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Nephroprotective effect of *Croton zambesicus* root extract against gentimicin-induced kidney injury

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ABSTRACT

Objective: To evaluate the kidney protective effect of ethanolic root extract of *Croton zambesicus (C. zambesicus)* against gentimicin-induced kidney injury in rats. **Methods:** The root extract (27 -81 mg/kg) was administered to rats for eight days with concurrent administration of gentimicin (100 mg/kg) daily for the same period of time. Protective effect of the extract was evaluated in serum levels of creatinine, urea, and uric acid as well as some ions like sodium, potassium and chloride. Histological examination of the kidneys from different treatment groups were also carried out. **Results:** Administration of the root extract significantly reduced histopathological changes in the kidneys of the extract-treated rats especially in the rats treated with lower doses of the extract (27 and 54 mg/kg). The levels of serum urea and creatinine were also reduced significantly ($P<0.01$) at these doses with no observable effect on the levels of uric acid and ions. **Conclusions:** The kidney - protective activity of this extract could be due to its antioxidant and free radical scavenging activities.

1. Introduction

Croton zambesicus Muell Arg. (Euphorbiaceace) (syn *C. amabilis* Muell. Arg. *C. gratissimus* Burch) (*C. zambesicus*) is an ornamental tree grown in villages and towns in Nigeria. It is a Guineo -Congolese species widely spread in tropical Africa. Ethnobotanically, the leaf decoction is used in Benin as anti-hypertensive and anti- microbial (urinary infections)[1] and in parts of Nigeria as antidiabetic and malarial remedy[2,3]. The roots are used as antimalarial, febrifuge and antidiabetic by the Ibibios of Niger Delta region of Nigeria[4]. Boyom *et al*. [5] studied the composition of essential oils from the leaves, stem and roots of *C. zambesicus* and found the three types of oils to be similar in composition, with those from the leaves and stem rich in monoterpenes, while that of the root bark contains sesquiterpenes. The root and stem bark oils were found to be rich in oxygen-containing compounds, with spathulenol and linalool as major components. Mohamed *et al*. [6] reported the isolation of lupeol, betulinic acid , betulin, lupenone , diterpene ent -kaurane-3_, 16_, 17-triol and vitexin from the seed of *C. zambesicus*. Okokon and Nwafor[4] reported that the root extract whose LD_{50} is 273.86 mg/kg contains alkaloids, saponins, terpenes, tannins, phlobatannins, anthraquinones and cardiac glycosides, while flavonoids were reported to be absent. Studies have reported on the antimicrobial properties of the leaf and stem [7]. The ethanolic leaf extract has been reported to possess antiplasmodial[2], antidiabetic[8], anti-inflammatory, analgesic and antipyretic activities[9], while the root extract has been reported to possess antimalarial[4], anticonvulsant and antiulcer activities[10].

Information on biological activity of the root are scarce. We therefore investigated the kidney-protective activity of the root extract of the *C. zambesicus* against gentimicin-induced kidney injury in rats to ascertain its medicinal potentials.

2. Materials and methods

2.1. Plant materials

The plant part (roots) was identified by Dr. Margaret

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Bassey, a taxonomist in the Department of Botany, University of Uyo, Uyo. The roots were collected from compounds in Uyo metropolis, Akwa Ibom State of Nigeria and were authenticated. A voucher specimen (DPNM. 31c) of the plant was deposited in the herbarium of Department of Pharmacognosy and Natural Medicine, University of Uyo, Uyo, Nigeria.

2.2. Extraction

The roots were shade dried for 2 weeks. The dried roots were further chopped into small pieces and reduced to powder. The powdered root was macerated in 97% ethanol for 72 hours to give the crude ethanolic extract. The liquid filtrates were concentrated and evaporated to dryness in vacuo at 40 ℃ using rotary evaporator. The yield of the extract was calculated. The dry extract was stored in a refrigerator at 4 ℃ until use for the proposed experiment.

2.3. Animals

Both male and female animals (mice and rats) that were used for these experiments were obtained from University of Uyo animal house. The animals were housed in standard cages and were maintained on a standard pelleted Feed (Guinea Feed) and water *ad libitum*. Permission and approval for animal studies were obtained from College of Health Sciences Animal Ethics committee, University of Uyo.

2.4. Animal treatment

A total of 30 rats of both sexes were weighed and divided into five groups of 6 animals and treated as follows: Groups A, B and C were respectively administered orally with 27, 54 and 81 mg/kg of *C. zambesicus* root extract daily for 8 days. Group D was administered with distilled water 10 mL/kg and Group E treated with paracalcitriol (100 mg/kg) for the same period of time. Gentimicin, 100 mg/kg, was administered daily to all the groups concommitantly with the above treatments for 8 days. At the end of the treatment period, the animals were weighed again and sacrificed under light chloroform vapour. Blood were collected by cardiac puncture and used immediately. Serum was separated from the blood and stored at −20 ℃ until used for biochemical determinations. The kidneys of the animals were surgically removed and fixed in 10% formaldehyde for histological processes.

2.5. Determination of the protective effect of the extract against gentimicin-induced kidney injury on Biochemical parameters and histology of kidney of rats

The various serum samples collected after treatment of the animals were analysed according to standard methods for effect of the extract on various biochemical parameters of rats such as uric acid, urea, creatinine as well as some ions like sodium, potassium and chloride. This analysis were done at Department of Chemical Pathology, University of Uyo Teaching Hospital, (UUTH), Uyo using various diagnostic kits like Randox Laboratory kits, Dialab diagnostic kits, HUMAN diagnostic kits and TECO analytical kits. The kidneys of the animals fixed in 10% formaldehyde were processed, sectioned and stained with Heamatoxylin and eosin (H&E) according to standard procedures at Department of Chemical Pathology, University of Uyo Teaching Hospital, Uyo.

2.6. Statistical analysis and data evaluation

Data obtained from this work were analyzed statistically using Students′ *t*-test and ANOVA (One- Way) followed by a post test (Tukey-kramer multiple comparison test). Differences between means will be considered significant at 1% and 5% level of significance i.e $P < 0.01$ and 0.05.

3. Results

Administration of the root extract (27 − 54 mg/kg) prior to treatment with gentamicin was found to protect the animals from kidney injuries. Serum creatinine and serum urea levels were found to be significantly increased in rats treated with only gentamicin; whereas treatment with the root extract significantly (P<0.05-0.01) lowered their levels in the treated animals though dose independently (Figures 1 and

Table 1
Effect of ethanolic root extract of *C. zambesicus* on some kidney function parameters of rat.

Extract	Dose	Na^+(mmol/L)	K^+(mmol/L)	CL^-(mmol/L)	HCO^-_3	Uric acid
Control (dist water)	10 mL/kg	139.75±2.65	3.83±0.17	98.00±0.54	26.75±1.02	0.30±0.02
C. zambesicus root extract	27 mg/kg	146.25±1.49	3.57±0.10	96.50±2.69	25.50±0.49	0.33±0.02
	54 mg/kg	146.00±2.13	3.84±0.14	97.20±1.58	25.60±0.46	0.31±0.02
	81 mg/kg	142.40±2.24	3.80±0.27	95.60±0.61	25.20±0.77	0.32±0.02
Paracalcitriol	100 mg/kg	145.33±1.84	3.63±0.25	96.33±0.56	25.00±0.36	0.31±0.03

Not significant when compared with control P>0.05. Data are expressed as mean ± SEM (n = 6).

2). The reductions in the levels of serum creatinine and urea were significantly (P<0.01 −0.01) prominent in the group treated with 54 mg/kg of the extract followed by that treated with 27 mg/kg of the extract. Paracalcitriol (a reference drug) also caused significant (P< 0.01− 0.001) reductions in the levels of serum creatinine and urea compared to the control (Figures 1 and 2). The highest dose of the root extract could not produce any significant reduction in the levels of these compounds. However, the levels of uric acid as well as some ions like sodium, potassium and chloride were not affected by the administration of the root extract (Table 1).

Histopathological study revealed that rats in the control group treated with gentimicin only as well as the groups treated with the root extract and paracalcitriol showed various degrees of injuries such as oedematous glomerular with periarteriolar haemorhage, oedematous interstitium, thyroidization(colloidal casts), atrophic and degenerated glomerular, inflammatory cells infiltrate, congested and dilated capillaries, ruptured and degenerated glomerular. Concurrent treatment with the ethanolic root extract of *C. zambesicusi* (27−18 mg/kg) were found to reduce such changes in kidney histology induced by gentamicin especially in rats treated with the lower doses (27 and 54 mg/kg) of the extract(Figure 3−7).

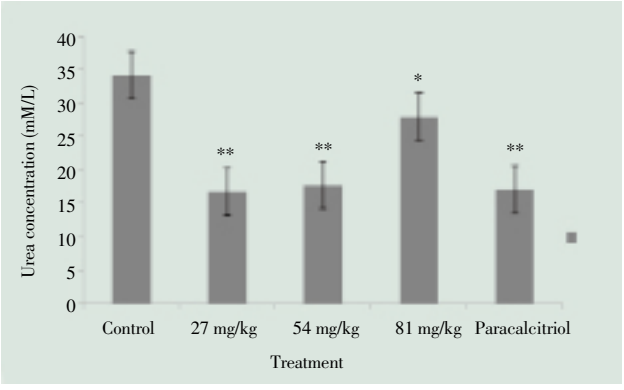

Figure 1. Effect of ethanolic root extract of *C. zambesicus* on urea level of rats.
*P<0.05, **P<0.01 comparing with the control group.

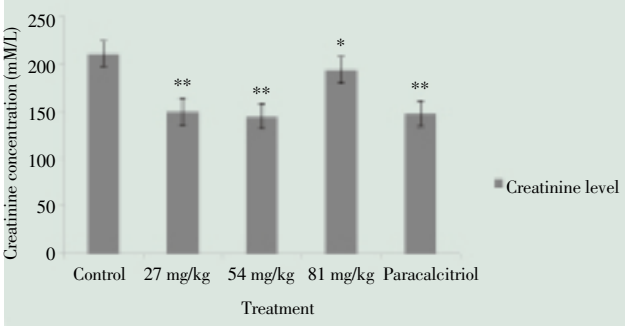

Figure 2. Effect of ethanolic root extract of *C. zambesicus* on creatinine level of rats.
*P<0.05, **P<0.01 comparing with the control group.

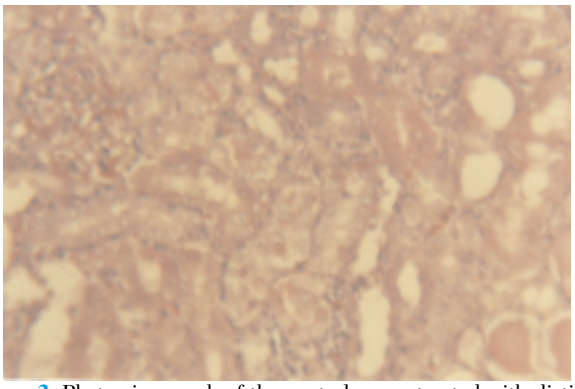

Figure 3. Photomicrograph of the control group treated with distilled water (10 mL/kg +gentimicin) X 200.

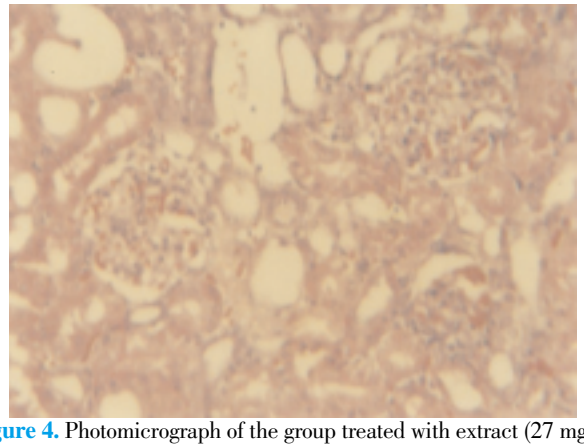

Figure 4. Photomicrograph of the group treated with extract (27 mg/kg +gentimicin) X 200.

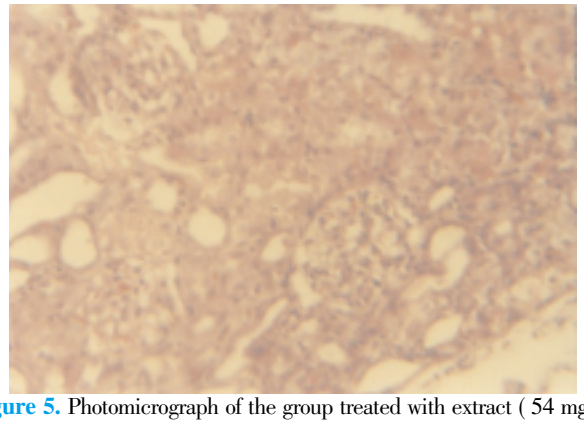

Figure 5. Photomicrograph of the group treated with extract (54 mg/kg +gentimicin) X 200.

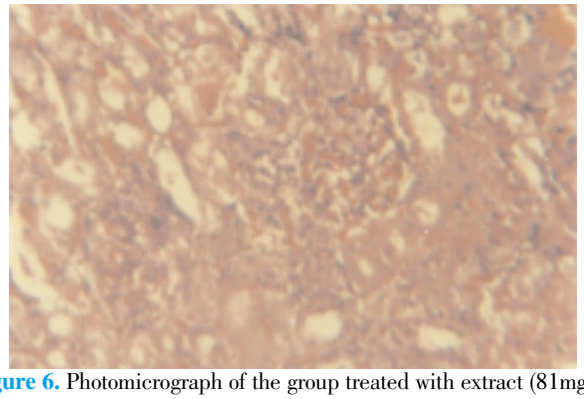

Figure 6. Photomicrograph of the group treated with extract (81mg/kg +gentimicin) X 200.

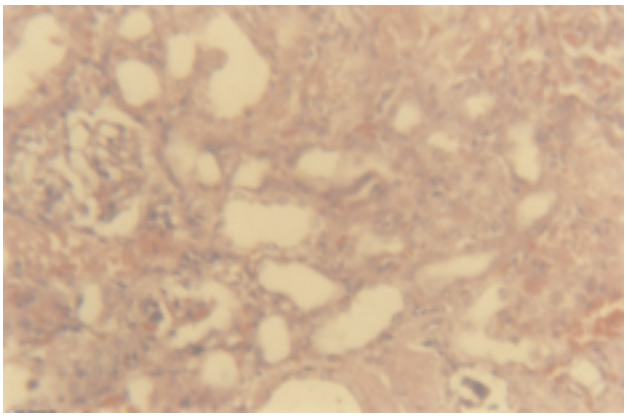

Figure 7. Photomicrograph of the group treated with extract (paracalcitriol +gentimicin) X 200.

4. Discussion

Gentimicin-induced kidney injury is characterized by increase in serum levels of creatinine, urea, uric acid as well as severe proximal renal tubular necrosis followed by renal failure[11]. Increase in the level of serum creatinine is indicative of glomerular filtration rate reduction which is often associated with increases in serum urea and uric acid[11]. The administration root extract of *C. zambesicus* produced prominent decreases in serum levels of creatinine and urea induced by gentimicin though the level of uric acid was not affected by the root extract. Also, lower doses of the root extract (27 and 54 mg/kg) were observed to be more protective than the high dose (81 mg/kg). This probably could have resulted from the toxic effect of the triterpenes content of the extract. The findings of this study corroborate earlier report by[12] Okokon on the nephroprotective effect of the root extract in diabetic rats. Reactive oxygen species (ROS) have been implicated in the pathogenesis of gentimicin-induced kidney injury[11]. This results in severe tissue damage and degeneration. The effect of ROS in the body is usually suppressed by antioxidant enzyme systems. The suppression of gentimicin-induced nephrotoxicity by the extract may have resulted from the antioxidant and free radical scavenging potentials of the extract. Secondary metabolites in plants like flavonoids have been reported to possess these activities and vitexin, a flavonoid glycoside, isolated from *C. zambesicus* has been reported to possess free radical scavenging and antioxidant activities[6]. Okokon and Nwafor[4] had reported the presence of flavonoids in the root extract of *C. zambesicus*. The polyphenolic compounds in this extract may have been responsible for the kidney protective activity observed with this extract.

The results of this study show that ethanolic root extract of *C. zambesicus* protects against gentimicin-induced kidney injury and this activity is due to the flavonoids content of the extract.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

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