

Original Research Article

Evaluation of Nephroprotective Activity of Aqueous and Hydroethanolic Extracts of *Trema guineensis* Leaves (Ulmaceae) Against Gentamicin-Induced Nephrotoxicity in Rats.

ABSTRACT

Aims: The present study investigated the potential nephroprotective activity of aqueous and hydroethanolic extracts of *Trema guineensis* leaves (Ulmaceae) and **determined** the most active extract in rat.

Study Design: *Trema guineensis* leaves were collected **from** Abobo in the district of Abidjan (Côte d'Ivoire). The plant was identified and authenticated by the Department of Botany, Felix Houphouet Boigny University of Abidjan (Côte d'Ivoire).

Place and Duration of Study: Analysis **of** the plant samples was done in pharmacodynamics Biochemistry Laboratory, Felix Houphouet Boigny University, the Laboratory of Organic Chemistry and Natural Substances, Felix Houphouet Boigny University and the Laboratory of Pharmacology, clinical and therapeutic pharmacy, UFR Pharmaceutical and Biologic Sciences, Felix Houphouet Boigny University between September and October 2016.

Methodology: The extracts obtained starting from 100 g of plant powder on the one hand by decoction in 1 Liter of distilled water and on the other hand by maceration in 1 Liter of ethanol-water mixture 70 % (70:30, v/v). The aqueous and hydroethanolic extracts at doses 100 and 200mg/kg body weight were studied in comparison with vitamin E (250 mg/kg body weight) against gentamicin-induced renal toxicity (80 mg/kg/day). **In assessing nephroprotective effect, rats were pretreated by oral gavage daily with vitamin E and extracts at different doses (100 and 200 mg/kg body weight) one hour before gentamicin intraperitoneal injection for seven days.**

Results: The administration **of** gentamicin through intraperitoneal route to rats **for** seven days, resulted **in** an increase in urea and **creatinine concentrations** as well as decrease of total protein concentration in the serum. The values of total protein and albumin **concentrations** increased in urine after **administration** of gentamicin. *Trema guineensis* aqueous and hydroethanolic extracts used to treat animals suffering from nephrotoxicity would have significantly reduced ($P < 0.001$ and $P < 0.01$) biochemical parameters considered as markers of nephrotoxicity. **Moreover, the aqueous extract (200 mg/kg body weight) and vitamin E restored the toxic effect of gentamicin into equal significance.**

Conclusion: The results of this study showed that the aqueous extract possesses a **nephroprotective** activity against gentamicin-induced kidney damage in rats. So aqueous extract can be utilized for preventives purposes.

Keywords: Nephroprotective, Gentamicin, *Trema guineensis*, Rat, **Vitamin E**

1. INTRODUCTION

The kidney is an organ that possesses several biological roles of which the most important is homeostatic balance of body fluids by cleaning and secreting metabolites like urea, uric acid creatinine and minerals from blood and excreting the nitrogenous wastes along with water, as urine [1]. They are often subjected to high concentrations of potentially toxic agents such as chemicals and drugs by accumulating and producing broad spectrum morphological and functional effects on the kidney [2]. Some reports suggest that between 5 to 20 % of cases of acute renal failure can be directly attributed to drugs and chemicals, although minor damage may pass undetected [3].

Gentamicin is one of the aminoglycosides; it is a very important agent for the treatment of Gram-negative bacterial infections. However; its clinical use is limited by its nephrotoxicity [4, 5]. It has been estimated that up to 30 % of patients treated with aminoglycosides for more than 7 days show some signs of nephrotoxicity [6].

Plants are important source of drugs. Many of the modern drugs that are currently available have also been derived directly or indirectly from herbal sources. Herbal medicines have been proved to be highly effective, economic and safe alternative tools for treatment and prevention of various human diseases in Africa and in developing countries [7, 8, 9].

Trema guineensis (Ulmaceae) is a plant distributed in the west central part of Côte d'Ivoire. The leaves of this plant are locally used for the treatment of various diseases including cardiac failure, constipation, pains, fever, cough, hypertension [9, 10, 11, 12]. Phytochemical study for *Trema guineensis* revealed the presence of sterol, terpen, polyphenol, flavonoid, tannin, saponosid and alkaloid which conferred antimicrobial, cholinergic, analgesic, antioxidant and anti-inflammatory properties on the plant leaves [13, 14, 15, 16, 17]. In view of *Trema guineensis* therapeutic advantages, the present study was carried out to evaluate the protective effect of its leaves aqueous and hydroethanolic extracts against gentamicin-induced nephrotoxicity.

2. MATERIALS AND METHODS

2.1 Samples Collection and Extraction

The fresh leaves of *Trema guineensis* were collected from Abobo (Abidjan). The plant species was later identified and authenticated by the Department of Botany, Felix Houphouet Boigny University of Abidjan.

It was dried at room temperature during two weeks and pulverized using an electric grinder (IKA-type MAG®). The powder of leaves obtained and constituted our sample to be analyzed.

2.1.1 Hydroethanolic Extract

100 g of powder of *Trema guineensis* leaves were macerated for 24 hours in 1 Liter of ethanol-water mixture 70 % (70:30, v/v). The obtained macerate was then filtered twice on white cotton and once on Whatman filter paper N°3. The filtrate was evaporated and dried at temperature of 40°C using a rotary evaporator type BUCHI 161 Water Bath [19].

2.1.1 Aqueous Extract

Concerning aqueous extract preparation, 100 g of *Trema guineensis* leaves powder were added to 1 Liter of boiling distilled water during 20 minutes. The decoction was filtered twice

on white cotton and once on Whatman filter paper N°3. The filtrate was dried under reduced pressure using a rotary flash evaporator and stored at a temperature of -4°C until use [20].

2.2 Experimental Animals

Healthy adult Wistar albino rats (*Rattus norvegicus*) weighing between 150-200 g were used for the study. The rats were provided and kept in the laboratory animal house of the Training and Research Unit of Pharmaceutical and Biologic Sciences, Felix Houphouet Boigny University, Côte d'Ivoire. The animals house was according to environmental standard temperature of 26±1°C and relative humidity 50±5% with 12 h light-dark cycle. The animals were housed in large spacious, hygienic plastic cages during the course of the experimental period. The rats fed with FACI® (Fabrication d'Aliments de Cote d'Ivoire) pellets and drank tap water.

2.3 Evaluation of nephroprotective activity in Gentamicin induced nephrotoxicity

The evaluation of the nephroprotective activity of *Trema guineensis* aqueous and hydroethanolic extracts was conducted using the method described by Paoulomi [21] with some modifications [22]. The animals were divided considering of their weight into seven groups consisting of 6 rats in each:

Group I (**Normal**): Normal control treated daily with distilled water and 0.9 % NaCl for 7 days

Group II (**Genta**): Negative control treated daily with distilled water and gentamicin (80 mg/kg) for 7 days

Group III (**Vit E**): Positive control treated daily with vitamin E (250 mg/kg) and gentamicin (80 mg/kg) for 7 days

Group IV (**EA 100**): Rats treated daily with aqueous extract of *Trema guineensis* (100 mg/kg) and gentamicin (80 mg / kg) for 7 days

Group V (**EA 200**): Rats treated daily with aqueous extract of *Trema guineensis* (200 mg/kg) and gentamicin (80 mg/kg) for 7 days

Group VI (**EE 100**): Rats treated daily with hydroethanolic extract of *Trema guineensis* (100 mg/kg) and gentamicin (80 mg / kg) for 7 days

Group VII (**EE 200**): Rats treated daily with hydroethanolic extract of *Trema guineensis* (200 mg/kg) and gentamicin (80 mg / kg) for 7 days

The test drug (*Trema guineensis*) and the control groups were given by oral gavage 60 minutes prior to the gentamicin intraperitoneal injection in the different groups.

After the last treatment, animals were placed in metabolic cages to collect their urine for 24 hours.

2.4 Collection and Storage of Blood and Organs

After 7th day of last dose, animals were sacrificed after blood collection under ether anesthesia. The both kidneys were removed, rinsed with normal saline, weighed and then fixed in aqueous Bouin. The blood of each animal was collected (tail vein) in a tube without anticoagulant before and after experiment. The blood was centrifuged at 3000 rpm for 10 minutes (Centrifuge B4i) to separate serum. Serum was kept at -20°C until the analysis. The

collected urines were quantified and a sample of each urine was stored in eppendorf tubes for the determination of certain biochemical parameters [23].

2.5 Biochemical Assays

The serum samples were used to assay the biochemical parameters such as: creatinine, total proteins and urea. The collected urine was used to assess the levels of albumin and total proteins in animals using an automatic analyzer (Cobas C 311, Hitachi Rock).

2.6 Statistical analysis

The values expressed as Mean $\pm$ standard error mean (SEM) from 6 animals. The statistical analysis was carried out using one way analysis of variance (ANOVA) followed by Dunnett's test. The value of $P < 0.05$ was considered significant.

3. RESULTS AND DISCUSSION

3.1 RESULTS

Before treatment, the biochemical parameters in serum were evaluated statistically equal by comparing each group with every other group.

After 7 days of treatment, it was observed that the gentamicin produced significant changes in serum urea, serum creatinine, serum total protein, urinary total protein and urinary albumin when compared with normal saline treated animals indicating gentamicin induced toxicity.

Serum urea was increased in rats treated with only gentamicin but treatment with hydroethanolic and aqueous extracts of *Trema guineensis* at doses 100 mg/kg and 200 mg/kg and vitamin E (positive control) significantly ($P < 0.001$) reversed the effect of gentamicin indicating nephroprotective activity (Figure 1).

The aqueous extract at dose 200 mg/kg and vitamin E significantly ($P < 0.01$) decreased serum creatinine levels in animals (Figure 2) compared to negative control group.

In all the groups, there are elevated serum total protein levels with ($P < 0.001$ and $P < 0.01$) significance compared with negative control group (Figure 3).

Concerning the urine total protein, the Figure 4 showed significant ($P < 0.001$) increase in total protein levels in negative control group and hydroethanolic-treated groups compared to normal group.

Urinary albumin concentration was also significantly increased ($P < 0.001$) in extracts hydroethanolic-treated groups and negative control group compared to normal group (Figure 5).

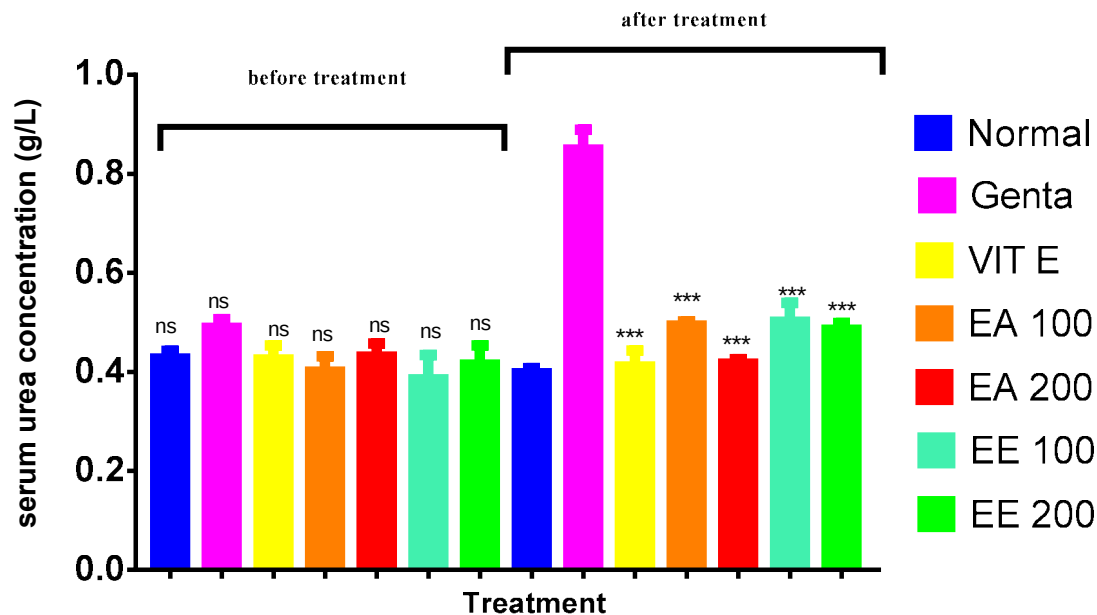


Figure 1: Effect of *Trema guineensis* extracts (aqueous and hydroethanolic) and vitamin E on serum urea in gentamicin-treated rats compared to negative control group.

Values are expressed as mean $\pm$ SEM (standard error mean) with n = 6 in each group

ns: No significant difference between the different groups before treatment. Significance ***P<0.001 compared to negative control group (Genta). **Normal group:** NaCl; **Genta:** gentamicin; **VIT E:** vitamin E + gentamicin; **EA 100:** aqueous extract (100 mg/kg) + gentamicin; **EA 200:** aqueous extract (200 mg/kg) + gentamicin; **EE 100:** hydroethanolic extract (100 mg/kg) + gentamicin; **EE 200:** hydroethanolic extract (200 mg/kg) + gentamicin.

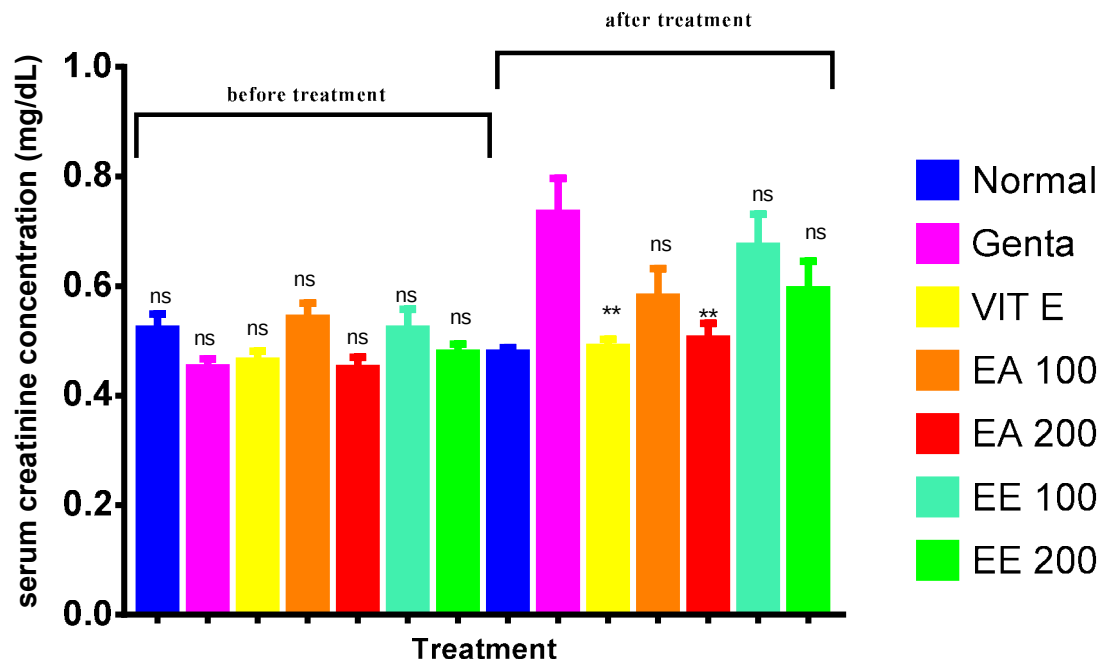


Figure 2: Effect of *Trema guineensis* extracts (aqueous and hydroethanolic) and vitamin E on serum creatinine in gentamicin-treated rats compared to negative control group.

Values are expressed as mean $\pm$ SEM (standard error mean) with n = 6 in each group
ns: No significant difference between the different groups before treatment. Significance: **P<0.01, **ns:** no significance compared to negative control group.. **Normal:** NaCl; **Genta:** gentamicin; **VIT E:** vitamin E + gentamicin; **EA 100:** aqueous extract (100 mg/kg) + gentamicin; **EA 200:** aqueous extract (200 mg/kg) + gentamicin; **EE 100:** hydroethanolic extract (100 mg/kg) + gentamicin; **EE 200:** hydroethanolic extract (200 mg/kg) + gentamicin.

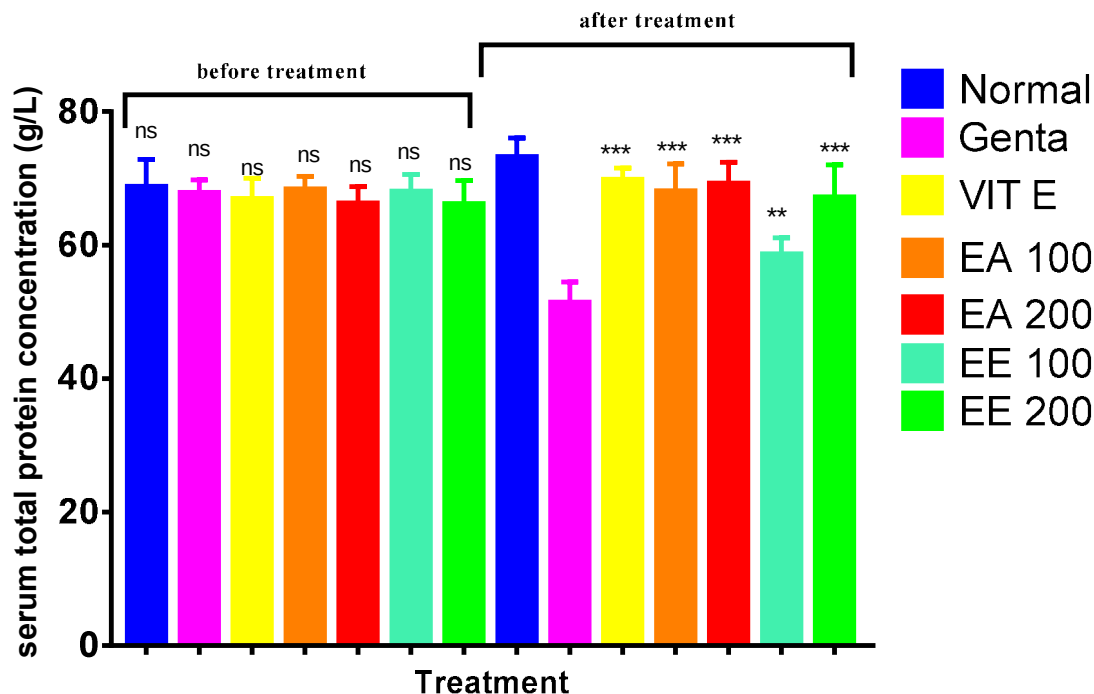


Figure 3: Effect of *Trema guineensis* extracts (aqueous and hydroethanolic) and vitamin E on serum total protein in gentamicin-treated rats compared to negative control group

Values are expressed as mean $\pm$ SEM (standard error mean) with n = 6 in each group
 ns: No significant difference between the different groups before treatment. Significance **P<0.01 and ***P<0.001 compared to negative control group (Genta). **Normal:** NaCl; **Genta:** gentamicin; **VIT E:** vitamin E + gentamicin; **EA 100:** aqueous extract (100 mg/kg) + gentamicin; **EA 200:** aqueous extract (200 mg/kg) + gentamicin; **EE 100:** hydroethanolic extract (100 mg/kg) + gentamicin; **EE 200:** hydroethanolic extract (200 mg/kg) + gentamicin.

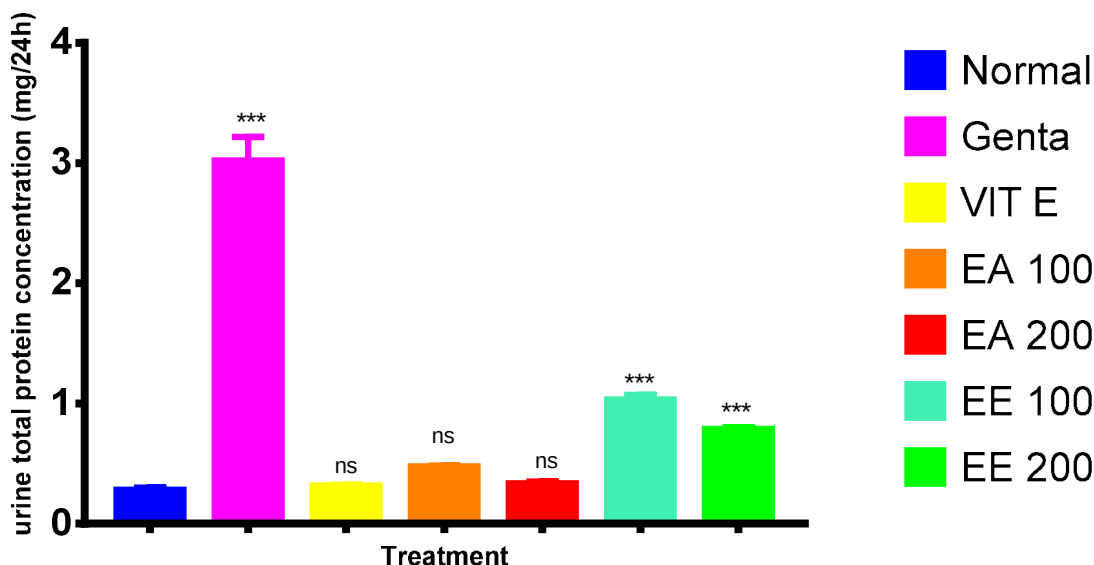


Figure 4: Effect of *Trema guineensis* extracts (aqueous and hydroethanolic) and vitamin E on urinary total protein in gentamicin-treated rats compared to normal control group

Values are expressed as mean $\pm$ SEM (standard error mean) with n = 6 in each group
Significance ***P<0.001 and ns: no significance compared to normal group. **Normal group:** NaCl; **Genta:** gentamicin; **VIT E:** vitamin E + gentamicin; **EA 100:** aqueous extract (100 mg/kg) + gentamicin; **EA 200:** aqueous extract (200 mg/kg) + gentamicin; **EE 100:** hydroethanolic extract (100 mg/kg) + gentamicin; **EE 200:** hydroethanolic extract (200 mg/kg) + gentamicin.

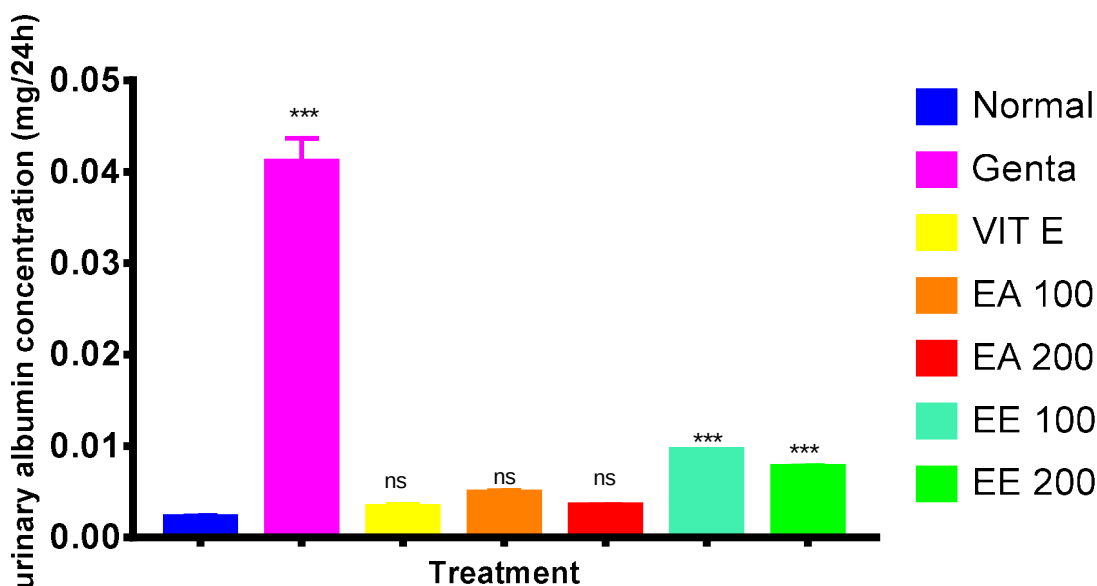


Figure 5: Effect of *Trema guineensis* extracts (aqueous and hydroethanolic) and vitamin E on albumin in gentamicin-treated rats compared to normal group

Values are expressed as mean $\pm$ SEM (standard error mean) with n = 6 in each group
Significance ***P<0.001 and ns: no significance compared to normal group. **Normal:** NaCl; **Genta:** gentamicin; **VIT E:** vitamin E + gentamicin; **EA 100:** aqueous extract (100 mg/kg) + gentamicin; **EA 200:** aqueous extract (200 mg/kg) +

gentamicin; **EE 100**: hydroethanolic extract (100 mg/kg) + gentamicin; **EE 200**: hydroethanolic extract (200 mg/kg) + gentamicin.

3.2 DISCUSSION

The kidney is an essential organ that plays a dominant role in homeostasis by excreting the metabolic waste products. It conserves necessary products depending on body needs. medicinal plants are commonly used in treating or preventing specific diseases and they are considered to play a beneficial role in health care [24, 25]. The present study was carried out to evaluate the protective effects of the aqueous and hydroethanolic extracts of *Trema guineensis* leaves on gentamicin-induced nephrotoxic in rats.

Nephrotoxicity is a poisonous effect of some substances, as chemicals and some drugs, causing damage to the kidneys [26]. Gentamicin is a commonly used aminoglycoside. Routine therapeutic use of gentamicin (80 mg/kg/day) for more than seven days has been a common cause of nephrotoxicity [27]. It has been shown that nephrotoxicity caused by gentamicin treatment is associated with increased of the release of the oxidants compounds, which might be the major contributing factor towards renal damage [28]. Gentamicin usually accumulates in renal proximal tubules and enhances hydrogen peroxide and oxygen free radicals generation [7, 29, 30]. Abnormal production of reactive oxygen species may result in cellular injury and necrosis through peroxidation of membrane lipids, protein denaturation and DNA damage [31]. Hydrogen peroxide generated during the gentamicin induced oxidative stress in mitochondrial membranes releases iron from the mitochondria. The released iron makes a complex with gentamicin and accelerates the oxidative stress [32].

Nephrotoxic effect is identified by estimating the biomarkers like serum creatinine and serum urea which are considered reliable markers [33]. Urea is the main product of protein catabolism. It is completely filtered by the glomerulus and passively excreted at high concentrations in the urine. The serum level of urea is used as an index of renal function [34]. Creatinine is an end product of muscle catabolism, which is removed at a constant rate by the kidneys. The serum creatinine concentration is an index of the renal function. The level of serum creatinine increases if the kidney does not work properly [34]. Thus, increases in serum levels of these markers are indicative of renal injury [33]. Therefore, in this study, the nephroprotective activity of our extracts was evaluated by the determination of certain biochemical parameters in both serum (urea, creatinine, total protein) and in the urine (total proteins and albumin) in animals.

Gentamicin-administered rats (negative control group) had encountered acute kidney dysfunction as evidenced by elevation of serum urea and creatinine with low total protein in serum. This result is in conformity with previous reports attributing these changes to nephrotoxicity induced by gentamicin [22, 35, 36]. Treatment with aqueous and hydroethanolic extracts of *Trema guineensis* at dose of 100 mg / kg body weight and 200 mg / kg body weight daily for seven days restored significantly ($P < 0.001$ and $P < 0.01$) creatinine and urea levels (**Figures 1 and 2**) compared to the negative group. This result is supported by Paoulomi *et al.* (2012) who indicated that supplementation of *Aloe barbadensis* restored the increased levels of urea and creatinine in gentamicin-induced rats. However, treatment with these extracts resulted in increased serum total protein levels significantly ($P < 0.001$) in comparison with negative control (**Figure 3**); this suggests that *Trema guineensis* contents protected the kidney tissue integrity. This result is reinforced by Bamba *et al.* (2016) studies.

Proteins are filtered by the glomerulus, but totally reabsorbed by the proximal tubule. They are not detectable in urine or present in very small quantities [37]. Proteinuria, usually reflecting the loss of normal glomerular filtration impermeability to plasma proteins is an

early sign of kidney disease [38]. Thus, detection of proteinuria is necessary for the recognition of most kidney disease [34].

The parameters evaluated in urine of rats were total protein and albumin. These parameters were found to be increased significantly ($P < 0.001$) in gentamicin induced rats and extracts hydroethanolic-treated groups (EE 100; EE 200) when compared to normal rats (**Figures 4 and 5**).

The increased concentration of urinary total protein and albumin observed in gentamicin treated in rats only (negative control) could be due to toxicity of free radical generated from gentamicin **damaging effect**.

There was no statistical difference between the animals groups treated with aqueous extracts (100 and 200 mg/kg body weight) and the group treated with the normal saline; that proves these aqueous extracts would have reduced the nephrotoxicity induced by gentamicin in rats, so that the urinary total protein and albumin concentrations would be close to those of untreated rats. Treatment by *Trema guineensis* normalized the levels of urinary total protein and albumin in gentamicin treated rats. This result is similar to Bamba *et al.* (2016) work. They showed that aqueous and ethanol extracts of *G. celosioides*, *C. nitida*, and *E. angolense* effectively mitigated the effects of gentamicin on proteinuria and albuminuria.

Natural antioxidants have a variety of biochemical actions such as inhibition of reactive oxygen species production, scavenging of free radicals [39]. **Many studies showed that the presence of antioxidant compounds in plants conferred them a nephroprotective activity** [40, 41, 42]. The phytochemical investigation of *Trema guineensis* revealed the presence of antioxidant compounds such as phenols, flavonoids, flavonols and sterols [16, 17]. The investigation revealed that *Trema guineensis* possessed protective effect against gentamicin induced nephrotoxicity.

4. CONCLUSION

The nephroprotective effect of *Trema guineensis* leaves extracts was evaluated. The present study indicated that the aqueous and hydroethanolic extracts of *Trema guineensis* leaves restored significant gentamicin-induced perturbation rates on biochemical parameters such as urea, creatinine and total protein in serum thus that total protein and albumin in urine. The aqueous extract at dose 200 mg/kg body weight possessed profound nephroprotective activity and also revealed that the nephroprotective activity of this extract was comparable to that of vitamin E.

ETHICAL APPROVAL

The experimental procedures were conducted after the approval of the Ethical Guidelines of University (Côte d'Ivoire) Committee on Animal Resources. All these procedures used, were in strict accordance with the guidelines for Care and Use of Laboratory Animals and the statements of the European Union regarding the handling of experimental animals (86/609/EEC).

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