

NEPHRO-PROTECTIVE EFFECT OF *ACACIA SENEGAL* AGAINST GENTAMYCIN-INDUCED RENAL DAMAGE IN RATS

Ebtihal El. Tahir¹, Sania A. Shaddad*², A. K. Muddathir³, Nuha M.E. Agabna²

¹Omdurman Maternity Hospital. (Master Student)

²Prof. of Pharmacology (supervisor), Dept. of Medicine (Pharmacology), Faculty of Medicine University of Khartoum P.O. Box 102. (Nuha Assistance Prof. of Pharmacology (Collaborator)).

³Prof. of Pharmacognasy (co-supervisor), Dept. of Pharmacognasy Faculty of Pharmacy University of Khartoum. P.O. Box 1996.

Article Received on
08 March 2016,

Revised on 29 March 2016,
Accepted on 20 April 2016

DOI: 10.20959/wjpr20165-6184

*Corresponding Author

Prof. Sania A. Shaddad

Prof. of Pharmacology
(supervisor), Dept. of
Medicine (Pharmacology),
Faculty of Medicine
University of Khartoum
P.O. Box 102.

ABSTRACT

Gum Arabic *Acacia Senegal* is a dietary fiber known to inhibit colonic bacterial ammonia generation and increase faecal nitrogen; it is thought to have protective action on the renal system. **Objectives:** To investigate the nephroprotective effect of *Acacia senegal* (A.S) extract on laboratory rats. **Methods:** Twenty four males and females *Albino rats* (150-220 gm) were divided into four groups, each of six rats. Group I was control group, group 2 received gentamicin (100 mg/kg) intramuscularly for induction of nephrotoxicity (8 days), group 3 received the aqueous extract of *Acacia Senegal* (0.67 gm/kg) orally (21 days), group 4 was concomitantly administered gentamicin and the aqueous extract of *Acacia Senegal* (8 day). Blood samples were collected for haematological parameters (Hb, RBC, MCHC, MCV and

PCV), biochemical studies (creatinine, urea, sodium, potassium, AST, ALT, albumin) and the kidney for histopathological studies. **Results:** the aqueous extract of *Acacia senegal* (0.67 gm/kg) given orally for three weeks caused insignificant change in serum creatinine, urea, sodium and potassium levels. There were insignificant changes in both AST and ALT levels. However, the extract resulted in significant increase in albumin level. Haematological parameters were not affected. However, in rats treated with I.M. gentamycin in a dose of (100 mg/kg), concomitantly with *Acacia senegal* for 8 days revealed a nephroprotective effect, that significantly lowered serum creatinine and urea level.

KEY WORDS: acacia senigal, gentamicin, renal failure.

INTRODUCTION

Renal failure could be acute (ARF) or chronic caused by a number of factors. In acute renal failure there is an abrupt deterioration in renal function occurring over a period of hours or days. The causes of ARF may be pre-renal resulting from decreased renal perfusion and undamaged parenchymal tissue, or intrinsic resulting from structural damage to the kidney most commonly the tubule from ischemic or toxic insult. Post-renal causes could be due to obstruction of urine flow downstream from the kidney.

Chronic renal failure develops over a period of weeks, months or years, as consequences of reduction of adequate renal function. It produces a variety of complications due to the retention of waste products, impaired water, electrolyte and acid-base balance, loss of erythropoietin production, impaired vitamin D metabolism, activation of rennin angiotensin system and the development of hypertension. (Tonelli, Marcello, et. al.2006).

Drug –induced interstitial nephritis is a well documented form of iatrogenic disease being described in association with an increasing number of drugs, most commonly antibiotics, NSAIDs and diuretics (Keller et al., 2007). The disease usually begins about 2 weeks after exposure to the drug and may be characterized by systemic illness such as fever and eosinophilia with or without skin rash. On histological examination of renal biopsies there is pronounced edema and infiltration of tubule and interstitium by lymphocytes and macrophages. Eosinophils and neutrophils may be present in insignificant numbers. Plasma cells are found in more long-standing cases. There is a variable degree of tubular damage and degeneration is usually evident. The glomeruli, for the most part are normal in acute tubule-interstitial nephritis. The clinical features and morphology suggest a hypersensitivity reaction which is not dose-related but rather is idiosyncratic (Perazella et al, 2010).

Nephrotoxicity has been reported in 1.7% to 58% patients receiving aminoglycoside therapy. The large variance is in part due to the use of different definitions of toxicity, variability between agent in the class, as well as the risk factors in the study population. A gradual rise in the serum creatinine concentration and decrease in creatinine clearance after 6 to 10 days of therapy are the initial clinical manifestations of toxicity (Derakhshanfar et al., 2008, Dipiro, 2005).

Other drugs are known to cause nephrotoxicity: radiographic contrast media nephrotoxicity, cisplatin and carboplatin, amphotericin B, foscarnet, osmotic diuretics (mannitol) and low – molecular weight dextran or drug vehicles, including sucrose and propylene glycol, angiotensin-converting enzyme inhibitors and angiotensin-II receptor blocker, cyclosporine and tacrolimus (Naughton *et al.*, 2008, Taber *et al.*, 2006).

There are many suggestions that explain how dietary fibers such as gum Arabic decreases serum urea nitrogen. It has been mentioned that colonic bacteria ferment dietary fibers to provide them with energy for growth and nitrogen excretion (Stephen and Cumming, 1980 and Cumming, 1983). Another suggestion in animal models of experimental chronic renal failure showed that consumption of diets containing fermentable carbohydrates results in a greater rate of urea nitrogen transfer from blood to the caecal lumen, where it is hydrolyzed by bacterial urease before subsequent microflora metabolism and proliferation. Therefore, this results in a greater fecal nitrogen excretion, coupled with a reduction in urinary nitrogen excretion and plasma urea concentration (Younges, *et al.*, 1999).

However, experimental feeding gum Arabic shows that a period of ingestion is required to develop evidence of fermentation of these fibers (McLean Ross *et al.*, 1983).

Badreldin. H. Ali and his collaborators (2008), made an animal model of chronic renal failure (feeding adenine at a concentration of 0.75% (W/W) for four weeks) to test the effect of gum Arabic on chronic renal failure. Gum Arabic 6% (W/V) and 12% (W/V) in drinking water for four consecutive weeks, significantly ameliorate the adverse biochemical alterations indicative of renal failure, abated the decrease in body weight and reduced glomerular and interstitial lesions induced by adenine. The mechanism(s) of this nephroprotection was uncertain but might involve anti-oxidant and/or anti-inflammatory actions.

A comparative study between the crude gum (*Acacia senegal*) and two fractions [highly protein fraction (H), low protein fraction (L)] carried out in the scope of their physico-chemical properties and their effect when 25g/day of each was given to chronic renal failure patients on a low protein diet and conservative management for four weeks. It showed that fraction L had a significant effect in decreasing the level of blood urea nitrogen, creatinine, uric acid and phosphate and also it had a significant effect in increasing the level of blood calcium if the patient consumed it for four weeks, (Intisar, 2006).

Objectives

This study aims to investigate the nephro-protective activity of *Acacia Senegal* against gentamicin-induced nephrotoxicity in albino rats.

Specific Objectives

1. To evaluate the effect of *Acacia Senegal* extract on renal function and liver function
2. To study the haematological parameters and histopathological effects of *Acacia Senegal* extract.

MATERIALS AND METHODS

1. Materials

1.1. Animals

Male and female albino rats, weighing 150 -220 gm were obtained from National Center for Research, Khartoum, Sudan and were kept at animal house of Faculty of Pharmacy, University of Khartoum, under standard conditions of animal keeping. All animals were kept at room temperature ($26\pm 1^{\circ}\text{C}$) with free access to food and water.

1.2. Drugs: Gentamycin injectable and diethyl ether were used in this study

1.3. Equipments: Sysmex KX -21 and Hitachi analyzer (Germany)

2. Methods

2.1. Preparation of plant materials

The gum was obtained from El Nuhood North Kordofan State Sudan. It was finely powdered and kept for further use.

2.2. Preparation of extracts: Twenty grams (20g) of the powdered gum was dissolved in 100 ml of cold distilled water, and the concentration of the solution was determined.

2.3. Assessment of nephroprotective activity against gentamicin-induced nephrotoxicity

Nephrotoxicity was induced by gentamicin (100mg/kg). Gentamicin was injected by intramuscular route concomitantly with plant's extract administered orally.

Blood samples were drawn from the orbital plexuses under light diethyl ether anesthesia into heparinized capillary tubes. Serum was separated by centrifugation for 15 minutes at 3000 rpm. The serum samples were kept frozen at -20°C until tested for measurement of renal and liver function.

2.4. Hematological methods

For hematological measurement, blood samples were collected from rats in dry clean bottles containing ethelene diamine tetra acetic acid (EDTA) as described by Schalm (1965).

Haemoglobin concentration (Hb), packed cell volume (PCV), red blood cell count (RBC), mean corpuscular volume (MCV), mean corpuscular heamoglobin concentration (MCHC) were determined using Sysmex KX-21.

2.5. Histopathological study

Kidney specimens were dissected and fixed in formalin 10%, then were cut and stained using haematoxyline and eosin, seen under microscope (in Department of Pathology, Faculty of Veterinary Medicine, U of K).

2.6. Experimental design

twenty four male and female *Albino rats* (150-220 gm) were divided into four groups, each of six rats. Group I was control group, group 2 received gentamicin (100 mg/kg) intramuscularly for induction of nephrotoxicity (8 days), group 3 was concomitantly administered gentamicin and the aqueous extract of A.S orally (8 day), group 4 received the aqueous extract of A.senegal (0.67 gm/kg) orally for 21 days.

2.7. Statistical analysis

The observation in each group were compiled and tabulated for the assessment of mean and standard error of mean (Mean \pm SEM). Statistical comparison between different groups was done using one-way analysis of variance ANOVA and T-test (SPSS soft ware version 17.0).

RESULTS

Table 1: the effect of gum arabic solution on renal function

Group		Creatinine mg/dl	Urea mg/dl	Na mmol/l	K mmol/l
Control	Day 0	0.39 \pm 0.01	51.33 \pm 1.17	141.0 \pm 0.37	4.73 \pm 0.22
	Day 8	0.40 \pm 0.0	49.67 \pm 1.05	141.67 \pm 0.21	4.43 \pm 0.18
Gentamycin	Day 0	0.33 \pm 0.05	67.17 \pm 3.63	137.33 \pm 3.29	4.57 \pm 0.19
	Day 8	0.63 \pm 0.02 *	79.0 \pm 1.32 *	142.33 \pm 0.84	3.77 \pm 0.27
Gentamycin + A. senigal	Day 0	0.73 \pm 0.02	65.80 \pm 3.24	145.33 \pm 0.21	3.90 \pm 0.10
	Day 8	0.4 \pm 1.0	44.00 \pm 8.59 *	141.67 \pm 0.92 *	3.33 \pm 1.15 *

Values were expressed as Mean \pm SEM, P<0.05* significant. N=6

Table 2: The effect of gum arabic solution on liver function

Group		AST (IU/ml)	ALT (IU/ml)	Albumin (g/dL)
Control	Day 0	93.87 ±5.2	53.39 ± 4.09	3.30 ±0.0
	Day 8	90.90±4.35	56.23 ± 2.38	3.67 ± 0.17
Gentamycin	Day 0	73.0 ±2.95	41.51 ± 4.45	3.70± 0.2
	Day 8	145.37 ± 4.83*	48.50 ± 7.18	3.27 ± 0.06
Gentamycin + A. senigal	Day 0	92.87 ± 7.29	48.07± 0.65	4.30 ± 0.06
	Day 8	174.93 ± 1.62*	49.43 ± 0.19	3.97 ± 0.49

Values were expressed as Mean ± SEM, P<0.05* significant. N=6

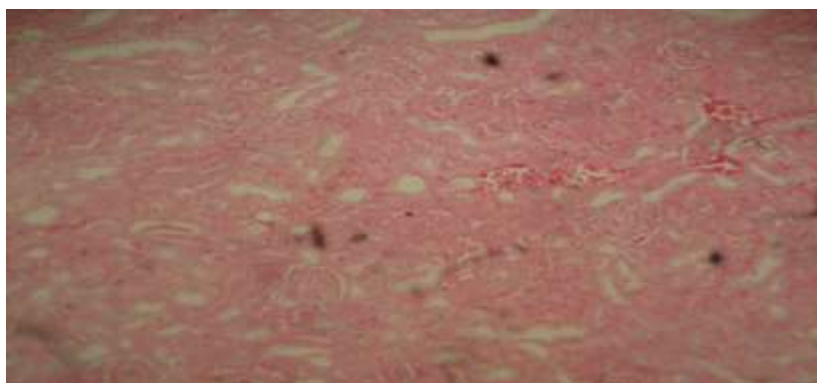
Table 3: the effect of gum arabic solution on haematological parameters

Group		Hb (g/dL)	RBC (X10 ⁶ μL)	MCHC (g/dL)	MCV (FL)	PCV (%)
Control	Day 0	13.77 ±0.25	7.47 ± 0.24	31.63 ± 0.33	58.47 ±0.52	43.60 ± 1.06
	Day 8	14.30±0.11	7.53 ± 0.01	32.23 ± 0.16	58.73 ± 0.09	44.35 ±0.13
Gentamycin	Day 0	13.30 ±0.57	7.83 ± 0.22	29.43 ± 1.06	58.03 ± 0.55	45.40 ± 1.11
	Day 8	11.77± 0.33*	6.80 ± 0.39*	30.17 ± 1.35	58.10 ±0.31	39.50 ± 2.36*
Gentamycin + A. senigal	Day 0	14.03 ±0.51	7.63 ±0.31	32.40 ± 0.26	56.80 ± 0.71	43.27 ±1.27
	Day 8	13.57 ± 0.19	7.18 ±0.14	31.87 ± 0.13	59.37 ± 1.09	42.60 ±0.66

Values were expressed as Mean ± SEM, P<0.05* significant. N=6

Table 4: the effect of gum arabic solution administered for 21 days on biochemical parameters

Parameter	Day 0	Day 21
Creatinine (mg/dl)	0.28 ± 0.01	0.28 ± 0.04
Urea mg /dl	53.60 ± 4.70	55.20 ± 4.23
AST (IU/ml)	132.20± 5.46	136.03 ± 5.77
ALT (IU/ml)	56.70 ± 7.83	55.63 ± 7.23
Albumin (g/dl)	3.70 ± 0.07	4.07 ± 0.06 *
Hb(g/dl)	12.84 ± 0.34	13.06 ± 0.18
MCV (FL)	60.04 ± 0.96	59.30 ± 0.80
PCV%	43.86 ± 0.64	40.88 ± 0.67*
MCHC (g/dl)	29.26 ± 0.65	31.98 ± 0.41 *
RBC × 10 ⁶ μL	7.29 ± 0.21	6.87 ± 0.08

**Figure 1: Kidney from rats received Gentamycin (100 mg/kg) for 8 days.**

Notice: Tubular destruction (coagulative necrosis), haemorrhage, glomeruli are less cellular, hyaline cast.



Figure 2: Kidney from rats received *Acacia senegal* (0.67 mg/kg) for 21 days.

Notice: normal kidney structure.

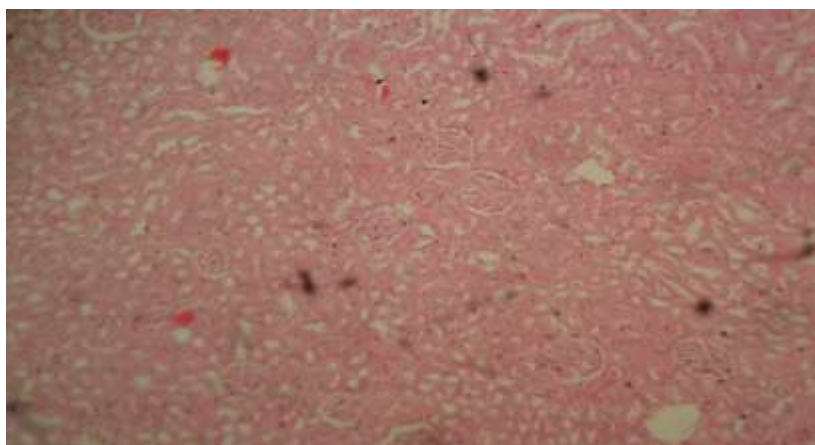


Figure 3 Kidney from rats received *Acacia senegal* (0.67 mg/kg) for 8 days, concomitantly with Gentamycin (100 mg/kg).

Notice: dilated tubules but glomeruli were not affected.

DISCUSSION

In this model of acute renal failure induced by gentamycin; gum arabic produced mild reduction in urea and creatinine serum levels (table1). This reduction was statistically significant for urea but not for creatinine. Sodium and potassium serum levels were significantly reduced in the treated group. In the group that received gum arabic solution alone for 21 days little variation in renal performance was observed. Similar results were obtained by Ali et al., 2005 using the acute renal failure model. However, Almajid et al,

2003., postulated that the nephroprotective value of gum arabic seen in rats treated with gentamycin was due to reduced lipid oxidation and antioxidant effect. Ali et al 2013.

The effect of gum arabic solution on electrolytes (sodium and potassium) was also observed by Nasir et al. 2008. They attributed the reduction to the high magnesium and calcium content in gum arabic solution which caused reduced urinary excretion of inorganic phosphates and reduced plasma concentration of 1-25 dihydroxy vitamin D, and increased sodium excretion.

Gum arabic possesses anti-inflammatory properties that seem to ameliorate the renal damage caused by gentamycin. This was documented by Mahmoud et al., 2012 who studied gum arabic on acute and chronic renal failure induced in rats. This group of researchers found that gum arabic attenuated c-reactive protein levels and increased renal superoxide dismutase activity.

Table 2 showed that gentamycin caused a significant elevation in liver enzyme AST both alone and with gum arabic solution. Other liver function parameters were not affected. The group that took gum arabic solution alone for 21 days displayed no variation in liver function. Haematological parameters were significantly affected by gentamycin which caused reduction in haemoglobin, red blood cells count, and packed cell volume. This effect was absent in the group that took gentamycin concurrently with gum arabic. This effect on haematology might in a way contribute to the protective action on kidneys.

Histopathological study of the kidneys from the different groups indicated that gum arabic attenuated the damage caused by gentamycin; as coagulative necrosis, haemorrhage and reduced cellularity were not seen when gum arabic was co-administered with gentamycin. The reduction in necrosis was also observed by Ali et al., 2003 in similarly treated animal groups.

From the above it is safe to conclude that gum arabic can have a positive value in renal failure. The clinical effect of gum arabic on renal patients on regular dialysis was assessed by Ali et al. 2008 and proved beneficial. Matsumoto 2006 found significant benefit renal patients.

REFERENCES

1. Ali, B. H. "The effect of treatment with gum Arabic on gentamicin nephrotoxicity in rats: a preliminary study." *Renal failure* 2003; 25.1: 15-20.

2. 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. *Natural product research*. 2008 Jan 10; 22(1): 12-21.
3. 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 Feb 1; 8(2): e55242.
4. Al-Majed AA, Abd-Allah AR, Al-Rikabi AC, Al-Shabanah OA, Mostafa AM. Effect of oral administration of arabic gum on cisplatin-induced nephrotoxicity in rats. *Journal of biochemical and molecular toxicology*. 2003 Jun 1; 17(3): 146-53.
5. Cummings, J. H. Dietary fibers and intestinal microflora in : Hallgren, B (ed.), XV Symposium of Swedish Nutrition Foundation. Stockholm: Almqvist and Wiskell International, 1983; 77-87
6. Derakhshanfar, A.; Bidarkosh, A and Hashempour Sadeghian, M. L-methionine attenuated gentamycin nephrotoxic in male Wister rate: pathological and biochemical findings. *Iranian J. Vet. Res.*, 2008; 10: 323 – 328.
7. Dipiro, J.T, Talbert, R.L, Yee, G.C; Matzke, G.R; Wells, B.G, Posey, L.M. *Pharmacotherapy, a pathophysiologic approach*, 6th edition, McGraw. Hill Companies, Inc United States America. 2005; 874-881.
8. Intisar Abd Alrahim Bashir (2006). Use of gum Arabic fraction supplement for chronic renal failure patients.(A thesis submitted to Ph.D, Faculty of pharmacy U.of Khartoum.)
9. Keller, M., Z. Spanou, and W. Pichler. "Drug-induced interstitial nephritis." 2007: 295-305.
10. Mahmoud MF, Diaai AA, Ahmed F. Evaluation of the efficacy of ginger, Arabic gum, and Boswellia in acute and chronic renal failure. *Renal failure*. 2012 Feb; 34(1): 73-82.
11. Matsumoto N, Riley S, Fraser D, Al-Assaf S, Ishimura E, Wolever T, Phillips GO, Phillips AO. Butyrate modulates TGF- β 1 generation and function: Potential renal benefit for Acacia (sen) SUPERGUMTM(gum arabic)?. *Kidney international*. 2006 Jan 1; 69(2): 257-65.
12. McLean Ross, A.H., Eastwood, M.A, Brydon, W.G, Anderson, J. R and Anderson, B. M. W. A study of the effects of dietary gum Arabic in Human. *Amer. J Clin. Nutr.*, 1983; 37: 368 –b 37515.
13. Nasir O, Artunc F, Saeed A, Kambal MA, Kalbacher H, Sandulache D, Boini KM, Jahovic N, Lang F. Effects of gum arabic (Acacia senegal) on water and electrolyte balance in healthy mice. *Journal of Renal Nutrition*. 2008 Mar 31; 18(2): 230-8.

14. Naughton, Cynthia A. "Drug-induced nephrotoxicity." *American family physician* 78.6 2008: 743-750.
15. Perazella, Mark A., and Glen S. Markowitz. "Drug-induced acute interstitial nephritis." *Nature Reviews Nephrology* 2010; 6.8: 461-470.
16. Schalm, O.W. (1965). *Verterinay heamatology*, 4th ed. Lea and Febiger. Philadelphia, U.S.A.
17. Stephen, A. M. and Cummings, J. H. Mechanism of action of dietary fiber in human colon. *Nature*, 1980b ; 284: 282-284.
18. Taber, Stephanie S., and Bruce A. Mueller. "Drug-associated renal dysfunction." *Critical care clinics* 2006; 22.2: 357-374.
19. Tonelli, Marcello, et al. "Chronic kidney disease and mortality risk: a systematic review." *Journal of the American Society of Nephrology*, 2006; 17.7: 2034-2047.
20. Younges H.; Alphones, J. C.; Behr S. R.; Demigne, C. and Remesy, C. Role of fermentable carbohydrate, supplementations with a low protein diet in the course of chronic renal failure: experimental basis. *Am. J. Kidney Dis.*, 1999; 33: 633-646 in14.