

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 to determine the most active extract in rat.

Study Design: *Trema guineensis* leaves were collected to 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 on 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).

Results: The administration with gentamicin through intraperitoneal route to rats during seven days, resulted an increase in urea and creatinine concentration as well as decrease of total protein concentration in the serum. The values of total protein and albumin concentration were also increased in urine after administering 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 to equal significance the toxic effect of gentamicin restoring in biochemical markers of kidney function evaluated.

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

Keywords: Nephroprotective, Gentamicin, *Trema guineensis*, Rat, Côte d'Ivoire

39 1. INTRODUCTION

40 The kidney is the main organ that possesses several biological roles. Its most important role is
41 homeostatic balance of body fluids by cleaning and secreting metabolites like urea, uric acid
42 and creatinine and minerals materials from the blood and excreting the nitrogenous wastes
43 along with water, as urine [1]. They are often subjected to high concentrations of potentially
44 toxic external agents such as environmental chemicals and drugs by accumulating and
45 producing broad spectrum of morphological and functional effects of kidney [2].

46 Gentamicin, being one of the aminoglycosides, is a very important agent for the treatment of
47 Gram-negative bacterial infections. However, its clinical use is limited by its nephrotoxicity
48 [3; 4].

49 Nephroprotection has known ample development lately and consists in measures which
50 protect the kidney against many aggressive factors [5].

51 Plants have always been an exemplary source of drugs. Many of the modern drugs that are
52 currently available have also been derived directly or indirectly from herbal sources. Herbal
53 medicines have been proved to be highly effective, economical and safe alternative tools for
54 the treatment and prevention of various human diseases in Africa and in developing countries
55 [6; 7; 8].

56 *Trema guineensis* (Ulmaceae) is a plant distributed in the west central part of Côte d'Ivoire.
57 The leaves of this plant are locally used for the treatment of various diseases including
58 cardiac failure, constipation, pains, fever, cough, hypertension [9; 10; 11; 12].

59 Scientifically, several studies were carried out on phytochemistry and biological properties of
60 various extracts of these plants. Among them we note analgesic, antioxidant and anti-
61 inflammatory properties [13; 14; 15; 16; 17].

62 In view of *Trema guineensis* therapeutic advantages, the present study was carried out to
63 evaluate the protective effects of its leaves aqueous and hydroethanolic extracts against
64 gentamicin-induced nephrotoxicity.

65 2. MATERIALS AND METHODS

66 2.1 Samples Collection and Extraction

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

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

72 2.1.1 Hydroethanolic Extract

73 100 g of powder of *Trema guineensis* leaves were macerated for 24 hours in 1 Liter of
74 ethanol-water mixture 70 % (70:30, v/v). The macerated obtained was then filtered twice on
75 white cotton and once on Whatman filter paper N°3. The filtrate was evaporated to dryness at
76 reduced pressure at temperature of 40°C using a rotary evaporator type BUCHI161 Water
77 Bath [18].

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79

80 2.1.1 Aqueous Extract

81 Concerning aqueous extract preparation, 100 g of *Trema guineensis* leaves powder were
82 added to 1 Liter of boiling distilled water during 20 minutes. The decoction was filtered twice
83 on white cotton and once on Whatman filter paper N°3. The filtrate is preserved at
84 temperature of 40°C in an oven for drying [19].

85 2.2 Experimental Animals

86 Healthy adult Wistar albino rats (*Rattus norvegicus*) of both sexes between weighing about
87 150-200 g were used for the study. The rats were provided and kept in the laboratory animal
88 home of the Training and Research Unity of Pharmaceutical and Biologic Sciences, Felix
89 Houphouet Boigny University, Côte d'Ivoire. The animals were housed according to
90 environmental standards temperature of 26±1°C and relative humidity 50±5% with 12 h
91 light-dark cycle. The rats were fed with FACI® (Fabrication d'Aliments de Cote d'Ivoire)
92 pellets and their drink was tap water.

93 2.3 Administration Method of Plant Extracts

94 The evaluation of the nephroprotective activity of *Trema guineensis* aqueous and
95 hydroethanolic extracts was conducted using the method described by Paoulomi [20] with
96 some modifications [21]. The principle of this method is based on a sequential process of first
97 treating animals with vitamin E (nephroprotective reference molecule) or with extracts. Then
98 one hour after, gentamicin was administered (nephrotoxic reference molecule).

99 Group I (**Normal**): Normal control treated with distilled water and 0.9 % NaCl.

100 Group II (**Genta**): Negative control treated with distilled water and gentamicin (80 mg/kg).

101 Group III (**Vit E**): Positive control treated with vitamin E (250 mg/kg) and gentamicin (80
102 mg/kg).

103 Group IV (**EA 100**): Rats treated with aqueous extract of *Trema guineensis* (100 mg/kg) and
104 gentamicin (80 mg / kg).

105 Group V (**EA 200**): Rats treated with aqueous extract of *Trema guineensis* (200 mg/kg) and
106 gentamicin (80 mg/kg).

107 Group VI (**EE 100**): Rats treated with hydroethanolic extract of *Trema guineensis* (100
108 mg/kg) and gentamicin (80 mg / kg).

109 Group VII (**EE 200**): Rats treated with hydroethanolic extract of *Trema guineensis* (200
110 mg/kg) and gentamicin (80 mg / kg).

111 The test drug (*Trema guineensis*) and the control groups were given 60 minutes prior to the
112 gentamicin injection in the different groups.

113 After treatment on day 7, the animals were placed in metabolic cages to collect their urine for
114 24 hours.

115 2.4 Collection and Storage of Blood and Organs

116 The rats were euthanized by cervical dislocation under mild ether anesthesia. These animals
117 were sacrificed and their both kidneys were removed, rinsed with normal saline, weighed and
118 then fixed in aqueous Bouin. The blood of each animal was collected (retro-orbital puncture)
119 in a tube without anticoagulant at Day 0 (D0) and at Day 8 (D8). The blood was centrifuged
120 at 3000 rpm for 10 min (Centrifuge B4i) to separate serum. This 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.

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

On Day 0 (D0), before treatment, the biochemical parameters in serum evaluated, were statistically identical ($p > 0.05$) in comparing each group with every other group.

After 8 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; treatment with hydroethanolic and aqueous extracts of *Trema guineensis* at doses 100 mg/kg and 200 mg/kg significantly ($p < 0.001$) reversed the effect of gentamicin indicating nephroprotective activity (**Figure 1**).

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

In all the groups, there have been 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 compared to normal group.

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

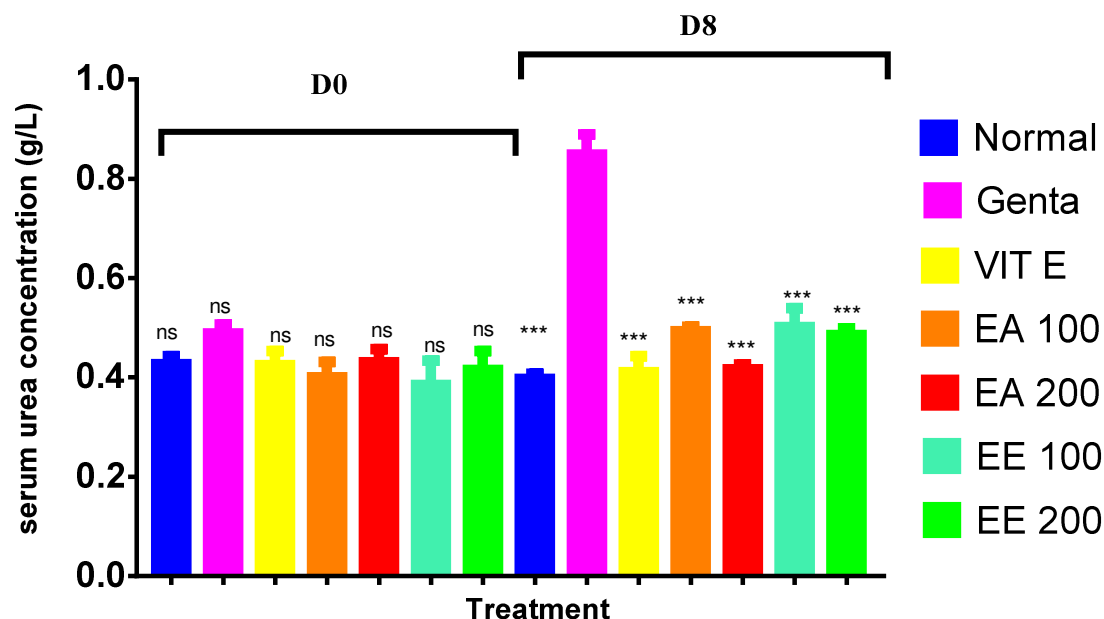


Figure 1: Effect of *Trema guineensis* extracts (aqueous and hydroethanolic) and vitamin E on serum urea in gentamicin-treated rats compared to negative control group.

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 the treatment (D0). Significance ***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. **D0:** Day 0 (before treatment); **D8:** Day 8 (after treatment)

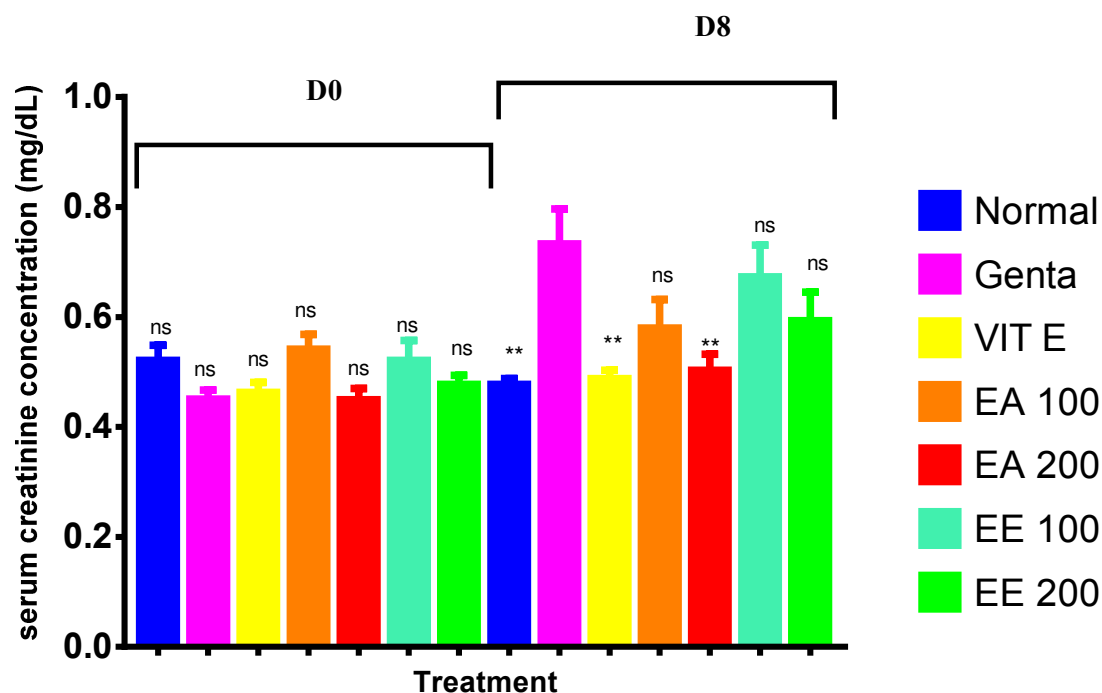


Figure 2: Effect of *Trema guineensis* extracts (aqueous and hydroethanolic) and vitamin E on serum creatinine in gentamicin-treated rats compared to negative control group.

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 the treatment (D0).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. **D0:** Day 0 (before treatment); **D8:** Day 8 (after treatment)

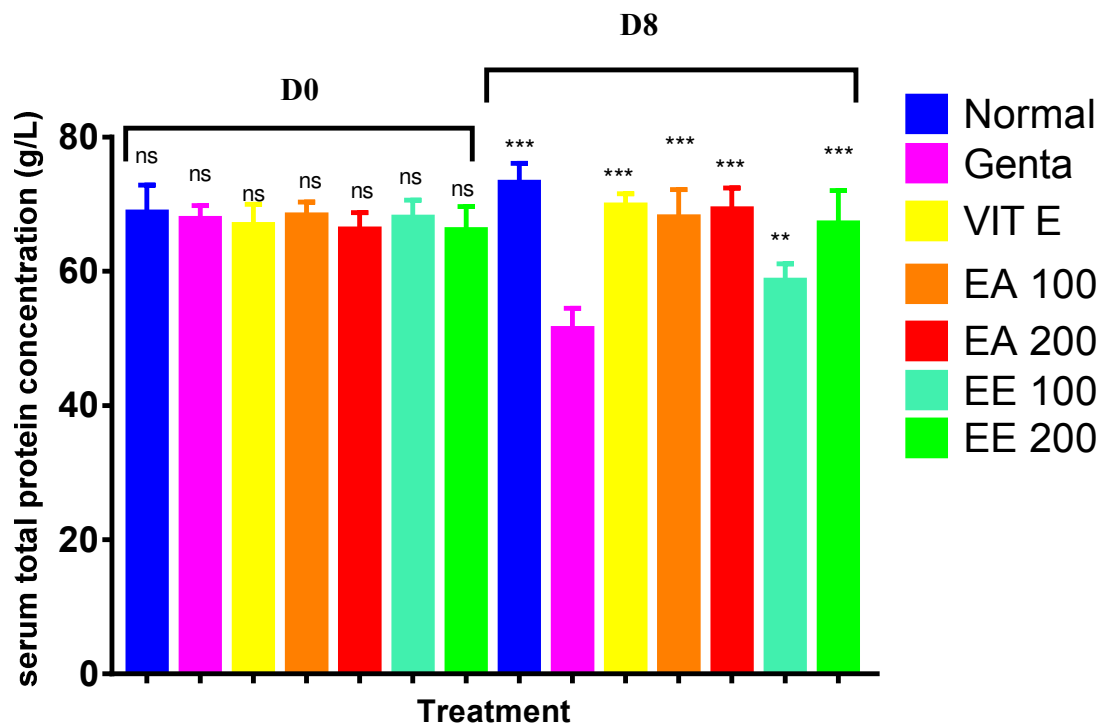


Figure 3: Effect of *Trema guineensis* extracts (aqueous and hydroethanolic) and vitamin E on serum total protein in gentamicin-treated rats compared to negative control group

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 the treatment (D0). 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. **D0:** Day 0 (before treatment); **D8:** Day 8 (after treatment)

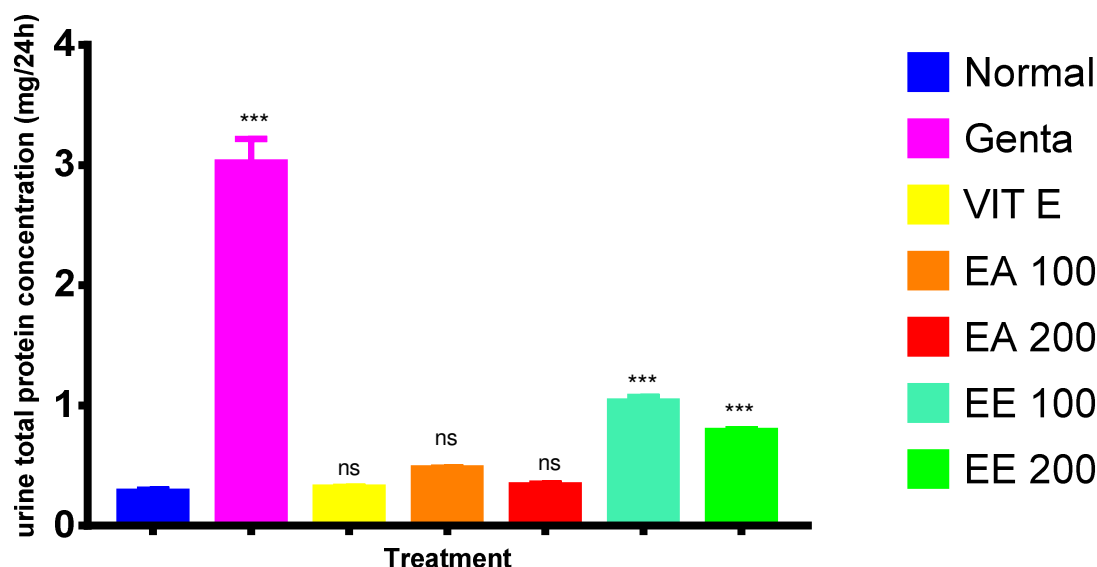


Figure 4: Effect of *Trema guineensis* extracts (aqueous and hydroethanolic) and vitamin E on urinary total protein in gentamicin-treated rats compared to normal control group

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:** 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.

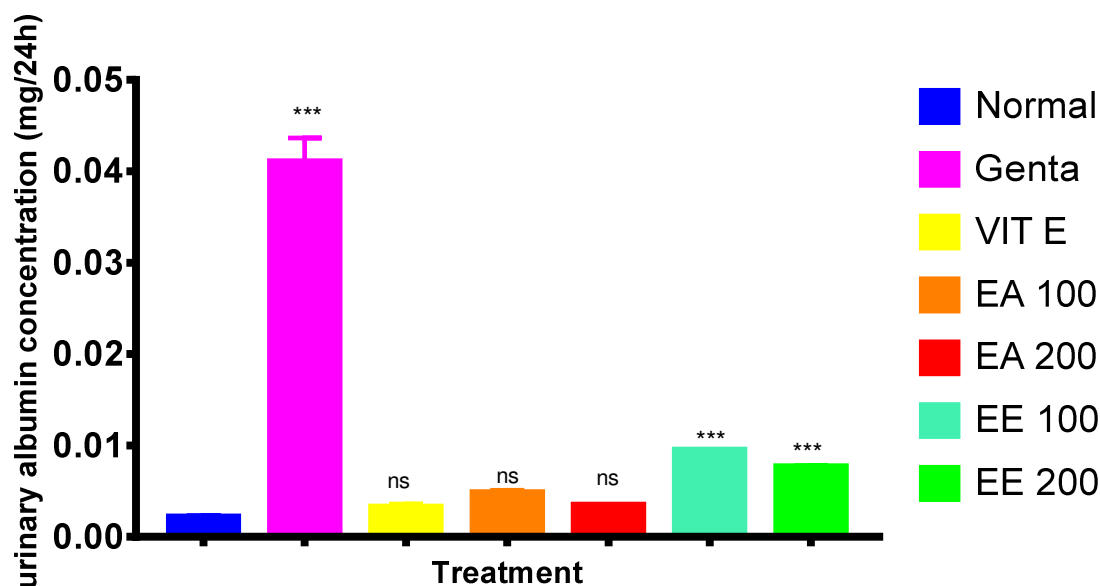


Figure 5: Effect of *Trema guineensis* extracts (aqueous and hydroethanolic) and vitamin E on albumin in gentamicin-treated rats compared to normal group

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 and excess necessary substances. 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 [22; 23]. 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, both toxic chemicals and medication on the kidneys, causing damage to the kidneys [24]. 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 [25]. It usually accumulates in renal proximal tubules. This toxicity has been induced because of the release of the oxidants in kidney. Thus damaging or destructing the nephrons which are the basic functional units of kidney [26].

Nephrotoxicity is identified by estimating the biomarkers like serum creatinine and serum urea which are considered reliable markers [27]. 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 [28]. Creatinine is an end product of muscle catabolism, which is removed at a constant rate by the kidneys. The serum creatinine concentration is the index most commonly used of the renal function. The level of serum creatinine increases if the kidney does not work properly [28]. Thus, increases in serum levels of these markers are indicative of renal injury [27]. 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 blood urea and serum creatinine and low of total protein in serum. This result is in conformity with previous reports attributing these changes to nephrotoxicity induced by gentamicin [21; 29; 30]. 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. However, this treatment with these extracts resulted in increased significant ($p < 0.001$) of serum total protein level in compared to the same control (**Figure 3**); this suggest that *Trema guineensis* contents would have protected the kidney tissue integrity.

Proteins are filtered by the glomerulus, but totally reabsorbed by the proximal bypass tubule. They are not detectable in urine or present in very small quantities [31]. Proteinuria is the most common urinary abnormalities. It may have a glomerular origin, tubular, pre-renal, or post-renal (cystitis) [24]. Proteinuria, usually reflecting the loss of normal glomerular filtration impermeability to plasma proteins is an early sign of kidney disease [32]. Thus, detection of proteinuria is necessary for the recognition of most kidney disease [28].

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 observed in gentamicin treated in rats only (negative control) could be due to toxicity of free radical generated from gentamicin which would have damaged nephron.

There was no statistical difference between the animal groups treated with aqueous extracts (100 and 200 mg/kg body weight) and the group treated with the normal control; that proves these aqueous extracts would have reduced the nephrotoxicity induced by gentamicin in rat, so that the urinary total protein and albumin concentrations would be close to those of untreated rats.

Oxidative stress is the main factor of the gentamicin-induced nephrotoxicity [27]. Natural antioxidants have a variety of biochemical actions such as inhibition of reactive oxygen species production, scavenging of free radicals [33]. Plants were a rich source of natural antioxidants which conferred a nephroprotective activity [34; 35; 36]. The phytochemical investigation of *Trema guineensis* revealed the presence of antioxidant compounds such as phenols, flavonoids, flavonols and sterols [15; 16]. Based on the above literature, it is proposed that the phytochemical constituents in *Trema guineensis* might be responsible of protective effects in gentamicin-induced nephrotoxicity.

4. CONCLUSION

The present study indicated that the aqueous and hydroethanolic extracts of *Trema guineensis* leaves restored significant gentamicin-induced perturbation of the biochemical parameters rates such as urea, creatinine and total protein decrease in serum just like total protein and albumin increase 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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