doi: 10.4149/gpb_2022008

Neonatal administration of zingerone prevents the subsequent development of high dietary fructose-induced early features of nephropathy in rats

Nasiru Muhammad[1,2], Busisani W. Lembede[1] and Kennedy H. Erlwanger[1]

[1] *School of Physiology, Faculty of Health Sciences, University of the Witwatersrand, Parktown, Johannesburg, South Africa*
[2] *Department of Physiology, College of Health Sciences, Federal University Birnin Kebbi, Birnin Kebbi, Nigeria*

Abstract. Excessive consumption of fructose-rich diets in early life stages increases the risk for developing nephropathy in adulthood. We investigated the potential preventive effects of neonatally administered zingerone on the development of dietary fructose-induced nephropathy. Four-day-old suckling male and female rat pups were orally gavaged (10 ml/kg) with: distilled water (Con group), 20% fructose solution (Fru group), 20% fructose solution + 40 mg/kg zingerone in distilled water (ZFru group), or 40 mg/kg of zingerone (Zgr group) for 14 days. Thereafter, Con and Zgr groups continued on plain drinking water while Fru and ZFru groups drank 20% fructose solution *ad libitum* for 10 weeks. The Fru group had significantly increased plasma concentration of the renal injury marker kidney injury molecule one (KIM-1) and decreased glomerular urinary space area compared to the controls in both sexes ($p < 0.05$). These alterations were prevented by neonatally administered zingerone. Zingerone administration neonatally is a potential prophylaxis for long-term high-fructose diet-induced nephropathy.

Key words: Fructose — Neonates — Nephropathy — Rats — Zingerone

Introduction

The global prevalence of chronic kidney disease is rapidly increasing, affecting 8–18% of the world population (Stern-Zimmer et al. 2020), and is projected to be among the five top causes of death by 2040 (Ramos et al. 2020). Chronic kidney disease is invariably associated with end stage renal disease and cardiovascular mortality (Ramos et al. 2020). Early postnatal life events can alter the structure and/or functions of the developing kidney leading to the development of kidney disease later in life (Tain and Hsu 2017). Physiologically, the developing kidney starts functioning only after birth and continues to develop and mature up to postnatal day 14 in rats (Tain and Hsu 2017; Rout and Dhar 2019). Thus, adverse events during the early lactation period may adversely affect nephrogenesis leading to epigenetic changes that can programme for chronic kidney disease in adult life (Tain and Hsu 2017). Early life malnutrition affects the metabolic function of kidneys (Safi-Stibler and Gabory 2020) leading to acute and reversible kidney diseases, which may progress to long-term irreversible chronic kidney diseases (Tain and Hsu 2017).

The rise in fructose consumption over the last century has paralleled the rising prevalence of kidney diseases, leading to the hypothesis that excessive fructose might be a causal factor for the development of chronic kidney disease (Nakagawa et al. 2020). The first step of fructose metabolism in the kidney involves phosphorylation by ketohexokinase which is heavily expressed in the proximal tubule of the kidney nephron (Nakayama et al. 2010). The phosphorylation of fructose causes adenosine triphosphate (ATP) depletion

Correspondence to: Nasiru Muhammad, School of Physiology, Faculty of Health Sciences, University of the Witwatersrand, 7 York Road, Parktown, Johannesburg 2193, South Africa
E-mail: nsmaaji@gmail.com

and subsequent generation of oxidative stress secondary to lipid peroxidation (Johnson et al. 2010; Mortera et al. 2019), thereby damaging the renal parenchyma (Nakagawa et al. 2020) and contributing significantly to the progression of nephropathy (Cui et al. 2018). Nakayama et al. (2010) previously reported that a high-fructose diet accelerated chronic renal disease *via* an increase in tubulointerstitial injury and glomerulosclerosis in rats. Furthermore, fructose is produced and metabolised endogenously by renal tissues, and this process might contribute to the development and progression of kidney disease (Nakagawa et al. 2020).

The gold standard for early diagnosis of renal diseases is kidney biopsy, which can also be used to determine the patterns and progression of the disease (Comai et al. 2019). Specific histopathology and morphometric changes such as alterations in glomerular urinary spaces, glomerular tuft and renal corpuscle areas are subjects of the kidney histological assessment (Rout and Dhar 2019; Wang et al. 2020). However, high costs and the invasive nature of kidney biopsy make it unaffordable to most patients, and thus it is not routinely performed in patients with kidney diseases (Glastras et al. 2016). It has been shown that metabolites measured in plasma or renal tissues can be used as kidney disease-relevant biomarkers for renal disease pathogenesis, progression, prediction, and diagnosis (Oraby et al. 2019). Kidney injury molecule 1 (KIM-1) and tissue malondialdehyde (MDA), have both been shown to be affected by high-fructose diets (Oraby et al. 2019) and therefore are potential viable markers of renal disease.

KIM-1 is a protein produced during toxic insults on the kidney and was observed as a sensitive and specific biomarker for early prediction of acute and chronic kidney damage in experimental animals (Rehman et al. 2018; Oraby et al. 2019). Additionally, KIM-1 is a potent target for therapeutic intervention of nephropathy in experimental models (Rehman et al. 2018), whilst MDA is a product of lipid peroxidation and thus a marker of oxidative stress (Rehman et al. 2018).

In the absence of any specifically approved drug treatment for kidney diseases, lifestyle modifications inclusive of regular exercise and good dietary habits are the first approaches in the early management of diet-induced nephropathy (Pereira et al. 2020). The use of synthetic pharmacological agents (both specific and non-specific) in the management of kidney diseases is limited to symptomatic treatment, and these agents are usually monotherapeutic and associated with adverse effects (Ramos et al. 2020). Additionally, the synthetic pharmacological agents may only slow the progression of nephropathy but do not completely stop it (Ramos et al. 2020). Advanced chronic kidney diseases pose a great economic burden on patients and government health care as dialysis and kidney transplants are the only alternative treatment options for late stages of the disease (Glastras et al. 2016).

Globally, communities are increasingly opting for products derived from nature including phytochemicals for their healthcare needs (Jamshidi-Kia et al. 2018; Rehman et al. 2018) in the belief that they provide a holistic, multi-spectrum nutraceutical value and are easily accessible (Rehman et al. 2018). Previous studies have used phytochemicals during the immediate postnatal period to programme for long-term metabolic health outcomes in fructose-fed rats. The development of hepatic lipid accumulation was prevented in adult rats fed high-fructose diets following postnatal administration of oleanolic acid (Nyakudya et al. 2018) and S-allyl cysteine (Lembede et al. 2017). In the current study, we investigated whether zingerone could be used to prevent fructose-induced nephropathy.

Zingerone is a phenolic alkanone found in the ginger (*Zingiber officinale*) plant (Ahmad et al. 2018). Previous studies have documented the antioxidant, anti-inflammatory and nephroprotective properties of zingerone in adult rats (Rehman et al. 2018) and mice (Cui et al. 2018) experimental models of diabetic nephropathy. The safety of zingerone has also been established with LD_{50} of 2580 mg/kg in rats (Rehman et al. 2018). Given the high burden that management of chronic kidney disease places on healthcare systems and the absence of specific treatments for the disease, there is need to develop alternative strategic prophylactic interventions. The biological properties of zingerone made it a suitable candidate phytochemical in the current study for administration during the neonatal period to explore its potential to protect against long-term development of nephropathy secondary to high-fructose diets. In that regard, due to close physiological relationship between rats and humans (Hashway and Wilding 2020), using a similar model of exploring zingerone neonatally could be translated in humans to manage diet-induced nephropathy; a strategy that could replace the use of animal models in exploring the health beneficial effects of zingerone.

Materials and Methods

Experimental setting and procedures

The study was approved by the Animal Research Ethics Committee (AREC) of the University of Witwatersrand (Clearance No: 2017/010/71/B). It was conducted at the Central Animal Service (CAS) of the University of Witwatersrand, Johannesburg, South Africa, using the internationally accepted principles for laboratory animal use and care, EU (86/609/EEC) guidelines, and the South African National Standard (SANS 10386:2008) and Animals Protection Act, 1962: Act No. 71. The protocol for the study was submitted to the AREC and has been archived there. The Animal Research Reporting of In Vivo Experiments (ARRIVE) guidelines were adopted while compiling the manuscript.

The study consisted of two stages: Pre-weaning (postnatal day 8–21) and post-weaning (postnatal day 22–91). Seven nulliparous dams (Sprague-Dawley rats), each with a litter consisting of 8–12 four-day-old suckling male and female pups (weighing 9.7 ± 1.1 g) were used in this study. The rats were obtained from the Central Animal Service, University of the Witwatersrand. The rat mating pairs were put together by animal attendants who were blinded to the study design. They then selected dams with the appropriate litter sizes and these were then availed to the researcher. On postnatal day 8 (after the pups were acclimatized to the environmental conditions for 4 days), the pups from each dam (n = 79; males 35, females 44) were randomly allocated to four treatment groups. The researcher created the allocation sequence. Rat pups from each dam were first stratified by sex. Thereafter they were placed in a cage and as taken out individually, they were proportionally and sequentially allocated to each treatment group, marked on their tails with non-toxic ink for identification and returned to their dams. Resultantly, pups from each litter were represented in each one of four treatment groups: Con group (negative control) received 10 ml/kg body weight (b.w.) of distilled water gavaged orally before weaning and *ad libitum* access to plain tap water to drink after weaning (n = 20; males 9, females 11); Fru group received 10 ml/kg b.w. of a 20% fructose solution gavaged orally before weaning and *ad libitum* access to a 20% fructose solution to drink after weaning (n = 20; males 9, females 11) to induce metabolic dysfunction; ZFru group received 10 ml/kg b.w. of a 20% fructose solution + zingerone at 40 mg/kg b.w. gavaged orally before weaning (to programme for protection against long-term high fructose consumption) and *ad libitum* access to a 20% fructose solution to drink after weaning (n = 21; males 9, females 12); Zgr group received 40 mg/kg b.w. of zingerone dissolved in 10 ml/kg b.w. of distilled water gavaged orally before weaning and *ad libitum* access to plain tap water to drink after weaning (n = 18; males 8, females 10) to assess the effects of neonatal intake of zingerone alone on renal metabolic health of the animals.

The dams with their respective litters were housed in Perspex cages containing wood shavings as bedding. The room temperature was maintained at 25 ± 1°C and a 12-h light and dark cycle with lights off from 7 p.m. to 7 a.m. The dams were supplied with commercially formulated standard rat chow (LabChef Research Nutrition, (Pty) Ltd, Stellenbosch, South Africa) and *ad libitum* water access throughout the suckling period. The pups were allowed to freely nurse from their respective dams until weaning on postnatal day 21. The dams were returned to stock after weaning of their pups. The weaned rats were then housed individually in Perspex cages having the same environmental conditions described earlier.

The fructose solution (Natures Choice, South Africa) was constituted to 20% by adding 20 g of fructose into 100 ml of water (Mamikutty et al. 2014), while food grade zingerone (W312401-1KG) was purchased from Sigma-Aldrich (USA), and it is ≥ 98% natural. The standard rat chow was provided by the LabChef Research Nutrition (Pty) Ltd, Stellenbosch, South Africa. The nutritional compositions of fructose and standard rat chow are shown in Table 1.

The rats were weighed daily during the pre-weaning stage, twice a week during the post-weaning stage and prior to termination by individually placing the rats in a pre-weighed cage on an electronic balance (Snowrex Electronic Scale, Clover Scales, Johannesburg, South Africa). This was to monitor the animals' growth and adjust treatment dosages.

Procedures at termination

After the 12-week intervention period, the rats were fasted from solid feed but provided plain drinking water overnight for 12 hours. Terminal body mass was then determined with an electronic balance (Snowrex Electronic Scale, Clover Scales, Johannesburg, South Africa) and the rats were then euthanized with sodium pentobarbitone (Centaur Laboratories, Johannesburg, South Africa) at 150 mg/kg b.w. intraperitoneally.

After euthanasia, a ventral midline incision was made through the thorax and abdomen; syringes with attached needles were used to withdraw blood samples from the heart.

Table 1. Nutritional composition of fructose and standard rat chow

Fructose rat chow	
Fructose nutritional analysis	Composition (*per* 100 g)
Energy	1680 KJ
Carbohydrates	99.8 g
Protein	0 g
Fats	0 g
Fibre	0 g
Sodium	0.2 mg
Standard rat chow	
Ingredients	Composition
Protein	220 g/kg
Moisture	100 g/kg
Oils and fats	50 g/kg
Linoleic acid	12 g/kg
Fibre	40 g/kg
Ash	70 g/kg
Calcium	12 g/kg
Phosphorous	7.5 g/kg
Vitamin E	100 g/kg
Vitamin A	16 000 IU/kg
Vitamin D	2000 IU/kg

The blood samples were transferred into heparinized tubes (BD Vacutainer, Plymouth, UK) and centrifuged (Rotofix 32A, Hettich Zentrifugen, Germany) at 3700 × *g* for 15 min at 20°C. The plasma was harvested and stored at −20°C for determination of KIM-1. The kidneys were carefully dissected out from the abdominal cavity, perirenal fat removed, and weighed using a Presica 310M balance (Precision Instruments, Johannesburg, South Africa), and then the weight of both kidneys relative to body mass was computed: Relative kidney weight (%) = [mass of kidneys (g)/terminal body mass (g)] × 100. The right kidney was preserved in 10% phosphate buffered formalin for histological analysis, while the left kidney was preserved at −20°C for tissue MDA determination.

Determination of plasma KIM-1 and kidney tissue MDA

Plasma KIM-1 was determined using a rat-KIM-1 Enzyme Linked Immuno-Sorbent Assay kit (Elabscience Biotechnology Co., Ltd, Wuhan, Hubei, China) according to manufacturer's instructions.

To determine kidney MDA, the frozen preserved kidneys were allowed to thaw at room temperature, and then homogenised in phosphate buffered saline with a glass homogeniser on ice. The cells were further fragmented by sonicating the suspension with an ultrasonic cell disrupter. The homogenates were centrifuged for 5 min at 5000 × *g* to obtain the supernatant. The tissue homogenate MDA was then determined using rat-MDA Enzyme Linked Immuno-Sorbent Assay kit (Elabscience Biotechnology Co., Ltd, Wuhan, Hubei, China) according to manufacturer's instructions.

Histological assessment of the kidney

The kidney samples that were preserved in 10% phosphate buffered formalin were processed using an automatic tissue processor (Microm STP 120, Thermo Scientific, MA, USA), embedded in paraffin wax, sectioned at 5 µm and then stained with haematoxylin and eosin (H&E) or Masson's trichrome (MT). The slides were viewed under a light microscope (Axioskop 2 microscope; Leica Biosystems, USA) mounted to an Axiocam HRc2 camera, which uses ZEISS ZEN microscope software for image capture. Measurements were taken on the photomicrographs using the Zen lite analysis software. The images at 40× magnification from the H&E stained slides were used to assess corpuscular and glomerular tuft areas under 40× magnification, and glomerular density under 10× magnification. Glomerular urinary space area was computed using the formula (Kashif et al. 2020): Glomerular urinary space area (μm^2) = renal corpuscular area (μm^2) – glomerular tuft area (μm^2). Glomerular density was computed using the following formula (Nigro et al. 2018): Glomerular density ($N/\mu m^2$) = number of renal corpuscle *per* section (N) ÷ total area of the section (μm^2).

The images at 40× magnification of the MT stained slides were used to assess collagen deposition and fibrosis.

Statistical analysis

Multiple-group data were analysed using Graphpad Prism 8 software (Graph-pad Software Inc., San Diego, USA), by one-way analysis of variance (ANOVA), and expressed as mean ± standard deviation (SD) followed by multiple-comparisons Tukey *post hoc* tests. Sex variations were analysed using two-way ANOVA. Statistical significance was considered when $p < 0.05$.

We used a previous study conducted in our research laboratory to calculate the sample size (8–12 rats *per* group) (Lembede et al. 2017) using the formula (Charan and Kantharia 2013): Sample size = $2\ SD^2\ (1.96 + 0.842)^2/d^2$, where SD is standard deviation from previous study; 1.96 is type 1 error of 5%; 0.842 is value at 80% power; d is difference between mean values.

Results

Kidney mass

The absolute and relative kidney masses of the study rats are shown at Table 2. Male rats had significantly greater absolute

Table 2. Effects of neonatally administered zingerone on the absolute and relative kidney masses of high-fructose diet fed rats

Parameter	Sex	Group				*p*
		Con	Fru	ZFru	Zgr	
Kidney mass (g)	Male	$2.40^{\alpha} \pm 0.21$	$2.40^{\alpha} \pm 0.24$	$2.30^{\alpha} \pm 0.19$	$2.30^{\alpha} \pm 0.18$	0.4644
	Female	1.60 ± 0.11	1.60 ± 0.05	1.50 ± 0.12	1.60 ± 0.18	0.0897
Relative kidney mass (% b.w.)	Male	0.63 ± 0.03	0.61 ± 0.03	0.61 ± 0.62	0.62 ± 0.02	0.5405
	Female	$0.65^{\beta} \pm 0.06$	$0.63^{\beta} \pm 0.04$	$0.62^{\beta} \pm 0.03$	$0.66^{\beta} \pm 0.05$	0.0759

Data expressed as mean ± standard deviation (SD) using analysis of variance (ANOVA). Con, rats received 10 ml/kg b.w. of distilled water (negative control); Fru, rats received 10 ml/kg b.w. of 20% fructose solution; ZFru, rats received 10 ml/kg b.w. of 20% fructose solution + 40 mg/kg b.w. zingerone; Zgr, rats received 40 mg/kg b.w. of zingerone; *p*, predictive value; % b.w., percentage body weight; $^{\alpha}$ male rats had higher absolute kidney masses than their female counterparts at $p < 0.0001$; $^{\beta}$ female rats had higher relative kidney masses than their male counterparts at $p = 0.0117$.

kidney mass than females ($p < 0.0001$ for sex effect, $p = 0.1740$ for treatment effect and $p = 0.7384$ for interaction effect), while female rats had significantly greater relative kidney masses than the males ($p = 0.0117$ for sex effect, $p = 0.0842$ for treatment effect and $p = 0.6807$ for interaction effect).

Plasma KIM-1 and kidney tissue MDA

The fructose only administered group (Fru) had significantly ($p = 0.0092$, males; $p < 0.0003$, females) increased levels of KIM-1 compared to the controls (Con) in both sexes (Table 3). Neonatally administered zingerone prevented the fructose-induced increase in the levels of KIM-1 such that rats administered combined fructose and zingerone (ZFru) had similar ($p = 0.9996$, males; $p = 0.3581$, females) concentration of KIM-1 when compared with the controls in both sexes.

Histomorphometry of the kidney

The fructose only administered group (Fru) had significantly ($p < 0.0076$, males; $p < 0.0067$, females) decreased glomerular urinary space area compared to the controls (Con) in both sexes (Fig. 1 and Table 4). Neonatally administered zingerone prevented the fructose-induced decrease in the glomerular urinary space area such that rats administered combined fructose and zingerone (ZFru) had similar ($p = 0.9878$, males; $p = 0.9696$, females) glomerular urinary space area when compared with the controls in both sexes. This demonstrated that zingerone prevented the development of early histological signs of nephropathy induced by high-fructose diet in the study rats.

For the Masson's trichrome histological sections (MT stain), there was no observable collagen deposition or fibrosis across the different treatment groups in both sexes (Fig. 2A, males; Fig. 2B, females).

Discussion

We investigated the potential protective effects of neonatal administration of oral zingerone on long-term dietary fructose-induced nephropathy later in adulthood. Increased levels of plasma KIM-1 and reduced urinary space area were observed in the adult male and female rats following long-term administration of the 20% fructose solution. Zingerone administered orally to the neonatal rats protected them against high-fructose diet-induced increased in KIM-1 and the reduced urinary space area later in adulthood in both sexes. Thus, neonatal administration of oral zingerone likely

Table 3. Effects of neonatally administered zingerone on the plasma levels of KIM-1 and kidney tissue MDA of high-fructose diet fed rats

Parameter	Sex	Group				*p*
		Con	Fru	ZFru	Zgr	
KIM-1 (pg/ml)	Male	$61.00^{a} \pm 30.00$	$104.00^{b} \pm 26.00$	$62.00^{a} \pm 20.00$	$70.00^{a} \pm 31.00$	0.0092
	Female	$50.00^{a} \pm 22.00$	$97.00^{b} \pm 34.00$	$67.00^{a} \pm 17.00$	$55.00^{a} \pm 19.00$	0.0003
MDA (ng/ml)	Male	61.00 ± 30.00	78.00 ± 22.00	66.00 ± 28.00	66.00 ± 17.00	0.5000
	Female	56.00 ± 18.00	67.00 ± 18.00	66.00 ± 21.00	52.00 ± 22.00	0.2123

Data expressed as mean ± SD using ANOVA. KIM-1, kidney injury molecule 1; MDA, malondialdehyde; ^{a,b} within row means with different superscript letters signifficantly different at $p < 0.05$. For other abbreviations, see Table 2.

Table 4. Effects of neonatally administered zingerone on kidney morphometry of the fructose-fed rats

Parameter	Sex	Group				*p*
		Con	Fru	ZFru	Zgr	
Corpuscular area (μm^2)	Male	15370.00 ± 4151.00	15016.00 ± 3877.00	16151.00 ± 4584.00	19928.00 ± 5384.00	0.1393
	Female	15702.00 ± 4000.00	16440.00 ± 3679.00	17339.00 ± 4316.00	16971.00 ± 3944.00	0.8579
Glomerular tuft area (μm^2)	Male	12614.00 ± 3521.00	14012.00 ± 3553.00	13557.00 ± 4433.00	16247.00 ± 4267.00	0.3252
	Female	12765.00 ± 3932.00	15046.00 ± 3636.00	14596.00 ± 3992.00	13857.00 ± 3996.00	0.6292
GUSA (μm^2)	Male	$2755.00^{a} \pm 1077.00$	$1005.00^{b} \pm 500.90$	$2594.00^{a} \pm 620.30$	$3681.00^{a} \pm 1465.00$	0.0076
	Female	$2937.00^{a} \pm 815.90$	$1394.00^{b} \pm 491.20$	$2744.00^{a} \pm 939.20$	$3114.00^{a} \pm 1090.00$	0.0067
G.D. (10^{-6} N/μm^2)	Male	3.36 ± 7.80	3.86 ± 7.09	3.23 ± 1.13	3.41 ± 3.41	0.4847
	Female	3.23 ± 8.97	3.38 ± 8.12	3.66 ± 1.04	3.63 ± 3.63	0.7067

Data expressed as mean ± SD using ANOVA. GUSA, glomerular urinary space area; G.D., glomerular density; ^{a,b} within row means with different superscript letters signifficantly different at $p < 0.05$. For other abbreviations, see Table 2.

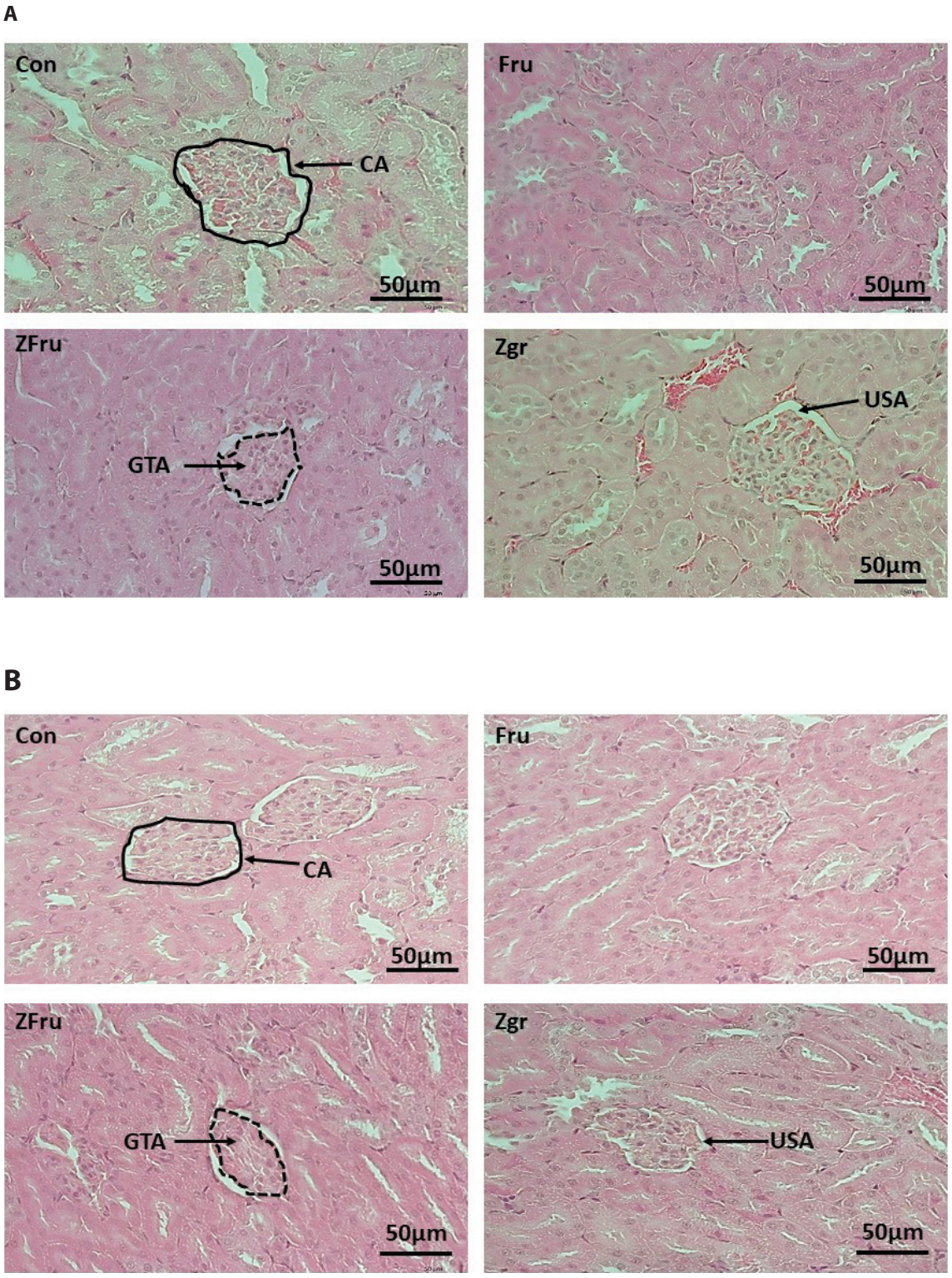

Figure 1. Representative photomicrographs of the male (**A**) and female (**B**) rat kidney sections stained with Haematoxylin and Eosin (×40). Scale bar = 50 µm. CA, corpuscular area; GTA, glomerular tuft area; USA, urinary space area. Groups: Con, rats received 10 ml/kg b.w. of distilled water (n = 20; 9 males, 11 females); Fru, rats received 10 ml/kg b.w. of 20% fructose solution (n = 20; 9 males, 11 females); ZFru, rats received 10 ml/kg b.w. of 20% fructose solution + 40 mg/kg b.w. zingerone (n = 21; 9 males, 12 females); Zgr, rats received 40 mg/kg b.w. of zingerone (n = 18; 8 males, 10 females).

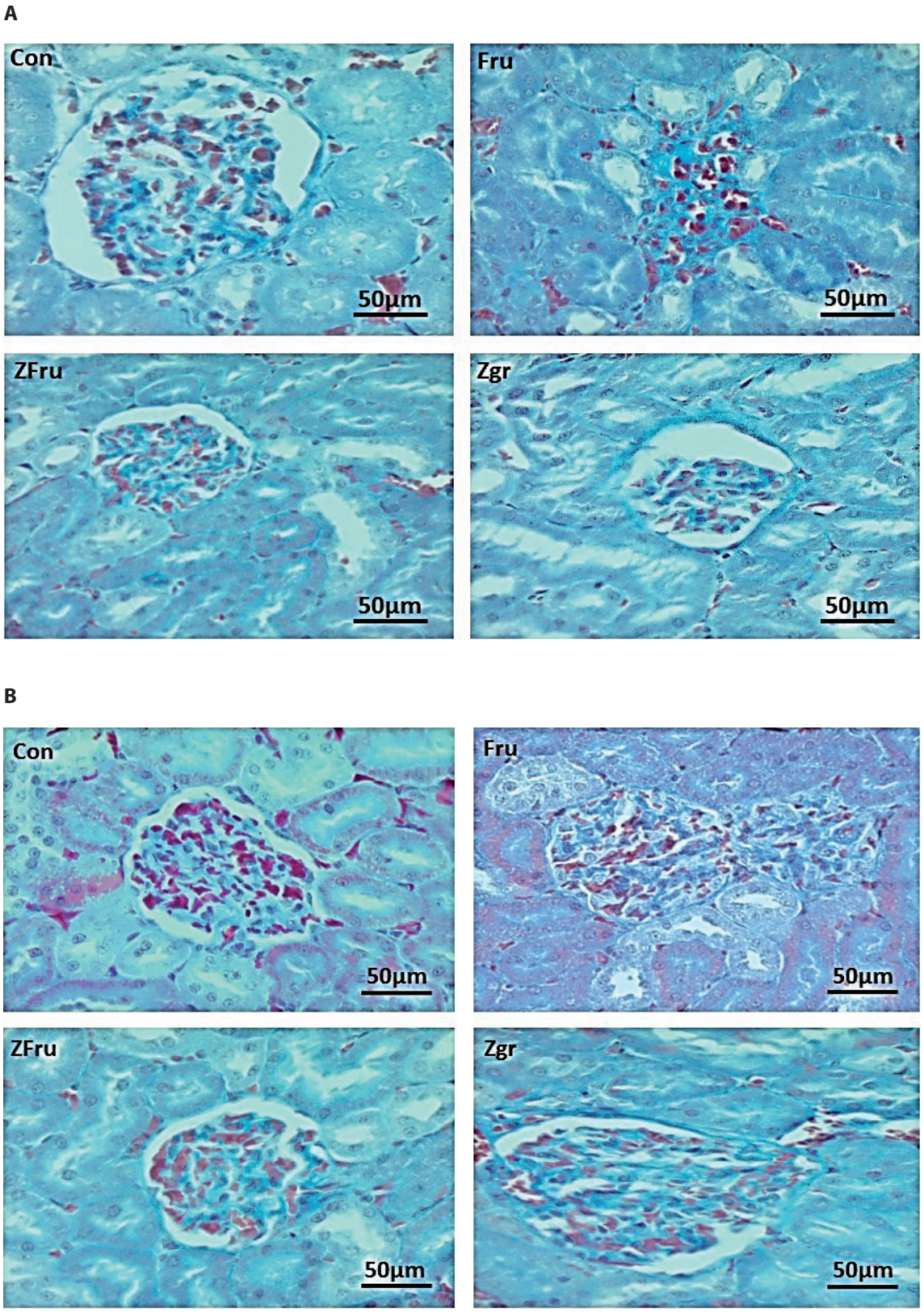

Figure 2. Representative photomicrographs of the male (**A**) and female (**B**) rat kidney sections stained with Masson's trichrome (magnification ×40). Scale bar = 50 µm. For abbreviations, see Figure 1.

attenuates the development of long-term fructose-induced nephropathy in both male and female rats in adult life. A sexually dimorphic response was observed only in kidney masses, where the absolute kidney masses were higher in males and the relative kidney masses were higher in females.

The weight of a kidney is an important parameter in evaluating renal tissue hypertrophy that may be associated with nephropathy (Cui et al. 2018). Additionally, changes in organ weights are used in toxicology to determine whether a particular substance has adverse effects which may cause atrophy (Shafaei et al. 2015). The current study did not note any differences in the absolute weight of the kidneys of the study rats within both sexes, implying that the interventions did not produce gross effects on the kidneys. The fact that male rats had greater absolute kidney weights than females could be attributed to inherent body sizes of the male rats compared for females (Wen et al. 2020), due to greater muscle mass (Virgen-Ortiz et al. 2018) and organ weight (Spritzer and Roy 2020) in males than female rats.

However, when scaled relative to body mass it was observed that the females had relatively heavier kidneys. This may be attributed to the fact that the kidneys stop growing at a particular age (Rout and Dhar 2019), and in male rats the body mass increase is relatively greater than female rats (Wen et al. 2020).

Renal function can be assessed using different parameters including glomerular filtration rate, blood urea and creatinine (Muller et al. 2019). Measurement of glomerular filtration rate and blood urea and creatinine levels is commonly employed to assess renal function; however, these measurements provide useful diagnostics only in cases of severe renal injury and have limitations in identifying and monitoring kidney diseases (Kim et al. 2016). Microalbuminuria, which is recognised as a characteristic feature for earliest stage of nephropathy, was not measured in this study because it has been observed that nephropathy can develop in patients with normoalbuminuria (Oraby et al. 2019). While increases in uric acid can also be diagnostic of impaired renal function in humans due to lack of the enzyme uricase, rats have the enzyme and hence are able to breakdown uric acid (El-Kafoury et al. 2019) hence it was not measured in the current study. It is therefore important to use more sensitive and direct assessments.

Indeed, a more comprehensive picture of early nephropathy could in principle be obtained by other markers, such as neutrophil gelatinase-associated lipocalin (NGAL), N-acetyl-β-D-glucosaminidase (NAG) and nephrin as well as urinary excretion of glucose (Oraby et al. 2019). However, due to technical challenges we did not assay for these and that is acknowledged as a limitation of the current study, and should be considered in future studies.

Metabolomics show that plasma (Rehman et al. 2018) and urinary (Ahmed and Hamed 2015) KIM-1 is affected in the early stages of nephropathy.

In the current study, we observed an increase in the plasma levels of KIM-1 in both male and female rats that received the long-term high fructose-diet only compared to the other groups. The fructose-induced increase in KIM-1 was prevented by neonatal administration of zingerone, meaning that neonatal zingerone had programmed protection against the renal injury secondary to high-fructose diet consumption. The finding of this study expands on that of a previous study that reported a decrease in KIM-1 following administration of 50 mg/kg b.w. of zingerone to streptozotocin-induced diabetic adult male Wistar rats for 16 weeks (Rehman et al. 2018). Thus, we have shown a prophylactic effect while Rehman et al. (2018) showed a therapeutic effect of zingerone. This beneficial effect of zingerone has been partially attributed to its antioxidant properties including suppression of MDA production, which is a lipid peroxidation product (Hosseinzadeh et al. 2020). In addition to attenuating oxidative stress, other mechanisms of zingerone's action include inhibition of inflammatory cells infiltration into the renal parenchyma as demonstrates by Hosseinzadeh et al. (2020). Zingerone is also reported to inhibit inflammatory processes associated with nuclear factor-kappa B activation (Rehman et al. (2019). Thus, future studies will be directed towards understanding these mechanisms of action of zingerone.

An increase in MDA level is an indicator of nephropathy-associated oxidative stress (Rehman et al. 2018). In the current study, neither administration of fructose nor that of zingerone had any effects on the kidney tissue level of MDA in the study rats in both sexes. This is in contrast to a previous report that demonstrated zingerone to inhibit oxidative stress *via* decreasing MDA in diabetic mice (Cui et al. 2018). The disparity could be that the high-fructose diet did not cause severe kidney damage (evident on histology) in the study rats that were not diabetic-induced.

Assessment of kidney histopathology is considered as the gold standard in the clinical diagnosis of nephropathy (Jin and Xie 2020). Additionally, histological lesions of kidneys may develop without derangement of metabolomics in nephropathy (Comai et al. 2019). In the present study, we observed a reduction in the glomerular urinary space area in the kidney sections of both male and female rats that received high fructose-diet only compared to the other groups. We acknowledge that although decreased glomerular urinary space area can be an early histologic feature of nephropathy that can lead to reduced glomerular filtration capacity and eventual kidney failure (Cui et al. 2018), the pathophysiological outcomes of decreased glomerular urinary space area are not well established. The observed fructose-induced histological lesions in both sexes were prevented by neonatal administration of zingerone in this study, implying that zingerone had programmed protection against the early histological features of nephropathy secondary to high-fructose

diet consumption. The observed histological outcomes in the current study require further investigations involving bio-histochemical assessments to establish the fundamental physiological mechanisms.

The absence of glomerular, tubular or interstitial lesions (particularly fibrosis) in the histological sections of the fructose-fed rats despite elevated KIM-1 further supported this assertion. Although Nakayama et al. (2010) previously reported that a high-fructose diet accelerated chronic renal disease *via* an increase in tubulointerstitial injury and glomerulosclerosis in rats, these histologic pathologies were not observed in the current study. This could be attributed to the lower concentration of fructose solution used in this study. We used 20% fructose solution while Nakayama et al. (2010) used 60% fructose solution. It is not surprising that the rats did not develop advanced nephropathy, since a significant proportion of individuals do have early histological features of nephropathy without affecting the tubules and surrounding interstitium (Comai et al. 2019).

Conclusively, early postnatal administration of zingerone together with fructose to rats prevented the long-term fructose-induced early nephropathy as measured with the early renal injury marker KIM-1 and confirmed by histological assessment in adulthood in both sexes. These findings present a potential alternative strategic prophylactic intervention to reduce the global burden of nephropathy in humans by targeting a critical period of developmental plasticity with this naturally derived phytochemical. This study has also shown that zingerone could be safe for administration in the neonatal period, despite reports that neonatal treatment with phytochemicals could cause toxicity (Sun et al. 2019) as most physiological systems are not fully developed (Ruggiero et al. 2019). Future studies will be directed towards understanding the mechanisms of action of zingerone that resulted in these long-term reno-protective effects.

Conflict of interest. The authors declare there is no conflict of interests.

Acknowledgements. Staff of the Central Animal Service, Faculty of Health Sciences, University of the Witwatersrand are hereby acknowledged for the care and handling of the animals. We also acknowledge the technical assistance received from Miss Monica Gomes, Miss Hasiena Ali and Dr. Abdurrahman Abdulkadir, with the histology and image capture.

Funding. This work was supported by the Medical Faculty Research Endowment Fund, Faculty of Health Sciences Research Committee and School of Physiology of the University of Witwatersrand (Grant Number: 001 254 8521101 5121105 000000 0000000000 5254); and the National Research Foundation (NRF) Thuthuka Fund (Grant Number: TTK170415227205). The Federal University Birnin Kebbi (Nigeria) and the Tertiary Education Trust Fund of Nigeria provided funding for the PhD candidate.

Data availability statement. The authors confirm that they are prepared to share all the data sets that support the findings of this study should the journal request it for verification during peer review.

References

Ahmad B, Rehman MU, Amin I, Mir R, Bilal S, Farooq A, Muzamil S, Hussain I, Masoodi M, Fatima B (2018): Zingerone (4-(4-hydroxy-3-methylphenyl) butan-2-one) protects against alloxan-induced diabetes via alleviation of oxidative stress and inflammation: probable role of NF-kB activation. Saudi Pharm. J. **26,** 1137-1145
https://doi.org/10.1016/j.jsps.2018.07.001

Ahmed SA, Hamed MA (2015): Kidney injury molecule-1 as a predicting factor for inflamed kidney, diabetic and diabetic nephropathy Egyptian patients. J. Diabetes Metab. Disord. **14,** 1-6
https://doi.org/10.1186/s40200-015-0131-8

Charan J, Kantharia N (2013): How to calculate sample size in animal studies? J. Pharmacol. Pharmacother. **4,** 303-306
https://doi.org/10.4103/0976-500X.119726

Comai G, Malvi D, Angeletti A, Vasuri F, Valente S, Ambrosi F, Capelli I, Ravaioli M, Pasquinelli G, D'Errico A, Fornoni A, Manna GL (2019): Histological evidence of diabetic kidney disease precede clinical diagnosis. Am. J. Nephrol. **50,** 29-36
https://doi.org/10.1159/000500353

Cui Y, Shi Y, Bao Y, Wang S, Hua Q, Liu Y (2018): Zingerone attenuates diabetic nephropathy through inhibition of nicotinamide adenine dinucleotide phosphate oxidase 4. Biomed. Pharmacother. **99,** 422-430
https://doi.org/10.1016/j.biopha.2018.01.051

El-Kafoury BMA, Ahmed MA, Hammouda GA, Elkady AH, Lasheen NN (2019): Possible role of L-carnitine in improvement of metabolic and hepatic changes in hyperuricemic and hyperuricemic-Fructose-supplemented rats. Physiol. Rep. **7,** 1-14
https://doi.org/10.14814/phy2.14282

Glastras SJ, Chen H, Teh R, Mcgrath RT, Chen J, Pollock A, Wong MG, Saad S (2016): Mouse models of diabetes, obesity and related kidney disease. PLoS One **11,** 1-15
https://doi.org/10.1371/journal.pone.0162131

Hashway SA, Wilding LA (2020): Translational potential of rats in research. In: The Laboratory Rats. pp. 77-88, Academic Press
https://doi.org/10.1016/B978-0-12-814338-4.00003-9

Hosseinzadeh A, Goudarzi M, Karimi MY, Khorsandi L, Mehrzadi S, Mombeini MA (2020): Zingerone ameliorates gentamicin-induced nephrotoxicity in rats. Comp. Clin. Path. **29,** 971-980
https://doi.org/10.1007/s00580-020-03129-5

Jamshidi-Kia F, Lorigooini Z, Amini-khoei H (2018): Medicinal plants: past history and future perspective. J. Herbmed Pharmacol. **7,** 1-7
https://doi.org/10.15171/jhp.2018.01

Jin L, Xie F (2020): Untargeted contrast-enhanced ultrasound versus contrast-enhanced computed tomography: A differential diagnostic performance (DDP) study for kidney lesions. Clinics **75,** 1-8
https://doi.org/10.6061/clinics/2020/e1489

Johnson RJ, Sanchez-Lozada LG, Nakagawa T (2010): The effect of fructose on renal biology and disease. J. Am. Soc. Nephrol. **21,** 2036-2039
https://doi.org/10.1681/ASN.2010050506

Kashif A, Verma N, Verma S, Boruah D, Sahu R, Kalra S, Malik A (2020): Utility of glomerular morphometry in diagnosing paediatric renal disease. Med. J. Armed Forces India **77,** 194-199
https://doi.org/10.1016/j.mjafi.2020.08.007

Kim SS, Kim JH, Kim IJ (2016): Current challenges in diabetic nephropathy: early diagnosis and ways to improve outcomes. Endocrinol. Metab. **31,** 245-253
https://doi.org/10.3803/EnM.2016.31.2.245

Lembede BW, Erlwanger KH, Nkomozepi P, Chivandi E (2017): Effect of neonatal orally administered S-allyl cysteine in high-fructose diet fed Wistar rats. J. Dev. Orig. Health Dis. **9,** 160-171
https://doi.org/10.1017/S2040174417000940

Mamikutty N, Thent ZC, Sapri SR, Sahruddin NN, Rafizul M, Yusof M, Suhaimi FH (2014): The establishment of metabolic syndrome model by induction of fructose drinking water in male Wistar rats. Biomed. Res. Int. **2014,** 1-8
https://doi.org/10.1155/2014/263897

Mortera RR, Bains Y, Gugliucci A (2019): Fructose at the crossroads of the metabolic syndrome and obesity epidemics. Front. Biosci. (Landmark Ed.) **24,** 186-211
https://doi.org/10.2741/4713

Muller CR, Leite APO, Yokota R, Pereira RO, Americo ALV, Nascimento NRF, Evangelista FS, Farah V, Fonteles MC, Fiorino P (2019): Post-weaning exposure to high-fat diet induces kidney lipid accumulation and function impairment in adult rats. Front. Nutr. **6,** 1-8
https://doi.org/10.3389/fnut.2019.00060

Nakagawa T, Johnson RJ, Andres-Hernando A, Roncal-Jimenez C, Sanchez-Lozada LG, Tolan DR, Lanaspa MA (2020): Fructose production and metabolism in the kidney. J. Am. Soc. Nephrol. **31,** 898-906
https://doi.org/10.1681/ASN.2019101015

Nakayama T, Kosugi T, Gersch M, Connor T, Sanchez-Lozada LG, Lanaspa MA, Roncal C, Perez-Pozo SE, Johnson RJ, Nakagawa T (2010): Dietary fructose causes tubulointerstitial injury in the normal rat kidney. Am. J. Physiol. Ren. Physiol. **298,** 712-720
https://doi.org/10.1152/ajprenal.00433.2009

Nigro M, Viggiano D, Ragone V, Trabace T, Di Palma A, Rossini M, Capasso G, Gesualdo L, Gigliotti G (2018): A cross-sectional study on the relationship between hematological data and quantitative morphological indices from kidney biopsies in different glomerular diseases. BMC Nephrol. **19,** 1-8
https://doi.org/10.1186/s12882-018-0846-0

Nyakudya TT, Mukwevho E, Nkomozepi P, Erlwanger KH (2018): Neonatal intake of oleanolic acid attenuates the subsequent development of high fructose diet-induced non-alcoholic fatty liver disease in rats. J. Dev. Orig. Health Dis. **9,** 500-510
https://doi.org/10.1017/S2040174418000259

Oraby MA, Marwa FE, Safar M, Assaf N, Ghoneim H (2019): Amelioration of early markers of diabetic nephropathy by linagliptin in type 2 diabetic rats. Nephron **141,** 273-286
https://doi.org/10.1159/000495517

Received: November 15, 2021
Final version accepted: January 18, 2022