



Evaluation of the antioxidant activity and nephroprotective Effects of *Guiera Senegalensis* Ethanolic Extract on Gentamicin-induced Nephrotoxicity in rats

Authors & Affiliation:

Hala, E.A., Samia, H. Abdelrahman.,
Samia.M.A Elbadwi. Sara, A.M., Halima,
M .O., ManaL, H .S., and Nuseiba, H.R.
Cent. Vet. Res. Lab, Khartoum Sudan

Corresponding Author:

Hala Elrayah Ahmed

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Caribbean Journal of Science and
Technology

ISSN 0799-3757

<http://caribjscitech.com/>

Abstract:

The present study was undertaken to evaluate *Guiera Senegalensis* protective effects on gentamicin-induced nephrotoxicity in rats. 40 albino rats of either sex, weighing 150-200g were divided into 4 groups; Control group, gentamicin 80 mg/kg, i.p., for 6 days, *Guiera Senegalensis* 250 and 500 mg/kg i.p., for 10 days, *Guiera Senegalensis* 4 days prior and concurrently with gentamicin for 6 days. Radical scavenging activity of *Guiera Senegalensis* was determined; Catalase and super oxide dismutase, urea and creatinine in serum and urine were analyzed. Gentamicin administration caused nephrotoxicity as evidenced by the remarkable elevation of urea and creatinine in serum and urine. Urea and creatinine in serum and urine were increased in response of gentamicin administration compared to the control group. (55.95 ± 2.40 mg/dl, 3.82 ± 0.33 mg/dl, 1219.2 ± 36.49 mg/dl and 139.4 ± 20.27 mg/dl respectively). Co-administration of *Guiera Senegalensis* with gentamicin decreased the rises of these parameters. To conclude, our data suggest that supplementation of *Guiera Senegalensis* may be useful in reducing gentamicin nephrotoxicity in rats.

Keywords: *Guiera Senegalensis*, nephroprotective, gentamicin, Urea, Creatinine.

Abbreviations: **Gs:** *Guiera senegalensis*, **DPPH:** 2,2-Di (4-tert-octylphenyl)-1-picryl-hydrazyl stable free radical, **ROS:** Reactive oxygen species, **CAT:** Catalase, **SOD:** Superoxide dismutase, **MAPRI:** Medicinal and Aromatic Plants Research Institute, **PG:** Propyl Gallate

Introduction:

Herbal and natural products of folk medicine have been used for centuries in every culture throughout the world. Scientists and medical professionals have shown increased interest in this field as they recognize the true health benefits of these remedies. Among the medicinal plants discovered by the ancestors, *Guiera senegalensis* (Gubeish) is one of the traditional folk medicinal plants that have been used for hundreded years.

Guiera senegalensis (Gs) occurs in the savanna zone from Senegal East to Sudan. (Sanogo, R., 2012). Several reports in the literature have described the use of Gs in traditional medicine for treatment of many diseases (Fiot et al., 2004). Gs extracts have been recognized as being useful against cough, respiratory congestion, and fever (Kerharo and Adam 1974). The Gs leave extract have been prescribed for treating cough, easing breathing and for treating lung and bronchial disorders (Diatta et al., 2007), and is also used against malaria (Azas et al., 2002). The branches, leaves, bark and roots of Gs are used for the treatment of stomach pains and dysenteric diarrhea (Aniagu et al., 2005). In addition to medicinal uses, Gs has also been used by Tukolor people of Senegal in diets prepared to enhance growth and increase body weight, reproductive capacity, and milk secretion in animals. Further, the plant extract has been applied as an antiseptic to help wound healing, and employed to help with stomatitis, gingivitis and syphilitic canker sores (Kerharo and Adam 1974).

An extract of the total phenolic content of the leaves showed significant anti-oxidant activity in vitro in a range of tests. The root extract was less active. A crude aqueous leaf extract showed moderate central nervous system depressant effects in guinea pigs. A hydroacetonc leaf extract showed significant antioxidant and anti-inflammatory activity in vitro (Sanogo, R., 2012). The present study was planned to examine the effect of the ethanolic extract of *Guiera senegalensis* leaves on Gentamicin induced nephrotoxicity in albino rats and its antioxidant activities.

Gentamicin induced renal damage is a popular model to study the effects of potential renoprotective drugs (Cuzzocrea.S et al 2002). A therapeutic approach to protect or reverse gentamicin-induced kidney injury would have significant clinical value in acute renal failure as well as drug induced renal damage. Nephrotoxicity induced by gentamicin is a complex phenomenon characterized by an increase in plasma creatinine and urea levels and severe proximal renal tubular necrosis, followed by deterioration and renal failure (Cuzzocrea.S et al 2002, A. Al-Majed et al 2002). The toxicity of gentamicin is believed to be related to the generation of reactive oxygen species (ROS) in the kidney (A. Al-Majed et al 2002, R .J. Reiter et al 2002).

The cellular antioxidant status determines the susceptibility to oxidative damage and usually alters in response to oxidative stress (B. Halliwell and J.M.C. Gutteridge 1999). Various studies have claimed antioxidant property of drugs for their nephroprotective effects in gentamicin induced renal damage (K.V. Kumar et al 2000, I.T.Abdel-Raheem et al 2009, I. Yaman and E. Balikci 2010, G V. Harlalka et al 2007).

Materials and Methods:

Animals: albino rats of either sex weighing 150-200 gram were used for this study. They were housed in clean polypropylene cages, 10 rats per cage; under controlled laboratory conditions and fed with standard rodent diet and

water *ad libitum*. The rodents were allowed to acclimatize to these conditions for one week prior to the commencement of the study.

Extract preparation:

Guiera senegalensis leaves were collected from West Sudan (North Kordofan). The plant material was taxonomically identified and authenticated by taxonomy expert at Herbarium of Medicinal and Aromatic Plants Research Institute (MAPRI), National Center for Research (NCR) Khartoum, Sudan where the voucher specimen has been deposited.

Extraction was carried out for the plants according to the method described by (Sukhdev *et. al.* 2008) Specific weight of each sample was grounded using mortar and pestle and extracted by soaking 80 % ethanol for about seventy two hours with daily filtration and evaporation. Solvent was evaporated under reduced pressure to dryness using rotary evaporator apparatus and the extract allowed to air till complete dryness.

Samples collection:

After acclimatization, the animals were divided randomly into four groups (n=10), and placed in metabolic cages separately for collecting 24-hour urine samples. After collecting the first urine samples, the animals were divided into the normal control group (fed with the standard diet and water *ad libitum*.), the gentamicin group (gentamicin 80mg/kg/day *i.p.*) and treated group (*Guiera Senegalensis* -250mg/kg/day and 500mg/kg/day, started 4 days prior orally and concurrently with Gentamicin 80 mg/kg *i.p.* for six days). Blood was collected in the first day (day 0) and then every five days from the orbital plexuses, Twenty-four hours after the last injection, urine samples were collected.

Anti-oxidant activities of *Guiera senegalensis*:

The DPPH radical scavenging was determined according to the method of Shimada *et.al.* (1992) with some modification. In 96-wells plate, the test samples were allowed to react with 2,2-Di (4-tert-octylphenyl)-1-picrylhydrazyl stable free radical (DPPH) for half an hour at 37°C. The concentration of DPPH was kept as (300µM). The test samples were dissolved in DMSO while DPPH was prepared in ethanol. After incubation, decrease in absorbance was measured at 517 nm using multi plate reader spectrophotometer. Percentage radical scavenging activity by samples was determined in comparison with a DMSO treated control group. All tests and analysis were run in triplicate.

Biochemical analysis:

At the end of the experimental period blood and urine were taken for analyses which include measurement of urea, creatinine and electrolytes using commercial kits (Biosystemm S.A Costa Barva 30, Barcelona Spain). Catalase was determined using commercial kits (Catalase (CAT) assay kit (visible light), A007-1, Nanjing Jiancheng Bioengineering Institute, Nanjing, China. spectrophotometric method. Superoxide dismutase (SOD) was determined in sera samples using commercial kits (SOD) typed assay kit (Hydroxylamine method), A001-2, Nanjing Jiancheng Bioengineering Institute, Nanjing, China). spectrophotometric method.

Statistical analysis:

Data were expressed as mean \pm standard error of mean (SEM). Statistical evaluation was done using SPSS (version 16.0). The differences among treated groups were analyzed by one-way ANOVA followed by Tukey's test. ($P < 0.05$) was considered statistically significant.

Results:**In vitro antioxidant activity of *Guiera senegalensis* ethanolic extract:**

The DPPH radical scavenging was determined in *Guiera senegalensis* (Gubeish) extract and the percentage of radical scavenging activity exhibited highly percentage (93%) (table1) when comparing with the standard drug propyl Gallate(PG) which found to be 95%.

Table1. The DPPH radical scavenging determination of *Guiera senegalensis* extract.

No	Sample code	% RSA \pm SD (DPPH)
1	<i>Guiera senegalensis</i> (Gubeish)	93 \pm 0.01
2	Propyl Gallate(PG)	95 \pm 0.01

Antioxidant activity of *Guiera senegalensis* (in vivo):

There was a significant decrease in Catalase and superoxide dismutase activities in genta mycin group (Table 2) while the administrations of *guiera senegalensis* prevent the inhibition of catalase and super oxide dismutase activity..

Table2. The activity of Catalase (U/ml) and Superoxide dismutase in rats' sera samples treated with (*Guiera senegalensis*) Gubiesh extract at different doses

Animal group	Catalase (Mean \pm SE)	Superoxide dismutase (Mean \pm SE)
Control	14.27 \pm 0.47b	92.42 \pm 4.82c
GM. 80mg	7.75 \pm 1.20a	42.97 \pm 9.66a
GM+250 Gs	13.97 \pm 1.31b	64.97 \pm 3.20ab
GM+500 Gs	13.44 \pm 0.64b	67.67 \pm 2.83b

Biochemical results:

In the present study, gentamicin (80 mg/kg) when injected for 6 consecutive days caused remarkable nephrotoxicity as evident from Table 3. There was a significant ($P < 0.05$) increase in urea and creatinine in serum and urine as compared to the negative control group.

The *Guiera Senegalensis* administered groups showed a significant nephroprotective effect as evidenced by a decrease in the renal parameters i.e. urea and creatinine in serum and urine when compared to the Gentamicin treated group. Moreover, the lower dose seems to be better in protecting the kidney from gentamicin damage.

Table3. Effect of *Guiera senegalensis* (Gs) on biochemical parameters in rats intoxicated with gentamicin

Animal group	Urea (serum) Mean±SE	Creatinine (serum) Mean±SE	Sodium (serum) Mean±SE	Potassium (serum) Mean±SE	Calcium Serum Mean±SE	Urea Urine Mean±SE	Creatinine Urine Mean±SE
Control	38.24 ± 1.86a	0.33 ± 0.03a	88.10 ± 1.10ab	2.86 ± 0.15b	9.30 ± 0.70a	919.1 ± 0.06a	86.98 ± 0.01a
GM. 80mg	55.95 ± 2.40b	3.82 ± 0.33c	105.1 ± 1.70c	2.22 ± 0.20a	9.55 ± 0.10a	1219.2 ± 36.49b	139.4 ± 20.27b
GM+250 Gs	51.07 ± 3.00ab	0.88 ± 0.03a	86.51 ± 3.29a	3.14 ± 0.16b	9.10 ± 0.22a	960.0 ± 51.96a	84.90 ± 1.67b
GM+500 Gs	50.42 ± 1.88ab	2.11 ± 0.35b	94.42 ± 0.00b	2.80 ± 0.15ab	9.50 ± 0.37a	1013 ± 58.31a	104.50 ± 4.91b

Discussion:

In the present study, we investigated the effect of *Guiera senegalensis* on gentamicin-induced nephrotoxicity. Results of this study confirmed that gentamicin at a dose of 80 mg/kg produces significant renotoxicity as evidenced by increase in urea and creatinine in serum and urine which corroborated with previous reports (A. Al-Majed et al 2002, K.V. Kumar et al 2000, I.T.Abdel-Raheem et al 2009, I. Yaman and E. Balikci 2010, G.V. Harlalka et al 2007, Jain Avijeet and A.K.Singhai 2010]. Pretreatment with *Guiera senegalensis* extract provided marked functional and protection against acute renal damage in rats treated with gentamicin. This study revealed that oral administration of *Guiera senegalensis* has a significant protective effect in gentamicin-induced nephrotoxicity in rats as evident by the significant decrease in urea and creatinine in serum and urine. A relationship between oxidative stress and nephrotoxicity has been well demonstrated in many experimental animal models (K.V. Kumar et al 2000, I.T.Abdel-Raheem et al 2009, I. Yaman and E. Balikci, 2010, G.V. Harlalka et al 2007). In gentamicin treated rats, a significant decrease in catalase and super oxide dismutase activities suggesting the involvement of oxidative stress. A role of lipid peroxidation in gentamicin-induced acute renal failure has also been described in previous studies (S.Cuzzocrea et al 2002). Moreover, pretreatment of rats with hydroxyl radical scavengers has shown protection against gentamicin induced acute renal failure (K.V. Kumar et al 2000). From this study it has been demonstrated that *Guiera senegalensis* has a strong antioxidant activity (RSA=93%) in vitro, Therefore, it is not unreasonable to assume that the nephroprotection shown by *Guiera senegalensis* extract in Gentamicin induced nephrotoxicity is mediated through its potent antioxidant effects that help to preserve intracellular catalase and super oxide dismutase levels. The antioxidant activity of *Guiera senegalensis* might have contributed to its nephroprotective effect by inhibiting gentamicin-induced lipid peroxidation. However other mechanisms of protection (Denis Beauchamp et al 1997) like inactivation of the aminoglycoside by electrostatic complex formation or preventing its binding to the brush border membrane or by forming complexes at acidic pH and preventing phospholipid overloading in lysosomes cannot be negated also. Therefore, further investigations should be conducted in order to better characterize the attenuation of gentamicin-induced nephrotoxicity by *Guiera senegalensis*.

Conclusion:

To conclude, this study provides scientific evidence of the nephroprotective effects of orally administration of *Guiera senegalensis* in toxicant that directly induces renal damage. It further proposes that observed protective effects of *Guiera senegalensis* in gentamicin nephrotoxicity could be attributed to its well-known antioxidant potential.

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