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Potential Role of Gum Arabic on Calcium Homeostasis in Adenine-Induced Chronic Renal Failure in Rats

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Abstract: The present study aimed to investigate the effect of gum Arabic (GA) on calcium (Ca) homeostasis in adenine (AD)-induced chronic renal failure (CRF) in male rats. Twenty four adult male rats were divided into four groups (six rats each): Control (C) group without treatment, GA (15%w/v) in drinking water treated group, AD (300 mg/kg/day) intragastric treated group, AD and GA treated group. All treatments continued for 4 weeks. The results showed that AD treatment caused renal disorders, resembling CRF represented by significant increase in serum creatinine, BUN and uric acid levels associated with increase in urine volume, kidney weights, the levels of microprotein and NAG activity in urine. Also, creatinine clearance and body weight were significantly decreased. Renal oxidative stress marker Malondialdehyde (MDA) was increased with significant reduction in the activities of superoxide dismutase (SOD), catalase (CAT), the level of reduced glutathione (GSH) and total antioxidant capacity (TAC). AD treatment caused hormonal disorders, elevated levels of parathormone (PTH) and calcitonin (CT), but calcitriol levels decreased. In addition, disturbances in serum and urine mineral levels (Ca, P, Na and K) were observed associated with histopathological changes in renal sections. Moreover, GA administration improved kidney function and calcium (Ca) homeostasis as it keeps the levels of the measured parameters tended to normal level and prevented the oxidative stress induced by AD.

keywords: chronic renal failure, calcium homeostasis, adenine, gum Arabic, electrolytes

1.Introduction

Chronic renal disease (CRD) is a progressive and potentially life threatening condition that can lead to complete renal failure, bone illness and cardiovascular disorder [1, 2].The number of patients with renal failure continues to increase in all countries, bringing a grave socioeconomic cost to humanity [3]. Chronic renal failure (CRF) is most often the result of a gradual decline in kidney function for a period more than 3 months, and the stage at which the kidneys are no longer able to adequately filter blood and maintain homeostasis [4].The prevalence and development of CRF is influenced by many reasons, such as increased blood pressure, diabetes mellitus and obesity [5].

Plasma calcium homeostasis is ultimately controlled by three important physiological homeostatic processes, represented in renal calcium excretion or re-absorption, intestinal calcium absorption and bone formation or resorption [6], via the changes of three hormones, parathormone (PTH), 1, 25-dihydroxycholecalciferol (Calcitriol) and Calcitonin (CT) [7].

Progressive deterioration of renal function in CRF causes decreased production of Calcitriol and disturbance in mineral homeostasis appeared mainly by an imbalance in serum calcium (Ca) and phosphorus (P) concentrations and increment of PTH levels [8].

The experimental CRF rat model can induce both metabolic and bone disorders that develop in CRF patients [9]. AD be able to used for induction of CRF model [10-12] and has been used to check the influence of numerous agents against experimental CRF [3, 13].

Gum Arabic (GA) also termed Gum acacia, a natural biopolymer resin, is a dietetic soluble fibrous secreted from either *Acacia Senegal* or *Acacia Seyal* trees [14,15] used for the treatment of CKD subjects in several Middle Eastern countries [16,17].

The objective of the current study was to clarify the mechanism that contributes to the disturbances of calcium homeostasis in CRF and to investigate whether the administration of gum Arabic (GA) has a protective effect in adenine-induced CRF.

2 .Materials and methods

Chemicals:

AD was purchased from agent of Lobachemie (India, 107 Wode House Road, Jehanghir Villa, Colaba, Mumbai). GA was obtained from local supplier from Saudi Arabia. All other chemicals were purchased locally and were of analytical reagent grade.

Animals

Male Wistar albino rats initially weighting 140-160 g were used in this study. Rats were purshased from the Animal house of Faculty of Pharmacy, Mansoura University, Mansoura, Egypt. They were putted in stainless cages at well ventilated animal house at temperature of 25±2°C, relative humidity of about 60%, with a 12 h light-dark cycle and free access to normal laboratory diet and water *ad libitum*. The animals care and experiments were complied with "Research Ethics Committee" Mansoura University, Egypt, in harmony with ethics of the foundation of Laboratory animal Resources, National Research Council (NRC) [18].

Experimental protocol

After one week of accommodation, animals were equally classified into four groups each of six animals as follows: 1) Normal control group.2) Gum Arabic (GA) treated group (15% w/v) in drinking water **[19].**3) Adenine (AD) administered group, (300 mg/kg B. wt) orally with gastric tube **[13, 20].**4) AD and GA treated group as described in the above groups.

All treatments were continued for four weeks. The gross weights of each rat were recorded weekly for each group during the experimental period. At the end of the experiment, animals were individually placed in metabolic cages for 24 h to collect urine. Thereafter, the animals were anesthetized by diethyl ether then sacrificed to obtain blood samples. The prepared urine and blood samples were centrifuged at 860 g for 15 min at 4°C. Both serum and urine specimens were kept frozen at −20°C until biochemical tests. The rats were dissected and kidneys were removed, dried on filter paper and weighed. A known weight from the left kidney was homogenized in ice-cold Tris buffer (pH 7.4) to give a 10% w/v homogenate. The renal homogenates were spinned at 860 g for 15 min at 4°C, and the supernatants were kept frozen at −20°C until used for biochemical analysis. The right kidney was placed in formal-saline for subsequent histopathological examination.

Biochemical analysis

Creatinine (Cr), blood urea nitrogen (BUN), uric acid (UA) levels, calcium (Ca), phosphorus (P), sodium (Na) and potassium (K) concentrations, renal malondialdhyde (MDA) concentration, superoxide dismutase (SOD) activity, catalase (CAT) activity, reduced glutathione (GSH) concentration and TAC, were estimated colorimetrically using kits purchased from Bio-Diagnostic Company, Giza, Egypt, according to the manufacturer's instructions. Creatinine clearance was calculated according to the equation of Duarte and Preuss [21].

$$\text{in)} = \frac{\text{urine cre; } \quad \text{l/24 hrs)}}{\text{serum creatinine (mg / dl) X 1440}}$$

Microproteins concentration in urine was measured spectrophotometrically according to the method of Watanabe et al [22] using kits obtained from BioSTC Company, Egypt. Urine N-acetyl-β-glucosaminidase (NAG) activity was estimated using rat ELISA kit Catalog NO. E0069rof EIaab Company, China. Parathormone (PTH) level was estimated in serum using Rat PTH Elisa kit catalog No. EIA-PTH, EIAM-PTH, EIAR-PTH of RayBiotech Company, USA. Calcitonin (CT) hormone concentration was estimated in serum using Rat CT Elisa kit catalogNo.CSB – E05132r of Cusabio Company, China. 1, 25-Dihydroxyvitamin D_3 hormone (calcitriol) level was estimated in serum using Rat1, 25-dihydroxyvitaminD_3 Elisa kit catalog No.MBS2601701 of MyBiosource Company, USA.

Histopathologicalexamination:

Formal saline fixed kidneys were dehydrated in an ascending series of ethanol then cleared with xylene and embedded in paraffin wax. The prepared tissue blocks were sectioned using microtome on glass slides (5 µm) and stained with either hematoxyline and eosin or alcian PAS. The prepared slides were examined for histopathological changes under light microscope [23].

Statistical analysis:

The obtained results of the present study were analyzed using SPSS statistical package, version 20.00 software. Comparisons between the four groups were performed by One Way Analysis of Variance (One–way ANOVA) and followed by Least Significant Difference (LSD) test. Each result was represented as means ± SEM. Statistically the values were considered significant at P≤ 0.05 [24].

3. Results and Discussion

The obtained results showed that, supplementation of GA to normal healthy rats did not cause any significant changes in the tested parameters compared with control. The appearance of the kidneys from the control and GA -administered rats was normal. On the other hand, the kidneys of AD-administered rats appeared pale, and some crystals were seen, mainly in the cortical region **(Fig1 & Fig2).** The appearance of the kidneys of rats treated with AD plus GA were ameliorated in comparison to AD- administered rat kidney

Fig. (1): Gross morphology of the kidney of rats: (A) control, (B) GA, (C) AD, (D) AD+GA

The results in table (**1)** revealed that administration of rats with AD for 4 weeks caused a significant reduction in the final body weight compared with the initial weight and compared with control at the end of the experiment. In addition, water intake and urine volume were significantly increased in AD treated rats. Also, the results showed that, at the end of the experiment, the absolute and the relative kidney weights were significantly increased in AD administered rats in comparison to control. However, supplementation of GA to AD administered rats ameliorated these effects comparing to AD treated rats, although still significantly compared to control

Table (1): Initial and final body weights, water intake, urine volume, absolute and relative kidney weights in control and different treated rat groups.

	Control	GA	AD	AD + GA
Initial B. wt(g)	166.17±0.79^{a}	163.33±1.05^{a}	167.33±0.33^{a}	168.83±1.64^{a}
Final B. wt (g)	203.66±3.31^{a}	95.17±2.89^{a}	130.83±3.73^{b}	148.50±1.28^{bc}
Water intake (ml/24h)	23.90 ± 0.45^{a}	22.66 ± 0.64^{a}	32.76 ± 1.52^{b}	28.78 ±1.20^{bc}
Urine volume(ml /24h)	4.74 ± 0.05^{a}	4.65 ± 0.09^{a}	15.72 ±0.50^{b}	10.10 ±0.58^{bc}
Absolute kidney wt (g)	0.60 ± 0.02^{a}	0.56 ± 0.02^{a}	2.28 ± 0.21^{b}	1.59 ± 0.12^{bc}
Relative kidney wt (%)	0.29 ± 0.01^{a}	0.28 ± 0.01^{a}	1.74 ± 0.13^{b}	1.07 ± 0.08^{bc}

Each value represents the mean ± SE of six rats. Values superscripts with various letters were significantly different at P≤ 0.05. AD = Adenine, GA = Gum Arabic**.**

In table **(2),** Serum Cr, BUN and UA levels were significantly increased in AD administered rats compared to control. Also significant increases were observed in the activity of NAG and microprotein concentration in urine, while Crcl was significantly decreased compared to control. Meanwhile, concomitant treatment of GA with AD caused significant improvement of these parameters towards the normal levels, but still significant compared to control

Table (2): Serum creatinine (SCr), blood urea nitrogen (BUN), uric acid (UA), urine creatinine levels (UCr), creatinine clearance (Crcl), urine N acetyl-β glucosaminidase (NAG) activity and microprotein concentration in control and different treated rat groups**.**

	Control	GA	AD	AD +GA
SCr (mg/dl)	0.88 ± 0.03^{a}	0.86 ± 0.02^{a}	3.15 ± 0.15^{b}	1.98 ± 0.15^{bc}
BUN (mg/dl)	13.81 ± 0.76^{a}	13.58 ± 0.90^{a}	37.39 ± 1.05^{b}	29.64 ± 0.89^{bc}
UA(mg/dl)	0.97 ± 0.03^{a}	0.91 ± 0.0^{a}	1.94 ± 0.0^{b}	1.44 ± 0.02^{bc}
UCr (mg/dl)	157.65 ± 2.52^{a}	157.20 ± 1.91^{a}	47.59 ± 1.95^{b}	100.09 ± 2.61^{bc}
Crcl (ml/min)	0.59 ± 0.02^{a}	0.58 ± 0.01^{a}	0.16 ± 0.01^{b}	0.36 ± 0.04^{bc}
Urine NAG (u/l)	0.09 ± 0.01^{a}	0.06 ± 0.001^{a}	0.42 ± 0.02^{b}	0.20 ± 0.01^{bc}
Microprotein(mg/24h)	3.50 ± 0.16^{a}	4.38 ± 0.15^{a}	21.54 ± 0.78^{b}	11.99 ± 0.88^{bc}

Each value represents the mean ± SE of six rats. Values superscripts with various letters were significantly different at P≤ 0.05. AD = Adenine, GA = Gum Arabic.

The results also indicated that MDA concentration was significantly increased while SOD and CAT activities as well as GSH and TAC were significantly reduced in AD administered rats in comparison to control. However, administration of AD with GA significantly reduced the elevations in renal MDA accompanied by a significant increase in the activities of SOD and CAT as well as GSH level and TAC compared to AD administered rats as shown in Table-

Table (3): Renal MDA concentration, GSH concentration, SOD activity, CAT activity and TAC in control and different treated rat groups**.**

	Control	GA	AD	AD + GA
MDA(nmol/g)	38.84 ± 1.17^{a}	36.02 ± 0.91^{a}	101.09 ± 1.58^{b}	66.34 ± 1.96^{bc}
GSH(mmol/g)	3.18 ± 0.12^{a}	3.37 ± 0.19^{a}	1.30 ± 0.09^{b}	2.30 ± 0.11^{bc}
SOD(u/mg)	63.86 ± 1.59^{a}	64.11 ± 1.01^{a}	21.59 ± 0.66^{b}	39.84 ± 1.75^{bc}
CAT(u/g)	4.04 ± 0.25^{a}	4.49 ± 0.22^{a}	0.76 ± 0.03^{b}	2.11 ± 0.12^{bc}
TAC(mM/mg)	0.85 ± 0.005^{a}	0.88 ± 0.01^{a}	0.34 ± 0.01^{b}	0.63 ± 0.05^{bc}

Each value represents the mean ± SE of six rats. Values superscripts with various letters were significantly different at P≤ 0.05. AD = Adenine, GA = Gum Arabic.

Table (4): Serum and urine, calcium (Ca), phosphorus (P), sodium (Na) and potassium (K) concentrations in control and different treated rat groups**.**

	Control	GA	AD	AD +GA
Serum Ca (mg/dl)	8.71 ± 0.16^{a}	9.09 ± 0.10^{a}	5.36 ± 0.14^{b}	7.03 ± 0.08^{bc}
Urine Ca(mg/24h)	50.77 ± 0.70^{a}	53.11 ± 1.48^{a}	109.85 ± 2.65^{b}	94.30 ± 5.13^{bc}
Serum P(mg/dl)	2.93 ± 0.08^{a}	3.31 ± 0.14^{a}	8.87 ± 0.25^{b}	6.17 ± 0.09^{bc}
Urine P(mg/24h)	55.36 ± 2.54^{a}	51.06 ± 1.78^{a}	18.16 ± 1.65^{b}	32.66 ± 1.76^{bc}
Serum Na(mmol/l)	139.95 ± 0.34^{a}	141.01 ± 0.58^{a}	123.54 ± 0.64^{b}	132.15 ± 0.58^{bc}
Urine Na (mmol/24h)	240.74 ± 7.70^{a}	236.45 ± 4.62^{a}	590.85 ± 9.46^{b}	447.12 ± 9.62^{bc}
Serum K(mmol/l)	4.07 ± 0.07^{a}	3.84 ± 0.12^{a}	6.68 ± 0.15^{b}	5.61 ± 0.06^{bc}
Urine K(mmol/24h)	40.28 ± 0.83^{a}	39.00 ± 1.14^{a}	10.65 ± 1.01^{b}	22.18 ± 1.04^{bc}

Each value represents the mean ± SE of six rats. Values superscripts with various letters were significantly different at P≤ 0.05. AD = Adenine, GA = Gum Arabic.

By the end of the 4th week, the concentrations of serum PTH and CT significantly increased, while serum calcitriol levels significantly decreased in AD administered rats in comparison to control rats. However, the concomitant treatment of rats with GA and AD showed significant reduction in PTH and CT and significant increase in calcitriol levels compared to AD-treated group (table- 5).

Table (5): Serum Parathormone, Calcitonin and Calcitriol hormone levels**.**

	Control	GA	AD	AD + GA
Parathormone (pg/ml)	0.09±0.004[a]	0.08±0.003[a]	0.31±0.01[b]	0.14±0.002[bc]
Calcitonin (pg/ml)	7.69±0.37[a]	6.91±0.24[a]	16.57±0.28[b]	13.05±0.44[bc]
Calcitriol (ng/ml)	0.30±0.005[a]	0.29±0.004[a]	0.10±0.002[b]	0.18±0.003[bc]

. Each value represents the mean ± SE of six rats. Values superscripts with various letters were significantly different at P≤ 0.05. AD = Adenine, GA = Gum Arabic

The histopathological observations revealed normal architecture of kidney in the control and GA-administered rats (Fig.2 A&B). However, the kidneys of AD-treated rats showed marked atrophy and proliferation of renal corpuscles, total hyalinization of many glomeruli, degeneration and necrosis of renal epithelial linings of proximal, distal and cortical collecting ducts as well as inflammatory interstitial infiltrate. Also the sections showed sever tubular congestion and most of tubules contain protein casts (Fig.2 C).Most of these effects were markedly ameliorated by supplementation with GA in concomitant with AD (Fig. 2D).

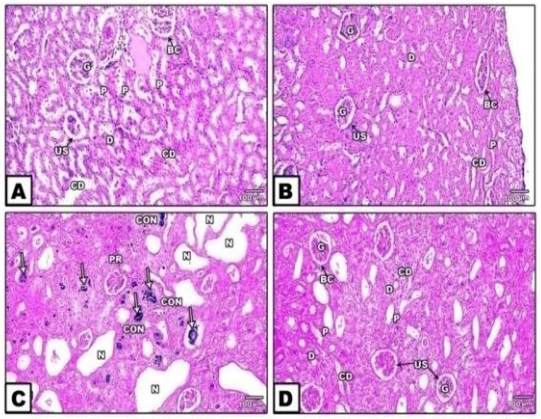

Figure (2) (A) and **(B):** Photomicrograph of a transverse s

ection through the cortical region of the kidney of control and gum Arabic (GA) supplemented rats respectively showing normal renal corpuscles formed of glomerulus (G) and Bowman capsule (BC) (parietal layer and visceral layer) and in between the urinary space (US) as well as the cross sections of the proximal (P), and collecting ducts (CD) with typical pattern of epithelial linings. H & E *100.

Figure (2) (C): Photomicrograph of a transverse section through the cortical region of the kidney of AD- treated rats showing obviously congestive glomeruli (CON), necrosis (N) of most renal epithelial linings of proximal (P), distal (D) and cortical collecting ducts. The white filled arrows point to examples of inflammatory interstitial infiltrates. H & E *100**.**

Figure (2) (D): Photomicrograph of a transverse section of the kidney of AD+GA treated rats showing nearly normal appearance of renal corpuscles, some are hypertrophied, others are regenerated while the renal epithelial linings of the renal tubules are necrotic. H & E* 100

Discussion

Chronic renal failure (CRF) is a worldwide health burden with a high economic cost to health systems and is an independent risk factor for cardiovascular disease (CVD), which is associated with increased risk of morbidity, premature mortality, and / or decreased quality of life [25].

Protection of the kidney using natural products may be a safe, effective, and cost–efficacious selection to preserve the kidneys against harmful agents, and to eliminate the progression of renal disorder to end stage renal disease (ESRD) where, either kidney transplantation or dialysis are the only available selections that are expensive and unavailable in the developing countries [26].Thus, there is a strong demand for studies exploring new therapeutic strategies and ameliorating agents in order to slow CRF progression and its complications [3].

In the current study, intragastric administration of AD caused a significant reduction in body weight, associated with a significant increase in urine volume and water intake. These results are in harmony with that of Abdel-Rahman et al and Nemmar et al [11, 27]. These changes could be attributed to malnutrition associated with AD treatment, mainly due to a reduction of food intake and increase excretion of protein as

albumin in urine [4, 28]. A result confirmed by the significant increase of microprotein in urine. The obtained results in this contest showed also that the absolute and relative kidney weights significantly higher in AD administered rats compared to control revealing the enlargement of kidneys. These results in agreement with results of Thakur et al [29], where intragastrically administered AD in rats, oxidized to 2, 8-dihydroxy adenine (DHA), which can precipitate forming crystals in renal tubules causing CRF [19]. In this concern, Zhang et al [9] reported that AD administration caused changes in the renal tubules in the kidneys of rats; there was considerable swelling and expansion of the renal tubules as shown in histopathological examination.

The results of the current study showed that GA can attenuate the increased kidney weights, water intake and urine volume of AD- administered rats in agreement with Al Za'abi et al [19]. The salutary action of GA in improvement of CRF caused by AD may be attributed to the antioxidant and anti-inflammatory properties [16, 17]. Recently, Al Za'abi et al [30] found that co-administration of GA with AD abated the increased water intake and urine volume in rats treated with AD. Moreover, GA can show binding of free water in the intestine, which resulted in a reduction of intestinal fluid absorption and urine volume [31].

In addition, urine protein concentration and NAG activity were significantly increased in AD treated rats. These data are in accordance with Abdel-Rahman et al and Ali et al [11, 32]. The elevated NAG activity in urine confirming kidney damage as NAG considers as a biomarker of the damage of proximal tubules and other parts of the nephron [33]. Moreover, the co-treatment of GA to AD administered rats caused significant reduction in protein concentration and NAG activity in urine compared to AD administered rats, indicating improvement in renal functions. These results agree with results of FadlAlla [34].

The obtained data indicated that intragastric administration of rats with AD (300 mg/kg for four weeks) significantly increased the markers of kidney function, serum Cr, BUN and UA while Crcl was significantly decreased compared to control. These data are in consistent with previous studies confirming that AD administration caused significant increase in concentrations of BUN and Cr in serum [29, 35] and significant decrease in Crcl (as a measure of GFR)] [36, 37]. These findings indicated that the obtained elevation in serum Cr, BUN and UA reflects a disease or damage in the kidneys [38]. Oral administration of AD causes obstruction of renal tubules which inhibits the excretion of nitrogenous substances causing a physiological and biochemical state resembling CRF in humans. Both AD and its oxidized form 2, 8-DHA can precipitate in the renal tubules forming crystals because both of them have low solubility [39].

Moreover, the co-treatment of GA to AD administered rats caused significant reduction in serum Cr, BUN and UA as well as significant increase in Crcl compared to AD administered rats, indicating improvement in renal functions. These results agree with Al Za'abi et al and Khojah [30, 40]. The effect may attributed to the formation of short-chain fatty acids such as butyrate [41], which are formed after GA degradation by intestinal bacteria and which can modify the propagation of the pro-fibrotic cytokine transforming growth factor-beta1 (TGF-β1) by epithelial cells of the kidney leading to increase GFR and renal blood flow [42]. Also, the ameliorating role on renal function of GA may be as urea-reducing effect by using the intestine as alternative kidney, stimulating the excretion of urea nitrogen (N) in the stool, associated with a reduction in urinary excretion of total N [43].

In the AD- CRF model used here, it have been shown that there is evidence of a marked increase in oxidative stress in renal tissue as reflected by increase in lipid peroxidation (LPO) marker MDA and significant decrease in antioxidant enzymes (SOD and CAT) as well as levels of GSH and TAC. These results are in accordance with previous findings by Ali et al, Kim et al and El-Habibi [32, 36, 44].In the metabolism of mammals, when there are excess amounts of AD, it become a suitable substrate for xanthene

dehydrogenase, an enzyme which converts AD to 2,8- DHA forming crystals in renal tubules [9] and could increase the releasing of reactive oxygen species (ROS) such as superoxide anion radicals and peroxides Nemmar et al [45] causing oxidative stress (OS). These produced reactive oxygen species (ROS) interact with renal epithelia causing damage of the renal membranes and lead to dysfunction and damage of the tubules [46].

On the other hand, the administration of GA significantly increased renal SOD and CAT activities as well as GSH and TAC levels. While a significant decrease in LPO marker (MDA) in renal tissues of AD treated rats was obtained. These results are in harmony with Kaddam et al and FadlAlla [14, 34].The antioxidant effect of GA may be directly by subsisting the ROS or through increasing the production of antioxidant molecules, because GA contains flavonoid and phenolic compounds [34] such as flavone, epicatechin, catechin, polyphenols, chalcones, tannins, flavonoids and alkaloids [2, 47]. These phytocomponents have been broadly reported to have antioxidant and anti-oxidant activities [48].GA also, contains various antioxidant molecules seem to be responsible for the antioxidant capacity of GA versus ROS such as tyrosine, lysine, methionine and histidine [17].

In the present study, the histological observations of the kidney in AD- treated rats showed marked atrophy and proliferation of renal corpuscles, degeneration and necrosis of renal epithelial linings of proximal, distal and cortical collecting ducts as well as inflammatory interstitial infiltrate. These observations are in harmony with those of Ali et al [3]

On the other hand, histological observations of AD plus GA treated rats showed nearly normal pattern of renal cortex and renal medulla with approximately normal configuration of renal corpuscles and moderately necrotic renal epithelial linings. These observations are in harmony with those of Al Za'abi et al [22, 34].

The results of the present study indicated that oral administration of AD led to perturbation of systemic mineral metabolism. Serum calcium (Ca) and sodium (Na) concentrations were significantly decreased, while serum phosphorus (P) and potassium (K) were significantly increased. On the other hand, the reverse was observed in urine. These data are in agreement with those of Kim et al [36].

CRF Patients suffer from hyperphosphatemia due to reduced renal filtration and have decreased kidney activation of 25-hydroxyvitamin D to 1, 25-dihydroxy vitamin D, leading to reduced calcitriol level and finally hypocalcaemia [49]. Regarding hypocalcaemia and hyperphosphatemia, the parathyroid gland properly augments PTH secretion to activate the release of bone calcium phosphate and reduce renal tubular re-absorption of phosphorus [50].

The results in the present study showed that PTH and CT levels significantly increased in AD-treated animals accompanied by significant decrease in calcitriol level compared with control rats. These data are in consistent with previous studies [9, 51].

The progressive decline in kidney function, as obtained in the present study and the loss of renal 1α-hydroxylase in CRF are accompanied by a gradual decline of serum 25-hydroxyvitamin D3 and 1, 25-dihydroxyvitamin D3 [52]. CRF Patients are often suffering from the deficiency of calcitriol due to the decrease of its precursor 25-hydroxyvitamin D3, and also due to the reduced activity of renal1α-hydroxylase enzyme, which activates 25-hydroxyvitamin D3 into 1, 25-dihydroxyvitamin D3 [53]. Decreased concentrations of 1, 25-dihydroxyvitamin D3 in CRF patients may attributed to reduced levels of urinary vitamin D binding protein [54], defective photoproduction in the skin after the exposure to ultraviolet β radiation and likely reduced nutritional intake and sun exposure [55] as well as because of adverse effects of the elevated fibroblast growth factor 23 (FGF23), the loss of renal mass [49, 56] and the loss of renal 1α-hydroxylase activity [57, 58].

Secondary hyperparathyroidism (SHPT) exists in most of CKD subjects due to reduced serum calcitriol level, hypocalcaemia, hyperphosphatemia, presence of uremic toxins and reduced levels of the PTH1 receptors [1, 59,60].

Although the kidney considers as the main site for metabolism of CT, its values are increased in uremic humans and animals [61]. Decreased bone mineral density (BMD) significantly related with CRF, and CRF subjects are at higher risk for osteoporosis [62].Physiologically, CT is mainly known as an inhibitor of bone resorption [63].The elevated CT level in CRF rats may be due to decreased BMD, to activate osteoblasts and inhibit osteoclasts. As osteoblasts facilitate bone formation, while osteoclasts break bone tissue and release calcium [64]. Serum CT was significantly elevated in CKD patients both on hemodialysis or non-dialyzed patients and there was a significant inverse relationship between serum CT and Crcl. Also, a positive correlation was found between CT and P level. Therefore, the elevated CT level in AD-induced CRF rats may be explained by a reduced renal degradation of CT and/or an increased production due to stimulation by hyperphosphatemia [65].On the other hand, Felsenfeld and Levine [63] stated that the reason of elevated CT level in CKD subjects remains unknown. A point which needs further investigation.

On other hand, concomitant treatment of GA with AD caused a significant increase in serum calcium (Ca) and sodium (Na) and urinary phosphorus (P) and potassium (K) levels and significant decrease in serum phosphorus (P) and potassium (K) and urinary calcium (Ca) and sodium (Na) levels. These data are in agreement with FadlAlla [34]. GA administration causes elevation in serum calcium concentration, which may be due to increased calcium absorption and retention as GA enrich in Ca^{+2} [66]. This assumption is in accordance with Dashtdar and Kardi [67] who illustrated that GA act as probiotics which can improve the absorption of minerals, especially calcium and helps to maintain a healthy balance of bacteria in the gastrointestinal tract (GIT).The results of the present study, revealed that GA ameliorated kidney function of rat treated with AD and GA, showing that the level of calcitriol significantly elevated and serum calcium increased, therefore, the level of PTH decreased but stilled significantly increased compared with control rats. Vitamin D also plays a key role in calcium/phosphate homeostasis and it is well reported that the progression of CRF inhibits vitamin D metabolism [10]. Therefore, when GA ameliorated the kidney function, calcium homeostasis also has been ameliorated.

In the present study, concomitant treatment of GA with AD caused a significant decrease in serum PTH and CT levels associated with significant increase in calcitriol level. As shown by the obtained results, GA treatment can indeed exert favorable effects, because it might partially ameliorates disturbances in calcium homeostasis. Consequently, in AD-CRF rats, hyperphosphatemia could contribute to increase levels of FGF23 which reduces the activity of 1 α hydroxylase enzyme (which activates calcitriol) causing hypocalcaemia and SHPT [7]. However, progressive loss of kidney function gradually increases FGF23 levels as well as SHPT causing reductions in calcitriol levels in CRF patients [9, 10].

In addition to an enhanced glomerular filtration rate (GFR) after GA supplementation, GA acts as phosphate binding agents, can treat hyperphosphatemia Dashtdar and Kardi [67] leading to decreased levels of FGF23, increased 1 α hydroxylase activities, increased levels of calcitriol, increased calcium absorption and decreased PTH and CT concentrations in serum. Accordingly, the net result is elevated serum Ca2+ concentration compared with AD treated rats. Moreover, these effects are expected to prove that GA can improve calcium homeostasis in CRF patients.

Conclusion

The present study suggests that gum Arabic (GA) may be useful as a kidney protective against CRF induced by AD administration and help in improvement of calcium homeostasis in CRF disease.

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