THE POSSIBLE PROTECTIVE EFFECTS OF GUM ARABIC IN KIDNEY INJURY INDUCED BY ADENINE

Athraa Shakir Dakhil and Layla Alhasan*
Biology Department, Education College for Pure Sciences, Thi-Qar University, Nasiriyah 64001, Thi-Qar, Iraq
*Corresponding author: layla.alhassan14@gmail.com

Abstract

The effect of treatment with gum acacia (GA), a prebiotic shown to ameliorate chronic kidney disease (CKD) in rats has been investigated using many immunological, oxidative stress, and histopathological markers. Rats were randomly divided into four groups, and given either normal food or food mixed with adenine (250mg/kg, for 28 days) to induce CKD. Two of these groups were also concomitantly treated orally with GA in the drinking water (15% w/w) for the same period. Rats fed adenine alone exhibited increasing in IL-6 and IL-10 inflammatory biomarkers, as well as indices of oxidative stress, and triggered histological damage in kidneys, while, fed mixed has led to decline in IL-6 and IL-10 inflammatory biomarkers and improved histopathological damage in animal models. GA enhances adenine-induced chronic renal failure (CRF) in male rats.

Keywords: Adenine, Gum acacia, Oxidative biomarkers, histopathological changes, Rats.

Introduction

Chronic kidney disease (CKD) is a health problem worldwide, is a slowly progressive disorder that might lead to end-stage renal disease. This increase tests the need for more incomes and the rising requirement on health-care systems already burdened by paucity of resources. Thus, there is a need for a new therapeutics and ameliorating strategies in particular for earlier stages of CKD so as to slow its progression towards end-stage renal disease. One of these ameliorating agents is gum acacia (GA).

Gum Arabic is widely employed in pharmaceutical; It is also used in the traditional treatment of patients with chronic kidney disease (CKD) (Ali et al., 2009). It has been also shown to increase fecal nitrogen excretion and decrease serum urea nitrogen concentration with patients CKD (Ali et al., 2008; Bliss et al., 1996). It is equally included in (traditional) medicine concoctions to address internal ailments such as cough, diarrhea, dysentery and hemorrhage and applied externally. It is also used in veterinary medicine, to treat skin diseases and inflammation (Kasper et al., 2005). Hence, our study is designed to investigate the possible protective effects of Arabic gum on kidney injury. The consumption of oral adenine thus might cause the occlusion of renal tubules which retards the excretion of nitrogenous substances leading to a biochemical and physiological status resembling CKD in humans (Nasir et al., 2012; Ali et al., 2010). Although it has been shown by some studies that the basis for the ameliorative effects of GA is via its anti-inflammatory and antioxidant actions (Ali et al., 2009; Ali et al., 2013; Gado and Aldahmash, 2013) and not only its possible local effect of excreting fecal nitrogen when administered with oral adenine, there still remains a need for further scientific work on the mechanism of action of GA as a nephron-protective agent. Our approach towards a better understanding of the mechanisms of action of GA led us to use the CKD inducing agent (adenine) by a systemic route, and this is, as far as the current study ware, the induction of CKD has been carried out by adding of adenine in feeding to produce a model of CKD. Furthermore, our aim shown that treatment of rats with Gum Arabic can be effective in ameliorating several biochemical and histopathological indices of adenine-induced CRF and Gum Arabic can be antioxidative agents as possible protective materials.

Materials and Methods

Experimental animals

Twenty-four Male wistar rats, type Rattus norvegicus the strain Balb/c (11 to 12 old, weighting 250 ± 10 gm) were used in the current study and obtained from the animal house of the Biology Department-College of Science, Thi-Qar University. Animals were given a sufficient amount of water and food from a local source (Wheat 34%, barley 20%, corn 25%, animal protein 10%, powdered milk 10%, salt 1%) these ingredient were grinded and mixed with some oil and water until they become a paste coherent (Vielhauer et al., 2005) and put in the designated place for the food in the cages for animal breeding.

Chemicals

GA and adenine were both obtained from Sigma (St. Louis, MO, USA). All other chemicals used were of Analytical Reagent grade.

Study Design

After an acclimatization period of seven days, rats were each divided into four equal groups, and each group contains six rats. The first group (control group) was received a normal feeding without treatment until the end of the study.

The second group (Adenine group) was administrated 250mg/kg in feeding for 28 days). The third group (Gum Arabic group) was given normal food and GA in drinking water at a concentration of 15%, w/v, for four weeks. The fourth group (Adenine + GA group) of was given adenine as in the second group, and GA in drinking water at a concentration of 15%w/v, for four weeks.

IL-6 and IL-10 cytokine assessment

A medical syringe drew blood samples via cardiac puncture. The blood samples were put in the Gel tubes, and it is left to clot at room temperature (25) C° for (20) minute and then put in a centrifuge at (3500) rpm for 10 minutes to separate the blood serum. Then separated serum was collected by a micropipette and kept into plastic tubes and frozen to at 20 C° for immunological tests. The ELISA kit
applied to determine (IL-10, IL-6) concentration in rat serum according to manufacturing instructions.

**Real time PCR assay**

After the dissection of animals (rats) and took the kidneys after drying with blotting paper and weighed with a delicate balance then took a small part of the kidney almost (40 mg) and placed in Eppendorf (contains 600 µL trizol) then use a parafilm and roll on the Eppendorf to keep the ingredients from leaking.

**RNA Extraction**

RNA was isolated from sample according to the protocol of TRIzol™ Reagent as the following steps. For each tube, 1mL from TRIzol™ Reagent was added per 50-100 mg of sample and gently mixed by vortex. A 0.2 mL of chloroform was added to the lysate, then the tube cap secured. All mixture were incubated for 2–3 minutes, and then centrifuged for 10 minutes at 12,000 rpm, the mixture was separate into a lower organic phase, interphase, and a colorless upper aqueous phase. The aqueous phase containing the RNA was transferred to a new tube. A 0.5 mL of isopropanol was added to the aqueous phase and incubated for 10 minutes then centrifuged for 10 minutes at 12,000 rpm. Total RNA was precipitate formed a white gel-like pellet at the bottom of the tube. Supernatant then discarded. A 0.5 mL of 70% ethanol was added and vortex briefly then centrifuge for 5 minutes at 10,000 rpm. Then, aspirated and air-dried the pellet. Pellet was rehydrated in 20-50 µL of Nuclease Free Water then incubated in a water bath or heat block set at 55–60°C for 10–15 minutes. All primers are designed by primer BLAST program, through the identification of the database and genetics, on the basis of which the primer is designed and request to manufacture them from Korea.

**Quantitation of RNA**

Quantus Florometer was used to detect the concentration of extracted RNA or cDNA in order to detect the goodness of samples for downstream applications. For 1 µl of RNA or cDNA, 199 µl of diluted QuantyFlour Dye was mixed. After 5min incubation at room temperature in dark room, RNA concentration values were detected.

**Gene Expression by Real-Time PCR**

OneTaq One-Step Reaction (2X) 25 µl and OneTaq One-Step Enzyme Mix (25X) 2 µl, GHS Gene-specific Forward Primer (10 µM) 2 µl at a sequences 5′-CCTGCTAGTGGATCTGTC3′- and Gene-specific reverse primer (10 µM) 2 µl at a sequences (5′-TCACTTGTGTGGATCTGTC3′), SOD Gene-specific forward primer at a sequences (5′-TCCATTTGGATTTGGAGAT3′) and SOD Gene-specific reverse primer at a sequences (5′-CCCCACCTTTCTGGGAT3′), B-actin Gene-specific forward primer at a sequences (5′-GGCTTGGGACACTCTTCC3′) and B-actin Gene-specific reverse primer at a sequences (5′-CGGATGGCACACTCTTCC3′), were put it in the sterile RNase-free microfuge tubes. The RNA template last was added, and reactions immediately were started, according to manufacturing instructions. Fold alterations in mRNA levels of the targeting gene relative to the endogenous B-Actin control were calculated. Briefly, the cycle threshold (–Ct) values of each target gene were subtracted from the Ct values of the housekeeping gene B-actin (∆Ct). Targeting gene ∆∆Ct was considered as ∆Ct of target gene minus ∆Ct of control. The fold change in mRNA expression was calculated as 2^−∆∆Ct following a previous study.

**Histopathology**

Rat kidneys were fixed in 10% neutral-buffered formalin, dehydrated in increasing concentrations of ethanol, cleared with xylene and embedded in paraffin. Four-micrometer (µm) sections were prepared from kidney paraffin blocks and stained with hematoxylin and eosin (Bancroft and Gamble, 2008).

**Statistical analysis**

Data analysis was carried out using GraphPad Prism 7.0 (GraphPad Software, SanDiego, CA, USA). Data are expressed as means ± SEM. Comparisons between the groups were performed by one-way analysis of variance followed by multiple comparison tests. P<0.05 was considered statistically significant.

**Results and Discussion**

The worldwide incidence of CKD is increasing (Locatelli et al., 2006), but access to renal replacement therapy, either transplantation or dialysis is limited in several regions of the world due to a lack of financial and clinical resources (Jain et al., 2012; Aviles-Gomez et al., 2006). Therefore, natural product may be the best therapy available for all patients around the world.

**Immunological Finding**

**IL-6**

Inflammatory mechanisms underlie the CKD model induced by adenine and are ameliorated by gum Arabic to confirm the role of inflammation in the development of CKD in rat, levels of IL-6 were significantly higher compared with the control group show in (Figure 1 and Table 1), gum Arabic treatment significantly reduced of these cytokines in the renal tissue.

Interleukin-6 is cytokine with a wide range of biological activities. On the immune system, it has a wide range of effect and can influence the equilibrium process by having hormone-like properties. It is widely used in clinical intervention because it has anti-inflammatory properties. It is activated during inflammation and maturation of B cells. It can act as a protein and can cause fever during infection, non-communicable diseases and autoimmune diseases. IL-6 is produced by macrophages and monocytes as a reaction to other inflammatory cytokines. It is also inflammation indicator within body. For the occurrence of bacteremia IL-6 can also use as investigative marker (Fuster and Walsh, 2014).

In this work opted the administration of adenine to rats in feeding diet for 28 days, which was found by some researchers to be an optimum duration of treatment that damages the kidneys but does not produce mortality (Ngai et al., 2005) Others have found that the same dose that employed (0.75% w/w) produced an ideal model resembling CRF. Another animal model with many similar characteristics of human CRF is the remnant kidney (Yokozawa et al., 1986). In a current work, employed the
latter model to test the reputed usefulness of GA in CRF. GA given in the drinking water found that GA significantly mitigated the extent of the biochemical, physiological and histo-pathological effects of the adenine. Presented results suggest that GA at the doses tested and for the duration used was, in fact, effective in significantly. Although statistically significant, a trend to an increase in the concentration of IL-6 was found in urine, suggesting that its level in plasma has decreased. This action was mitigated by GA treatment, a possible indication that the adenine-induced CRF involves an inflammatory response.

### Table 2: Effect of treatment of rats with adenine in feeding diet with addition of gum Arabic (15%, w/v) in the drinking water on some immunological markers.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>IL-6</th>
<th>IL-10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>32.82±2.596</td>
<td>32.92±2.44</td>
</tr>
<tr>
<td>A</td>
<td>180.88±16.010</td>
<td>123.27±10.843</td>
</tr>
<tr>
<td>GA</td>
<td>31.83±6.50</td>
<td>34.86±2.956</td>
</tr>
<tr>
<td>A+GA</td>
<td>39.49±4.999</td>
<td>67.23±9.149</td>
</tr>
</tbody>
</table>

#### IL-10

IL-10 played an important role in normal kidney physiology, as well as during severe kidney injury, and in the development of chronic kidney failure. The main function of IL-10 is to control inflammation and direct adaptive immune responses. IL-10 prevents activation and labeling of antigen presenting cells, such as stem cells and macrophages.

In the CRF model used in the present study, adenine is given mixed with the feed at a concentration of 250 mg/kg, for 4 weeks. Treatment with GA significantly abated the adenine effect. The concentrations of the anti-inflammatory cytokine IL-10 were detectable with low percentage in rats treated with water (controls) (Figure 2). However, the concentration of this cytokine was significantly increased in the adenine-treated rats (p< 0.05) compared to the control and the GA-treated rats. Both of these two possible mechanisms need to be investigated in more detail by measuring more cytokines and reactive oxygen species.

#### Oxidative Stress Markers Study by Real-Time PCR

Oxidative stress is already found in early stages of renal disease and increases with declining kidney function (Dounousi et al., 2006). In adenine-induced CRF, until now oxidative stress was demonstrated in the heart and in the vasculature (Goux et al., 2011; Zhao et al., 2011), so this is requiring more investigation to study the increased glutathione and superoxide production in the kidneys. Hence assessed in the presented study.

### Glutathione

Signs of oxidation, including GSH, were high in treated adenine groups (Figure 3 and Table 3) where p <0.05 was considered a significant. General appearance, the kidneys of the control rats appeared normal. However, the kidneys of adenine-treated animals were pale, and a few adenine crystals were seen, mainly in the cortex area. The kidneys of the groups that had been treated with adenine and GA together visually appeared improved compared with those of the kidneys of groups treated with adenine alone (Results not shown).

Adenine has a tendency to cause several oxidative and inflammatory reactions in renal tissues, which might cause an increase in several oxidative and inflammatory markers such as GSH, as seen in the present and other studies (Ali et al., 2013; Ali et al., 2014; Waring and Moonie, 2011; Baumgarten et al., 2011). Recently, GA has been found in several studies to have anti-inflammatory and anti-oxidative properties making it an apoptosis scavenger (Ali et al., 2013; Ali et al., 2010). In this study concomitant treatment with GA significantly reduced the inflammatory and oxidative stress induced by the administration of adenine as shown in Real time results, it was reduced in glutathione expression both GA and GA and Adenine together compared with adenine alone,

These findings suggest the need for further in vivo studies on a molecular basis for the systemic effects associated with the use of GA and perhaps suggest the need for a translational move towards a small-scale human study.
Superoxide Dismutase

In the present work the administration of adenine was administrated in feeding diet, has led to a significant increases in water intake and urine and feces production as seen from a daily observation of treated rats. In addition, the morphological and histo-pathological alterations in kidneys of adenine groups confirm the development of CKD. Adenine has a tendency to cause several oxidative and inflammatory reactions in renal tissues which might cause an increase in several oxidative and inflammatory markers such as, SOD, as demonstrated in the current and other studies (Ali et al., 2013; Ali et al., 2014; Waring and Moonie, 2011; Baumgarten et al., 2011). The increase in the oxidative derivative of deoxyguanosine, 8-OHdG, one of the major DNA oxidative products, in the adenine treated groups also indicates oxidative stress within the cells. These oxidative biomarkers in the long run, as reported previously, might have systemic toxicity potentially causing damage to several other organs such as liver and heart (Fraga et al., 1990; Astor et al., 2012).

A treatment with GA significantly declined oxidative stress marker (SOD) induced by the adenine administration. These beneficial effects suggest that the anti-oxidative and anti-inflammatory properties possessed by oral GA are the main mechanism for its salutary action in adenine-induced CKD as shown in (Figure 4 and Table 4).

Table 4: Mean with different letters within each column significantly different (P< 0.05).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>GSH</th>
<th>SOD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.752±0.051*</td>
<td>1.0000±0.0000*</td>
</tr>
<tr>
<td>A</td>
<td>2.030±0.167*</td>
<td>0.535±0.09732a</td>
</tr>
<tr>
<td>GA</td>
<td>0.655±0.099a</td>
<td>0.9800±0.034a</td>
</tr>
<tr>
<td>A+GA</td>
<td>0.609±0.452a</td>
<td>0.892±0.0690a</td>
</tr>
</tbody>
</table>

Figure 3: Fold change in Glutathione gene expressions in untreated rats, and rats treated with adenine (250 mg/kg in the feed) and gum Arabic (15%, w/v) in the drinking water. Each column represents mean ± SD (n=6). Differences between the groups were assessed by analysis of variance, followed Duncan’s tests. P<0.05 was considered significant.

Figure 4: Fold change in Glutathione gene expressions in untreated rats, and rats treated with adenine (250 mg/kg in the feed) and gum Arabic (15%, w/v) in the drinking water. Each column represents mean ± SD (n=6). Differences between the groups were assessed by analysis of variance, followed Duncan’s test. P<0.05 was considered significant.
seen in the adenine treated group to normal in about 80% of the examined tissue fields. There were few foci showing tubular injury with neutrophil polymorph infiltration, tubular necrosis, tubular atrophy and interstitial fibrosis, which were less intense than those seen in the adenine-treated group and the adenine plus GA-treated rats, and were seen in about 20% of the examined tissue field.

Compared with control rats in the present study, the adenine-induced CRF rats provided experimental evidence that GA attenuated adenine-induced renal dysfunction, suggesting a promising potential of GA in protecting against renal failure progression.

Fig. 5: Healthy glomerular (Red arrow), Bowman capsule (Orange arrow) in control group, H&E 100x.

Fig. 6: Representative photograph of sections after hematoxylin and eosin (H&E 40x) of renal tissue of rats treated (control group), normal saline showing normal histological architecture there are many of bowman’s capsules (Orange arrow) with healthy glomerulus (Red arrow).

Fig. 7: Representative photograph of sections after hematoxylin and eosin staining (H&E). Adenine treated groups, showing severe hyperemia in the renal tissue (Orange arrow), degeneration of glomerular and shrinking glomerulus (Red arrow).

Fig. 8: Renal tubules represent as cyst (Orange arrow) due to severe degeneration of epithelial layer also, glomerulus necrosis, degeneration to renal glomeruli (Red arrow), cystic dilation of renal tubules (black arrow) in adenine group.
Fig. 9: Photograph showing diffuse tubular injury, renal tubules represent as cyst due to severe degeneration of epithelial layer also, necrosis epithelial cell (Red arrow), cystic dilation of cortical renal tubule (Black arrow) in adenine treated group.

Fig. 10: Representative photograph of sections after hematoxylin and eosin staining (H&E,100x) of renal tissue under light microscope showing degeneration of tubules (Red arrow), bowman’s capsules underwent atrophy in Adenine group.

Fig. 11: Photograph showing diffuse tubules injury and necrosis (Black arrow) with neutrophil polymorph infiltration, tubular atrophy, interstitial inflammatory cells infiltration, bleeding (Red arrow) in adenine treated group.

Figure 12: Representative photograph of section after hematoxylin and eosin staining (H&E100x) of renal tissue architecture and histology cortical tubules and peritubular capillaries with no pathogenic changes, parenchyma with normal glomeruli (red arrow), tubular epithelial changes in the form of cloudy swelling in gum Arabic group.
Figure 13: Representative photograph of sections after hematoxylin and eosin staining (H&E 100x) showing histological improvement histological appearance components nearby to normal renal tissue, normal glomerular, normal tubules seen in Adenine + GA group.

Figure 14: Bowman’s capsules (Black arrow) appeared with normal shape and increasing glomerular size as well as there are numerous normal proximal and distal renal tubules with non-degenerative epithelial layer, with less dilated tubules, less interstitial inflammation and less atrophic tubules, severe necrosis of tubules (Red arrow) in Adenine + GA group.

Conclusions
This study concludes that oral GA significantly ameliorated the effects associated with use of adenine given in feeding to rats. The administration of Gum Arabic can successfully replace the oral model of induction of chronic kidney disease.

References


