specimens at 24 h after ischemia/reperfusion (IR) procedure (PAS dye × 400). Representative light photomicrograph are depicted from (A) sham-operated rats, (B) obese sham-operated rats: showing positive PAS reaction with thin basement membrane of Bowman's capsules (arrow) and thin tubular basement membranes (double arrows), (C) obese rats underwent IR only: showing strong positive PAS reaction in a shrunken glomerulus with a very thick basement membrane of Bowman's capsule (arrow). Strong PAS reaction with thick basement membrane can be also detected in renal tubules (double arrows), (D) obese renal ischemic rats pretreated with meloxicam (3 mg kg^{-1}, p.o.): showing positive PAS reaction in a relatively thin basement membrane of Bowman's capsule (arrow) and relatively thin tubular basement membranes (double arrows), (E) obese rats underwent IR procedure and pre-conditioned with garlic (500 mg kg^{-1}, p.o.): showing strong positive PAS reaction with a very thick basement membrane of Bowman's capsule (arrow). Strong PAS reaction with thick basement membrane in renal tubules (double arrows) can be also detected, (F-I) obese IR group gavaged with telmisartan (5,10 mg kg^{-1}) alone and in combination with garlic (500 mg kg^{-1}) 4 wk prior to IR procedure: showing positive reaction of PAS in a relatively thin basement membrane of Bowman's capsule (arrow) and relatively thin tubular basement membranes (double arrows).

increased the risk of lipid peroxidation decreasing the activity of cytoprotective enzymes. It can induce lipid peroxidation by the increased pressure of large body mass resulting in cumulative and progressive cell injury. Cell injury elicits also the release of cytokines, especially TNF-α which generates ROS from the tissues inducing lipid peroxidation [55].

There are several mechanisms explaining the reduction of antioxidants enzymatic (CAT, GPx, Mn-SOD) activities and GSH levels in obese rats. The increased lipid peroxidation leads to inactivation of antioxidant enzymes by MDA; this will cause an increased accumulation of superoxide, H_2O_2 and hydroxyl radicals which could further stimulate lipid peroxidation. Another explanation is the rapid consumption and exhaustion of storage of these antioxidant enzymes in fighting free radicals generated during the development of obesity. This may probably contribute to the additional progression of obesity related problems [56].

Our findings also indicated that obese sham-operated rats showed a marked increase in the transcription of peri-renal adipose tissue macrophages M1 and M2 polarization markers (CD11c and CD206) as well as renal IL-10 genes.

Adipose tissue macrophages play an important role in the establishment of the chronic inflammatory state and metabolic dysfunction associated with obesity [57]. Recent findings show that adipose tissue macrophages

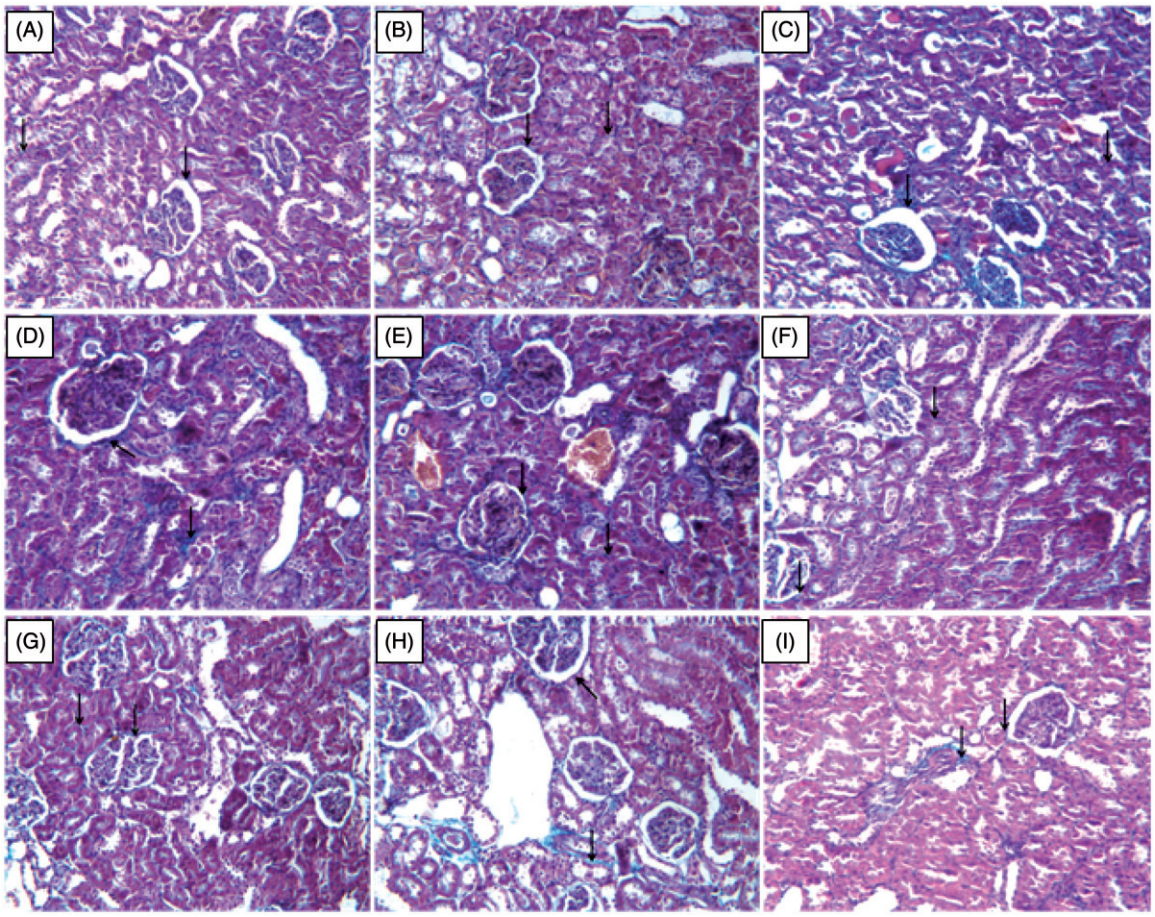

Figure 6. Histological examination of renal tissue specimens at 24 h after ischemia/reperfusion (IR) procedure (Mallory trichrome dye ×200). Representative light photomicrograph are depicted from (A) sham-operated rats, (B) obese sham-operated rats: showing normal distribution of collagen fibers around renal glomeruli and renal tubules (arrows, collagen fibers are stained blue), (C) obese rats underwent IR only, (D) obese renal ischemic rats pretreated with meloxicam (3 mg kg^{-1}, p.o.): showing a marked increase in collagen fibers deposition around renal glomeruli and renal tubules (arrows), (E) obese rats underwent IR procedure and pre-conditioned with garlic (500 mg kg^{-1}, p.o.): showing a massive increase in collagen fibers deposition around renal glomeruli and renal tubules (arrows), (F–I) obese IR group gavaged with telmisartan (5,10 mg kg^{-1}) alone and in combination with garlic (500 mg kg^{-1}) 4 wk prior to IR procedure: showing a moderate increase in collagen fibers deposition around renal glomeruli and renal tubules (arrows).

from lean organisms express markers of the M2 macrophage, whereas obesity leads to a reduction of these markers and an increase of genes associated with the M1 macrophage [58,59]. M1 macrophages up-regulate pro-inflammatory cytokines, including TNF-α, IL-6, and IL-12, increasing the production of reactive oxygen and nitrogen intermediates [60]. M2 macrophages secrete the anti-inflammatory cytokine IL-10 and down-regulate the production of pro-inflammatory cytokines [59].

Since our data indicated that feeding HFD for 16 wk not only stimulated classical M1 polarization marker (CD11c) expression in peri-renal adipose tissue, but also elicited a mixed M1/M2-like pattern of gene expression characterized by enhanced expression of CD206 (M2 polarization marker), we assumed that there was no phenotypic shift or conversion of adipose tissue macrophages from M2 to an M1-dominant state. However, our findings could be explained on the basis of *de novo* recruitment of circulating monocytes to adipose tissues followed by their differentiation into M1 and M2 macrophages [61]. Another mechanism for the increment macrophage polarization markers genes expressions with HFD was likely to be a direct response to peri-renal adipose tissue expansion [62] coupled with the accumulation of mixed M1/M2-polarized (anti-inflammatory) adipose tissue macrophages [63].

In our study, the up-regulation of macrophages polarization markers mRNA expression in peri-renal adipose tissue was coincident with increased TNF-α content (M1 derived) and up-regulated IL-10 (M2 derived) genes expression in renal tissues. These results are in agreement with the findings of Fujisaka et al. [61] and Shaul et al. [64]. They suggested that the enhanced expression of IL-10 is involved in the recruitment of M2

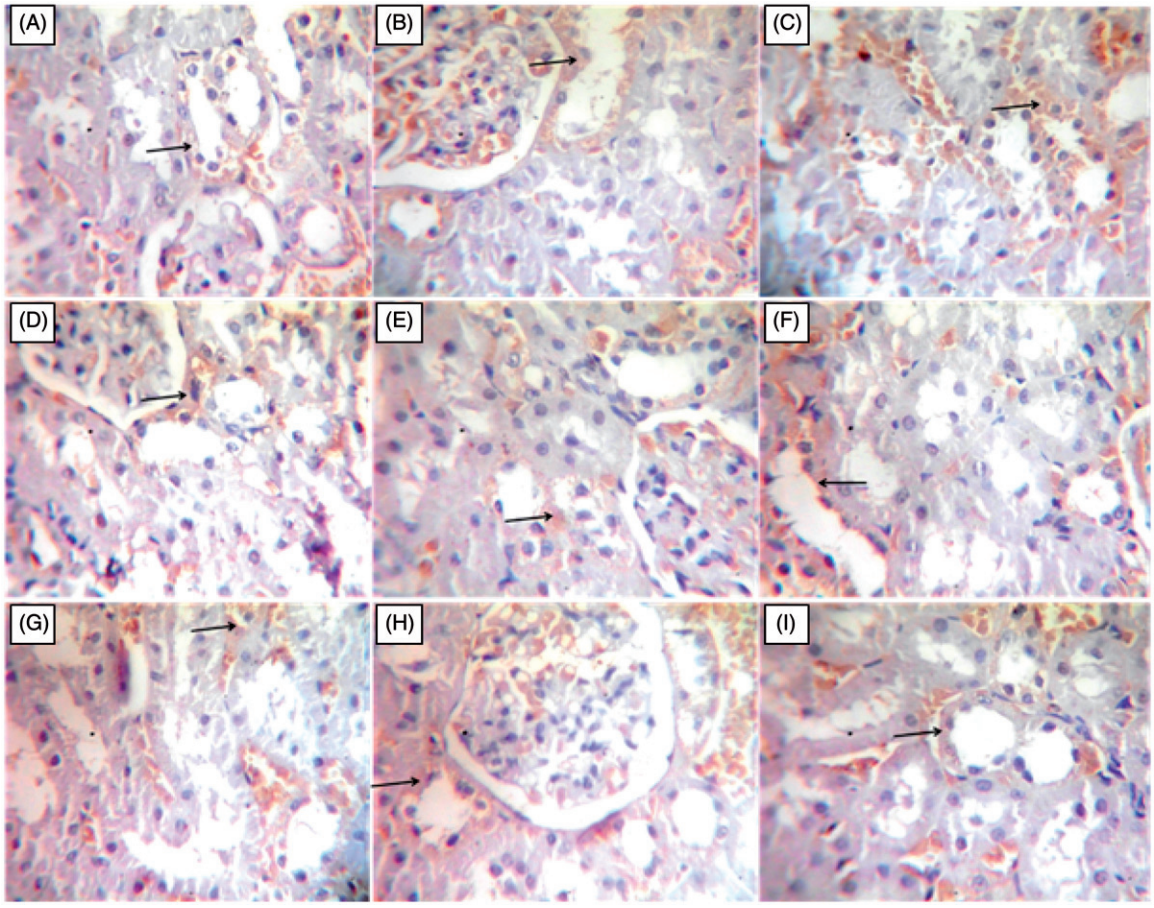

Figure 7. Histological examination of renal tissue specimens at 24 h after ischemia/reperfusion (IR) procedure (Avidin-biotin peroxidase dye with Mayer's hematoxylin counter stain x400). Representative light photomicrograph are depicted from (A) sham-operated rats: showing a very-weak immunoreaction for NF-κB (arrow), (B) obese sham-operated rats: showing a moderate immunoreaction for NF-κB (arrow), (C) obese rats underwent IR only: showing a strong immunoreaction for NF-κB (arrow), (D) obese renal ischemic rats pretreated with meloxicam (3 mg kg^{-1}, p.o.): showing a mild immunoreaction for NF-κB (arrow), (E) obese rats underwent IR procedure and pre-conditioned with garlic (500 mg kg^{-1}, p.o.): showing a weak immunoreaction for NF-κB (arrow), (F–I) obese IR group gavaged with telmisartan (5,10 mg kg^{-1}) alone and in combination with garlic (500 mg kg^{-1}) 4 wk prior to IR procedure: showing a mild (TeL) to weak immunoreaction for NF-κB (arrow).

macrophages in larger adipose tissue under HFD feeding conditions.

Under several pathological conditions, including cardiovascular diseases and AKI, obesity-related hyperinflammation might even cause aggravated organ injury response rather than beneficial pre-conditioning against stress conditions. Preoperative fasting protects against renal IR injury in both male and female aged obese mice [6].

The deterioration of kidney function in obese renal ischemic in the current was evident from the significant elevation of serum creatinine and urea levels, which are widely used to assess kidney function, compared to sham-operated obese animals. An increase in serum creatinine level is used clinically to identify AKI [65]. The elevation of kidney function markers could be attributed to the sudden decline in glomerular filtration rate (GFR) induced by AKI.

Our histopathological examination revealed that the deterioration of kidney function was associated with renal architecture damage along with increasing fibrotic area%. Taken together, these changes confirm the proper induction of AKI in our experiment. Renal IR-induced oxidant injury could also result in impairment of kidney function, leading to a marked elevation in serum creatinine and urea levels [26]. The deterioration of kidney function and associated injury here were significantly suppressed by individual pre-treatment with Melo, G, TeL and TeH or the combination forms (G + TeL and G + TeH). In addition, these treatments ameliorated the structural damage caused by IR injury in the kidney and peri-renal adipose tissues, suggesting their renoprotective potential.

The current study proved that the renal IR procedure significantly increased TNF-α content and NF-κB optical density as well as the transcription of IL-10 gene in the

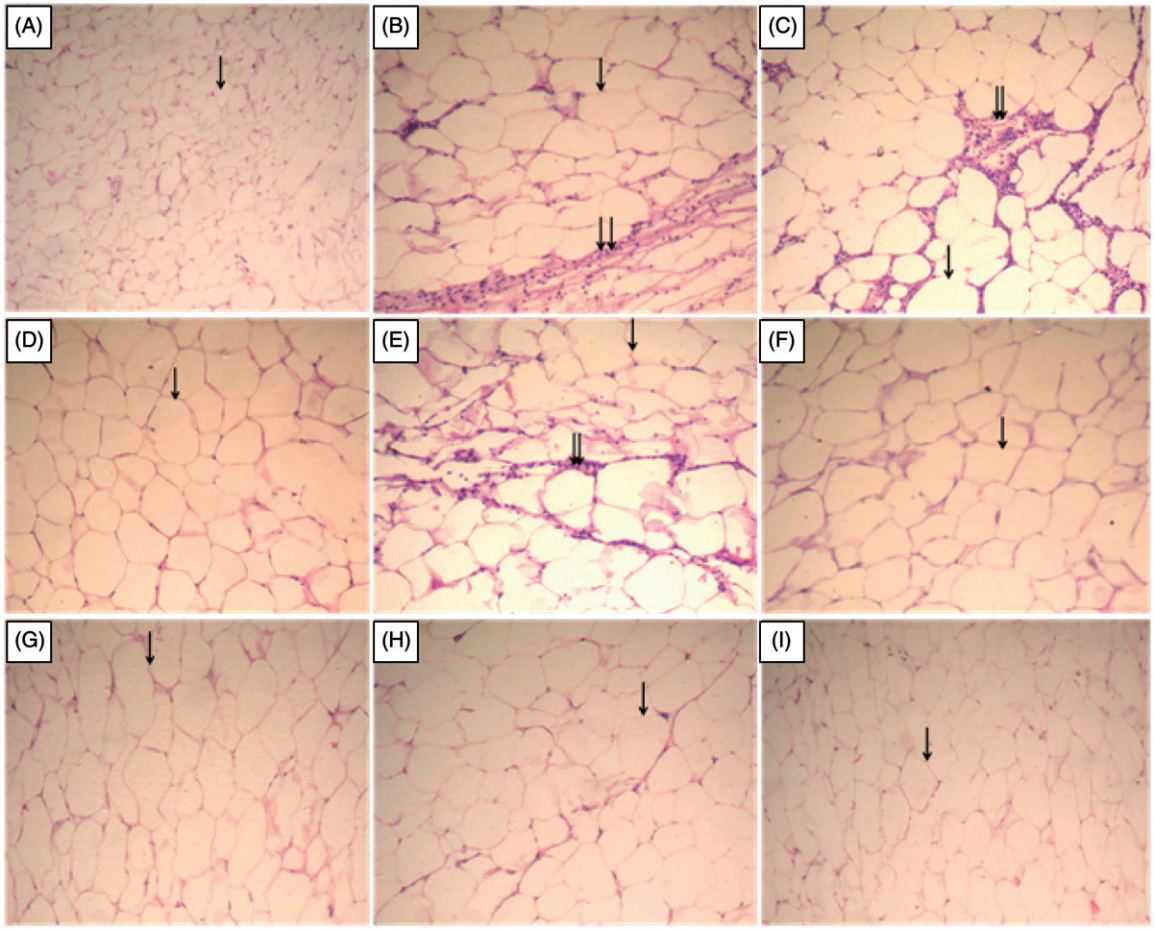

Figure 8. Histological examination of peri-renal adipose tissue specimens at 24 h after ischemia/reperfusion (IR) procedure (H&E dye × 200). Representative light photomicrograph are depicted from (A) sham-operated rats: showing many adipocytes (fat cells-arrow) with thin rim of acidophilic cytoplasm and large central empty space which represents the dissolved fat globule, (B) obese sham-operated rats, (C) obese rats underwent IR only: showing many enlarged adipocytes (arrow) and marked mononuclear cellular infiltration (double arrows), (D) obese renal ischemic rats pretreated with meloxicam (3 mg kg^{-1}, p.o.): showing some enlarged adipocytes (arrow), (E) obese rats underwent IR procedure and pre-conditioned with garlic (500 mg kg^{-1}, p.o.): showing some enlarged adipocytes (arrow) and limited mononuclear cellular infiltration (double arrows), (F,G) obese IR group gavaged with telmisartan (5, 10 mg kg^{-1}) 4 wk prior to IR procedure: showing some enlarged adipocytes (arrow), and (H,I) obese rats subjected to renal IR and received both garlic (500 mg kg^{-1}) and telmisartan (5,10 mg kg^{-1}) orally 4 wk prior to IR procedure: showing few enlarged adipocytes (arrow).

left ischemic kidneys and macrophage polarization markers in fat tissues around the ischemic kidneys of obese rats as compared to obese sham-operated ones. This is consistent with previous studies [66,67]. It is obvious that renal tubular cell damage induced by ischemia activates the release TNF-α [68], which in turn stimulates NF-κB pathway leading to its translocation to the nucleus [69]. Consequently, NF-κB- mediated inflammatory response in the vascular endothelium results in migration and adhesion of leukocytes, which is accelerated by over-expression of adhesion molecules [70]. The activation of leukocytes leads to obstruction of capillaries, additional production of cytokines, and worsening of the inflammatory response [71].

Indeed, we detected a marked mononuclear cell infiltration in peri-renal adipose tissue at 24 h following IR. The observed up-regulation of IL-10 gene expression in the ischemic kidney here could reflect a protective response initiated by this organ after the initial injury. This response, however, is not strong enough to halt renal inflammation and fibrosis. Our data corroborate previously-described observations [72]. Increased renal IL-10 mRNA expression here coincided with up-regulation of M2 polarization marker in peri-renal adipose tissues, suggesting that IL-10 was derived mainly from M2. The increase in M2 polarization marker expression in our study could also be just to compensate for the increase in M1 polarization marker in peri-renal adipose tissues. In the current study, the inflammatory markers and macrophage polarization markers were significantly reduced by ischemic pre-treatment with meloxicam and garlic, telmisartan (both doses) or their combination.

The combination of both garlic and telmisartan (high dose) totally inhibited the elevation of theses markers that they approached normal levels.

The up-regulation of macrophages M1 and M2 polarization markers genes expressions in the present experiment was accompanied by increased collagen deposition in ischemic kidneys of obese rats. Supporting this notion, there is a strong correlation between macrophage accumulation and renal fibrosis in kidney diseases [73,74]. Macrophages are greatly involved in the kidney homeostasis. M1 (pro-inflammatory) macrophages can induce acute tissue injury, but M2 (pro-fibrotic) macrophages can initiate the fibrotic response secondary to continuous tissue injury. Macrophages cause fibrosis *via* the recruitment, activation and proliferation of fibroblasts. Moreover, there is a plethora of evidence supporting the direct fibrotic role of macrophages through transition into myofibroblasts. This process is termed as macrophage–myofibroblast transition (MMT) renal diseases [75]. Furthermore, the tight co-localization of proliferative macrophages and alpha smooth muscle actin (α-SMA) myofibroblasts in areas of severe renal damage suggests a close connection between macrophages and renal fibrosis in CKD [76].

Our results demonstrated that renal IR injury enhanced oxidative stress and lipid peroxidation in the kidney of HFD-fed rats compared to obese sham ones. This was manifested by increased formation of MDA. These results are in harmony with previous studies, which demonstrated the crucial role of oxidative stress in acute renal ischemia [77,78]. Angiotensin-II is a well-known oxidative stress inducer, and ROS are involved in many of the angiotensin-II signaling pathways. Angiotensin-II, through the AT1 receptor, stimulates the NADPH oxidase enzyme with increased generation of superoxide anion, hydrogen peroxide, and hydroxyl radicals [79]. Generation of ROS during IR activates inflammatory cascades with increased production of pro-inflammatory cytokines particularly TNF-α leading to neutrophil infiltration into inflammatory organs, thereby renal tissue injury [25,36,80]. Similarly, the current and previous [36,81] studies demonstrated that acute renal IR caused an intense inflammatory reaction with over-expression of TNF-α in the kidney tissue. This can be explained by rapid up-regulation of mRNA expression of TNF-α and inducible nitric oxide synthase (iNOS) during IR [36,81].

Furthermore, our results revealed that IR injury in obese rats impaired both enzymatic and non-enzymatic anti-oxidant protective mechanisms in the kidney as manifested by the significant reduction of GSH content and SOD activity in obese renal ischemic rats compared to obese sham-operated ones. Under normal physiological conditions, antioxidants can scavenge ROS, however, during ischemia supplies of these endogenous antioxidants are depleted by ROS leading to cellular injury. This hypothesis is supported by the findings of other studies showing a reduction of cellular stores of these scavengers during brain [82] and heart [83] ischemia. In accordance with these results, previous studies demonstrated that acute renal IR injury caused intense over-expression of iNOS mRNA and nitric oxide (NO) in the kidney tissue [81,83]. Excess NO depletes intracellular GSH increasing the susceptibility to oxidative stress [83,84].

Individual administration of G, TeL and TeH or their combination significantly reduced oxidative stress and inflammation induced by renal IR injury and enhanced the activity of the anti-oxidative enzymes in the kidney. Interestingly, Melo produced equivalent antioxidant and anti-inflammatory effects to the G + TeH group. This, in part, had a positive impact on improving the kidney function and reducing associated injury. Induction of SOD activity might be a potential therapeutic approach to limit oxidative stress-induced injury during renal IR [85]. Thus, enhancing SOD activity by different therapeutic agents in this study could contribute to their protective effect against renal IR-induced oxidative damage.

The cyto-protective effect of G against injury in kidney tissues can be attributed to the antioxidant properties of its components including S-allylcysteine and S-allylmercaptocysteine [86,87]. In this regard, our results agree with Bagheri et al. [15] who reported that G juice reduced oxidative stress in renal tissue in the setting of IR injury *via* its antioxidant and anti-inflammatory properties. The anti-inflammatory effect of G may be caused by other components comprising 1,2-vinyldithiin and alliin. 1,2-vinyldithiin reducing the secretion of IL-6 and MCP-1,-2 in preadipocytes treated with macrophage factors [88].

Pre-ischemic treatment with telmisartan also significantly reduced serum creatinine and urea values through improving antioxidant capacity and combating oxidative stress [25,26]. Telmisartan, a selective AT1 receptor antagonist, was proved to be effective in reducing the generation of ROS, over-expression of TNF-α and excess NO production observed with oxidative and inflammatory tissue injuries [89]. The antioxidant and anti-inflammatory effects of telmisartan are also related to its ability to suppress the protein expression for NADPH oxidase, TNF-α and iNOS by inhibiting the phosphorylation of p38 mitogen-activated protein kinase. Telmisartan, therefore, prevents the activation of

NF-κB signaling pathway, which promotes the transcription of NADPH oxidase, TNF-α and iNOS genes [90].

Telmisartan also acts as a partial agonist of peroxisome proliferator-activated receptor gamma (PPARγ) [91]. The activation of PPARγ nuclear receptors by telmisartan may explain partly the observed anti-hypertriglyceridemic effect and the reduction of peri-renal adipocytes size of this AT1 receptor antagonist in the current study, since these receptors regulate the expression of target genes involved in lipid metabolism [92]. Consequently, telmisartan significantly reduced HFD-induced adipogenesis [39]. The hypocholesterolemic potential of telmisartan in the present work might be due to inhibition of cholesterol absorption since it was reported previously that it lowered cholestanol, a cholesterol absorption marker, along with TC and LDL-C [93]. The antifibrotic effect of telmisartan in renal ischemic tissues here could be also attributed to inhibition of transforming growth factor-beta1 (TGF-β1)-induced α-SMA expression and collagen IV secretion in mesangial cells *via* the activation of PPAR-γ [94,95].

Meloxicam (Melo) was used in the present study as a reference anti-inflammatory agent. Although non-steroidal anti-inflammatory drugs (NSAIDs) cause acute renal failure through cyclooxygenase (COX) inhibition impairing renal perfusion [96], parecoxib (a selective COX-2 inhibitor) protects the kidney from injury induced by IR in rats [97]. Similarly, another recent study has shown that an inhibition of inducible COX-2 with Melo (a selective COX-2 inhibitor) ameliorated renal damage and improved renal function, potentially through the inhibition of oxidative stress responses [98]. Therefore, in the current work, we hypothesized that COX-2 contributes to a cascade of persistent inflammation and oxidative stress during IR, since inflammatory and oxidative stress markers were suppressed by Melo pre-treatment, affording renal protection.

We found that pre-treatment with Melo, G and/or telmisartan (5 or 10 mg/kg) markedly reduced the mRNA expression of macrophages M1 and M2 polarization markers (CD11c and CD206) in peri-renal adipose tissues of obese renal ischemic rats. This may be explained on the basis of the anti-inflammatory potential of these agents reducing macrophage infiltration. In contrast, another gene expression study showed that telmisartan tended to decrease the expressions of some M1 macrophage markers and to increase the expressions of the M2 markers in epididymal fat tissue. This was associated with a reduction in the number of CD11c-positive M1 macrophages and the M1 to M2 ratio, suggesting modulating macrophage polarization from M1 to M2 [92]. However, it should be noted that similar to our findings in the same previous study when telmisartan treatment decreased the number of the M1 macrophages, it did not increase but tended to decrease the number of M2 macrophages. These phenomena were probably associated with the general loss of the total adipose tissue macrophages [92].

Moreover, we found that telmisartan at both doses reduced adipocytes size, resulting in the further decrease in the number of M1 and M2 macrophages. In the current study, telmisartan failed to up-regulate IL-10 gene expression in renal ischemic tissues of obese rats but instead it down-regulated this gene. One of the reasons for the failure of telmisartan to increase M2 markers (CD206 and IL-10) genes expressions may be its weaker activity as a PPARγ agonist.

Similar to our findings, increased expression of pro-inflammatory cytokines (TNF-α and MCP-1) as well as AdipoR1, hypertriglyceridemia and increased infiltration by immunocompetent cells have been observed in adipose tissue of obese patients with CKD. This up-regulated inflammation may contribute to the induction of a systemic pro-inflammatory state in those patients accelerating the progression of renal dysfunction [99]. The increase in these markers, including AdipoR1 could be explained on the basis of their decreased degradation in the kidney due to renal insufficiency in addition to their increased systemic production because of subclinical inflammation.

We also reported that the increase in renal content of TNF-α (a pro-inflammatory cytokine) and expression of NF-κB in obese renal ischemic rats was accompanied by increased transcription of AdipoR1 in the kidney. Our results are in agreement with a previous clinical study which suggested that up-regulation of AdipoR1 gene expression in patients with CKD could represent a compensatory anti-inflammatory response to the uremic milieu [100].

Jin et al. [101] have also tested the novel hypothesis that adiponectin is a mediator of IR injury in a mouse model. In contrast to prior reports of adiponectin's effects in the heart and brain, adiponectin deficiency protected mice from renal IR injury, which was demonstrated by lower serum creatinine and histologic injury score. Adiponectin- knockout mice had also reduced up-regulation of NF-κB-related p65, apoptosis-related Bax and caspase-3, and inflammatory cytokines. In wild type mice, adiponectin mRNA was up-regulated in injured kidneys. Thus, the target disruption of adiponectin, suppresses the NF-κB activation of infiltrating inflammatory cells into the ischemic kidney protecting the kidney from IR injury. Interestingly, it was found that adiponectin significantly enhanced tissue expression of AdipoR1 and AdipoR2 beyond the levels

observed in HFD-fed mice [51]. Adiponectin promotes macrophage migration *in vitro* studies of AKI [102].

It has also been reported that adiponectin elicits the M2 phenotype by examining the expression of macrophage polarization surface markers [103]. Accordingly, we reported that over-expression of AdipoR1 in renal ischemic tissues was coupled with increased inflammatory response in these tissues along with up-regulation of M1 and M2 polarization markers genes expressions in peri-renal adipose tissues. Thus, all treatments used in our study seemed to improve renal function and reduce inflammation in renal tissues of obese IR group through down-regulation of AdipoR1 gene expression, confirming the idea that adiponectin receptor antagonism could be a therapeutic target for suppression of the inflammatory cascade during renal IR injury.

One of the critical findings in our study was that TeH provided a superior nephroprotective effect against acute renal IR injury over G and Melo. This may be due to its particular renal benefits comprising higher selectivity and greater binding affinity to the AT1 receptor, prolonged duration of action and partial agonism of PPAR-γ (mixed agonists/antagonists), which produce additional antioxidant and anti-inflammatory effects [23]. Moreover, blocking AT1 receptor by telmisartan, allows angiotensin II to further interact with the angiotensin-II type 2 receptor which, in contrast, affords cellular protective action against oxidative stress and excitotoxic damage [104]. This, in turn, effectively prevents activation of renal caspase-3 and cellular apoptosis [25,94,104]**,** allowing TeH to further improve renal functions and to diminish the inflammatory/oxidative stress cascade induced by renal IR injury in obese rats better than G and Melo.

Another critical finding in our study is the approximately complete blockade of IR-induced damage in the kidney by G + TeH. The protection exerted by this combination form was superior to the other treated groups. This was evident from the normalization of the majority of measured parameters and histological structure. This seems to be a result of the additive or synergistic effects of both drugs through modulation of different mechanistic pathways.

In conclusion, G and telmisartan monotherapy significantly knocked down inflammation, macrophages polarization and lipid peroxidation, thus protecting from renal injury associated with HFD-IR. Moreover, pretreatment with a combined form of G and telmisartan synergistically improved kidney function and structure in a dose-dependent manner *via* a potentiative reduction in inflammation and oxidative stress associated with synergistic down-regulation of macrophages (M1 and M2) polarization markers genes expressions in peri-renal adipose tissues as well as Adipo R1 and IL-10 genes expressions in the kidney, combating HFD-IR-induced kidney injury. These results may be a promising renoprotective strategy for managing obesity related AKI. However, this application needs further large scale experimental and clinical studies.

Acknowledgments

The authors acknowledge Biochemistry department technicians, Faculty of Pharmacy, Zagazig University for their help in the adjustment of laboratory instruments.

Disclosure statement

The authors have no conflicts of interest to declare.

References

1. Hafner S, Hillenbrand A, Knippschild U, Radermacher P. The obesity paradox and acute kidney injury: beneficial effects of hyper-inflammation? Crit Care 2013;17:1023.
2. Othman M, Kawar B, El Nahas AM. Influence of obesity on progression of non-diabetic chronic kidney disease: a retrospective cohort study. Nephron Clin Pract 2009;113:c16–c23.
3. Kelz RR, Reinke CE, Zubizarreta JR, Wang M, Saynisch P, Even-Shoshan O. et al. Acute kidney injury, renal function, and the elderly obese surgical patient: a matched case-control study. Ann Surg 2013;258:359–363.
4. Lafrance JP, Miller DR. Acute kidney injury associates with increased long-term mortality. Am J Soc Nephrol JASN 2010;21:345–352.
5. Ploeg RJ, van Bockel JH, Langendijk PT, Groenewegen M, van der Woude FJ, Persijn GG, et al. Effect of preservation solution on results of cadaveric kidney transplantation. The European Multicentre Study Group. Lancet 1992;340:129–137.
6. Jongbloed F, de Bruin RW, Pennings JL, Payán-Gómez C, van den Engel S, van Oostrom CT, et al. Preoperative fasting protects against renal ischemia-reperfusion injury in aged and overweight mice. PLoS One 2014;9:e100853.
7. Korkmaz A, Kolankaya D. The protective effects of ascorbic acid against renal ischemia-reperfusion injury in male rats. Ren Fail 2009;31:36–43.
8. Agarwal KC. Therapeutic actions of garlic constituents. Med Res Rev 1996;16:111–124.
9. Augusti KT. Therapeutic values of onion (*Allium cepa* L.) and garlic (*Allium sativum* L.). Indian Exp J Biol 1996;34:634–640.
10. Kyo E, Suzuki A, Kakimoto M, Ushijima M, Kasuga S, Itakura Y. Immunomodulation and antitumor activities of aged garlic extract. Phytomedicine 1998;5:259–267.
11. Moriguchi T, Takashina K, Chu PJ, Saito H, Nishiyama N. Prolongation of life span and improved learning in the senescence accelerated mouse

produced by aged garlic extract. Biol Pharm Bull 1994;17:1589–1594.
12. Lawson LD. Garlic: a review of its medicinal effects and indicated active compounds. In: Lawson LD, Bauer R, eds. Phytomedicines of Europe: chemistry and biological activity. Washington DC: American Chemical Society; 1998: 176–209.
13. Chung LY. The antioxidant properties of garlic compounds: allyl cysteine, alliin, allicin, and allyl disulfide. Med J Food 2006;9:205–213.
14. Savas M, Yeni E, Ciftci H, Yildiz F, Gulum M, Keser BS, et al. The antioxidant role of oral administration of garlic oil on renal ischemia-reperfusion injury. Ren Fail 2010;32:362–367.
15. Bagheri F, Gol A, Dabiri S, Javadi A. Preventive effect of garlic juice on renal reperfusion injury. Iran J Kidney Dis 2011;5:194–200.
16. Seujange Y, Eiam-Ong S, Tirawatnapong T, Eiam-Ong S. Role of angiotensin II on dihydrofolate reductase, GTP-cyclohydrolase 1 and nitric oxide synthase expressions in renal ischemia-reperfusion. Am Nephrol J 2008;28:692–700.
17. Makino H, Haneda M, Babazono T, Moriya T, Ito S, Iwamoto Y, et al. The telmisartan renoprotective study from incipient nephropathy to overt nephropathy – rationale, study design, treatment plan and baseline characteristics of the incipient to overt: angiotensin II receptor blocker, telmisartan, investigation on type 2 diabetic nephropathy (INNOVATION) study. Int J Med Res 2005;33:677–686.
18. Barnett A. Preventing renal complications in type 2 diabetes: results of the diabetics exposed to telmisartan and enalapril trial. Am J Soc Nephrol 2006;17: S132–S135.
19. Aranda P, Segura J, Ruilope LM, Aranda FJ, Frutos MA, Lopez V, de Novales E. Long-term renoprotective effects of standard versus high doses of telmisartan in hypertensive nondiabetic nephropathies. Am Kidney J Dis 2005;46:1074–1079.
20. Wienen W, Richard S, Champeroux P, Audeval-Gerard C. Comparative antihypertensive and renoprotective effects of telmisartan and lisinopril after long-term treatment in hypertensive diabetic rats. Renin J Angiotensin Aldosterone Syst 2001;2:31–36.
21. Mandarim-de-Lacerda CA, Pereira LM. Effects of telmisartan on preexistent cardiac and renal lesions in spontaneously hypertensive mature rats. Histol Histopathol 2004;19:727–733.
22. Tsunenari I, Ohmura T, Seidler R, Chachin M, Hayashi T, Konomi A, et al. Renoprotective effects of telmisartan in the 5/6 nephrectomised rats. J Renin Angiotensin Aldosterone Syst 2007;8:93–100.
23. Benson SC, Pershadsingh HA, Ho CI, Chittiboyina A, Desai P, Pravenec M, et al. Identification of telmisartan as a unique angiotensin II receptor antagonist with selective PPARγ-modulating activity. Hypertension 2004;43:993–1002.
24. Cianchetti S, Del Fiorentino A, Colognato R, Di Stefano R, Franzoni F, Pedrinelli R. Anti-inflammatory and anti-oxidant properties of telmisartan in cultured human umbilical vein endothelial cells. Atherosclerosis 2008;198:22–28.
25. Fouad AA, Qureshi HA, Al-Sultan AI, Yacoubi MT, Al-Melhim WN. Nephroprotective effect of telmisartan in rats with ischemia/reperfusion renal injury. Pharmacology 2010;85:158–167.
26. Tawfik MK. Renoprotective activity of telmisartan versus pioglitazone on ischemia/reperfusion induced renal damage in diabetic rats. Eur Rev Med Pharmacol Sci 2012;16:600–609.
27. Kocak C, Kocak FE, Akcilar R, Bayat Z, Aras B, Metineren MH, et al. Effects of captopril, telmisartan and bardoxolone methyl (CDDO-Me) in ischemia-reperfusion-induced acute kidney injury in rats: an experimental comparative study. Clin Exp Pharmacol Physiol 2016;43:230–241.
28. Harirforoosh S, Aghazadeh-Habashi A, Jamali F. Extent of renal effect of cyclo-oxygenase-2-selective inhibitors is pharmacokinetic dependent. Clin Exp Pharmacol Physiol 2006;33:917–924.
29. Shiju TM, Rajesh NG, Viswanathan P. Renoprotective effect of aged garlic extract in streptozotocin-induced diabetic rats. Indian J Pharmacol 2013;45:18–23.
30. Al-Qattan K, Thomson M, Ali M, Mansour M, Divya J. Garlic attenuate hypertension and renal clearance in streptozotocin-diabetic rats: a possible role of plasma and kidney angiotensin converting enzyme 1. FASEB J 2014;28:689.
31. Omotoso GO, Saibu SA, Akinlolu A, Kadir E. Effects of aqueous extract of garlic and vitamin C on the kidney of albino rats. Asian Exp J Biol Sci 2011;2:455–461.
32. Huang JH, Huang XH, Chen ZY, Zheng QS, Sun RY. Dose conversion among different animals and healthy volunteers in pharmacological study. Chin J Clin Pharmacol Ther 2004;9:1069–1072.
33. Zhang Q, Xiao X, Li M, Li W, Yu M, Zhang H, et al. Telmisartan improves kidney function through inhibition of the oxidative phosphorylation pathway in diabetic rats. Mol J Endocrinol 2012;49:35–46.
34. Williams P, Lopez H, Britt D, Chan C, Ezrin A, Hottendorf R. Characterization of renal ischemia-reperfusion injury in rats. J Pharmacol Toxicol Methods 1997;37:1–7.
35. Youssef MI, Mahmoud AA, Abdelghany RH. A new combination of sitagliptin and furosemide protects against remote myocardial injury induced by renal ischemia/reperfusion in rats. Biochem Pharmacol 2015;96:20–29.
36. Neto JS, Nakao A, Kimizuka K, Romanosky AJ, Stolz DB, Uchiyama T, et al. Protection of transplant-induced renal ischemia-reperfusion injury with carbon monoxide. Am Physiol J Renal Physiol 2004;287:F979–F989.
37. Alzoubi KH, Abdul-Razzak KK, Khabour OF, Al-Tuweiq GM, Alzubi MA, Alkadhi KA. Adverse effect of combination of chronic psychosocial stress and high fat diet on hippocampus-dependent memory in rats. Behav Brain Res 2009;204:117–123.
38. Mohamed HE, El-Swefy SE, Rashed LA, Abd El-Latif SK. Biochemical effect of a ketogenic diet on the brains of obese adult rats. Clin J Neurosci 2010;17:899–904.
39. Goyal A, Goyal R, Sharma PL, Malik ZA. Beneficial effect of combination of metformin and telmisartan

on high fat diet-induced obesity in Wistar rats. Asian J Pharm Clin Res 2011;4:106–111.
40. Oh J, Rabb H. Adiponectin: an enlarging role in acute kidney injury. Kidney Int 2013;83:546–548.
41. Wahba IM, Mak RH. Obesity and obesity-initiated metabolic syndrome: mechanistic links to chronic kidney disease. Clin Am J Soc Nephrol 2007;2:550–562.
42. Fantuzzi G. Adiponectin and inflammation: consensus and controversy. Allergy J Clin Immunol 2008;121: 326–330.
43. Kappes A, Loffler G. Influences of ionomycin, dibutyryl-cycloAMP and tumour necrosis factor-alpha on intracellular amount and secretion of apM1 in differentiating primary human preadipocytes. Horm Metab Res 2000;32:548–554.
44. Li L, Yang G, Shi S, Yang M, Liu H, Boden G. The adipose triglyceride lipase, adiponectin and visfatin are downregulated by tumor necrosis factor-alpha (TNF-alpha) in vivo. Cytokine 2009;45:12–19.
45. van Stijn CMW, Kim J, Barish GD, Tietge UJF, Tangirala RK. Adiponectin expression protects against angiotensin II-mediated inflammation and accelerated atherosclerosis. PLoS one 2014;9:e86404.
46. Tilg H, Moschen AR. Adipocytokines: mediators linking adipose tissue, inflammation and immunity. Nat Rev Immunol 2006;6:772–783.
47. Toussirot E, Binda D, Gueugnon C, Dumoulin G. Adiponectin in autoimmune diseases. Curr Med Chem 2012;19:5474–5480.
48. Perri A, Vizza D, Lupinacci S, Toteda G, De Amicis F, Leone F, et al. Adiponectin secreted by tubular renal cells during LPS exposure worsens the cellular inflammatory damage. Nephrol J 2016;29:185–194.
49. Pan QR, Ren YL, Zhu JJ, Hu YJ, Zheng JS, Fan H, Xu Y, Wang G, Liu WX. Resveratrol increases nephrin and podocin expression and alleviates renal damage in rats fed a high-fat diet. Nutrients 2014;6:2619–2631.
50. Gao G, Zhang B, Ramesh G, Betterly D, Tadagavadi RK, Wang W, Reeves WB. TNF-α mediates increased susceptibility to ischemic AKI in diabetes. Am Physiol J Renal Physiol 2013;304:F515–F521.
51. Wu Y, Liu Z, Xiang Z, Zeng C, Chen Z, Ma X, Li L. Obesity-related glomerulopathy: insights from gene expression profiles of the glomeruli derived from renal biopsy samples. Endocrinology 2006;147:44–50.
52. Chow FY, Nikolic-Paterson DJ, Ma FY, Ozols E, Rollins BJ, Tesch GH. Monocyte chemoattractant protein-1-induced tissue inflammation is critical for the development of renal injury but not type 2 diabetes in obese db/db. Diabetologia 2007;50:471–480.
53. Olusi SO. Obesity is an independent risk factor for plasma lipid peroxidation and depletion of erythrocyte cytoprotectic enzymes in humans. Int Obes J Relat Metab Disord 2002;26:1159.
54. Vincent HK, Taylor AG. Biomarkers and potential mechanisms of obesity-induced oxidant stress in humans. Int Obes J (Lond) 2006;30:400–418.
55. Lachieitner M, Koch T, Harold M, Dzien A, Hoppiahler F. Tumour necrosis factor-alpha plasma level in patients with type 1 diabetes mellitus and its association with glycaemic control and cardiovascular risk factors. Intern J Med 2000;248:67–76.
56. Noeman SA, Hamooda HE, Baalash AA. Biochemical study of oxidative stress markers in the liver, kidney and heart of high fat diet induced obesity in rats. Diabetol Metab Syndr 2011;3:17.
57. Xu H, Barnes GT, Yang Q, Tan G, Yang D, Chou CJ, et al. Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. Clin J Invest 2003;112:1821–1830.
58. Lumeng CN, Bodzin JL, Saltiel AR. Obesity induces a phenotypic switch in adipose tissue macrophage polarization. Clin J Invest 2007;117:175–184.
59. Ohashi K, Parker JL, Ouchi N, Higuchi A, Vita JA, Gokce N, et al. Adiponectin promotes macrophage polarization toward an anti-inflammatory phenotype. Biol J Chem 2010;285:6153–6160.
60. Gordon S. The macrophage: past, present and future. Eur Immunol J 2007;37:S9–S17.
61. Fujisaka S, Usui I, Bukhari A, Ikutani M, Oya T, Kanatani Y, et al. Regulatory mechanisms for adipose tissue M1 and M2 macrophages in diet-induced obese mice. Diabetes 2009;58:2574–2582.
62. Linden MA, Pincu Y, Martin SA, Woods JA, Baynard T. Moderate exercise training provides modest protection against adipose tissue inflammatory gene expression in response to high-fat feeding. Physiol Rep 2014;2:e12071.
63. Zeyda M, Farmer D, Todoric J, Aszmann O, Speiser M, Gyori G, et al. Human adipose tissue macrophages are of an anti-inflammatory phenotype but capable of excessive pro-inflammatory mediator production. Int Obes J (Lond) 2007;31:1420–1428.
64. Shaul ME, Bennett G, Strissel KJ, Greenberg AS, Obin MS, Dynamic. M2-like remodeling phenotypes of CD11c + adipose tissue macrophages during high-fat diet–induced obesity in mice. Diabetes 2010;59: 1171–1181.
65. Kellum JA, Levin N, Bouman C, Lameire N. Developing a consensus classification system for acute renal failure. Curr Opin Crit Care 2002;8:509–514.
66. Kelly KJ. Distant effects of experimental renal ischemia/reperfusion injury. Am J Soc Nephrol 2003;14:1549–1558.
67. Chen YT, Tsai TH, Yang CC, Sun CK, Chang LT, Chen HH, et al. Yip HExendin K-4 and sitagliptin protect kidney from ischemia-reperfusion injury through suppressing oxidative stress and inflammatory reaction. Translational J Med 2013;11:270.
68. Burne-Taney MJ, Kofler J, Yokota N, Weisfeldt M, Traystman RJ, Rabb H. Acute renal failure after whole body ischemia is characterized by inflammation and T cell-mediated injury. Am Physiol J Renal Physiol 2003;285:F87–F94.
69. Karin M, Ben-Neriah Y. Phosphorylation meets ubiquitination: the control of NF-[kappa]B activity. Annu Rev Immunol 2000;18:621–663.
70. Rao RM, Yang L, Garcia-Cardena G, Luscinskas FW. Endothelial-dependent mechanisms of leukocyte recruitment to the vascular wall. Circ Res 2007;101:234–247.
71. Bonventre JV, Zuk A. Ischemic acute renal failure: an inflammatory disease. Kidney Int 2004;66:480–485.

72. Feitoza CQ, Goncalves GM, Semedo P, Cenedeze MA, Pinheiro HS, Beraldo FC, et al. Inhibition of COX 1 and 2 prior to renal ischemia/reperfusion injury decreases the development of fibrosis. Mol Med 2008;14:724–730.
73. Nikolic-Paterson DJ, Atkins RC. The role of macrophages in glomerulonephritis. Nephrol. Dialysis Transplant 2001;16:3–7.
74. Eardley KS, Kubal C, Zehnder D, Quinkler M, Lepenies J, Savage CO, et al. The role of capillary density, macrophage infiltration and interstitial scarring in the pathogenesis of human chronic kidney disease. Kidney Int 2008;74:495–504.
75. Nikolic-Paterson DJ, Wang S, Lan HY. Macrophages promote renal fibrosis through direct and indirect mechanisms. Kidney Int Suppl 2014;4:34–38.
76. Yang N, Wu LL, Nikolic-Paterson DJ, Ng YY, Yang WC, Mu W, et al. Local macrophage and myofibroblast proliferation in progressive renal injury in the rat remnant kidney. Nephrol Dialysis Transplant 1998;13:1967–1974.
77. Noiri E, Nakao A, Uchida K, Tsukahara H, Ohno M, Fujita T, et al. Oxidative and nitrosative stress in acute renal ischemia. Am Physiol J Renal Physiol 2001;281: F948–F957.
78. Canacankatan N, Sucu N, Aytacoglu B, Gul OE, Gorur A, Korkmaz B, et al. Affirmative effects of iloprost on apoptosis during ischemia-reperfusion injury in kidney as a distant organ. Ren Fail 2012;34:111–118.
79. Cai H, Griendling KK, Harrison DG. The vascular NAD (P)H oxidases as therapeutic targets in cardiovascular diseases. Trends Pharmacol Sci 2003;24:471–478.
80. Bhalodia Y, Sheth N, Vaghasiya J, Jivani N. Role of fenofibrate alone and in combination with telmisartan on renal ischemia/reperfusion injury. Renal Fail 2010;32:1088–1094.
81. Volti GL, Sorrenti V, Murabito P, Galvano F, Veroux M, Gullo A, et al. Pharmacological induction of heme oxygenase-1 inhibits iNOS and oxidative stress in renal ischemia-reperfusion injury. Transplant Proc 2007;39:2986–2991.
82. Flamm ES, Demopoulos HB, Seligman ML, Poser RG, Ransohoff J. Free radicals in cerebral ischemia. Stroke; J Cereb Circ 1978;9:445–447.
83. Guarnieri C, Flamigni F, Caldarera CM. Role of oxygen in the cellular damage induced by re-oxygenation of hypoxic heart. Mol J Cell Cardiol 1980;12:797–808.
84. Guven A, Uysal B, Akgul O, Cermik H, Gundogdu G, Surer I, et al. Scavenging of peroxynitrite reduces renal ischemia/reperfusion injury. Ren Fail 2008;30:747–754.
85. Honma S, Takahashi N, Shinohara M, Nakamura K, Mitazaki S, Abe S, et al. Amelioration of cisplatin-induced mouse renal lesions by a cyclooxygenase (COX)-2 selective inhibitor. Eur Pharmacol J 2013;715:181–188.
86. Segoviano-Murillo S, Sanchez-Gonzalez DJ, Martinez-Martinez CM, Cruz C, Maldonado PD, Pedraza-Chaverri J. S-allylcysteine ameliorates ischemia and reperfusion induced renal damage. Phytother Res 2008;22:836–840.
87. Pedraza-Chaverri J, Barrera D, Maldonado PD, Chirino YI, Macías-Ruvalcaba NA, Medina-Campos ON, et al. S-allylmercaptocysteine scavenges hydroxyl radical and singlet oxygen in vitro and attenuates gentamicin-induced oxidative and nitrosative stress and renal damage in vivo. BMC Clin Pharmacol 2004;4:5.
88. Keophiphath M, Priem F, Jacquemond-Collet I, Clément K, Lacasa D. 1,2-vinyldithiin from garlic inhibits its differentiation and inflammation of human preadipocytes. Nutr J 2009;139:2055–2060.
89. Munger MA. Use of Angiotensin receptor blockers in cardiovascular protection: current evidence and future directions. P T 2011;36:22–40.
90. Vaziri ND, Bai Y, Ni Z, Quiroz Y, Pandian R, Rodriguez-Iturbe B. Intra-renal angiotensin II/AT1 receptor, oxidative stress, inflammation, and progressive injury in renal mass reduction. Pharmacol J Exp Ther 2007;323:85–93.
91. Arab HH, Al-Shorbagy MY, Abdallah DM, Nassar NN. Telmisartan attenuates colon inflammation, oxidative perturbations and apoptosis in a rat model of experimental inflammatory bowel disease. PLoS One 2014;9: e97193. e97193.
92. Fujisaka S, Usui I, Kanatani Y, Ikutani M, Takasaki I, Tsuneyama K, et al. Telmisartan improves insulin resistance and modulates adipose tissue macrophage polarization in high-fat-fed mice. Endocrinology 2011;152:1789–1799.
93. Inoue T, Taguchi I, Abe S, Toyoda S, Sakuma M, Node K. Inhibition of intestinal cholesterol absorption might explain cholesterol-lowering effect of telmisartan. Clin J Pharm Ther 2011;36:103–110.
94. Baden T, Yamawaki H, Saito K, Mukohda M, Okada M, Hara Y. Telmisartan inhibits methylglyoxal-mediated cell death in human vascular endothelium. Biochem Biophys Res Commun 2008;373:253–257.
95. Yao Y, Zou R, Liu X, Jiang J, Huang Q, He Y, et al. Telmisartan but not valsartan inhibits TGF-beta-mediated accumulation of extracellular matrix via activation of PPARgamma. Huazhong J Univ Sci Technol Med Sci 2008;28:543–548.
96. Nakamura T, Fujiwara N, Sato E, Ueda Y, Sugaya T, Koide H. Renoprotective effects of various angiotensin II receptor blockers in patients with early-stage diabetic nephropathy. Kidney Blood Press Res 2010;33:213–220.
97. Calistro Neto JP, Torres Rda C, Goncalves GM, Silva LM, Domingues MA, Módolo NS, et al. Parecoxib reduces renal injury in an ischemia/reperfusion model in rats. Acta Cir Bras 2015;30:270–276.
98. Musu M, Finco G, Antonucci R, Polati E, Sanna D, Evangelista M, Ribuffo D, Schweiger V, Fanos V. Acute nephrotoxicity of NSAID from the foetus to the adult. Eur Rev Med Pharmacol Sci 2011;15:1461–1472.
99. Schneider MP, Sullivan JC, Wach PF, Boesen EI, Yamamoto T, Fukai T, Harrison DG, Pollock DM, Pollock JS. Protective role of extracellular superoxide dismutase in renal ischemia/reperfusion injury. Kidney Int 2010;78:374–381.
100. Teplan V Jr, Vyhnánek F, Rürlich G, Haluzík M, Racek J, Vyhnankova I, et al. Increased proinflammatory cytokine production in adipose tissue of obese patients

with chronic kidney disease. Wien Klin Wochenschr 2010;122:466–473.
101. Jin X, Chen J, Hu Z, Chan L, Wang Y. Genetic deficiency of adiponectin protects against acute kidney injury. Kidney Int 2013;83:604–614.
102. Cheng CF, Lian WS, Chen SH, Lai PF, Li HF, et al. Protective effects of adiponectin against renal ischemia-reperfusion injury via prostacyclin-PPARα-heme oxygenase-1 signaling pathway. Cell J Physiol 2012;227:239–249.
103. Mandal P, Pratt BT, Barnes M, McMullen MR, Nagy LE. Molecular mechanism for adiponectin-dependent M2 macrophage polarization: link between the metabolic and innate immune activity of full-length adiponectin. Biol J Chem 2011;286:13460–13469.
104. Mehta PK, Griendling KK. Angiotensin II cell signaling: physiological and pathological effects in the cardiovascular system. Am Physiol J Cell Physiol 2007;292: C82–C97.