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EFFECTS OF EXERCISE AND STEVIA ON RENAL ISCHEMIA/ REPERFUSION INJURY IN RATS

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ABSTRACT

Background. The present work was designed to study the effects of methanolic stevia extracts and aerobic exercise and combination of both on renal I/R injury in male rats.
Methods. 60 adult male Sprague-Dawley rats were subdivided into five equal groups as sham, control, exercise, stevia, and stevia plus exercise group. After 5 weeks of exercise and stevia, animals were exposed to 45 min of left renal ischemia and right nephrectomy followed by reperfusion. Serum creatinine, creatinine clearance, fractional Na excretion (FE_{Na^+}), malondialdehyde (MDA), reduced glutathione (GSH) and catalase (CAT) levels in kidney tissues were measured. Also, renal histopathology and the expression of caspase-3 by immunohistochemical examination were done.
Results. The results showed that stevia, exercise or combination of stevia and exercise caused a significant decrease in serum level of creatinine ($p < 0.001$) and FE_{Na^+} ($p < 0.001$) and an increase in creatinine clearance ($p < 0.001$). Moreover, this caused a significant decrease in (MDA; $p < 0.046$) and an increase in GSH ($p < 0.01$) and CAT ($p < 0.01$), as well as causing a significant decrease in caspase 3 expression compared to the control group.
Conclusion. Pretreatment with either stevia or exercise of combination of both seem to have protective effects on renal I/R injury. However, the protective effect of exercise against renal I/R injury seems to be less than stevia. These effects might be due to attenuation of oxidative stress and apoptosis in kidney tissues.

Keywords: renal ischemia/reperfusion, oxidative stress, *Stevia rebaudiana*, exercise, caspase 3

INTRODUCTION

Acute kidney injury (AKI) is a serious problem in hospitalized patients with high mortality rates (Palant et al., 2017) due to tubular, glomerular, interstitial and vascular damage (Zuk and Bonventre, 2016). Renal ischemia/reperfusion (I/R) injury is often seen in intensive care units, secondary to trauma, shock, sepsis, renal transplantation, cardiovascular and urologic surgery (Ahmadiasl et al., 2014; Sancaktutar et al., 2014). In renal transplantation, I/R injury usually leads to primary renal dysfunction, delayed graft failure, increased late acute rejection and allograft dysfunction (Fadili et al., 2013), as well as in the case of renal vascular surgery such as partial nephrectomy and removal of renal tumors in which the renal arteries are clamped and the kidney are exposed to I/R injury leading to functional renal tissue damage (Martin et al.,

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2012). Renal I/R injury is an obvious cause of acute renal failure (ARF) and chronic renal failure (CRF) (Malek and Nematbakhsh, 2015).

Renal I/R injury is a complex inflammatory process in which imbalance between local tissue oxygen supply and demand results in the accumulation of metabolites that could harm the tubular epithelial cells, eventually leading to death by apoptosis and necrosis (Bonventre and Yang, 2011). One important pathway that contributes to the pathogenesis of renal I/R injury is oxidative stress (Nath and Norby, 2000). The excessive production of reactive oxygen species (ROS) during renal I/R causes lipid peroxidation, DNA mutation, and induced apoptotic and necrotic cascades ultimately results in cell death in various ways (Feng et al., 2011; Tsuda et al., 2012). Shokeir et al. (2014) and Hussein et al. (2016) demonstrated a significant increase in the expression of caspase-3 (marker of apoptosis) in kidney tissues during renal I/R injury and its attenuation by ischemic preconditioning and stem cell therapy.

Physical activity is recognized as an important component of a healthy lifestyle and recommended throughout life by scientists and clinicians (Donaldson, 2000). Clarkson (1995) reported a significant increase in the antioxidant enzymes by exercise. Gündüz et al. (2004) also reported that regular swimming muscle exercise reduced oxidative stress and increase the antioxidants enzymes such as SOD, catalase and glutathione peroxidase (GPx) enzyme in different organs such as the liver, kidney, heart and lungs. Herbal supplements and other alternative medicine are necessary for prevention and treatment. *Stevia rebaudiana* (Bertoni) is commonly used as an alternative to artificial sweeteners in many regions of the world such as Canada, Asia and Europe (Lemus-Mondaca et al., 2012). In addition to their sweetening properties, stevia extracts possess other therapeutic effects such anti-hyperglycemic, anti-hypertensive, anti-inflammatory, anti-tumor, anti-diarrhea, diuretic and immunomodulatory effects (Chatsudthipong and Muanprasat, 2009). Stevia also contains phytochemical compounds e.g. stevioside, rebaudioside A and steviol that help to reduce blood sugar, cholesterol and blood pressure, and flavonoids and phenolics compounds such as SR-3, SR-1, SR-5, SR-2 are known to possess potential antioxidant properties (Ramya et al., 2014). The present work was therefore designed to study the effects of muscle exercise and methanolic stevia extracts and a combination of both on the outcome of renal I/R injury in a rat model.

MATERIALS AND METHODS

Experimental animals

The material for this work comprised of sixty adult male Sprague-Dawley rats, aged 4–6 months and weighing 180–200 g, which were bred and housed in separate cages in the animal house at Al Wasemy Center at Qesm Alhadaaq-Hadaaq Alqubba-Cairo at temperature 20–25°C – and light-controlled environment under a 12-h light, 12-h dark cycle. The rats were fed on normal standard lab chow and had free access to water. Our local IRB committee of Mansoura Faculty of Medicine (#ms.17.02.260) approved all experimental procedures and protocols.

Study design

The animals were randomly allocated to 5 groups (each 12 rats):

1. group I (sham group), in which right nephrectomy was done without left renal ischemia
2. group II (control group), in which right nephrectomy was done with left renal ischemia for 45 min without administering a drug
3. group III (stevia group), similar procedure to group II, but the animals were given stevia (200 mg/kg b.w. methanolic extract of stevia leaves) orally by gastric gavage once daily for 5 weeks before renal ischemia (El-Mousalamy et al., 2018); details of the preparation of the methanolic stevia extract were mentioned in our previous study (El-Mousalamy et al., 2018); the extract was dissolved in saline before administration
4. group IV (exercise group), like group II with swimming exercise for 5 weeks before renal ischemia
5. group V (combined group), like group II with combination of methanolic stevia extract and muscle exercise for 5 weeks before renal ischemia.

Experimental model of renal I/R injury

A well-known model of renal I/R injury was produced in the present study in which left renal ischemia was done for 45 min with contralateral right nephrectomy

(Hussein et al., 2016). Briefly, after induction of anesthesia, abdominal midline laparotomy was performed, then the left renal pedicle was dissected and clamped for 45 min, before the edges of the abdominal incision were approximated to each other and covered by a piece of gauze soaked with warm isotonic saline (37°C) to prevent undue loss of body fluids. Five minutes prior to removal of the vascular clamp, the right kidney was exposed and a thread ligature was tied securely around the renal blood vessels and ureter and the blood vessels were dissected next to the kidney, after which the kidney was removed.

Exercise protocol

Rats were allowed to swim individually into a container 30 cm high and 18 cm in diameter containing 20 cm of water at 25°C. At the start, the duration of swimming was 5 min and the duration increased gradually every day until it reached 15 min. An exercise protocol was completed 5 days per week, and not on every day of the week. Exercise continued for 5 weeks before renal ischemia.

Collection of blood and urine samples

Blood and urine samples were collected on days 0 (basal before operation), 2 and 7 (after renal ischemia). The blood sample was collected from the ophthalmic venous plexus by using a fine-walled Pasteur pipette under light anesthesia using halothane (Waynforth and Flecknell, 1998). Blood was left to clot then serum was obtained by centrifugation and stored at –20°C until the time of analysis of serum sodium and creatinine. Moreover, the rats were placed in metabolic cages for 24 hours in order to collect 24-hour urine. The urine volume was measured, and then a sample was taken for estimation of urine sodium and creatinine (Waynforth and Flecknell, 1998).

Harvesting of kidney specimens

The animal was anaesthetized again by sodium thiopental (12 mg/kg b.w.) intraperitoneally (Waynforth and Flecknell, 1998), then the abdomen was opened and the left kidney was perfused briefly with phosphate-buffered saline (PBS) through a cannula inserted into the abdominal aorta to rinse out the blood. The kidney was removed rapidly and cut into two equal halves by a scalpel. One half of the kidney was rapidly placed in a container containing 10% neutral buffered formalin for histopathological examination and immunostaining and the other half was rapidly frozen in liquid N_2, and stored at –72°C until biochemical assay.

Assessment of renal functions

Serum and urine creatinine and sodium concentrations were measured by commercially available kits according to the manufacturer's instructions (Bio-Diagnostics, Dokki, Giza, Egypt). The creatinine clearance was calculated from the following formula:

$$\text{Creatinine clearance} = \frac{\text{urine creatinine concentration, mg/dl} \times \text{urine volume, ml/24 h}}{\text{serum creatinine concentration, mg/dl} \times 1440, \text{ min}} = \text{ml/min}$$

Fractional sodium excretion (FE_{Na+}) was calculated from the following formula:

$$FE_{Na+} = \frac{\text{serum creatinine concentration, mg/dl} \times \text{urine sodium concentration, mEq/L}}{\text{serum sodium concentration, mEq/L} \times \text{urine creatinine concentration, mg/dl}} \times 100$$

Assay of oxidative stress markers in kidney tissues

In brief, the renal cortex was separated from the medulla, weighed (75 mg), minced, homogenized in 0.02 M sodium phosphate buffer, pH 7.4 (1:4 wt/vol) using a Ultra-Turrax smooth glass homogenizer with a motor driven Teflon pestle and then centrifuged at 3000 rpm for 20 min at 4°C. Malondialdehyde (MDA), reduced glutathione (GSH) and catalase enzyme activity in the supernatant of kidney homogenates were measured using a colorimetric method in accordance with the manufacturer's instructions (Bio-Diagnostics, Dokki, Giza, Egypt).

Histopathological examination

After the removal of the left kidney, half was immediately fixed in 10% neutral buffer formalin. The kidney specimens were processed for paraffin blocks and sections of 3-µm thickness were made and stained with hematoxylin and eosin (H&E) stain. The severity of necrotic lesions was scored as follows: 1–5 necrotic tubules / HPF = (1), 5–10 necrotic tubules / HPF = (2),

10–15 necrotic tubules / HPF = (3) and more than 15 necrotic tubules / HPF = (4). So, activity necrotic tubules scoring index is (4). On the other hand, a regeneration scoring index was calculated as follows: (A) solid sheets of cells in interstitium; 1 = 1–2 / HPF, 2 = 3–5 / HPF and 3 = >5 / HPF; (B) intraepithelial tubular proliferation (solid tubules); 1 = 1–2 / HPF, 2 = 3–5 / HPF in cortex and 3 = >5 / HPF in outer medulla (outer and inner strip); (C) mitotic figures inner medulla 1 = 1–2 / 10 HPF, 2 = 3–5 / 10 HPF in cortex and 3 = >5 / 10 HPF; (D) tubules with long vesicular nuclei, 0 = absence and 1 = presence and (E) dilated tubules with festooned nuclei, 0 = absence and 1 = presence. Therefore, the regenerating tubules scoring index was 11.

Immunohistochemical examination for caspase-3 expression

Immunostaining was performed using the Avidin-Biotin complex (ABC) method according to the manufacturer's instructions. Kidney tissue slides were deparaffinized before staining to remove embedding media, as incomplete removal of paraffin results in non-specific staining. Slides were deparaffinized in xylol and rehydrated in descending grades of alcohol. Blocking endogenous peroxidase using 30% hydrogen peroxide in methanol for 10 minutes was done followed by washing in PBS solution. Antigen retrieval was performed in a citrate buffer solution at 95°C to induce refolding of target antigen (cleaved caspase). Slides were allowed to cool then washed in PBS. A serum blocking solution was added for 10 minutes then rinsed without washing. The cleaved caspase-3 rabbit monoclonal antibody (Cell Signaling Technology® Cat. # 9664) was applied for 1 hour at room temperature followed by washing in PBS 3 times (the dilution of this antibody in PBS is (1:400)). This was followed by secondary antibody for 10 minutes then washed in PBS. Next, the slides were covered by avidin enzyme conjugate 10 minutes then washed – PBS Diaminobenzidine (DAB) was used as a chromogen for 5 minutes at room temperature. The DAB chromogen yielded a reddish brown reaction product at the site of target antigen. Slides were counterstained with Meyer's hematoxylin, dehydrated and covered. The cellular localization of this antibody is cytoplasmic and sometimes nuclear.

Statistical analysis

All these statistics were carried out using SPSS/PC package version 11.0. All data were presented as mean ±standard deviation (SD) of the mean. One-way ANOVA test with Tukey's post hoc test were used to establish the significant difference among different groups of the same time intervals. $p \leq 0.05$ was considered significant.

RESULTS

Effects of methanolic stevia extract and exercise on renal functions in a rat model of renal I/R injury

Serum creatinine level and FE_{Na+} were significantly higher in the control group than the sham group, while creatinine clearance was significantly lower in the control group than the sham group on days 2 and 7 after renal ischemia ($p < 0.05$). On the other hand, the treated groups (exercise, stevia and combined) showed significant improvements in serum creatinine, FE_{Na+} and creatinine clearance compared to the control group with a more significant improvement in the stevia and combined groups ($p < 0.05$; Fig. 1A–1C).

Effects of methanolic stevia extract and exercise on oxidative stress in kidney in a rat model of renal I/R injury

MDA concentrations in kidney tissues showed a significant increase in the control group compared to the sham group ($p < 0.001$). On the other hand, MDA levels were significantly attenuated in all treated groups (exercise, stevia and combined groups) compared to the control group ($p \leq 0.048$) with more significant attenuation observed in the combined group. Moreover, there was no statistically significant difference between the sham and combined groups. On the contrary, GSH concentration and CAT activity in kidney tissues showed a significant decrease in the control group compared to the sham group ($p < 0.001$), while their levels were significantly increased in all treated groups (exercise, stevia and combined groups) compared to the control group ($p < 0.001$) with a more significant increment observed in the combined group. Moreover, there was no statistically significant difference between the sham and combined groups (Table 1).

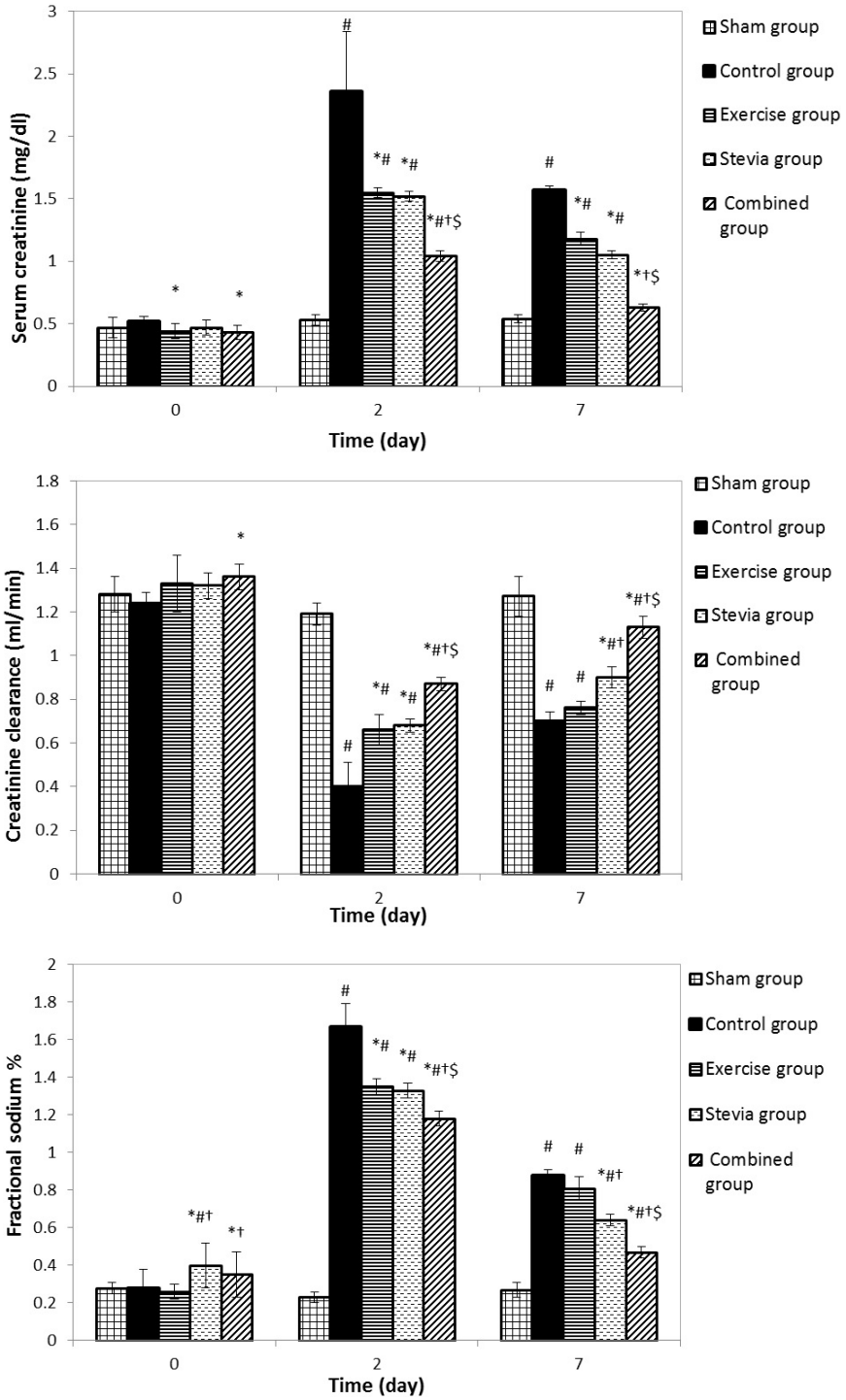

Fig. 1. Effect of methanolic stevia extracts, muscle exercise and combination of both on (A) serum creatinine, mg/dl, (B) creatinine clearance, ml/min, (C) fractional excretion of sodium (FE_{Na+}): # – signficant vs sham group, * – significant vs control group, † – significant vs exercise group, $ – significant vs stevia group

Table 1. Effects of exercise and methanolic stevia extracts on markers of oxidative stress (MDA, GSH and CAT) in kidney tissues

	Sham group	Control group	Exercise group	Stevia group	Combined group
MDA, nmol/mg protein	1.89 ±0.31	5.20 ±1.07 P1 =.000	4.30 ±0.36 P1 = .000 P2 =.046	2.92 ±0.16 P1 =.023 P2 =.000 P3 =.001	1.75 ±0.16 P1 = NS P2 = .000 P3 = .000 P4 = .008
GSH, nmol/mg protein	8.65 ±0.57	3.38 ±0.36 P1 =.000	5.98 ±0.26 P1 =.000 P2 =.000	7.17 ±0.64 P1 =.000 P2 =.000 P3 =.001	8.94 ±0.33 P1 = NS P2 = .000 P3 = .000 P4 = .000
CAT, unit/mg protein	34.66 ±3.14	14.0 ±2.36 P1 = .000	21.66 ±3.14 P1 = .000 P2 =.001	27.66 ±3.72 P1 =.004 P2 =.000 P3 =.015	35.0 ±2.36 P1 = NS P2 = .000 P3 = .000 P4 = .002

All results are expressed as mean ±SD. *P* value – independent samples T test (significance at $p \leq 0.05$), *P* value – one-way ANOVA: P1 vs sham group, P2 vs control group, P3 vs exercise group, P4 vs stevia group. MDA – malondialydehyde, GSH – reduced glutathione, CAT – catalase, NS – non-significant.

Effects of methanolic stevia extract and exercise on necrotic and regeneration scores in kidneys in a rat model of renal I/R injury

By means of histopathological examinations, the tubular necrotic score showed a significant increase in the control group compared to the sham group ($p = 0.002$), while the score was significantly attenuated in all treated (exercise, stevia and combined group) groups compared to the control group with no statistically significant difference among all treated groups (Fig. 2A). Figures 2B–2G are representative samples from different groups showing normal kidney morphology in the sham group, tubular necrosis and inflammatory cell infiltrates in the control group, dilated renal tubules in the exercise group, intratubular casts in the stevia group and nearly normal glomeruli and tubules in the combined group.

On the other hand, the tubular regeneration score shows a significant increase in the control group compared to the sham group ($p = 0.002$) and a more significant increase in the regeneration score in the treated (exercise, stevia and combined) groups compared to the control group ($p < 0.05$; Fig. 3A). Figures 3B–3G are representative samples from different groups showing signs of regeneration in different groups.

Effects of methanolic stevia extract and exercise on caspase-3 expression in kidney in a rat model of renal I/R injury

Apoptotic index (expression of caspase-3 by immunostaining) was significantly higher in the control group compared to the sham group ($p < 0.001$). On the other hand, caspase-3 expression was significantly attenuated in all treated groups (exercise, stevia and combined groups) compared to the control group ($p < 0.001$), with more significant attenuation in the combined compared to other treated groups ($p < 0.05$; Fig. 4A). Fig. 4B–4F are representative samples from different groups with minimal expression in the sham group, marked cytoplasmic expression in proximal tubules and glomeruli, moderate expression in the exercise and minimal expression in the stevia and combined groups.

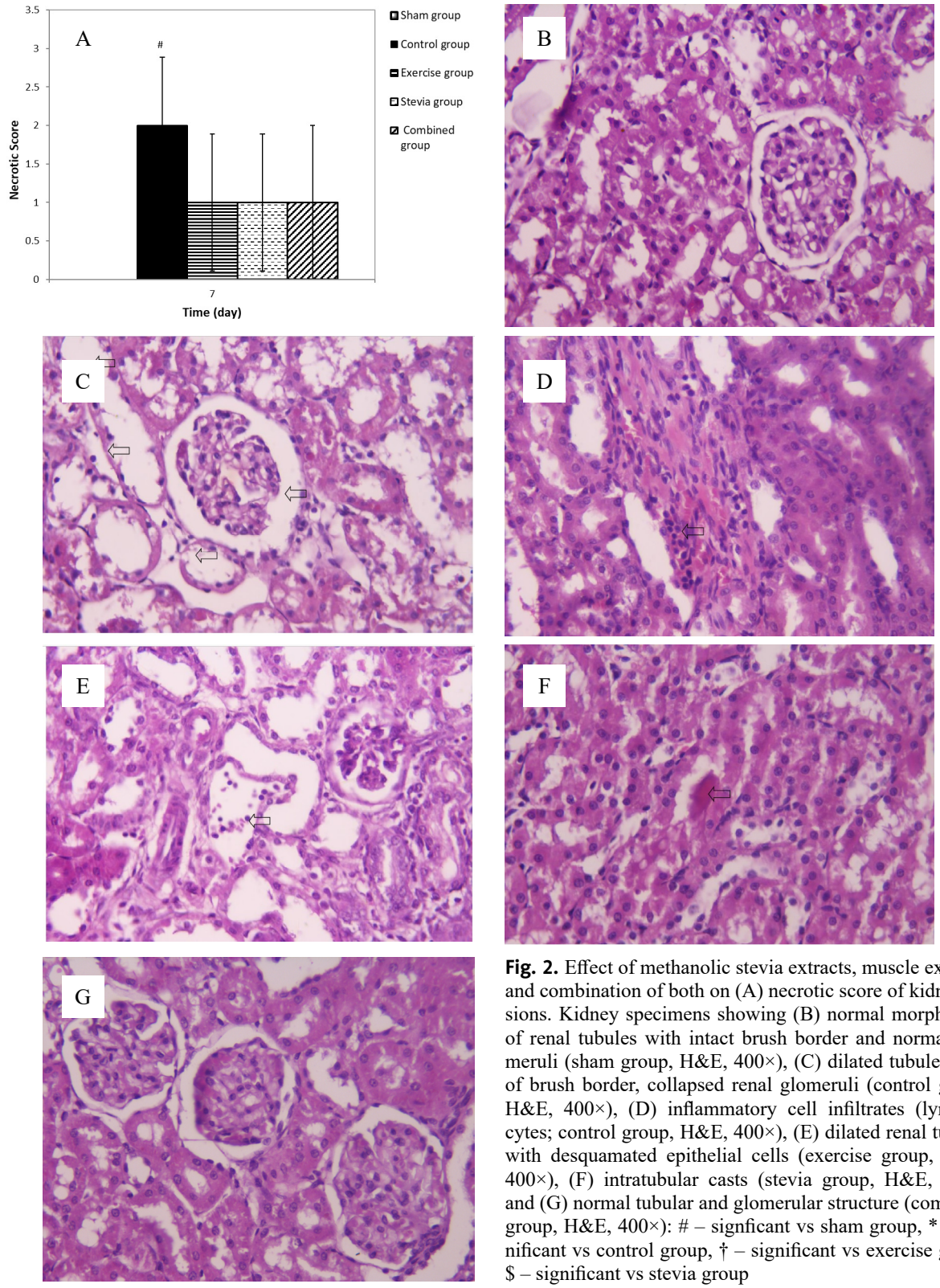

Fig. 2. Effect of methanolic stevia extracts, muscle exercise and combination of both on (A) necrotic score of kidney lesions. Kidney specimens showing (B) normal morphology of renal tubules with intact brush border and normal glomeruli (sham group, H&E, 400×), (C) dilated tubules, loss of brush border, collapsed renal glomeruli (control group, H&E, 400×), (D) inflammatory cell infiltrates (lymphocytes; control group, H&E, 400×), (E) dilated renal tubules with desquamated epithelial cells (exercise group, H&E, 400×), (F) intratubular casts (stevia group, H&E, 400×) and (G) normal tubular and glomerular structure (combined group, H&E, 400×): # – signficant vs sham group, * – significant vs control group, † – significant vs exercise group, $ – significant vs stevia group

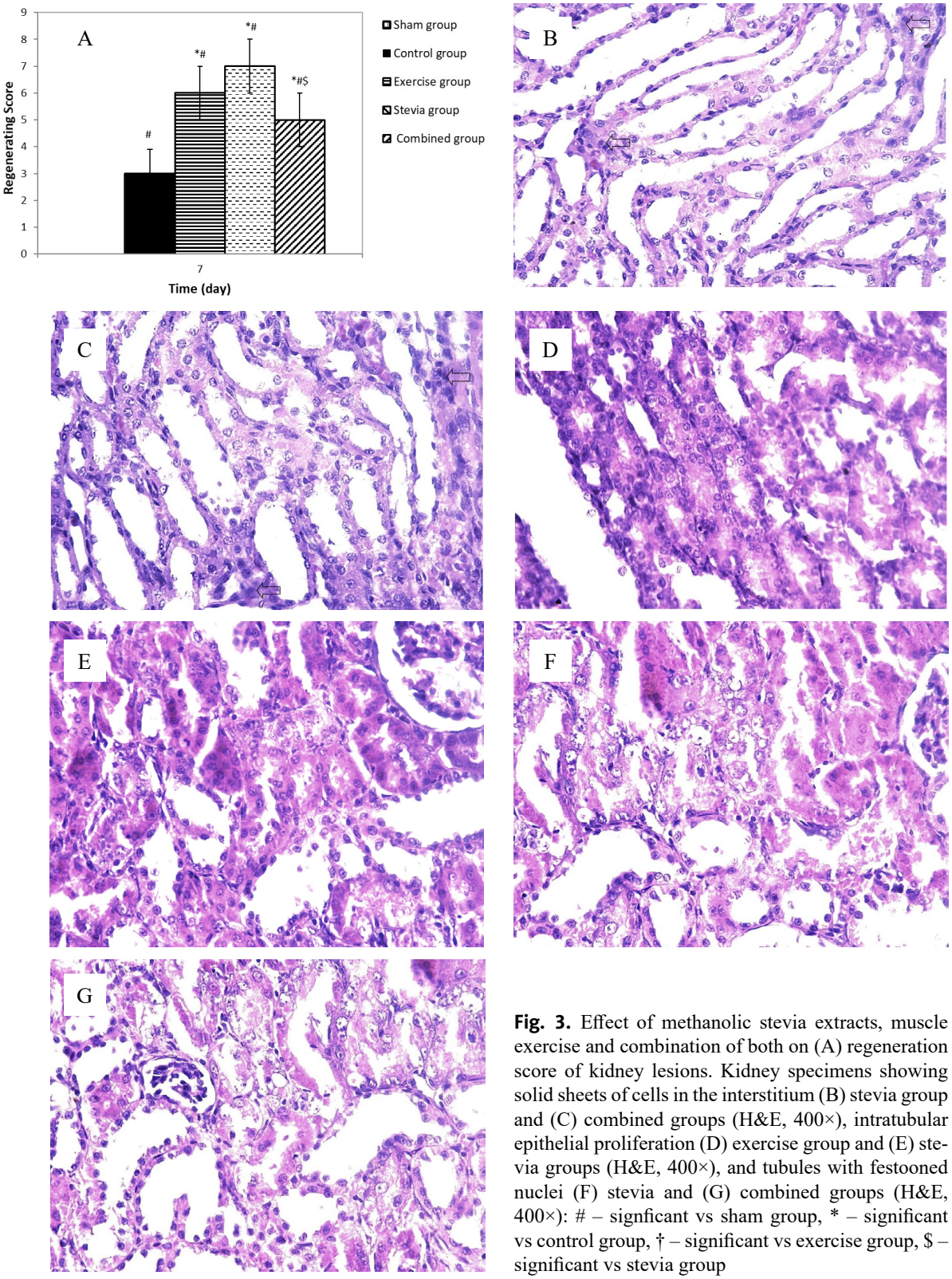

Fig. 3. Effect of methanolic stevia extracts, muscle exercise and combination of both on (A) regeneration score of kidney lesions. Kidney specimens showing solid sheets of cells in the interstitium (B) stevia group and (C) combined groups (H&E, 400×), intratubular epithelial proliferation (D) exercise group and (E) stevia groups (H&E, 400×), and tubules with festooned nuclei (F) stevia and (G) combined groups (H&E, 400×): # – signficant vs sham group, * – significant vs control group, † – significant vs exercise group, $ – significant vs stevia group

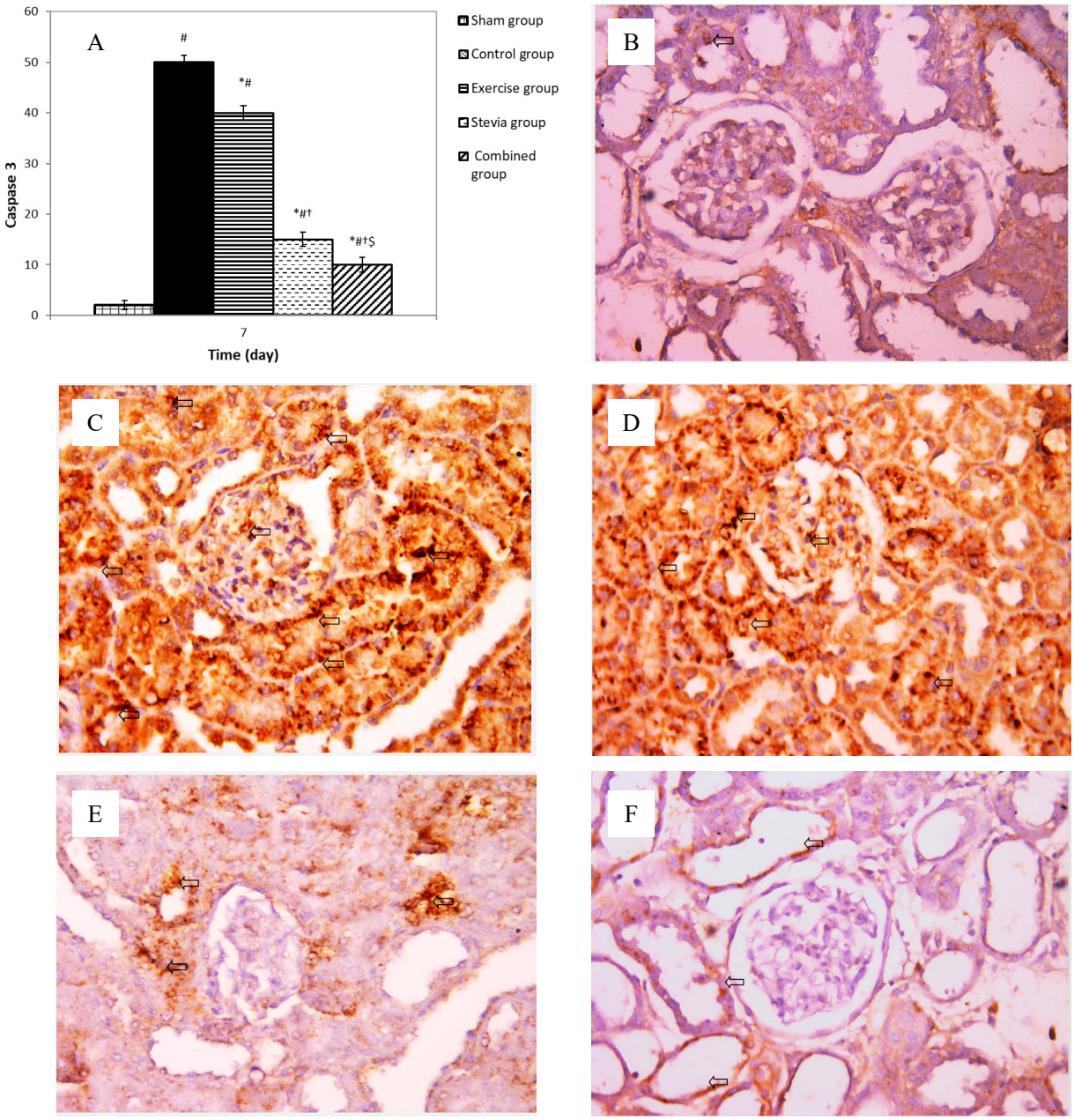

Fig. 4. Effect of methanolic stevia extracts, muscle exercise and combination of both on (A) caspase-3 expression by immunostaining. Kidney specimens showing (B) minimal cytoplasmic expression of caspase-3 in proximal tubules with negative expression in glomeruli (arrows) (sham group, immunoperoxidase DAB, 400×), (C) marked cytoplasmic expression of caspase-3 in proximal tubules with minimal expression in glomeruli (arrows) (control group, immunoperoxidase DAB, 400×), (D) marked cytoplasmic expression of caspase-3 in proximal tubules with minimal expression in glomeruli (arrows) (exercise group, immunoperoxidase DAB, 400×), (E) moderate cytoplasmic expression of caspase-3 in proximal tubules with negative expression in glomeruli (arrows) (Stevia group, immunoperoxidase DAB, 400×) and (F) minimal cytoplasmic expression of caspase-3 in proximal tubules with negative expression in glomeruli (arrows) (combined group, immunoperoxidase DAB, 400×): # – signficant vs sham group, * – significant vs control group, † – significant vs exercise group, $ – significant vs stevia group

DISCUSSION

Renal ischemia/reperfusion (I/R) injury is an inevitable sequale of kidney transplantation, shock with resuscitation, aortic cross-clamping and renal vascular surgery. Reactive oxygen species (ROS), apoptosis, cytokines, chemokines, and leukocytes activation play an important role in its underlying mechanisms (Jang and Rabb, 2009; Sharfuddin and Molitoris, 2011), therefore, it is imperative to find effective therapies and elucidate molecular mechanisms by which renal I/R injury may be attenuated. Antioxidants such as sulforaphane, oxymatrine and gelsemine have been demonstrated to protect murine kidneys against I/R injury (Shokeir et al., 2015; Jiang et al., 2015; Lin et al., 2015). These findings indicated that renal I/R injury might be ameliorated via targeting oxidative stress. Therefore, our intention hypothesis was to investigate a combination of muscle exercise and *Stevia rebaudiana* extracts in the prevention of I/R injury, through their potent antioxidant and antiapoptotic properties. The rational for using a combination therapy in the present study is based on the fact that multiple deleterious processes in different cell types of the kidney get initiated during renal I/R injury and ultimately all of them contribute synergistically towards the irreversible injury.

The effect of renal I/R on renal functions was studied by comparing the sham and the control groups on various days. The present study showed that renal ischemia caused a significant increase in serum creatinine and a significant decrease in creatinine clearance on the 2 time points of follow-up (days 2 and 7 after ischaemia), suggesting a significant impairment in glomerular function. Moreover, renal I/R resulted in a significant increase in FE_{Na+} on the 2 time points of follow-up after ischaemia, suggesting a significant impairment of tubular function. These findings confirm that I/R injury to the kidney causes both glomerular and tubular dysfunctions, and are in agreement with those reported by others (Bussmann et al., 2014; Castro et al., 2014; Malek and Nematbakhsh, 2015; Punuru et al., 2014). Renal I/R injury is characterized by decreases in the glomerular filtration rate (GFR), tubular and glomerular damage, impairment in hemodynamic regulation and energy depletion (Malek and Nematbakhsh, 2015).

In the present study, the ischemic kidneys showed characteristic morphological changes of acute tubular necrosis (ATN), such as tubular dilatation, cellular vacuolization, necrosis, intratubular detachment of cells with loss of integrity of brush border cell membrane. The forms of tubular epithelial cell death include a combination of necrosis and apoptosis. These findings are in agreement with previous studies reporting that renal I/R injury initiates a complex cascade of events that eventually result in injury and subsequently in necrotic and/or apoptotic death of renal cells (Malek and Nematbakhsh, 2015; Punuru et al., 2014; Rovcanin et al., 2016).

Lameire et al. (2005) reported that the primary histological changes of renal tubular cells are characterized with sloughing of tubular epithelial cells, brush border loss, tubular lumen dilatation, and the formation of tubular casts caused by necrosis and apoptosis. Rather than necrosis, apoptosis of renal tubular cells may be in charge of the dominant mode of injury. Therefore, the prevention of apoptosis of renal tubular cells is important to cure AKI caused by I/R injury (Lin et al., 2014; Yang et al., 2016; Zhang and Shu, 2016). Punuru et al. (2014) also found that the histological changes in the ischemia reperfusion groups included degeneration of tubular architecture, tubular dilation, proteinaceous debris, swelling and necrosis and luminal congestion with a loss of brush border indicating severe renal injury

Apoptosis and inflammation have been proposed as mechanisms for renal I/R injury. Caspases (e.g., cysteine proteases) are the executors of apoptosis (Hussein et al., 2016; Shokeir et al., 2014). Cell damage induced by prolonged ischemia-reperfusion injury may lead to apoptosis, autophagy, necrosis, and necroptosis (Ling et al., 2017; Lopez-Neblina et al., 2005; Zhu et al., 2016). The present work demonstrates that renal I/R injury caused a marked increase in apoptotic cell death in proximal and distal tubular cells as determined by caspase 3 as a marker for apoptosis, and this result was in line with those reported by others (Hussein et al., 2016; Shokeir et al., 2014).

In this model of renal I/R injury, renal I/R injury caused a significant increase in (oxidant) malondialdehyde (MDA) content of the tissue (indicating increased lipid peroxidation) and a significant decrease in (antioxidants) catalase (CAT) and in reduced glutathione

(GSH) after renal ischaemia. These findings suggest that there is a state of oxidative stress during renal I/R and provide good evidence for the role of ROS in the pathophysiology of renal ischemia/reperfusion injury and this finding agrees with other studies showing that renal I/R-induced oxidative stress generates high levels of reactive oxygen species (ROS). Consequently, overproduction of ROS results in lipid peroxidation, DNA mutation, apoptosis and necrosis, thus leading to cell death in various ways (Feng et al., 2011; Tsuda et al., 2012).

Malek and Nematbakhsh (2015) found that the most obvious causes of acute renal failure is ischemia reperfusion (I/R) injury due to the formation of reactive oxygen species (ROS). Reactive oxygen species – ROS lead to lipid peroxidation and decrease antioxidant defense, impair renal endothelial cells and decrease nitric oxide (NO) production (Chatauret et al., 2014). Malondialdehyde (MDA), which arises from the breakdown of lipid peroxyl radicals, is one of the indicators of oxidative stress. Malondialdehyde is also important, in which it can cause further oxidative injury by oxidizing protein molecules (Erkasap et al., 2004). Malondialdehyde as a marker of lipid peroxidation increased after renal I/R, as proved by Malek and Nematbakhsh (2015).

Previous studies mostly emphasize suppression of nearly all antioxidant defense mechanisms, including lower activities of SOD and catalase and a lower GSH level after I/R. On the other hand, one of the mechanisms of ROS-induced IR injury is lipid peroxidation and the malondialdehyde (MDA) level is a good indicator of this process (Li and Jackson, 2002). Other studies indicated that major antioxidants of renal tissue, CAT and GSH as well as total antioxidant capacity were lower after I/R (Bhalodia et al., 2009; Sener et al., 2006; Tugtepe et al., 2007). Punuru et al. (2014) found that during reperfusion, large amount of free radicals are generated and groups treated with *Murraya koenigi* showed a significant increase in endogenous anti-oxidants such as SOD, CAT, GSH with a reduction in MDA levels compared with the control groups.

The second aim of the present study was to investigate the effect of exercise for 5 weeks before renal ischemia on renal functions after renal I/R injury. In the present study, we found that regular exercise for 5 weeks before renal ischemia significantly improved the kidney functions and morphology, suggesting protective potential for muscle exercise against renal I/R injury. In agreement with our study, Saad (2014) demonstrated that regular exercise before the induction of renal I/R injury significantly improved renal function. Also, the renoprotective role of exercise against renal I/R injury were reported by dos Santos et al. (2012) and Leite and Rombaldi (2015). Moraes et al. (2014) reported that exercise training improved endothelium-dependent and endothelium independent kidney vasodilation through NO and Ex induced adaptation in vascular endothelium (Padilla et al., 2011). Exercise also increased renal antioxidant capacity (Moningka, 2011) and enzymes (Moningka et al., 2010). In this study, oxidative stress caused by renal I/R was significantly reduced by regular exercise for 5 weeks before renal ischemia as evidenced by a significant reduction in MDA and a significant increase in catalase (CAT) and reduced glutathione (GSH) in the kidney tissues of the exercise group. These findings are agreement with previous studies (Moningka, 2011; Moningka et al., 2010; 2013; de Moraes et al., 2014).

Recently, Bouzid et al. (2018) reported that physical activity improves antioxidant defenses and lowers lipid peroxidation levels both in adults and in aged individuals. Moreover, exercise has the potential to reduce apoptosis through upregulation of protective stress-sensitive proteins including nuclear factor kappa B (NF-kB), insulin-like growth factor (IGF-1), and heat shock proteins (Hsp90 and Hsp70) (Frost and Lang, 2003; Milne and Noble, 2002; Morton et al., 2009; Naito et al., 2001). In the present study, immunohistochemical examination for the expression of caspase-3 revealed a significant reduction in cytoplasmic expression for caspase-3 in proximal and distal tubular cells compared to the control group. Moreover, Kwak (2013) showed that aging resulted in increases in mitochondrial-mediated apoptotic pathways including pro-apoptotic protein levels such as Bax, Bax/Bcl-2 ratio, caspase-9, and cleaved caspase-3 and apoptosis. However, endurance exercise training reversed the elevation of apoptotic signaling and apoptosis, suggesting that exercise training protects the heart against apoptosis. In disagreement with our study, a study conducted by Yavari et al. (2015) showed that exercise tends to increase oxidative stress due to higher oxygen

consumption. Furthermore, Vafamand et al. (2017) demostrated that exercise increased serum MDA and oxidative stress, because the antioxidant system was not adapted to excessive production of ROS due to I/R injury.

The third aim was to investigate the effect of administration of methanolic stevia extract on the outcomes of renal I/R injury. In the present study, we found that administration of 200 mg\kg stevia extracts for 5 weeks before renal ischemia caused a marked improvement in renal function compared to the ischaemic group, as evidenced by a significant increase in creatinine clearance and a significant decrease in the serum creatinine and FE_{Na+}. In addition, a histopathological examination showed that stevia reduced the renal tubular damage (ATN) score and also increased regenerating activity score. These findings suggest that administration of stevia extract improved both the glomerular and the tubular injury induced by severe I/R injury in the rat. Thus, our study provides convincing evidence that stevia extract reduces I/R injury and possess a renoprotective effect, and this is in agreement with other studies which proved that different components of stevia extraction exert renoprotective action (Kujur et al., 2010; Lou et al., 2016; Nishi and Kumar, 2013; Shivanna et al., 2013). Moreover, the present study demonstrated more powerful renoprotective effects than muscle exercise against renal I/R injury.

The present study also provides strong evidence that methanolic stevia extract has powerful antioxidant action, as evidenced by the significant reduction in (MDA) and significant rise in (CAT) and (GSH). The antioxidant action of stevia extract was proved in different tissues by other researchers (Ahmad et al., 2011; Criado et al., 2013; John et al., 2011; Rao et al., 2014; Shivanna et al., 2013). Aqueous and methanolic extracts of stevia leaves have antioxidant properties equivalent to gallic acid and butylated hydroxyanisole (Abou-Arab and Abu-Salem, 2010). Stevia also increased the level of antioxidant enzymes and could be effective through scavenging these free radicals (Wu and Ng, 2008). In 2016, Assaei et al. (2016) reported that stevia could reduce the MDA level and raise the catalase activity in the pancreas supernatant of STZ--induced diabetic rats (40 mg/kg b.wt.). In 2017, Abd Elwahab et al. investigated the impact of stevia leaf extract on oxidative stress in the liver tissue of alloxan monohydrate-induced diabetic rats, and after six weeks, the results showed that stevia had significant effects on the MDA level in the liver homogenate. In the present study, we found that pretreatment with stevia extract caused a significant reduction in caspase 3 and apoptosis. In agreement with this finding, Wang et al. (2014) reported that in diabetic rats, Stevia extract inhibits the release of cytokines, caspase-3 and Bax versus a significant increase in bcl-2 level Thus, consumption of Stevia could maintain cell survival and counteract apoptosis. Recently, Zhong et al. (2019) found that Isosteviol sodium protects neural cells against hypoxia-induced apoptosis through inhibiting MAPK and NF-κB Pathways.

The last point investigated in this study was the effect of a combination of exercise and methanolic stevia extract on renal I/R injury. This study demonstrated that a combination of stevia and exercise caused a significant increase in creatinine clearance and a significant decrease in the serum creatinine and FE_{Na+} on various days after ischaemia. Consistent with the laboratory findings, this combination improved the renal tubular damage (ATN) score and increased regenerating activity score after ischaemia. These findings suggest that administration of a combination of stevia and exercise before the onset of ischemia is protective in renal I/R injury and reduces both the glomerular and the tubular dysfunction. Concerning the effect of this combination on oxidant and antioxidant systems in renal ischaemia, it was found that this combination caused a significant decrease in MDA and a significant increase in GSH and in CAT. These findings suggested that the combination of stevia and exercise improves the state of oxidative stress caused by renal I/R injury. Simioni et al. (2018) suggest that an antioxidant supplementation with natural compounds, accompanied by a constant physical exercise session, represents a useful mean to reduce oxidative stress and protect the body against oxidative damage, which was in agreement with our findings.

The present work demonstrates that a combination of stevia and exercise inhibits apoptotic cell death in proximal and distal tubular cells as determined by caspase 3 and protects the kidney from apoptosis caused by I/R injury. The results of this study demonstrate the levels of creatinine clearance, serum creatinine and FE_{Na+} in stevia alone and exercise alone groups on the

day 2 after ischaemia, but on day 7 after ischaemia, the results showed a significant difference in creatinine clearance, serum creatinine and FE_{Na+}. Stevia caused a significant decrease in MDA and a significant increase in GSH and in CAT and less apoptotic cell death and more regenerating activity than exercise, although necrosis was similar in both. This means that stevia was a more powerful antioxidant and antiapoptotic than exercise. By comparing the effect of this combination with the effect of stevia alone and exercise alone, it was found that a combination of stevia and exercise caused a significant improvement in glomerular and tubular function more than stevia alone or exercise alone. Moreover, it caused a significant decrease in MDA and a significant increase in GSH and in CAT and less apoptotic cell death and more regenerating activity than stevia alone or exercise alone. This means that a combination of stevia and exercise has a more protective effect on the kidneys after renal I/R injury.

In the present study, we did not measure the ratio of Bcl-2 to Bax that determines a cell's susceptibility to apoptosis. Bcl-2 and Bax are cellular proteins that play important roles in the regulation of apoptosis. This is considered one of the limitations of the present study. I/R-induced increases of nuclear factor erythroid-2-related factor 2 (Nrf2) and anti-oxidant enzyme heme oxygenase-1 (HO-1), although we did not measure this, which was another limitation. Exercise has the potential to reduce apoptosis through upregulation of protective stresssensitive proteins, including nuclear factor kappa B (NF-kB), insulin-like growth factor (IGF-1), Mn isoform of superoxide dismutase (Mn-SOD), extracellular receptor kinase (ERK), and heat shock proteins (Hsp90 and Hsp70). We did not investigate these stress-sensitive proteins and this was other limitation of our study. However, this limitation can be overcome by further investigations in future studies.

CONCLUSIONS

We concluded that 45 min left renal ischemia caused significant deteriorations in kidney functions and morphology with enhanced oxidative stress and apoptosis in kidney tissues and pretreatment with either exercise or stevia or combination of them caused a significant improvement in these deteriorations. The protective effects of stevia extract and exercise might be due to the attenuation of oxidative stress and apoptosis. Stevia alone or in combination with exercise offer a more protective effect than exercise alone.

REFERENCES

AbdElwahab, A. H., Yousuf, A. F., Ramadan, B. K., Elimam, H. (2017). Comparative effects of *Stevia rebaudiana* and aspartame on hepato-renal function of diabetic rats: Biochemical and histological approaches. J. Appl. Pharm. Sci., 7(08), 034–042.

Abou-Arab, E. A., Abu-Salem, F. M. (2010). Evaluation of bioactive compounds of *Stevia rebaudiana* leaves and callus. Afr. J. Food Sci., 4, 627–634.

Ahmad, N., Fazal, H., Abbasi, B. H., Inayat-UrRahman Anwar, S., Khan, M. A., …, Khan, K. Y. (2011). DPPH--scavenging antioxidant potential in regenerated tissues of *Stevia rebaudiana*, *Citrus sinensis* and *Saccharum officinarum*. J. Med. Plant Res., 5(14), 3293–3297.

Ahmadiasl, N., Banaei, S., Alihemmati, A., Baradaran, B., Azimian, E. (2014). The anti-inflammatory effect of erythropoietin and melatonin on renal ischemia reperfusion injury in male rats. Adv. Pharm. Bull., 4, 49–54.

Assaei, R., Mokarram, P., Dastghaib, S., Darbandi, S., Darbandi, M., Zal, F. (2016). Hypoglycemic effect of aquatic extract of Stevia in pancreas of diabetic rats: PPAR-gamma-dependent regulation or antioxidant potential. Avic. J. Med. Biotechnol., 8(2), 65–74. PMID 27141265. PMCID: PMC4842244.

Bhalodia, Y., Kanzariya, N., Patel, R., Patel, N., Vaghasiya, J., Jivani, N., Raval, H. (2009). Renoprotective activity of benincasa ceriferafruit extract on ischemia/reperfusion-induced renaldamage in rat. Iran J. Kidney Dis., 3, 80–85.

Bonventre, J. V., Yang, L. (2011). Cellular pathophysiology of ischemic acute kidney injury. J. Clin. Invest., 121(11), 4210–21.

Bouzid, M. A., Filaire, E., Matran, R., Robin, S., Fabre, C. (2018). Lifelong voluntary exercise modulates age-related changes in oxidative stress. Int. J. Sport. Med., 39, 21–28.

Bussmann, A. R., Filho, M. A. M., Módolo, M. P., Módolo, R. P., Amado, P., Domingues, M. A. C., …, Módolo, N. S. P. (2014). Effect of allopurinol on the kidney function, histology and injury biomarker (NGAL, IL 18) levels in uninephrectomised rats subjected to ischaemia-reperfusion injury. Acta Cir. Bras., 29(8), 51521. http://dx.doi.org/10.1590/S0102-86502014000800006

Castro, B. B. A., Colugnati, F. A. B., Cenedeze, M. A., Suassuna, P. G. A., Pinheiro, H. S. (2014). Standardization of renal function evaluation in Wistar rats (*Rattus norvegicus*) from the Federal University of Juiz de Fora's colony. J. Bras. Nefrol., 36(2), 139–149. http://dx.doi.org/10.5935/0101-2800.20140023

Chatauret, N., Badet, L., Barrou, B., Hauet, T. (2014). Ischemia-reperfusion: From cell biology to acute kidney injury. Progr. Urol., 24 (Suppl. 1), S4–S12.

Chatsudthipong, V., Muanprasat, C. (2009). Stevioside and related compounds: Therapeutic benefits beyond sweetness. Pharmacol. Ther., 121, 41–54.

Clarkson, P. M. (1995). Antioxidants and physical performance. Crit. Res. Food Sci. Nutr., 35, 131–141.

Criado, M. N., Barba, F. J., Frígola, A., Rodrigo, D. (2013). Effect of *Stevia rebaudiana* on oxidative enzyme activity and its correlation with antioxidant capacity and bioactive compounds. Food Bioproc. Technol., 7(5), 1518–1525.

de Moraes, R., Valente, R., Leon, I., Trugilho, M., Pacheco, A., Nobrega, A. (2014). Alterations of the kidney cortex proteome in response to exercise training in normoglycemic and hyperglycemic conditions. Curr. Topic. Med. Chem., 14(3), 450–461.

Donaldson, L. J. (2000). Sport and exercise: the public health challenge. Brit. J. Sports Med., 34, 409–415.

dos Santos Silva, K. A., da Silva Luiz, R., Rampaso, R. R., de Abreu, N. P., Moreira, É. D., Mostarda, C. T. (2012). Previous exercise training has a beneficial effect on renal and cardiovascular function in a model of diabetes. PloS One, 7(11), e48826.

El-Mousalamy, A. M. D., Hussein, S. A. M., Hussein, A. M., Mahmoud, S. A., El Azab, K. M., (2018). Reno protective effect of methanolic *Stevia rebaudiana* Bertoni leaves extract and its phenolic compounds in type-1-diabetes. Egypt. J. Chem., 61, 4, 609–615.

Erkasap, S., Erkasap, N., Koken, T., Kahraman, A., Uzuner, K., Yazihan, N., Ates, E. (2004). Effect of leptin on renal ischemia-reperfusion damage in rats. J. Physiol. Biochem., 60, 79–84.

Fadili, W., Habib Allah, M., Laouad, I. (2013). Chronic renal allograft dysfunction: risk factors, immunology and prevention. Arab. J. Nephrol. Transplant., 6(1), 45–50.

Feng, L., Ke, N., Cheng, F., Guo, Y., Li, S., Li, Q., Li, Y. (2011). The protective mechanism of ligustrazine against renal ischemia/reperfusion injury. J. Surg. Res., 166(2), 298–305.

Frost, R. A., Lang, C. H. (2003). Regulation of insulin-like growth factor-1 in skeletal muscle and muscle cells. Minerv. Endocrinol., 28, 53–73.

Gündüz, F., Sentürk, U. K., Kuru, O., Aktekin, B., Aktekin, M. R. (2004). The effect of one year's swimming exercise on oxidant stress and antioxidant capacity in aged rats. Physiol. Res., 53(2), 171–176.

Hussein, A. M., Barakat, N., Awadalla, A., Gabr, M. M., Khater, S., Harraz, A. M., Shokeir, A. A. (2016). Modulation of renal ischemia/reperfusion in rats by a combination of ischemic preconditioning and adipose-derived mesenchymal stem cells (ADMSCs). Can. J. Physiol. Pharmacol., 94(9), 936–946.

Jang, H. R., Rabb, H. (2009). The innate immune response in ischemic acute kidney injury. Clin. Immunol., 130, 41–50.

Jiang, G., Liu, X., Wang, M., Chen, H., Chen, Z., Qiu, T. (2015). Oxymatrine ameliorates renal ischemia-reperfusion injury from oxidative stress through Nrf2/HO-1 pathway. Acta Cir. Bras., 30, 422–429.

John K. M. M., Rajesh, J., Mandal, A. K. A., Natarajan, S. (2011). Antioxidantand antimicrobial activity of individual catechin molecules: a comparative study between gallated and epimerized catechin molecules. Eur. J. Exp. Biol., 1(3), 145–153.

Kujur, R. S., Singh, V., Ram, M., Yadava, H. N., Singh, K. K., Kumari, S., Roy, B. K. (2010). Antidiabetic activity and phytochemical screening of crudeextract of *Stevia rebaudiana* in alloxan-induced diabetic rats . Pharmac. Res., 2(4), 258–263.

Kwak, H. B. (2013). Effects of aging and exercise training on apoptosis in the heart. J. Exerc. Rehabil., 9(2), 212–219.

Lameire, N., Van Biesen, W., Vanholder, R. (2005). Acute renal failure. Lancet, 365, 417–430.

Leite, C. F., Rombaldi, A. J. (2015). Effect of carbohydrate intake and physical exercise on glycogen concentration. Rev. Bras. Cineantrop. Desemp. Hum., 17(1), 62–72.

Lemus-Mondaca, R., Vega-Gãlvez, A., Zura-Bravo, L., Ah-Hen, K. (2012). *Stevia rebaudiana* Bertoni, source of a high-potency natural sweetener: A comprehensive review on the biochemical, nutritional and functional aspects. Food Chem., 132, 1121–1132.

Lin, L., Zheng, J., Zhu, W., Jia, N. (2015). Nephroprotective effect of gelsemine against cisplatin-induced toxicity is mediated via attenuation of oxidative stress. Cell. Biochem. Biophys., 71, 535–541.

Lin, M., Li, L., Li, L., Pokhrel, G., Qi, G., Rong, R., Zhu, T. (2014). The protective effect of baicalin against renal ischemia-reperfusion injury through inhibition of inflammation and apoptosis. BMC Compl. Altern. Med., 14, 19. http://dx.doi.org/10.1186/1472-6882-14-19

Ling, Q., Yu, X., Wang, T., Wang, S. G., Ye, Z. Q., Liu, J. H. (2017). Roles of the exogenous H2S-mediated SR-A signaling pathway in renal ischemia/reperfusion injury in regulating endoplasmic reticulum stress-induced autophagy in a rat model. Cell. Physiol. Biochem., 41, 2461–74.

Lopez-Neblina, F., Toledo, A. H., Toledo-Pereyra, L. H. (2005). Molecular biology of apoptosis in ischemia and reperfusion. J. Invest. Surg., 18, 335–350.

Lou, J., Gu, X., Xing, Y., Cui, J., Lv, W., Zhang, Y. (2016). Chlorogenic acid slows down proteinuriaand renalfbrosis in 5/6-nephrectomized rats byanti-oxidation and inhibiting accumulation ofextracellular matrix. Int. J. Clin. Exp. Med., 9(8),15719–727.

Malek, M., Nematbakhsh, M. (2015). Renal ischemia/reperfusion injury; from pathophysiology to treatment. J. Renal Injur. Prev., 4(2), 20–27.

Martin, G. L., Warner, J. N., Nateras, R. N., Andrews, P. E., Humphreys, M. R., Castle, E. P. (2012). Comparison of total, selective, and nonarterial clamping techniques during laparoscopic and robot-assisted partial nephrectomy. J. Endourol., 26, 152–156.

Moningka, N. C. (2011). Impact of exercise on renal nitric oxide and antioxidant systems. Proquest Dissertations Publishing. University of Florida.

Moningka, N. C., Cunningham Jr, M. W., Sterling, M., West, C. A., Verlander, J. W., Croker, B. P., …, Baylis, C. (2013). Effects of voluntary wheel running on the kidney at baseline and after ischaemia – reperfusion-induced acute kidney injury: Astrain difference comparison. J. Physiol., 591(5), 1313–1324.

Moningka, N., Cunningham, M., Sterling, M., Baylis, C. (2010). Impact of 12 wks exercise on renal nitric oxide and antioxidant status: a strain difference comparison. FASEB J., 24 (1 Suppl.), 1059.1.

Morton, J. P., Kayani, A. C., McArdle, A., Drust, B. (2009). The exercise-induced stress response of skeletal muscle, with specific emphasis on humans. Sports Med., 39, 643–662.

Naito, H., Powers, S. K., Demirel, H. A., Aoki, J. (2001). Exercise training increases heat shock protein in skeletal muscles of old rats. Med. Sci. Sports Exerc., 33, 729–734.

Nath, K. A., Norby, S. M. (2000). Reactive oxygen species and acute renal failure. Am. J. Med., 109(8), 665–678.

Nishi Ahad, A., Kumar, P. (2013). Protective effect of chlorogenic acid against diabetic nephropathy in high fat diet/streptozin induced type-2 diabetic rats. Int. J. Pharm. Pharm. Sci., 5 (Suppl. 2), 489–495.

Padilla, J., Simmons, G. H., Bender, S. B., ArceEsquivel, A. A., Whyte, J. J., Laughlin, M. H. (2011). Vascular effects of exercise: endothelial adaptations beyond active muscle beds. Physiology, 26(3), 132–145.

Palant, C. E., Amdur, R. L., Chawla, L. S. (2017). Long-term consequences of acute kidney injury in the perioperative setting. Curr. Opin. Anesthesiol., 30(1), 100–104.

Punuru, P., Sujatha, D., Kumari, B. P., Charisma, V. V. (2014). Evaluation of aqueous extract of *Murraya koenigii* in unilateral renal ischemia reperfusion injury in rats. Indian J. Pharmacol., 46(2), 171–175. http://dx.doi.org/10.4103/0253-7613.129310

Ramya, M., Manogaran, S., Joey, K., Keong, T., Katherasan, S. (2014). Studies on biochemical and medicinal properties of *Stevia rebaudiana* grown in vitro. Int. J. Res. Ayurv. Pharm., 5(2), 169–174.

Rao, G. N., Rao, P. P., Balaswamy, K., Satyanarayana, A. (2014). Antioxidant activity of stevia (*Stevia rebaudiana* L.) leaf powder anda commercial stevioside powder. J. Food Pharm. Sci., 2, 32–38.

Rovcanin, B., Medic, B., Kocic, G., Cebovic, T., Ristic, M., Prostran, M. (2016). Molecular dissection of renal ischemia-reperfusion: oxidative stress and cellular events. Curr. Med. Chem., 23(19), 1965–1980.

Saad, R. A. (2014). Long term exercise preconditioning protects against renal dysfunction after ischemia reperfusion injury in rat kidneys. J. Am. Sci., 10(6), 154–161.

Sancaktutar, A. A., Bodakci, M. N., Hatipoglu, N. K., Soylemez, H., Basarılı, K., Turkcu, G. (2014). The protective effects of pomegranate extracts against renal ischemia-reperfusion injury in male rats. Urol. Ann., 6, 46–50.

Sener, G., Sehirli, O., Velioglu-Ogunc, A., Cetinel, S., Gedik, N., Caner, M., …, Yegen, B. C. (2006): Montelukast protects against renal ischemia/reperfusion injury in rats. Pharmacol. Res., 54, 65–71.

Sharfuddin, A. A., Molitoris, B. A. (2011). Pathophysiology of ischemic acute kidney injury. Nat. Rev. Nephrol., 7, 189–200.

Shivanna, N., Naika, M., Khanum, F., Kaul, V. K. (2013). Antioxidant, antidiabetic and renal protective properties of *Stevia rebaudiana*. J. Diab. Compl., 27, 103–113.

Shokeir, A. A., Barakat, N., Hussein, A. M., Awadalla, A., Harraz, A. M., Khater, S., …, Kamal, A. I. (2015). Activation of Nrf2 by ischemic preconditioning and sulforaphane in renal ischemia/reperfusion injury: A comparative experimental study. Physiol. Res., 64, 313–323.

Shokeir, A. A., Hussein, A. M., Barakat, N., Abdelaziz, A., Elgarba, M., Awadalla, A. (2014). Activation of nuclear factor erythroid 2-related factor 2 (Nrf2) and Nrf-2-dependent genes by ischaemic pre-conditioning and

postconditioning: new adaptive endogenous protective responses against renal ischaemia/reperfusion injury. Acta Physiol., 210(2), 342–353.

Simioni, C., Zauli, G., Martelli, A. M., Vitale, M., Sacchetti, G., Gonelli, A., Neri, L. M. (2018). Oxidative stress: role of physical exercise and antioxidant nutraceuticals in adulthood and aging. Oncotarget., 9(24), 17181–198. http://dx.doi.org/10.18632/oncotarget.24729

Tsuda, H., Kawada, N., Kaimori, J. Y., Kitamura, H., Moriyama, T., Rakugi, H., …, Isaka, Y. (2012). Febuxostat suppressed renal ischemia-reperfusion injury via reduced oxidative stress. Biochem. Biophys. Res. Commun., 427(2), 266–272.

Tugtepe, H., Sener, G., Biyikli, N. K., Yuksel, M., Cetinel, S., Gedik, N., Yegen, B. C. (2007). The protective effect of oxytocin onrenal ischemia/reperfusion injury in rats. Regul. Peptid., 140, 101–108.

Vafamand, E., Bolboli, L., Talebi, A., Nematbakhsh, M. (2017). The role of L-arginine and aerobic exercise in experimental renal ischemia reperfusion injury in male and female rats. Hormozgan Med. J., 21(1), 20–27.

Wang, T., Song, X., Zhang, Z., Guo, M., Jiang, H., Wang, W., …, Zhang, N. (2014). Stevioside inhibits inflammation and apoptosis by regulating TLR2 and TLR2--related proteins in *S. aureus*-infected mouse mammary epithelial cells. Int. Immun. Pharmacol., 22, 192–199.

Waynforth, H. B., Flecknell, P. A. (1998). Experimental and surgical technique in the rat (2nd ed., pp. 66–113). London–San Diego: Academic Press.

Wu, S., Ng, L. (2008). Antioxidant andfree radical scavenging activities of wildbitter melon (*Momordica charantia* Linn. var. *abbreviata* Ser.) in Taiwan. LWT – Food Sci. Technol., 41, 323–330.

Yang, Y., Song, M., Liu, Y., Liu, H., Sun, L., Peng, Y., …, Dong, Z. (2016). Renoprotective approaches and strategies in acute kidney injury. Pharmacol. Therapeut., 163, 58–73.

Yavari, A., Javadi, M., Mirmiran, P., Bahadoran, Z. (2015). Exercise-induced oxidative stress and dietary antioxidants. Asian J. Sports Med., 6(1).

Zhang, W., Shu, L. (2016). Upregulation of miR-21 by ghrelin ameliorates ischemia/reperfusion-induced acute kidney injury by inhibiting inflammation and cell apoptosis. DNA Cell Biol., 35, 417–425.

Zhong, K. L., Lu, M. Y., Liu, F., Mei, Y., Zhang, X. J., Zhang, H., …, Tan, W. (2019). Isosteviol sodium protects neural cells against hypoxia-induced apoptosis through inhibiting MAPK and NF-κB pathways. J. Stroke Cerebrov. Dis., 28(1), 175–184.

Zhu, J., Yao, K., Wang, Q., Guo, J., Shi, H., Ma, L., …, Ge, J. (2016). Ischemic postconditioning-regulated miR-499 protects the rat heart against ischemia/reperfusion injury by inhibiting apoptosis through PDCD4. Cell Physiol. Biochem., 39, 2364–80.

Zuk, A., Bonventre, J. V. (2016). Acute kidney injury. Ann. Rev. Med., 67, 293–307.