European Review for Medical and Pharmacological Sciences 2015; 19: 498-506

Influence of treatment with gum acacia on renal vascular responses in a rat model of chronic kidney disease

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Abstract. – OBJECTIVE: This study was conducted in order to investigate the effects of adenine-induced chronic kidney disease (CKD) on renal blood flow and biochemical changes in rats, and to assess the effect of treatment with gum acacia (GA) thereon.

MATERIALS AND METHODS: CKD was induced by feeding rats with adenine (0.25% w/w, five weeks). Concomitantly, some of these rats were also given gum acacia (GA) (15% w/v in the drinking water). Before animals were sacrificed, changes in renal blood flow (RBF) were monitored in anaesthetized rat preparations. Several biochemical and histological renal function tests were also conducted.

RESULTS: Adenine-induced CKD significantly impaired the vasopressor actions of acetylcholine, sodium nitroprusside and phenylephrine and concomitant treatment with GA abated these responses. Additionally, plasma concentrations of urea, creatinine, uric acid, indoxyl sulfate, nitrite and nitrate and urinary excretion of protein were all significantly increased by adenine. GA significantly mitigated the severity of adenine – induced changes.

CONCLUSIONS: Adenine-induced CKD in rats significantly impaired renal vascular responses to acetylcholine, sodium nitroprusside and phenylephrine and this was mitigated by treatment with GA. This provides another experimental evidence for the usefulness of GA in the amelioration of CKD.

Key words:
Adenine, Rats, Gum acacia, Hemodynamics, Chronic kidney disease.

Introduction

The number of patients in need of kidney transplantation worldwide is increasing[1]. The main attributing factor of this is the development of chronic kidney disease (CKD). The disease is characterized by reduced glomerular filtration rate and increased proteinuria associated with massive damage to the kidney structure including tubular atrophy, tubulointerstitial fibrosis and glomerulosclerosis[2,3]. One of the serious complications of CKD is the development of cardiovascular disease including hypertension and cardiac hypertrophy[4,5,6]. Several clinical and experimental studies have demonstrated an alteration in vascular responses in end-stage renal disease caused by changes in endothelial and smooth muscle function[6,7,8,9].

There have been two major animal models of the disease that have facilitated understanding of the possible mechanisms that lead to CKD and its complications and, therefore, improve therapeutic options. One is the surgical (nephrectomy) model and the other is the chemical model (addition of adenine in the diet). Cardiovascular changes in the latter model were first reported in 1980[10]. Several recent studies have also utilized the adenine-induced CKD model to investigate the mechanisms that might contribute to the associated cardiovascular changes[3,4,11-13]. One of the suggested mechanisms of adenine-caused kidney damage is the development of oxidative stress inflammation and DNA damage[2].

Gum acacia (GA) is an edible, dried gummy exudate from the stems and branches of *Acacia Senegal* and *Acacia seyal*. Supplementation with GA fiber improves renal function in patients with CKD. The latter action and the other biological actions of GA have been reviewed[14]. Experimentally, GA ameliorates biochemical, physiological and behavioral changes in adenine-induced CKD[2,11,12,15], and the effect of GA on blood pressure in rats with adenine-induced CKD has been recently investigated[4,12]. However, up to now the-

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re are no published studies focusing on changes in regional blood flow in this model and the effect of GA thereon. Therefore, to obtain further information on this, the present study was conducted to investigate the changes that might be observed in renal blood flow (RBF) as well as changes in biochemical markers in a rat model of adenine-induced CKD. Furthermore, a possible protective effect of GA on these changes was also investigated.

Materials and Methods

Animals and ethics

Thirty-two male Wistar rats, initially weighing 150-200 g, were obtained from the Animal House of Sultan Qaboos University (SQU). They were maintained under 12:12 h light:dark cycle (light on at 6:00) and fed with standard laboratory diet (Oman Flour Mills, Muscat, Oman) and water *ad libitum*. Animal procedures were approved by the Animal Ethical Committee of SQU and were carried out in accordance with the NIH Guide for the Care and Use of Laboratory Animals (NIH Publications No. 85-23, 1985).

Experimental design

The rats were randomly divided into four groups (n=8 in each group). The first group continued to receive the same diet without treatment and served as negative control. The second group was switched to a powder diet containing adenine (0.25%, w/w). The third group was given normal diet with GA (15% in distilled water) in the drinking water and the fourth group was given adenine in the feed and GA as above. The treatment was carried out for five consecutive weeks.

Hemodynamic study

At the end of the treatment period, the rats were anaesthetized with sodium pentobarbital (60 mg/kg, i.p.). PE_{50} cannulae, filled with heparinized normal saline (25 IU/ml in 0.9% NaCl), were inserted into the right carotid artery for the measurement of blood pressure by a pressure transducer (TSD104A, Biopac Systems, Santa Barbara, CA, USA), and into the right jugular vein for the administration of drugs. An ultrasonic probe (1RB, Hughes Sacks Electronik-Harvard Apparatus, March-Hugstetten, Germany) was placed around the left renal artery to measure renal blood flow (RBF) and was connected to a flow meter (Hughes Sacks Electronik-Harvard Apparatus, March-Hugstetten, Germany).

After a 20 min stabilization period, baseline blood pressure and RBF were monitored on a data acquisition system (MP 150, Biopac Systems, Santa Barbara, CA, USA). The following substances were administered dose-dependently, each dose given at an interval of 3 min: acetylcholine (ACh; 0.1, 0.2, 0.4 and 0.8 μg/kg), sodium nitroprusside (SNP; 1, 2 and 4 μg/kg) and phenylephrine (0.5, 1, 2 and 4 μg/kg). The magnitudes of the responses were expressed as % change.

Biochemical measurements

The animals were placed in metabolic cages one day before sacrifice and the urine voided in 24 h was collected. Blood was collected from the inferior vena cava of anaesthetized rats in heparinized tubes, and centrifuged (together with collected urine) at 900g at 4°C for 15 min, and the obtained plasma and urine were kept frozen (−80°C) pending analysis. The kidneys were removed, cleaned off fat, blotted on filter paper, weighed, and placed in a fixative buffer pending for histological analysis. The kidney relative weight was calculated as kidney weight/body weight × 100. Concentrations of creatinine in plasma and urine and urea in plasma were measured spectrophotometrically using commercially available kits (Human GmbH, Heidelberg, Germany). Concentrations of plasma nitrite and nitrate were measured using ELISA kits from R&D Systems (Bristol, UK) as described in the manufacturer's protocol. Uric acid concentration was measured using Beckman Coulter Automated Clinical Chemistry Analyzer, Synchron CX5 (Brea, CA, USA). Vascular endothelial growth factor (VEGF) and endothelin-1 were measured in the plasma using an ELISA method (Abcam, Cambridge, MA, USA).

Measurement of indoxyl sulfate in plasma

Concentrations of Indoxyl sulfate in plasma were measured quantitatively using a validated high-performance liquid chromatography-fluorescence method developed in our laboratory[16].

Histopathology

Parts of kidneys were sectioned, fixed in 10% neutral phosphate-buffered formaldehyde, and embedded in paraffin. Sections (5 μm thick) were cut and stained with hematoxylin and eosin. Histopathological evaluation was carried out by

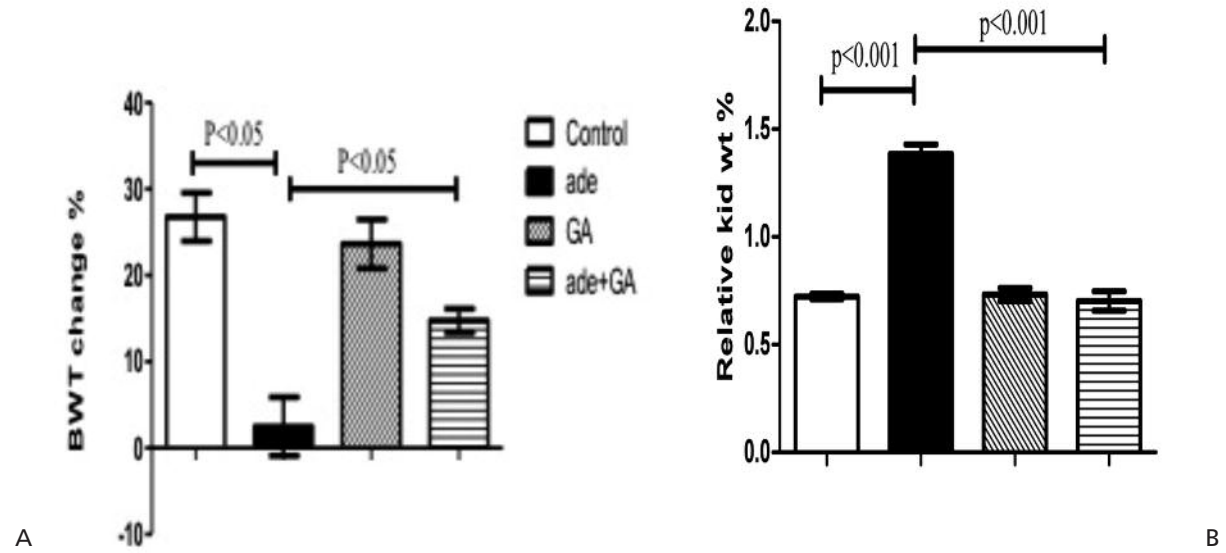

Figure 1. Effect of treatment of rats with saline (control), GA, adenine and adenine + GA for 5 weeks on body weight changes (final weight – initial weight x 100) (**A**) and kidney relative weight (kidney weight /final body weight x 100) (**B**). GA (15%) was given in the drinking water and adenine (0.25% w/w) in the feed. Data are mean ± SEM (n = 6-8).

an observer unaware of the treatments, and assigned a score, which represents the approximate extent of necrotic area in the cortical tubules on a scale of 0-4 (0 - no necrosis; 1 - a few local necrotic spots; 2 - necrotic area was about one half; 3 - tubular necrosis > 60%; 4 - nearly the entire area was necrotic).

Statistical Analysis

Values were expressed as mean ± SEM and n represents number of rats. The data from hemodynamic study were analyzed using two-way analysis of variance (ANOVA), and those obtained from other studies were analyzed using one-way ANOVA for the whole data set, and determination of statistical significance among individual groups was carried out using Tukey's multiple comparison tests. $p < 0.05$ was selected as the criterion for statistical significance. Statistical analyses were performed using GraphPad Prism version 4.03 (San Diego, CA, USA).

Results

General effects and biochemical parameters

Adenine feeding (0.25%, w/w) for 5 weeks caused a significant reduction in the body weight and an increase in kidney relative weight. Concomitant treatment with GA prevented the effects (Figure 1). Figure 2 shows that adenine feeding caused significant increase in the plasma concentrations of urea and creatinine, and significant decrease in the creatinine clearance. These effects were significantly abated with concomitant treatment with GA. The plasma concentrations of nitrite and nitrate were significantly elevated in adenine-fed group as compared with the control, and that treatment with GA prevented the rise in nitrite (Figure 3). The urinary total protein excretion (in mg/24h) in adenine –treated rats (100.3 ± 9.0) was significantly higher than in the control (18.2 ± 07), GA – treated rats (19.5 ± 0.8) or in rats treated with adenine +GA (55.7 ± 5.7). Adenine feeding caused dramatic increase in the plasma concentrations of the uremic toxin indoxyl sulfate, and no noticeable rise was observed in other groups (Figure 3). The concentration of endothelin-1 was insignificantly increased in adenine-treated rats compared to the other groups, and no detectable concentrations of VEGF were found in any of the rats in the four groups.

Hemodynamics

Adenine produced significant reduction (50 ± 4%, n=6) in RBF, but no significant effect on blood pressure as compared with the control (Table 1). GA showed slight inhibition of adenine-induced reduction in RBF but it was not statisti-

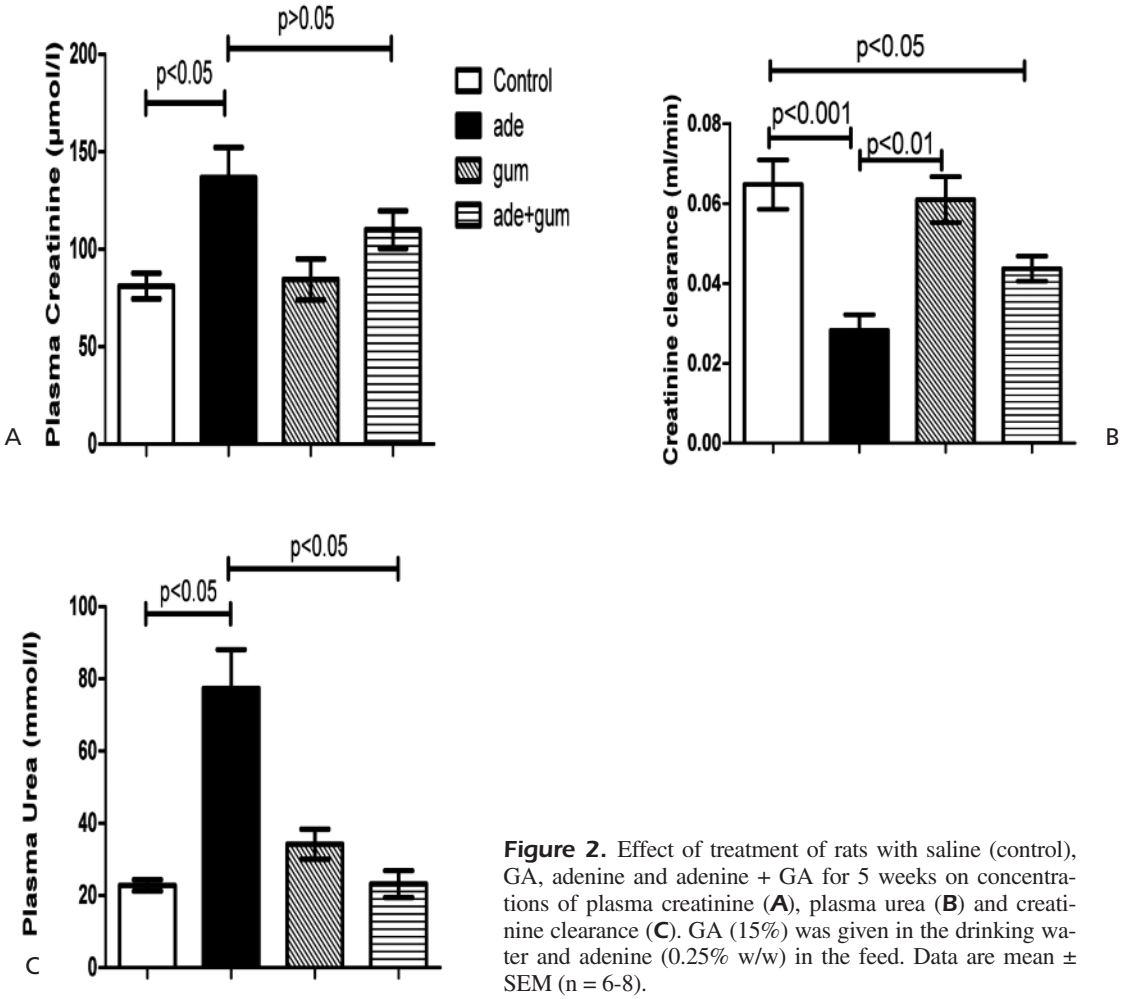

Figure 2. Effect of treatment of rats with saline (control), GA, adenine and adenine + GA for 5 weeks on concentrations of plasma creatinine (**A**), plasma urea (**B**) and creatinine clearance (**C**). GA (15%) was given in the drinking water and adenine (0.25% w/w) in the feed. Data are mean ± SEM (n = 6-8).

cally significant (Table I). Intravenous administration of either acetylcholine (Ach) or sodium nitroprusside produced dose-dependent decreases in RBF in all studied groups (Tables II and III). This decrease was significantly potentiated in adenine-treated rats as compared with the control group. Treatment with GA significantly abated the effect of adenine. Phenylephrine given intravenously caused dose-dependent decreases in RBF (Table IV). The vasoconstrictor effect of phenylephrine was potentiated in the adenine-treated group, while GA significantly abated the effect (Table IV).

Histopathology

Histological observations of renal sections revealed remarkable renal damage in adenine-fed rats characterized by necrosis and vacuolization (Table V and Figure 4). Treatment with GA ameliorates the observed damage.

Discussion

The current study is, as far as we are aware, the first to report changes in regional blood flow in adenine-induced CKD in anaesthetized rats. In this model, there was a decrease in baseline RBF. The vascular actions of acetylcholine, sodium nitroprusside and phenylephrine were impaired. Plasma concentrations of creatinine and urea increased and creatinine clearance decreased. The uremic toxin indoxyl sulfate concentrations in plasma were elevated, so were concentrations of nitrite and nitrates. GA (15% for 5 weeks) significantly abated the adenine effects. Experimental CKD induced by adenine (with or without GA) was not associated with any significant change in the concentrations of VEGF or endothelin-1.

The induction of renal failure in rats fed with adenine 0.25% for 5 weeks was confirmed by both biochemical and histopathological changes.

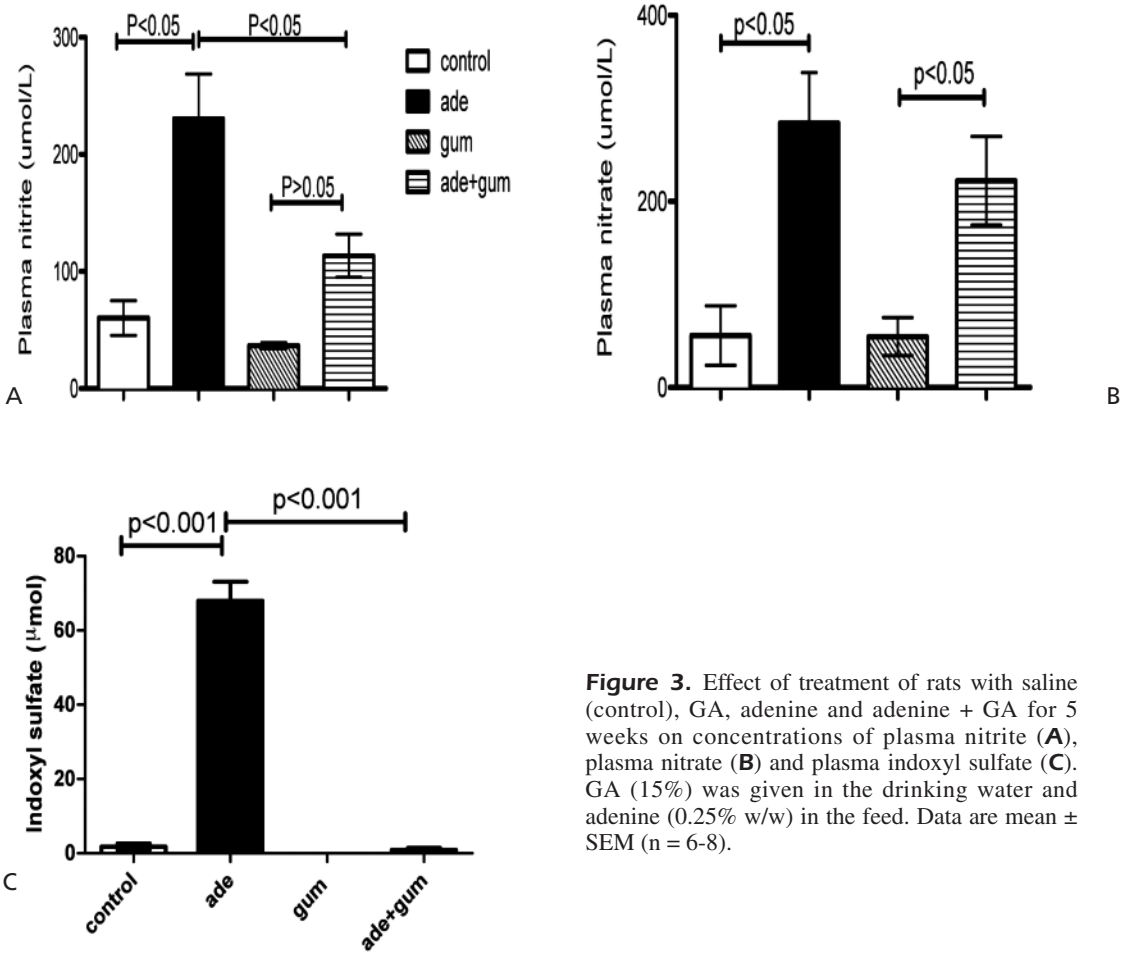

Figure 3. Effect of treatment of rats with saline (control), GA, adenine and adenine + GA for 5 weeks on concentrations of plasma nitrite (**A**), plasma nitrate (**B**) and plasma indoxyl sulfate (**C**). GA (15%) was given in the drinking water and adenine (0.25% w/w) in the feed. Data are mean ± SEM (n = 6-8).

Table I. Baseline changes in mean arterial blood pressure (MAP) and renal blood flow (RBF) in untreated (control) rats and rats treated with 0.25% adenine, 15% GA or adenine plus GA. All treatments were carried out for 5 weeks. Data were obtained in sodium pentobarbital (60 mg/kg) anaesthetized rats.

Groups	N	Baseline MAP (mmHg)	Baseline RBF (ml/min)
Control	8	123 ± 5	6.2 ± 0.6
Adenine	6	119 ± 4	3.1 ± 0.3[a]
GA	5	108 ± 6	4.9 ± 0.8
Adenine + GA	6	103 ± 8	4.4 ± 0.2

Values are mean ± SEM. [a]Significantly different from control group ($p < 0.05$).

Table II. Effect of treatment with adenine (0.25% in the feed, 5 wks), GA (15% in drinking water) or adenine plus GA on mean decreases in renal blood flow (%) following intravenous administration of acetylcholine (ACh) in sodium pentobarbital (60 mg/kg) anaesthetized rats (n = 5-8).

Group	0.1 µg/kg	0.2 µg/kg	0.4 µg/kg	0.8 µg/kg
Control	-20 ± 3	-24 ± 3	-35 ± 3	-44 ± 5
Adenine	-35 ± 7	-51 ± 6[a]	-61 ± 6[a]	-64 ± 3[a]
GA	-20 ± 4	-30 ± 5	-41 ± 6[b]	-51 ± 4
Adenine + GA	-26 ± 4	-36 ± 4	-44 ± 4[c]	-54 ± 3

Values are mean ± SEM. [a]Significantly different from control group ($p < 0.05$): [b]Significantly different from adenine group ($p < 0.05$); [c]Significantly different from adenine group ($p < 0.05$).

Table III. Effect of treatment with adenine (0.25% in the feed, 5 wks), GA (15% in drinking water) or adenine plus GA on mean decreases in renal blood flow (%) following intravenous administration of Na nitroprusside in sodium pentobarbital (60 mg/kg) anaesthetized rats (n = 5-8).

Group	1 µg/kg	2 µg/kg	4 µg/kg
Control	-17 ± 1	-23 ± 2	-37 ± 4
Adenine	-28 ± 5	-48 ± 8a	-62 ± 8a
GA	-19 ± 7	-29 ± 7b	-38 ± 8
Adenine + GA	-16 ± 2	-28 ± 2c	-38 ± 4c

Values are mean ± SEM. [a]Significantly different from control group ($p < 0.05$); [b]Significantly different from adenine group ($p < 0.05$); [c]Significantly different from adenine group ($p < 0.05$).

Table V. Effect of treatment with adenine (0.25% in the feed, 5 wks), gum acacia (15% in drinking water) or adenine plus gum acacia on the rat renal histology.

Groups	Score/renal cortex
Control	0 = no damage
Gum acacia	0 = no damage
Adenine	4 = nearly all tubules showed microcytic dilatation, extensive chronic interstitial nephritis and fibrosis; some tubules appear shrunken and atrophic and PAS-positive; materials filling tubular epithelium; thickened and wrinkled basement membrane
Gum acacia + adenine	1 = nodular desquamation of tubules and slight chronic interstitial nephritis

Renal sections were stained with haemotoxylin and eosin and the scores were made "blindly" without prior knowledge of treatment.

Several studies have indicated a possible association between serum concentrations of indoxyl sulfate and progression of CKD[17,18]. This chemical is a uremic toxin produced from tryptophan by intestinal flora and primarily excreted via tubular secretion[19]. In the current model, the concentrations of indoxyl sulfate in plasma were significantly elevated, supporting the notion that this chemical plasma concentration is highly elevated in renal failure, and is a useful index for evaluating the degree of renal damage.

The study further investigated the possible changes in the concentrations of nitric oxide (NO) in renal failure. The total amount of NO in plasma can be assessed using the stable oxidation metabolites of NO, nitrite and nitrate[20]. Plasma concentrations of both metabolites were significantly increased in adenine-fed rats. While there are reports of NO deficiency in CKD patients[21], 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[22]. The increase in the activity of TNF-α in subjects with renal failure has been suggested as one of the possible mechanisms of the increase in NO plasma content[3,23]. In the current study, GA prevented the increase in NO metabolites in rats with renal failure, and this might resulted from the anti-inflammatory and anti-oxidative effect of this product that have been reported before[2].

Additionally, the study investigated certain hemodynamic changes, namely mean arterial blood pressure (MAP) and RBF. Recently, several studies have drawn attention to the possible alterations in cardiovascular system in chronic renal failure. Diwan et al[3] found cardiovascular changes in rats with adenine-induced chronic renal failure, including increased ventricular fibrosis, systolic blood pressure and left ventricular stiffness and impaired vascular responses. Previous to that, an experimental study[12] reported an increase in both systolic and diastolic blood pressure in rats with adenine-induced CKD. Other studies on similar animal model have shown similar findings[4,13]. However, in the current *in vitro* study adenine failed to show significant changes

Table IV. Effect of treatment with adenine (0.25% in the feed, 5 wks), GA (15% in drinking water) or adenine plus GA on mean decreases in renal blood flow (%) following intravenous administration of phenylephrine in sodium pentobarbital (60 mg/kg) anaesthetized rats (n = 5-8)

Group	0.5 µg/kg	1 µg/kg	2 µg/kg	4 µg/kg
Control	-15 ± 2	-30 ± 4	-48 ± 5	-69 ± 3
Adenine	-17 ± 2	-36 ± 4	-60 ± 2a	-81 ± 2a
GA	-10 ± 2	-25 ± 1	-43 ± 3b	-69 ± 5b
Adenine + GA	-20 ± 3	-34 ± 3	-47 ± 3	-70 ± 3

Values are mean ± SEM. [a]Significantly different from control group ($p < 0.05$); [b]Significantly different from adenine group ($p < 0.05$).

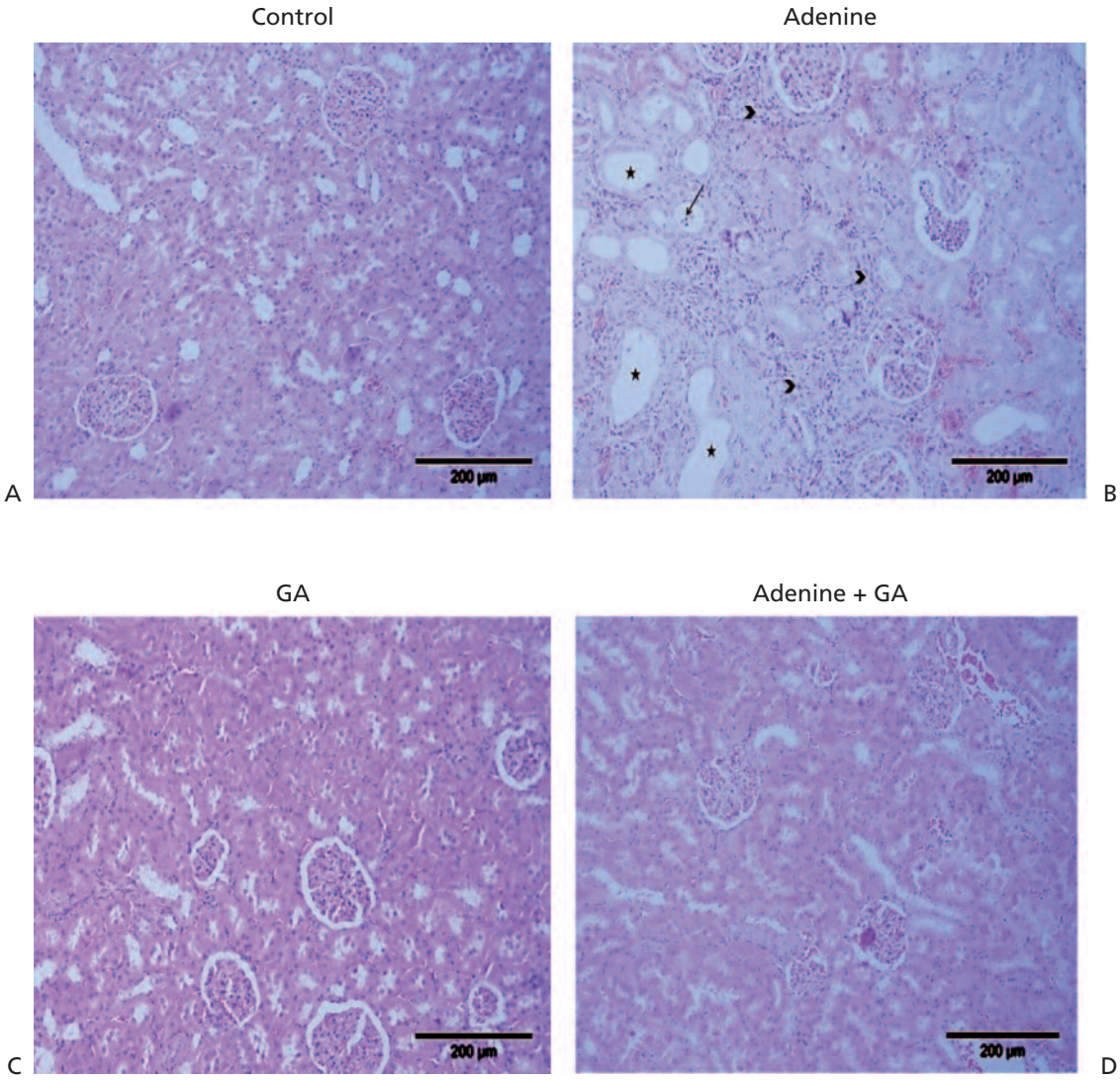

Figure 4. Representative photographs of sections of renal tissue under light microscope of rats that have been treated with saline (control), adenine, gum acacia (GA, 15% in water), and adenine + GA after hematoxylin and eosin staining (H & E, 200 ×). **A**, Control group, showing normal kidney architecture and histology; **B**, Adenine-treated group, showing chronic interstitial nephritis characterized by extensive infiltration of lymphocytes and macrophages (arrow), microcystic dilatation of tubules (★) and desquamation of tubular epithelium (›); **C**, GA-treated group, showing normal kidney architecture and histology; **D**, Adenine + GA 15% treated group, showing considerable improvement in the structural and histological appearance of the kidney sections.

in blood pressure. This might be attributed to the way blood pressure was measured as in those studies despite causing other biochemical changes consistent with renal damage, blood pressure was measured non-invasively, while in this study it was detected invasively in anaesthetized rat preparations. Furthermore, adenine was given at lower concentration and to slightly a longer period of time as compared to the previous studies.

Currently, there are no published reports on changes in regional blood flow in adenine-induced chronic renal failure. Therefore, the current study went further and investigated changes in RBF. Rats fed with 0.25% adenine showed a significant decrease in their RBF and impairment of vascular actions of ACh, SNP and phenylephrine. Treatment with GA slightly prevented the alterations. In line with these findings, Manivannan et al[24] demonstrated impairment in vascular responses to NE, Ach and SNP in isolated aortic rings of rats with adenine-induced chronic renal failure. The study suggested that increased uric acid formation is a likely pathogenic factor. In support to this, the current study also showed in-

creased plasma uric acid concentrations in adenine-fed rats. The study also examined whether or not induction of renal failure had any effect on angiogenesis. Concentrations of endothelin-1 and the angiogenic factor VEGF were measured in plasma of rats treated with adenine, with and without GA, and no detectable concentrations of VEGF in any of the groups including the controls were found (data not shown). This may indicate that there were no new formations of blood vessels in any of the rats.

Conclusions

Adenine-induced CKD in rats significantly impaired renal vascular responses to Ach, sodium nitroprusside and phenylephrine and this was mitigated by treatment with GA. This gives another experimental evidence for the usefulness of GA in the amelioration of CKD, as has been shown in previous work.

Acknowledgements

This work was financially supported by a grant from the Research Council of Oman (RC/Med/Phar/10/01), and Sultan Qaboos University (SQU).

Conflict of interest

The authors have declared that there is no conflict of interest.

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