Original Paper

Gum Acacia Improves Renal Function and Ameliorates Systemic Inflammation, Oxidative and Nitrosative Stress in Streptozotocin-Induced Diabetes in Rats with Adenine-Induced Chronic Kidney Disease

Mohammed Al Za'abi^a Suhail Al Salam^b Yousuf Al Suleimani^a
Priyadarsini Manoj^a Abderrahim Nemmar^c Badreldin H. Ali^a

^aDepartment of Pharmacology and Clinical Pharmacy, College of Medicine and Health Sciences, Sultan Qaboos University, Al Khoudh, Oman, ^bDepartment of Pathology, College of Medicine and Health Sciences, United Arab Emirates University, Al Ain, ^cDepartment of Physiology, College of Medicine and Health Sciences, United Arab Emirates University, Al Ain, United Arab Emirates

Key Words

Adenine • Chronic kidney disease • Diabetes • Gum acacia • Rats

Abstract

Background/Aims: The effect of treatment with gum acacia (GA), a prebiotic shown previously to ameliorate chronic kidney disease (CKD), in diabetic and non – diabetic rats with adenine – induced CKD has been investigated using several conventional and novel physiological, biochemical, and histopathological parameters. **Methods:** Diabetes mellitus was induced in rats by a single injection of streptozotocin (STZ). Diabetic and non - diabetic rats were randomly divided into several groups, and given either normal food or food mixed with adenine (0.25% w/w, for five weeks) to induce CKD. Some of these groups were also concomitantly treated orally with GA in the drinking water (15% w/w). **Results:** Rats fed adenine alone exhibited physiological (decreased body weight, increased food and water intake and urine output), biochemical (increase in urinary albumin/creatinine ratio, plasma urea and, creatinine, indoxyl sulfate and phosphorus), inflammatory biomarkers (increased in neutrophil gelatinase-associated lipocalin, transforming growth factor beta -1, tumor necrosis factor alpha, adiponectin, cystatin C and interleukin-1 β), oxidative biomarkers (8-isoprostane, 8 -hydroxy -2-deoxy guanosine), nitrosative stress biomarkers (nitrite and nitrate) and histopathological (increase in tubular necrosis and fibrosis) signs of CKD. STZ - induced diabetes alone worsened most of the renal function tests measured. Administration of adenine in STZ – diabetic rats further worsened the renal damage induced by adenine alone. GA significantly ameliorated the renal actions of adenine and STZ, given either singly or in combination, especially with regards to the histopathological damage. **Conclusion:** GA is a useful dietary agent in attenuating the progression of CKD in rats with streptozotocin-induced diabetes.

© 2018 The Author(s)
Published by S. Karger AG, Basel

Mohammed Al Za'abi

Department of Pharmacology and Clinical Pharmacy,
College of Medicine and Health Sciences, Sultan Qaboos University,
P. O. Box 35 Al Khoudh, Postal code 123 (Oman), E-Mail zaabi@squ.edu.om

Introduction

Chronic kidney disease (CKD) is a significant public health problem worldwide with a global prevalence of 11% to 13% [1, 2], as it can lead to a high prevalence of morbidity and mortality. CKD is associated with inflammation and premature aging [3, 4]. It usually progresses to end stage renal disease (ESRD), necessitating dialysis or renal transplantation [5].

Diabetes mellitus (DM) is a well-known chronic metabolic disease, causing hyperglycemia, which can cause many complications, including nephropathy that can lead to ESRD [6]. Diabetic patients on dialysis can only be rescued by renal transplantation. This is a very effective therapy, but it is expensive and extremely limited by the availability of donor kidneys [7].

Adenine – induced CKD is a well – tested animal model [8-11], and has been used to investigate the effectiveness of several agents against experimental CKD [12, 13].

The pathophysiological basis of CKD and its complications include inflammation, oxidative stress and apoptosis, features that are always seen in human beings and animals with CKD [14-16]. There are also major mediators of the disease such as C-reactive protein, tumor necrosis factor and other cytokines, and several markers of nitrosative and oxidative stress, exerting similar effects in CKD models in rats [16-19].

The prebiotic gum acacia (GA) is a dietary soluble fibrous and complex heteropolysaccharide obtained from either *Acacia senegal* or *A. seyal* trees [20, 21]. Since the first report about the usefulness of GA in patients with CKD [22], several experimental and clinical publications have confirmed the salutary actions of GA in CKD [23-26]. GA has also been reported to be useful in mice with diabetic nephropathy [26] and in alloxan – induced diabetes in rats [27].

Thus, our aim here was to investigate the possible ameliorative effect of GA on several parameters of renal structure and function in streptozotocin-induced diabetes in rats with adenine-induced CKD using several traditional and the more sensitive novel biomarkers [28].

Materials and Methods

Animals

Male Wistar rats (9–10 weeks old, initially weighting about 200 g) were provided by the Animal House of Sultan Qaboos University (SQU) and accommodated in a room at a temperature of $22 \pm 2^\circ\text{C}$ and relative humidity of about 60%, with a 12 h light–dark cycle (lights on at 6:00 am). Rats were given free access to a standard pellet chow diet containing 0.85% phosphorus, 1.12% calcium, 0.35% magnesium, 25.3% crude protein and 2.5 IU/g vitamin D3 (Oman Flour Mills, Muscat, Oman) and tap water. The study was approved by the University Animal Ethical Committee (SQU/AEC/13/01) and was conducted in conformity with international laws and policies (EEC Council directives 86/609, OJL 358, 1, December 12, 1987; NIH Guide for the Care and Use of Laboratory Animals, NIH Publications No. 85-23, 1985).

Experimental design

After an acclimatization period of one week, rats ($n = 48$) were randomly divided into eight equal groups and treated for 35 consecutive days as follows: The 1st group (con) was fed a normal diet until the end of the study (control group). The 2nd group (A) was treated as above, but fed a diet containing adenine (0.25% w/w in feed). The 3rd (GA) group was given normal feed and GA in drinking water at a concentration of 15% w/v. The 4th group (A+GA) was given adenine in the feed as in group two, plus GA in drinking water as above. The 5th (STZ), 6th (A+STZ), 7th (GA+STZ) and 8th (A+GA+STZ) groups were treated in the same manner as the 1st, 2nd, 3rd and 4th groups, respectively, except that rats in these latter four groups were made diabetic by injecting them intraperitoneally with streptozotocin (STZ), as described below. The doses of adenine and GA were chosen from previous reports [24, 29].

During the treatment period, the rats were weighed weekly. One day before the last day of experiment rats were placed singly in metabolic cages to collect the urine voided in the last 24 h. Twenty-four hours

after the end of the treatment, the rats were anesthetized with ketamine (75 mg/kg) and xylazine (5 mg/kg) intraperitoneally, and blood (about 4–5 mL) was collected and centrifuged at 900 *g* at 4 °C for 15 min to separate plasma. Urine was similarly centrifuged. The plasma and urine were stored at –80 °C to await analysis. The animals were sacrificed by an overdose of the anesthetic, and the kidneys were removed, blotted on filter paper, and weighed. Part of the right kidney was placed in formalin for subsequent histopathological examination, as reported earlier. The remainder of the right and left kidneys were individually wrapped in aluminum foil and then dipped in liquid nitrogen and stored at –80°C, pending analysis within 10 days or less [30, 31].

Induction of diabetes

Diabetes was induced after 18 h fasting by intraperitoneal (i.p.) injection of STZ, at a dose of 50 mg/kg, dissolved in 0.1 M citrate buffer (pH 4.5). Other groups of rats were injected with citrate buffer. Two days thereafter, the blood glucose (BG) concentration was checked using a OneTouch® UltraMini® Meter (LifeScan, Milpitas, CA, USA) using a drop of blood obtained by pricking the tail tip. Rats with blood glucose concentrations ≥ 14 mmol/L were considered diabetic. Adenine and GA were given 3 weeks after either STZ or citrate buffer injections. Body weight was measured daily, fasting blood glucose concentration weekly, and blood insulin concentrations once at the end of the experiment.

Insulin resistance (HOMA-IR) was estimated using the homeostatic model assessment, using the formula $\text{HOMA-IR} = \text{fasting glucose (mmol/L)} \times \text{fasting insulin (mU/L)} / 22.5$ [32].

Biochemical methods

Creatinine and urea in plasma and creatinine and albumin in urine were measured with an autoanalyzer. Plasma concentration of indoxyl sulfate (IS) was assayed using an HPLC method as previously described [33]. Plasma concentrations of neutrophil gelatinase-associated lipocalin (NGAL), transforming growth factor beta -1 (TGF- β 1), tumor necrosis factor alpha (TNF- α), adiponectin, cystatin C, interleukin-1 β (IL-1 β) and interleukin-10 (IL-10), as well as the renal oxidative of 8-Isoprostane and 8-Hydroxy -2-deoxy Guanosine (8-OHdg) were measured using ELISA kits. Urinary *N*-acetyl- β -D-glucosaminidase (NAG) activity was determined with a colorimetric kit. Osmolality was measured by freezing point depression using the Osmomat 3000 (Gonotec GmbH, Berlin, Germany).

Histopathological methods

The methods used here were identical to those reported before [17, 24]. Briefly, the kidneys were first weighed and then sampled and fixed in 10% neutral-buffered formalin for 24–48 hrs, dehydrated in increasing concentrations of ethanol, cleared with xylene and embedded in paraffin. Four-micrometer (μ m) sections were prepared from kidney paraffin blocks and stained with hematoxylin and eosin (H & E). The microscopic scoring of the kidney sections was carried out in a blinded fashion by a pathologist who was unaware of the treatment groups, and assigned a score which represents the approximate extent of the necrotic area in the cortical and medullary tubules, and assigned a score on a scale of 0–4 (0, no necrosis; 1, a few focal necrotic areas of $\leq 25\%$ of the kidney; 2, necrotic area was about 26–50% of kidney; 3, necrotic area was 51–75% of kidney; 4, nearly the entire area was necrotic, with the necrotic area being 76–100% of kidney). The size of the necrosis was also estimated, and values were presented as means $\pm$ SEM. The used scoring system was adapted with modification from a previously published system [34].

Four- μ m sections were prepared from paraffin blocks and stained with Sirius Red stain to assess the degree of interstitial fibrosis. Image J software (NIH, USA) was used to measure the extent of necrosis and fibrosis.

Drugs and chemicals

Adenine, GA and STZ were bought from Sigma (St. Louis, MA., USA). Plasma urea, creatinine and urine albumin were measured using standard laboratory methods by an LX20 multiple automated analyzer (Beckman Coulter, CA, USA). NAG was determined using a colorimetric kit from Diazyme (Poway, CA, USA). Plasma NGAL, TGF- β 1, IL-10, nitrite, nitrate, and IL-1 β were measured using ELISA kits from R & D (Minneapolis, MN, USA). Urine kidney injury molecule (KIM -1) and vanin -1 (VNN1) were determined using ELISA kits (Cloud Clone corp, TX, USA). TNF- α , cystatin C and adiponectin were measured using ELISA kits from Abcam (Cambridge, UK). 8-Isoprostane and 8-OHdg were quantified using kits from Cayman (Ann

Arbor, MI, USA). The insulin ELISA kit was bought from Bertin pharma (Montigny le bretonneux, France). The rest of the chemicals were of the highest purity grade available.

Statistical analysis

Data were expressed as means $\pm$ SEM and were analyzed with GraphPad Prism Version 5.03 for Windows software (Graphpad Software Inc., San Diego, USA). Comparisons between the eight groups were performed by analysis of variance (ANOVA), followed by Bonferroni multiple comparison tests. *P* values < 0.05 were considered significant.

Results

Nutrition and glucose metabolism

The physiological data of the eight groups of rats in the experiment are shown in Table 1. GA, adenine and STZ, given separately or together, significantly reduced the weight gain of rats. When given either separately or together, adenine and STZ increased the absolute and relative kidney weight, feed and water intake and urine output.

Fig. 1 depicts the concentrations of fasting glucose and insulin, and the insulin resistance ratio in control and diabetic rats given adenine and / or STZ. No change in the blood glucose concentration was noted in CON group, and in A, GA or GA+A groups. STZ, either alone or together with either adenine or GA significantly elevated the fasting glucose concentrations when compared with the above four groups. However, the elevation was highest in GA+STZ group, and lowest in A+STZ group. Rats in A+GA+STZ group had a glucose concentration not significantly different from that of rats in A, GA and STZ groups.

Kidney function

Table 2 depicts the effects of adenine, STZ and GA, each given alone or in combination on renal function tests in plasma. A group had a significantly higher concentration of creatinine, urea, NGAL and IS (*P* < 0.05) than CON group. Rats with STZ – induced diabetes (STZ, A+STZ, GA+STZ, A+GA+STZ) had significantly higher values of creatinine, urea, IS and cystatin C than CON group (*P* < 0.05). GA did not significantly affect any of these analytes when compared with the CON group (*P* > 0.1). Combination of adenine and STZ aggravated the effect of either on urea level, but not the other analytes. The values in rats concomitantly given GA and adenine (A+GA group) were significantly lower than in rats given adenine alone (A group). Administration of GA to rats given adenine and STZ (A+GA+STZ) mitigated their actions on IS.

The effect of GA administration on diabetic and non –diabetic rats with adenine – induced CKD on some urinary analytes are shown in Table 3. Adenine significantly increased the albumin – creatinine ratio, NAG activity, and the concentrations of KIM-1 and VNN1, and

Fig. 1. Effect of gum acacia (GA, 15% w/v) on fasting blood glucose, plasma insulin and insulin resistance in control rats (CON), and rats concomitantly given adenine (A, 0.25% w/w) to induce chronic kidney disease and streptozotocin (STZ, 50 mg/kg) to induce diabetes. Each column and vertical bar represents mean $\pm$ SEM (*n* = 6). Different superscripts indicate significance as follows: ^adenotes significance of different groups vs. Control group: where ^a*P* < 0.05, ^bdenotes significance of group treated with A alone vs. A+ other groups: where ^b*P* < 0.05, ^cdenotes significance of group treated with GA alone vs. GA + other groups: where ^c*P* < 0.05, ^ddenotes significance of group treated with STZ alone vs. STZ + other groups: where ^d*P* < 0.05.

Table 1. Effect of gum acacia (GA) treatment on some physiological characteristics in control rats (CON), and rats administered concomitantly adenine (A) and /or streptozotocin (STZ). Values in the table are means $\pm$ SEM (n = 6). CKD was induced by including A in the feed (0.25 % w/w for 35 days); GA (15% w/v) was given to rats concurrently in the drinking water. STZ (50 mg/kg) was intraperitoneally injected once to induce diabetes. On the 35th day of treatment, rats were placed in metabolic cages to collect urine. Different superscripts indicate significance as follows: ^adenotes significance of different groups vs. Control group: where ^aP < 0.05, ^bdenotes significance of group treated with A alone vs. A+ other groups: where ^bP < 0.05, ^cdenotes significance of group treated with GA alone vs. GA + other groups: where ^cP < 0.05, ^ddenotes significance of group treated with STZ alone vs. STZ + other groups: where ^dP < 0.05

Parameters/Treatments	CON	A	GA	A+GA	STZ	A+STZ	GA+STZ	A+GA+STZ
BWT change (%)	54.2 $\pm$ 1.1	0.8 $\pm$ 1.3 ^a	20.7 $\pm$ 1.0 ^a	$\pm$ 2.8 ^{ac}	- 31.4 $\pm$ 3.5 ^a	- 43.4 $\pm$ 3.9 ^{ab}	- 35.3 $\pm$ 5.5 ^{ac}	- 42.5 $\pm$ 2.8 ^{abc}
Relative kidney weight (%)	0.63 $\pm$ 0.01	1.55 $\pm$ 0.02 ^a	0.66 $\pm$ 0.02	0.89 $\pm$ 0.03 ^{abc}	0.99 $\pm$ 0.06 ^a	1.26 $\pm$ 0.12 ^{ab, d}	0.99 $\pm$ 0.04 ^{ac}	0.86 $\pm$ 0.04 ^b
Water intake (mL)	18.6 $\pm$ 1.3	59.6 $\pm$ 4.6 ^a	20 $\pm$ 1.7	33.4 $\pm$ 3.6 ^b	100 $\pm$ 0.0 ^a	100 $\pm$ 0.0 ^{ab}	66.6 $\pm$ 1.6 ^{ac, d}	57.7 $\pm$ 4.4 ^{abc, d}
Urine output (mL)	8.8 $\pm$ 1.05	41.3 $\pm$ 3.4 ^a	3.8 $\pm$ 0.5 ^a	20 $\pm$ 1.1 ^{abc}	70 $\pm$ 0.00 ^a	70 $\pm$ 0.00 ^{ab}	32.5 $\pm$ 3.4 ^{ac, d}	37.5 $\pm$ 7.6 ^{abc, d}
Food intake (g)	26.9 $\pm$ 0.8	20.5 $\pm$ 2.2 ^a	23.4 $\pm$ 0.5	14.8 $\pm$ 1.5 ^{abc}	20.9 $\pm$ 0.5 ^a	23 $\pm$ 0.7	9.7 $\pm$ 1.5 ^{ac, d}	29.6 $\pm$ 0.5 ^{bc, d}
Faecal output (g)	6.9 $\pm$ 0.6	8.3 $\pm$ 1.2	12.7 $\pm$ 1.7 ^a	13.0 $\pm$ 1.4 ^a	6.8 $\pm$ 0.7	8.6 $\pm$ 0.8	5.3 $\pm$ 0.8 ^c	5.0 $\pm$ 0.8 ^c

Table 2. Effect of gum acacia (GA) treatment on creatinine, urea, neutrophil gelatinase-associated lipocalin (NGAL), indoxyl sulfate and cystatin C, in serum of control rats (CON), and rats administered concomitantly adenine (A) and /or streptozotocin (STZ). Values in the tables are means $\pm$ SEM (n = 6). CKD was induced by including A in the feed (0.25 % w/w for 35 days); GA (15% w/v) was given to rats concurrently in the drinking water. STZ (50 mg/kg) was intraperitoneally injected once to induce diabetes. On the 35th day of treatment, rats were sacrificed to collect blood. Different superscripts indicate significance as follows: ^adenotes significance of different groups vs. Control group: where ^aP < 0.05, ^bdenotes significance of group treated with A alone vs. A+ other groups: where ^bP < 0.05, ^cdenotes significance of group treated with GA alone vs. GA + other groups: where ^cP < 0.05, ^ddenotes significance of group treated with STZ alone vs. STZ + other groups: where ^dP < 0.05

Parameters/Treatments	CON	A	GA	A+GA	STZ	A+STZ	GA+STZ	A+GA+STZ
Creatinine (μ mol/L)	15.2 $\pm$ 0.6	73.8 $\pm$ 11.7 ^a	16.0 $\pm$ 0.6 ^a	40.1 $\pm$ 7.8 ^{ac}	67.5 $\pm$ 3.9 ^a	68.8 $\pm$ 2.8 ^a	69.7 $\pm$ 1.9 ^a	67.8 $\pm$ 0.8 ^{ac}
Urea (mmol/L)	5.1 $\pm$ 0.3	21.3 $\pm$ 0.5 ^a	4.7 $\pm$ 0.4	10.6 $\pm$ 0.7 ^{abc}	17.4 $\pm$ 1.9 ^a	20.4 $\pm$ 2.7 ^a	14.8 $\pm$ 1.2 ^{ac}	19.5 $\pm$ 1.7 ^{ac}
NGAL (ng/mL)	33.2 $\pm$ 1.2	65.9 $\pm$ 3.5 ^a	31.6 $\pm$ 1.6	55.7 $\pm$ 1.4 ^{ac}	47.9 $\pm$ 3.0	58.4 $\pm$ 3.2	42.4 $\pm$ 3.5	52.8 $\pm$ 2.5
Indoxyl sulfate (μ mol/L)	1.8 $\pm$ 0.8	68.0 $\pm$ 5.1 ^a	0.0 $\pm$ 0.0	0.9 $\pm$ 0.5 ^b	4.4 $\pm$ 1.1 ^a	4.9 $\pm$ 1.3 ^b	0.0 $\pm$ 0.0 ^d	1.1 $\pm$ 0.6 ^d
Cystatin C (ng/mL)	9.4 $\pm$ 0.3	18.2 $\pm$ 1.1 ^a	8.5 $\pm$ 0.2	16.3 $\pm$ 0.2 ^{ac}	13.6 $\pm$ 0.3 ^a	16.2 $\pm$ 0.5 ^{ad}	12.9 $\pm$ 0.2 ^{ac}	12.8 $\pm$ 0.5 ^{abc}

Table 3. Effect of gum acacia (GA) treatment on creatinine clearance, albumin/creatinine ratio, N-acetyl-beta-D-glucosaminidase (NAG), kidney injury molecule -1(KIM-1), vanin and osmolality, in urine of control rats (CON), and rats administered concomitantly adenine (A) and /or streptozotocin (STZ). Values in the tables are means $\pm$ SEM (n = 6). CKD was induced by including A in the feed (0.25 % w/w for 35 days); GA (15% w/v) was given to rats concurrently in the drinking water. STZ (50 mg/kg) was intraperitoneally injected once to induce diabetes. On the 35th day of treatment, rats were placed in metabolic cages to collect urine. Different superscripts indicate significance as follows: ^adenotes significance of different groups vs. Control group: where ^aP < 0.05, ^bdenotes significance of group treated with A alone vs. A+ other groups: where ^bP < 0.05, ^cdenotes significance of group treated with GA alone vs. GA + other groups: where ^cP < 0.05, ^ddenotes significance of group treated with STZ alone vs. STZ + other groups: where ^dP < 0.05

Parameters/Treatments	CON	A	GA	A+GA	STZ	A+STZ	GA+STZ	A+GA+STZ
Creatinine clearance (mL/min)	2.9 $\pm$ 0.3	0.3 $\pm$ 0.03 ^a	3.2 $\pm$ 0.3	1.3 $\pm$ 0.2 ^{abc}	0.4 $\pm$ 0.01 ^a	0.4 $\pm$ 0.02 ^a	1.1 $\pm$ 0.04 ^{ac, d}	0.4 $\pm$ 0.03 ^{ac}
Albumin/creatinine ratio	1.1 $\pm$ 0.1	7.3 $\pm$ 0.8 ^a	0.2 $\pm$ 0.04	1.9 $\pm$ 0.01 ^{abc}	3.3 $\pm$ 0.1 ^a	4.4 $\pm$ 0.1 ^{ab}	1.9 $\pm$ 0.1 ^{ac, d}	2.9 $\pm$ 0.01 ^{abc}
NAG (IU/L)	14.6 $\pm$ 1.1	40.8 $\pm$ 4.5 ^a	17.6 $\pm$ 2.4	33.7 $\pm$ 1.6 ^a	28.8 $\pm$ 1.9 ^a	42.7 $\pm$ 3.6 ^a	21.7 $\pm$ 1.1	25.6 $\pm$ 5.4 ^b
KIM (pg/mL)	43.1 $\pm$ 1.9	307.9 $\pm$ 17.6 ^a	41.2 $\pm$ 4.8	296.8 $\pm$ 3.5 ^{ac}	127.4 $\pm$ 8.4 ^a	190.3 $\pm$ 7.9 ^{ab}	129.3 $\pm$ 14.2 ^{ac}	137.2 $\pm$ 6.6 ^{abc}
Vanin (ng/mL)	0.6 $\pm$ 0.1	1.1 $\pm$ 0.04 ^a	0.5 $\pm$ 0.03	0.8 $\pm$ 0.02 ^{abc}	0.9 $\pm$ 0.06 ^a	1.0 $\pm$ 0.02 ^a	0.6 $\pm$ 0.04 ^a	0.9 $\pm$ 0.05 ^{abc}
Osmolality (mosmol/Kg)	2455.2 $\pm$ 82.6	373.5 $\pm$ 20.1 ^a	1922.2 $\pm$ 165.6	901.7 $\pm$ 41.1 ^{abc}	781.3 $\pm$ 61.9 ^a	695 $\pm$ 14.4 ^a	1038 $\pm$ 41.0 ^{ab}	775.8 $\pm$ 59.9 ^{ac}

decreased creatinine clearance and osmolality in A, A+GA, A+STZ and A+GA+STZ groups. GA administered groups (A+GA, A+GA+STZ) ameliorated these adenine-induced effects.

Kidney histology

The general appearance of the diabetic and non-diabetic rats with adenine - induced CKD was subjectively judged to be improved by GA treatment. Grossly, the kidneys from the CON and GA groups appeared normal. However, the kidneys of adenine-administered rats (A

Fig. 2. Representative photographs of H & E sections of renal tissues from control rats, and rats treated with adenine, streptozotocin (STZ), gum acacia (GA), alone or in different combinations. The control (CON) group showed normal kidney architecture and histology. The STZ group showed normal kidney architecture with foci of clear tubular epithelial cells (thin arrow). The adenine (A) and adenine-STZ (A+STZ) groups showed tubular necrosis with dilated tubules (thin arrow) and interstitial inflammation (thick arrow). The adenine group (A) also showed intratubular deposition of adenine (arrow head). The GA (GA) and GA-STZ (GA+STZ) groups showed normal kidney architecture and histology. The adenine-GA (A+GA) group showed an improvement in the histologic appearance with focal areas of tubular necrosis (thin arrow) and less intratubular deposition of adenine (arrow head). The adenine-STZ-GA (A+GA+STZ) group showed an improvement in the histologic appearance with focal areas of tubular necrosis (thin arrow), and had less dilated tubules, interstitial inflammation and less atrophic tubules.

Fig. 3. Representative photographs of Sirius red staining on sections of renal tissues from control rats, and rats treated with adenine, streptozotocin (STZ), and gum acacia (GA), alone or in different combinations. The control (CON) and STZ (STZ) groups showed normal kidney architecture and histology. The STZ (STZ) group showed no increase in interstitial fibrosis and had normal perivascular fibrous tissue (thin arrow). The adenine (A) and adenine-STZ (A+STZ) groups showed an increase in interstitial fibrosis (thin arrow). The GA (GA) and GA-STZ (GA+STZ) groups showed normal kidney architecture and histology, with normal perivascular fibrous tissue (thin arrow). The adenine-GA (A+GA) group showed an improvement in the histologic appearance and a significant decrease in interstitial fibrosis (thin arrow) when compared with the adenine group (A). The adenine-STZ-GA (A+GA+STZ) group showed an improvement in the histologic appearance and a significant decrease in interstitial fibrosis (thin arrow) when compared with the adenine-STZ group (A+STZ).

group) were pale and with white crystals, similar to those described before [15]. The gross appearance of the kidneys of the diabetic and non-diabetic rats A+GA group was improved compared with the kidneys of rats given adenine and STZ, alone and in combination (A, A+STZ, STZ groups).

Table 4 and Fig. 2 and 3 show the effect of GA administration on histopathology of kidney section of diabetic and non-diabetic rats with adenine-induced CKD.

The CON and STZ groups showed normal kidney architecture and histology (score 0 for necrosis, (Fig. 2 CON&STZ). Sirius red stain indicated no evidence of fibrosis (score 0; Fig. 3 CON&STZ). The A group had diffused tubular necrosis in $52.2 \pm 5.3\%$ of the examined tissue areas (score 3) showing tubular distention with necrotic material involving tubules, with deposition of adenine crystals, loss of brush border of proximal tubules, dilatation of large number of tubules, mixed inflammatory cells infiltration of the interstitium, focal tubular atrophy (Fig. 2 A), and significant interstitial fibrosis ($41.6 \pm 3.8\%$, score 2; Fig. 3 A).

The A+STZ group exhibited diffuse tubular necrosis in $37.8 \pm 6.5\%$ of examined tissue areas (score 2) showing tubular distention with necrotic material involving loss of brush border of proximal tubules, dilatation of a large number of tubules, mixed inflammatory

Fig. 4. Effect of gum acacia treatment (GA, 15% w/v) on the plasma concentrations of tumor necrosis factor - α (TNF- α), interleukin-10 (IL-10), interleukin-1 β (IL-1 β), transforming growth factor beta -1 (TGF- β 1) and adiponectin in control rats (CON), and rats concomitantly given adenine (A, 0.25% w/w) to induce chronic kidney disease and streptozotocin (STZ, 50 mg/kg) to induce diabetes. Each column and vertical bar represents mean $\pm$ SEM (n = 6). Different superscripts indicate significance as follows: ^adenotes significance of different groups vs. Control group: where ^aP<0.05, ^bdenotes significance of group treated with A alone vs. A+ other groups: where ^bP<0.05, ^cdenotes significance of group treated with GA alone vs. GA + other groups: where ^cP<0.05, ^ddenotes significance of group treated with STZ alone vs. STZ + other groups: where ^dP<0.05.

cells infiltration of the interstitium, focal tubular atrophy (Fig. 2 A+STZ), and significant interstitial fibrosis (30.1 $\pm$ 4.1%; Fig. 3 A+STZ). The GA and GA+STZ groups showed normal kidney architecture and histology (score 0, Fig. 2 GA&GA+STZ). Sirius red stain showed no increase in fibrosis (Fig. 3 GA&GA+STZ). However, the A+GA group showed a significant improvement in the histological appearance when compared with the A group. The areas of tubular necrosis involved 19.2 $\pm$ 2.2% of the examined areas (score 1, Fig. 2 A+GA); there was less dilatation of the tubules, less interstitial inflammatory cells infiltration, less tubular atrophy, and less interstitial fibrosis (22.8 $\pm$ 3.2%, score 1, Fig. 3 A+GA).

The A+GA+STZ group showed significant improvement in the histological appearance when compared with the A+STZ group. They had focal areas of tubular necrosis involving only 16.0 $\pm$ 1.6% of the examined areas (score 1, Fig. 2 A+GA+STZ), less dilatation of the tubules, less interstitial inflammatory cells infiltration, less tubular atrophy, and less interstitial fibrosis (14 $\pm$ 1.2%, score 1, Fig. 3 A+GA+STZ).

Systemic inflammation

Fig. 4 shows the effect of GA administration on diabetic and non-diabetic rats with adenine-induced CKD on the concentrations of some proteins and plasma inflammatory and anti-inflammatory cytokines. TNF- α , IL-1 β , TGF- β 1, and adiponectin were significantly increased, and IL-10 significantly decreased, by adenine administration (A group). GA and STZ, each given alone, were without any significant effect on any of these analytes (GA and STZ groups). When GA was given concomitantly with adenine (A+GA group), it blunted significantly the actions of adenine on the measured indices. STZ together with adenine (A+STZ) produced variable effects on these cytokines and proteins. Compared with rats given adenine alone (A group), the value of IL-10 was significantly higher in A+STZ group, while the values of TGF- β 1 and adiponectin were slightly lower. In comparison with values from rats given GA alone (GA group), in the rats given both GA and STZ (GA+STZ group) the values of TNF- α and TGF- β 1 were significantly higher. However, there was no significant difference between the two groups in the values of IL-10, IL-1 β and adiponectin. In rats given the three agents together (A+GA+STZ) the values of TNF- α , IL-1 β and adiponectin were significantly lower than those in the group given adenine alone (A group), and the value of IL-10 was significantly higher.

Oxidative and nitrosative stress

The effect of administration of GA on some oxidative and nitrosative indices (8-OHdg, 8 isoprostane, nitrite and nitrate) in plasma of diabetic and non-diabetic rats with CKD

Fig. 5. Effect of gum acacia (GA, 15% w/v) treatment on indices of oxidative and nitrosative stress in plasma of control rats (CON), and rats concomitantly administered adenine (A, 0.25% w/w) to induce chronic kidney disease and streptozotocin (STZ, 50 mg/kg) to induce diabetes. Each column and vertical bar represents mean $\pm$ SEM ($n = 6$). Different superscripts indicate significance as follows: ^adenotes significance of different groups vs. Control group: where ^a $P < 0.05$, ^bdenotes significance of group treated with A alone vs. A + other groups: where ^b $P < 0.05$, ^cdenotes significance of group treated with GA alone vs. GA + other groups: where ^c $P < 0.05$, ^ddenotes significance of group treated with STZ alone vs. STZ + other groups: where ^d $P < 0.05$.

is shown in Fig 5. GA or STZ given singly or together (GA, GA+STZ and STZ groups), did not affect any of the four indices measured. Adenine (A group) significantly increased, to a similar degree, the concentrations of the four indices measured. Combination A+GA+STZ significantly reduced the concentrations of the four indices, when compared with the data obtained A group.

Discussion

In this work, our aim was to evaluate the possible impact of experimental diabetes on the renal damage induced by adenine feeding, and the effect of administration of GA thereon. The validity of feeding adenine as a rodent model for human CKD has been confirmed before by several authors [10]. Recently a new model of CKD, using spontaneously diabetic Torii rats has been reported, and was found to be particularly useful in CKD with mineral bone disorder [35].

In 2016, the International Society of Nephrology identified ten themes that need to be addressed regarding CKD, and one of these was to develop novel therapeutic interventions to slow CKD progression and abate its complications [36]. Previous results obtained by us, and by others [26] have confirmed the beneficial effects of the novel prebiotic GA in CKD in human CKD patients [22, 23], and in rats with adenine – induced CKD [29], but not rats subjected to 5/6 nephrectomy [37].

As the prevalence of CKD of stages 3-5 (glomerular filtration rate [GFR] < 60 mL/min) is about 30 % in patients with type 2 DM [38], it was deemed of interest to find out experimentally if GA administration would benefit diabetic rats with CKD.

Nutrition and glucose metabolism

The mechanisms by which GA abates the studied parameters of CKD may include, among other possible factors [39], its ability to decrease the inflammatory cytokines and oxidative

Table 4. Effect of treatment with gum acacia (GA) on histopathological assessment of kidney sections in control rats, and rats treated concomitantly with adenine (A) and/or streptozotocin (STZ). Values in the tables are means $\pm$ SEM ($n = 6$). CKD was induced by including A in the feed (0.25 % w/w for 35 days); GA (15% w/v) was given to rats concurrently in the drinking water. STZ (50 mg/kg) was intraperitoneally injected once to induce diabetes. On the 35th day of treatment, rats were sacrificed to collect kidneys. Different superscripts indicate significance as follows: ^adenotes significance of different groups vs. Control group: where ^a $P < 0.05$, ^bdenotes significance of group treated with A alone vs. A + other groups: where ^b $P < 0.05$, ^cdenotes significance of group treated with GA alone vs. GA + other groups: where ^c $P < 0.05$, ^ddenotes significance of group treated with STZ alone vs. STZ + other groups: where ^d $P < 0.05$.

Assessment/Treatment	Tubular necrosis		Interstitial fibrosis	
	%	Score	%	Score
Control	0.00 $\pm$ 0.00	0	0.00 $\pm$ 0.00	0
A	52.2 $\pm$ 5.3 ^a	3	41.6 $\pm$ 3.8 ^a	2
GA	0.00 $\pm$ 0.00	0	0.00 $\pm$ 0.00	0
A + GA	19.2 $\pm$ 2.24 ^{abc}	1	22.8 $\pm$ 3.17 ^{abc}	1
STZ	0.00 $\pm$ 0.00	0	0.00 $\pm$ 0.00	0
A + STZ	37.8 $\pm$ 6.5 ^{abcd}	2	30.1 $\pm$ 4.1 ^{abcd}	2
GA + STZ	0.00 $\pm$ 0.00	0	0.00 $\pm$ 0.00	0
A + GA + STZ	16 $\pm$ 1.58 ^{abcd}	1	14 $\pm$ 1.2 ^{abcd}	1

and nitrosative stress markers that are significantly elevated by adenine feeding [29]. Another possible factor in the salutatory action of GA may be obesity, which is known to be a significant problem and a modifiable risk factor in patients with many diseases, including CKD, and the prevalence of obesity is progressively increasing in the general population, including CKD patients [40]. GA has been reported to reduce body weight of normal rats and obese humans [26, 30, 41]. Therefore, it is also likely that the salutary effect of GA might be related, among other factors, to the body weight reduction observed in rats affected with CKD. Interventions made to reduce body weight in patients with CKD have been confirmed to benefit glycemic control, body mass index and body composition, functional status and quality of life, without causing harmful effects [42].

Compared to control, STZ as expected caused less insulin and insulin resistance ratio and a higher fasting glucose (Figure). GA ameliorated the effect of STZ by causing more insulin secretion and a higher insulin resistance as also demonstrated in figure. However, we noticed that contrary to rise in insulin, fasting glucose was higher in GA+STZ than in STZ group alone. This finding requires further exploration to see whether this is a constant effect of GA with STZ or experimental error/artificial.

Renal structure and function

Beside the traditional biomarkers of renal damage (such as plasma urea and creatinine), we measured in this work the concentration and activity of some novel and more sensitive biomarkers of renal dysfunction. For example, we used sclerostin concentration as a novel plasma biomarker to assess CKD. Plasma NGAL concentration was initially introduced as a novel biomarker for acute kidney injury [43] and we have used it here, and in recently published work [44] as a biomarker for CKD in rats. Gil et al., have recently verified the use of NGAL as a reliable renal index in adenine – induced CKD. Other novel markers include KIM-1, cystatin C and VNN1 [45]. The reliability, efficacy, sensitivity and specificity of these renal markers have been shown in recent literature [28].

Oxidative stress, nitrosative stress and systemic inflammation

Oxidative stress and inflammation represent constant pathophysiological features in CKD [46, 47], and agents that can reduce these pathophysiological processes may be beneficial in preventing, treating or delaying the progression of the disease. The mechanisms by which GA abates the studied parameters of CKD may include, among other possible factors [39], its ability to decrease the inflammatory cytokines and oxidative and nitrosative stress markers that are significantly elevated by adenine feeding [29].

In addition, we investigated also the possible changes in the concentrations of nitric oxide (NO) in CKD. The total amount of NO in plasma can be measured using the stable oxidation metabolites of NO, nitrite and nitrate. The plasma levels of both metabolites were significantly elevated in rats given adenine. While there are reports of NO deficiency in CKD patients [48], the current data are in harmony with findings of a previous study, which indicated a possible enhancement of NO synthesis in uremic patients, as well as in cultured endothelial cells subjected to uremic plasma [49].

Increase in the concentration of the pro-inflammatory cytokine, TNF- α in subjects with renal failure has been suggested as one of the possible mechanisms of the increase in NO plasma content [50]. In the current study, GA prevented the increase in NO metabolites in rats with renal failure, and this might have resulted from the anti-inflammatory and anti-oxidative effects of this product that have been previously reported [21].

In line with our previous results [51], adenine induced a significant elevation in the concentration of the uremic toxin IS in plasma. In adenine – fed rats, GA significantly antagonized that action. Probably, the concentration of IS was reduced by GA via its interference with the bacterial degeneration of indole or by absorbing the latter within the intestine, as was suggested earlier as a mechanism of action of IS [52].

It should be noted with regard to the measurement of oxidative stress biomarkers (especially catalase) using ELISA kits that these kits might not be specific, and are likely to pick up other peroxidase activities that contribute to the overall catalase – like activity

measured here [53]. Probably in future work, these biomarkers should be determined using a more sensitive method for these particular enzymes, such as liquid chromatography–mass spectrometry.

It is of interest to mention here that STZ – induced diabetes has previously been shown to ameliorate the signs of gentamicin - induced acute kidney injury in rats [54]. This was assumed to be related to decreased cortical accumulation of the antibiotic. These results are in contrast to those obtained with the adenine –induced model of CKD here. It would be of interest to measure in future experiments the concentration of adenine or its metabolite in the urine and renal tissues of diabetic and control rats fed adenine.

Conclusion

STZ –induced diabetes worsened some of the physiological, biochemical and histological indices of renal damage induced by adenine. Concomitant treatment with GA abated the effects of adenine and STZ, given either singly or in combination. These results might have a great potential for translation to humans and the obtained data might set the stage for clinical trials investigating the effects of GA in patients with CKD and diabetes.

Acknowledgements

This study was financially supported by Sultan Qaboos University and by a grant from The Research Council of Oman (RC/MED/PHARM/13/01). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. We thank Professor G. Blunden for critically reading the manuscript, and Ms. A. Ramkumar and Mr. M. Ashique for their technical help.

Disclosure Statement

No conflict of interests exists.

References

- Okpechi IG, Bello AK, Ameh OI, Swanepoel CR: Integration of care in management of CKD in resource-limited settings. *Semin Nephrol* 2017;37:260-272.
- Temgoua MN, Danwang C, Agbor VN, Noubiap JJ: Prevalence, incidence and associated mortality of cardiovascular disease in patients with chronic kidney disease in low- and middle-income countries: a protocol for a systematic review and meta-analysis. *BMJ Open* 2017;7:e016412.
- Kaur J, Young BE, Fadel PJ: Sympathetic overactivity in chronic kidney disease: Consequences and mechanisms. *Int J Mol Sci* 2017;18, E1682.
- Kooman J, Dekker M, Usvyat LA, Kotanko P, Van der Sande F, Schalkwijk CG, Shiels PG, Stenvinkel P: Inflammation and premature aging in advanced chronic kidney disease. *Am J Physiol Renal Physiol* 2017;313:F938-F950.
- Collister D, Ferguson T, Komenda P, Tangri N: The patterns, risk factors, and prediction of progression in chronic kidney disease: A narrative review. *Semin Nephrol* 2016;36:273-282.
- Hass VM: Updated management of chronic kidney disease in patients with diabetes. *JAAPA* 2014;27:17-22.
- Jeon HJ, Koo TY, Han M, Kim HJ, Jeong JC, Park H, Ha J, Kim SJ, Ahn C, Park JB, Yang J: Outcomes of dialysis and the transplantation options for patients with diabetic end-stage renal disease in Korea. *Clin Transplant* 2016;30:534-544.
- Akchurin O, Sureshbabu A, Doty SB, Zhu YS, Patino E, Cunningham-Rundles S, Choi ME, Boskey A, Rivella S: Lack of hepcidin ameliorates anemia and improves growth in an adenine-induced mouse model of chronic kidney disease. *Am J Physiol Renal Physiol* 2016;311:F877-F889.
- Claramunt D, Gil-Peña H, Fuente R, García-López E, Loredó V, Hernández-Frías O, Ordoñez FA, Rodríguez-Suárez J, Santos F: Chronic kidney disease induced by adenine: a suitable model of growth retardation in uremia. *Am J Physiol Renal Physiol* 2015;309:F57-62.
- Diwan V, Brown L, Gobe GC: Adenine-induced chronic kidney disease in rats. *Nephrology (Carlton)* 2018;23:5-11.

- 11 Ferrari GO, Ferreira JC, Cavallari RT, Neves KR, dos Reis LM, Dominguez WV, Oliveria EC, Gracioli FG, Passlick-Deetjen J, Jorgetti V, Moysés RM: Mineral bone disorder in chronic kidney disease: head-to-head comparison of the 5/6 nephrectomy and adenine models. *BMC Nephrol* 2014;15:69.
- 12 Ali BH, Inuwa I, Al Za'abi M, Al Bahlani S, Al Issaei H, Ramkumar A, Madanagopal T, Nemmar A, Malheiros DM, Zatz R: Renal and myocardial histopathology and morphometry in rats with adenine - induced chronic renal failure: influence of gum acacia. *Cell Physiol Biochem* 2014;34:818-828.
- 13 Tatsumoto N, Yamada S, Tokumoto M, Eriguchi M, Noguchi H, Torisu K, Tsuruya K, Kitazono T: Spironolactone ameliorates arterial medial calcification in uremic rats: the role of mineralocorticoid receptor signaling in vascular calcification. *Am J Physiol Renal Physiol* 2015;309:F967-979.
- 14 Ali BH, Cahliková L, Opletal L, Karaca T, Manoj P, Ramkumar A, Al Suleimani, Al-YM, Al Za'abi M, Nemmar A, Chocholousova-Havlikova L, Locarek M, Siatka T, Blunden G: Effect of aqueous extract and anthocyanins of calyces of *Hibiscus sabdariffa* (Malvaceae) in rats with adenine-induced chronic kidney disease. *J Pharm Pharmacol* 2017;69:1219-1229.
- 15 Al Za'abi M, Al Busaidi M, Yasin J, Schupp N, Nemmar A, Ali BH: Development of a new model for the induction of chronic kidney disease via intraperitoneal adenine administration, and the effect of treatment with gum acacia thereon. *Am J Transl Res* 2015;7:28-38.
- 16 Kurose Y, Wada J, Kanzaki M, Teshigawara S, Nakatsuka A, Murakami K, Inoue K, Terami T, Katayama A, Watanabe M, Higuchi C, Eguchi J, Miyatake N, Makino H: Serum galectin 9 levels are elevated in the patients with type 2 diabetes and chronic kidney disease. *BMC Nephrol* 2013;14:23.
- 17 Ali BH, Alza'abi M, Ramkumar A, Al-Lawati I, Waly MI, Beegam S, Nemmar A, Brand S, Schupp N: The effect of activated charcoal on adenine-induced chronic renal failure in rats. *Food Chem Toxicol* 2014;65:321-328.
- 18 Ali BH, Al-Salam S, Al Za'abi M, Al Balushi KA, Ramkumar A, Waly MI, Yasin J, Adham SA, Nemmar A: Does swimming exercise affect experimental chronic kidney disease in rats treated with gum acacia? *PLoS One* 2014;9:e102528.
- 19 Correa-Costa M, Braga TT, Felizardo RJ, Andrade-Oliveira V, Perez KR, Cuccovia IM, Hiyane MI, da Silva JS, Câmara NO: Macrophage trafficking as key mediator of adenine -induced kidney injury. *Mediators Inflamm* 2014;2014:291024.
- 20 Calame W, Weseler AR, Viebke C, Flynn C, Siemensma AD: Gum arabic establishes prebiotic functionality in healthy human volunteers in a dose-dependent manner. *Br J Nutr* 2008;100:1269-1275.
- 21 Ali BH, Ziada A, Blunden G: Biological effects of gum arabic: a review of some recent research. *Food Chem Toxicol* 2009;47:1-8.
- 22 Bliss DZ, Stein TP, Schleifer CR, Settle RG: Supplementation with gum arabic fiber increases fecal nitrogen excretion and lowers serum urea nitrogen concentration in chronic renal failure patients consuming a low-protein diet. *Am J Clin Nutr* 1996;63:392-398.
- 23 Ali AA, Ali KE, Fadlalla AE, Khalid KE: The effects of gum arabic oral treatment on the metabolic profile of chronic renal failure patients under regular haemodialysis in Central Sudan. *Nat Prod Res* 2008;22:12-21.
- 24 Ali BH, Al-Salam S, Al Husseni I, Kayed RR, Al-Masroori N, Al-Harathi T, Al Zaabi M, Nemmar A: Effects of Gum Arabic in rats with adenine-induced chronic renal failure. *Exp Biol Med (Maywood)* 2010;235:373-382.
- 25 Elamin S, Alkhawaja MJ, Bukhamsin AY, Idris MAS, Abdelrahman MM, Abutaleb NK, Housawi AA: Gum arabic reduces C- reactive protein in chronic kidney disease patients without affecting urea or indoxyl sulfate levels. *Int J Nephrol* 2017;2017:9501470.
- 26 Nasir O: Renal and extrarenal effects of gum arabic (*Acacia senegal*)—what can be learned from animal experiments? *Kidney Blood Press Res* 2013;37:269-279.
- 27 Ahmed AA, Fedail JS, Musa HH, Kamboh AA, Sifaldin AZ, Musa TH: Gum Arabic extracts protect against hepatic oxidative stress in alloxan-induced diabetes in rats. *Pathophysiology* 2015;22:189-194.
- 28 Rysz J, Gluba-Brzózka A, Franczyk B, Jabłonowski Z, Ciałkowska-Rysz A: Novel biomarkers in the diagnosis of chronic kidney disease and the prediction of its outcome. *Int J Mol Sci* 2017;18:E1702.
- 29 Ali BH, Al-Husseni I, Beegam S, Al-Shukaili A, Nemmar A, Schierling S, Queisser N, Schupp N: Effect of gum arabic on oxidative stress and inflammation in adenine-induced chronic renal failure in rats. *PLoS One* 2013;8:e55242.
- 30 Ali BH, Al Balushi K, Al-Husseini I, Mandel P, Nemmar A, Schupp N, Ribeiro DA: Gum acacia mitigates genetic damage in adenine-induced chronic renal failure in rats. *Eur J Clin Invest* 2015;45:1221-1227.

- 31 Al Za'abi M, Shalaby A, Manoj P, Ali BH: The *in vivo* effects of adenine-induced chronic kidney disease on some renal and hepatic function and CYP450 metabolizing enzymes. *Physiol Res* 2017;66:263-271.
- 32 Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC: Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985;28:412-419.
- 33 Al Za'abi M, Ali BH, Al Toubi M: HPLC-fluorescence method for measurement of the uremic toxin indoxyl sulfate in plasma. *J Chromatogr Sci* 2013;51:40-43.
- 34 Servais A, Meas-Yedid V, Buchler M, Morelon E, Olivo-Marin JC, Lebranchu Y, Legendre C, Thervet E: Quantification of interstitial fibrosis by image analysis on routine renal biopsy in patients receiving cyclosporine. *Transplantation* 2007;84:1595-601.
- 35 Watanabe K, Fujii H, Goto S, Nakai K, Kono K, Watanabe S, Shinohara M, Nishi S: Newly developed rat model of chronic kidney disease-mineral bone disorder. *J Atheroscler Thromb* 2018;25:170-177.
- 36 Levin A, Tonelli M, Bonventre J, Coresh J, Donner JA, Fogo AB, et al: Global kidney health 2017 and beyond: a roadmap for closing gaps in care, research, and policy. *Lancet* 2017;390:1888-1917.
- 37 Ali BH, Alqarawi AA, Ahmed IH: Does treatment with gum Arabic affect experimental chronic renal failure in rats? *Fundam Clin Pharmacol* 2004;18:327-329.
- 38 Scheen AJ: Pharmacokinetics and clinical use of incretin-based therapies in patients with chronic kidney disease and type 2 diabetes. *Clin Pharmacokinet* 2015;54:1-21.
- 39 Matsumoto N, Riley S, Fraser D, Al-Assaf S, Ishimura E, Wolever T, Phillips GO, Phillips AO: Butyrate modulates TGF-beta1 generation and function: potential renal benefit for Acacia (sen) SUPERGUM (gum arabic)? *Kidney Int* 2006;69:257-265.
- 40 Mallamaci F, Tripepi G: Obesity and CKD progression: hard facts on fat CKD patients. *Nephrol Dial Transplant* 2013;28 Suppl 4:iv105-108.
- 41 Babiker R, Merghani TH, Elmusharaf K, Badi RM, Lang F, Saeed AM: Effects of gum Arabic ingestion on body mass index and body fat percentage in healthy adult females: two-arm randomized, placebo controlled, double-blind trial. *Nutr J* 2012;11:111.
- 42 Navaneethan SD, Yehner H, Moustarah F, Schreiber MJ, Schauer PR, Beddhu S: Weight loss interventions in chronic kidney disease: a systematic review and meta-analysis. *Clin J Am Soc Nephrol* 2009;4:1565-1574.
- 43 Ronco C, Cruz D, Noland BW: Neutrophil gelatinase-associated lipocalin curve and neutrophil gelatinase-associated lipocalin extended-range assay: a new biomarker approach in the early diagnosis of acute kidney injury and cardio-renal syndrome. *Semin Nephrol* 2012;32:121-128.
- 44 Ali BH, Al-Salam S, Al Suleimani Y, Al Kalbani J, Al Bahlani S, Ashique M, et al: Curcumin ameliorates kidney function and oxidative stress in experimental chronic kidney disease. *Basic Clin Pharmacol Toxicol* 2018;122:65-73.
- 45 Gil A, Brod V, Awad H, Heyman SN, Abassi Z, Frajewicki V: Neutrophil gelatinase-associated lipocalin in a triphasic rat model of adenine-induced kidney injury. *Ren Fail* 2016;38:1448-1454.
- 46 Akchurin OM, Kaskel F: Update on inflammation in chronic kidney disease. *Blood Purif* 2015;39:84-92.
- 47 Pedraza-Chaverri J, Sánchez-Lozada LG, Osorio-Alonso H, Tapia E, Scholze A: New pathogenic concepts and therapeutic approaches to oxidative stress in chronic kidney disease. *Oxid Med Cell Longev* 2016;2016:6043601.
- 48 Reddy YS, Kiranmayi VS, Bitla AR, Krishna GS, Rao PV, Sivakumar V: Nitric oxide status in patients with chronic kidney disease. *Indian J Nephrol* 2015;25:287-291.
- 49 Baylis C: Nitric oxide deficiency in chronic kidney disease. *Am J Physiol Renal Physiol* 2008;294:F1-9.
- 50 Diwan V, Mistry A, Gobe G, Brown L: Adenine-induced chronic kidney and cardiovascular damage in rats. *J Pharmacol Toxicol Methods* 2013;68:197-207.
- 51 Ali BH, Al Za'abi M, Adham SA, Yasin J, Nemmar A, Schupp N: Therapeutic Effect of Chrysin on Adenine-Induced Chronic Kidney Disease in Rats. *Cell Physiol Biochem* 2016;38:248-257.
- 52 Vanholder R, Schepers E, Pletinck A, Nagler EV, Glorieux G: The uremic toxicity of indoxyl sulfate and p-cresyl sulfate: a systematic review. *J Am Soc Nephrol* 2014;25:1897-1907.
- 53 Cipak Gasparovic A, Zarkovic N, Zarkovic K, Semen K, Kaminsky D, Yelisseyeva O, et al: Biomarkers of oxidative and nitro-oxidative stress: conventional and novel approaches. *Br. J. Pharmacol* 2017;174:1771-1783.
- 54 Ali BH, Bashir AK, Mugamer IT, Tanira MO: Gentamicin nephrotoxicity in the rat: influence of age and diabetes mellitus. *Hum Exp Toxicol* 1996;15:51-55.