EVALUATION OF NEPHROPROTECTIVE PROPERTIES OF AQUEOUS AND HYDROETHANOLIC EXTRACTS OF CRINUM SCILIFOLIUM AGAINST GENTAMICIN INDUCED RENAL DYSFUNCTION IN THE ALBINO RATS.

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ABSTRACT

Aims : Gentamycin, a widely used aminoglycoside antibiotic, is recognized as possessing significant nephrotoxic potential in human beings. The aims of this study was to determine the protective effect of aqueous and hydroethanolic extracts of Crinum *scilifolium* on gentamicin induced nephrotoxicity using biochemical approaches and determined the most active extract in rat.

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

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Place and Duration of Study: Analysis of the plant samples was done in pharmacodynamics Biochemistry Laboratory, Felix Houphouet Boigny University and the Laboratory of Organic

Chemistry and Natural Substances, Felix Houphouet Boigny University between July and August 2018.

Methodology: The extractions were performed by macerating 100 g of plant dry powder in 1 liter of distilled water (aqueous extraction) or in 2 liters of water / ethanol mixture (30/70 V / V) (ethanol extraction). The nephroprotective properties were evaluated by comparing the activity of the extracts (100 and 200mg / kg) to that of vitamin E (250 mg / kg) against gentamicin-induced renal toxicity by (80 mg / kg). Gentamicin is administered to the animals one hour after treatment with the extracts 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. Aqueous and hydroethanolic extracts of Crinum *scilifolium* 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.

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

KEYWORDS: Crinum scilifolium, extracts, nephrotoxicity, gentamicin

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1. INTRODUCTION

Kidney is the major organ in urinary system that eliminates waste material from the blood and excretes out from body via urine. Kidney plays a major in maintaining electrolyte balance, fluid homeostasis and blood pressure. In case any kidney injury occurs, our body fails to clear body wastes, excess urine and blood electrolytes such us potassium and magnesium. Among kidney associated problems, Nephrotoxicity is a common disorder and is usually observed when body is exposed some drug or toxin [1].

Renal dysfunction is a term that represents the failure of kidney to excrete the waste. Nitrogenous waste products formed through the metabolic reactions are retained in the blood [2]. In addition to that, fluid and electrolyte balance gets disturbed with endocrine dysfunction. Generally, renal failure is of two types, Acute and chronic renal failure [3].

Gentamicin (GM), an aminoglycoside class of bactericidal antibiotic, it is effective against Gram-negative bacterial infections [4].. However, the clinical use of GM is limited by its major drawback, acute renal failure (ARF), accounting for 10–20% of all cases of nephrotoxicity [5].. GM-induced nephrotoxicity is characterized by increased levels of serum creatinine and blood urea nitrogen, decreased glomerular filtration rate and morphological alterations [6].. A growing body of experimental evidence both in vitro and in vivo demonstrates that GM-induced renal injury is believed to involve the generation and release of reactive oxygen species (ROS) in the renal cortex [7].. This is considered as one of the important mechanisms for GM-induced nephrotoxicity and other deleterious effects [8]..

Medicinal plants have been identified and used throughout human history. Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions, and to defend against attack from predators such as insects, fungi and herbivorous mammals. At least 12,000 such compounds have been isolated so far; a number estimated to be less than 10% of the total. [9, 10].

Crinum scillifolium belongs to the family Amaryllidaceae. In Côte d'Ivoire Crinum scillifolium is found in the southwest and in the center precisely in the clearings of the dense forest. The leaves of this plant are locally used for the treatment of epilepsy and relieving pain. Phytochemical study of Crinum scillifolium revealed the presence of terpen, polyphenol, flavonoid, saponosid and alkaloid [11].. Some studies have shown that the bulbe of Crinum scillifolium have anticonvulsant and analgesic properties [12 ; 13].. However there were no any scientific reports available in support of its traditional claim of nephroprotective potential.

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therefore, present study was designed to evaluate the effect of Crinum scilifolium leaves aqueous and hydroethanolic extracts against gentamicin induced renal damage in experimental animals.

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2. MATERIALS AND METHODS

2.1 Samples Collection and Extraction

The plant material consists of leaves of Crinum scilifolium. A sample of the plant was authenticated by the Laboratory of Botany and Plant Biology of the UFR (Training and Research Unit) of Biosciences at Felix Houphouet Boigny University of Cocody-Abidjan. It was dried at room temperature during two weeks and pulverized using an electric grinder (IKA-type MAG ®). The powder of leaves served as our sample to be analyzed.

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2.1.1 Hydroethanolic extract

100 g of powder of Crinum scilifolium 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 . The filtrate was evaporated and dried at temperature of 40°C using a rotary evaporator type BUCHI 161 Water Bath [14].

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2.1.2 Aqueous extract

100 g of Crinum scilifolium leaves powder were added to 1 Liter of boiling distilled water for twenty 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 [15].

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 by the laboratory of Animal Physiology of the Félix Houphouet Boigny University. Acclimatized for two weeks at the pet store of the of ENS (Higher Normal School) Abidjan Cocody. The animals are housed according to environmental standards, fed with a standard rodent diet, water ad libitum, with conventional treatment and care conditions.

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2.3 Evaluation of Nephroprotective Activity in Gentamicin Induced Nephrotoxicity

The evaluation of the nephroprotective activity of Crinum scilifolium aqueous and hydroethanolic extracts was conducted using the method described by Paoulomi [16] with some modifications [17]. The animals were divided according to weight in seven groups each of six rats:

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 Crinum scilifolium (100mg/kg) and gentamicin (80 mg / kg) for 7 days.

Group V (EA 200): Rats treated daily with aqueous extract of Crinum scilifolium (200mg/kg) and gentamicin (80 mg/kg) for 7 days.

Group VI (EE 100): Rats treated daily with hydroethanolic extract of Crinum scilifolium (100mg/kg) and gentamicin (80 mg / kg) for 7 days.

Group VII (EE 200): Rats treated daily with hydroethanolic extract of Crinum scilifolium (200mg/kg) and gentamicin (80 mg / kg) for 7 days.

The test drug (Crinum scilifolium) 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.

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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 [18].

2.5 Biochemical Assays

The serum samples were used to analyse 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.

2.6 Statistical Analysis

The values expressed as Mean ± standard deviation (SD) 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.

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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 groups. 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 Crinum scilifolium at doses 200 mg/kg and vitamin E (positive control) significantly ($P < 0.001$ and $p < 0.01$) reversed the effect of gentamicin indicating nephroprotective activity (Fig. 1).

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The aqueous extract at dose 200 mg/kg and vitamin E significantly ($P < 0.001$) decreased serum creatinine levels in animals (Fig. 2) compared to negative control group. And The hydroéthanolic extract at dose 200 mg/kg significantly ($P < 0.01$) decreased this levels.

Serum total protein levels was elevated with ($P < 0.001$ and $P < 0.01$) in groups treated with aqueous extract at dose of 200 mg/kg, vitamin E and hydroethanolic extract at dose of 200 mg/kg compared with negative control group (Fig. 3).

Concerning the urine total protein, the Fig. 4 showed significant ($P < 0.001$ and $P< 0.01$) increase in total protein levels in negative control group, aqueous-treated groups and hydroethanolic treated- groups at dose of 100 mg/kg compared to normal group.

Urinary albumin concentration was also significantly increased ($P < 0.001$) in negative control group, aqueous-treated groups and hydroethanolic treated groups at dose of 100 mg/kg compared to normal group (Fig. 5).

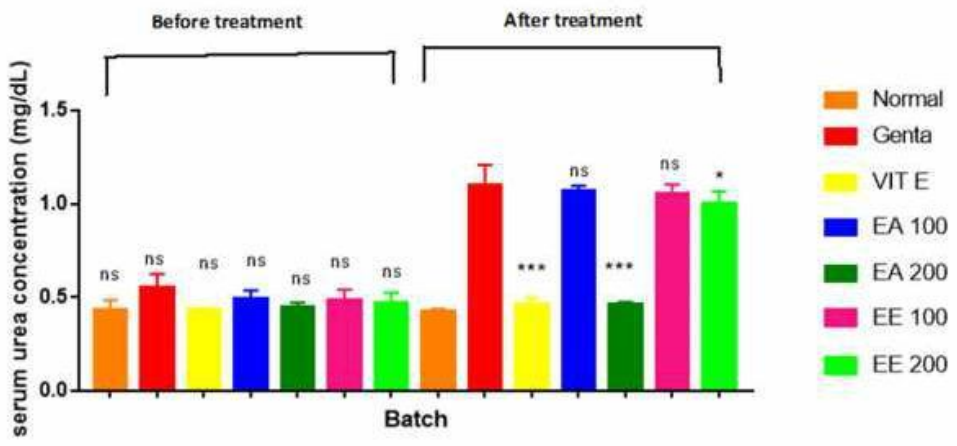

Fig. 1. Effect of Crinum *scilifolium* extracts (aqueous and hydroethanolic) and vitamin E on serum urea in gentamicin-treated rats compared to negative control group

Values are expressed as mean ± SD (standard devi ation) with n = 6 in each group. ns: No significant difference between the different groups before treatment. Significance ***P<0.001 and *P< 0.05 compared to negative control group (Genta). ns: no significance compared to negative control 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.

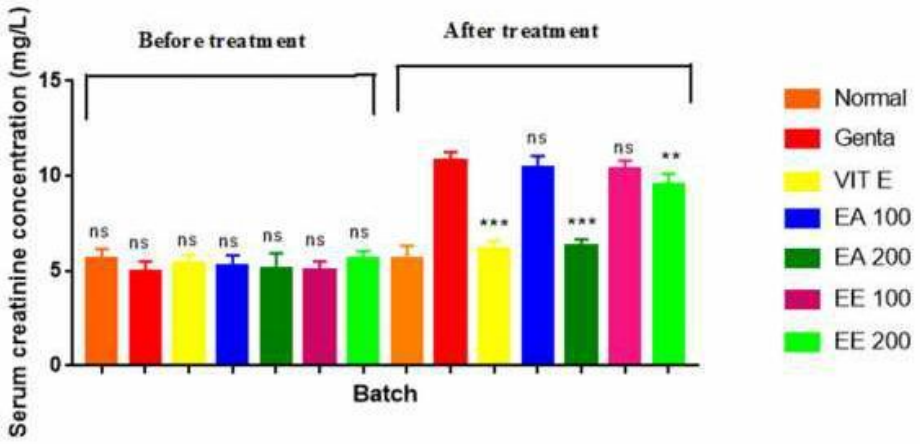

Fig. 2. Effect of Crinum *scilifolium* extracts (aqueous and hydroethanolic) and vitamin E on serum creatinine in gentamicin-treated rats compared to negative control group

Values are expressed as mean ± SD (standard devi ation) 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). 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.

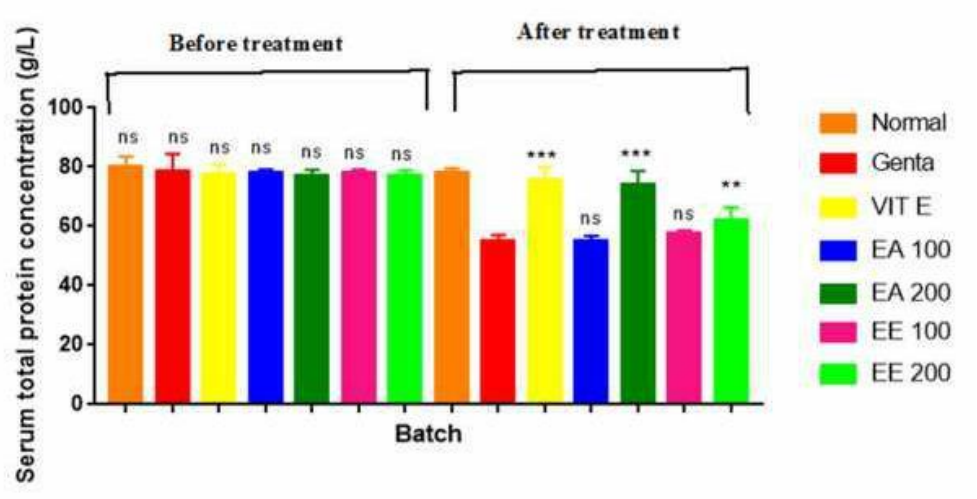

Fig. 3. Effect of Crinum *scilifolium* extracts (aqueous and hydroethanolic) and vitamin E on serum total protein in gentamicin-treated rats compared to negative control group

Values are expressed as mean ± SD (standard devi ation) 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). 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.

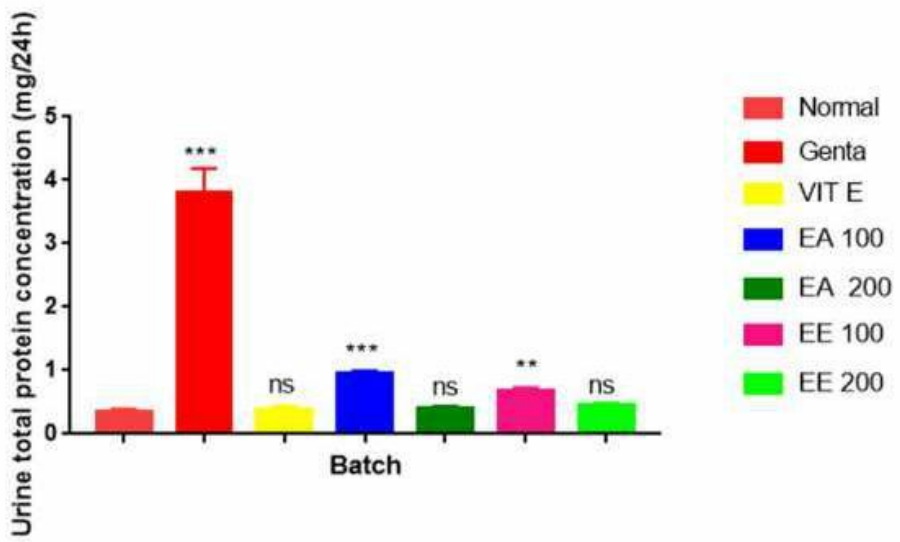

Fig. 4. Effect of Crinum *scilifolium* extracts (aqueous and hydroethanolic) and vitamin E on urinary total protein in gentamicin-treated rats compared to normal control group

Values are expressed as mean ± SD (standard devi ation) with n = 6 in each group. Significance ***P<0.001 and **P<0.01 compared to normal group. 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.

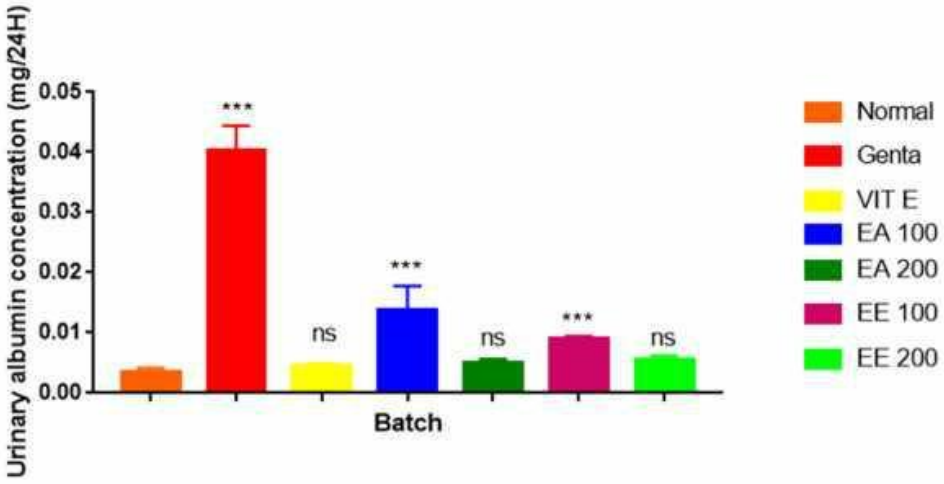

Fig. 5. Effect of Crinum *scilifolium* extracts (aqueous and hydroethanolic) and vitamin E on albumin in gentamicin-treated rats compared to normal group

Values are expressed as mean ± SD (standard deviation) 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 [19,20]. The present study was carried out to evaluate the protective effects of the aqueous and hydroethanolic extracts of Crinum scilifolium leaves on gentamicin-induced nephrotoxic in rats.

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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 [21]. 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 [22]. Gentamicin usually accumulates in renal proximal tubules and enhances hydrogen peroxide and oxygen free radicals generation [23,24]. Abnormal production of reactive oxygen species may result in cellular injury and necrosis through peroxidation of membrane lipids, protein denaturation and DNA damage [25]. 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 [26].

Nephrotoxic effect 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 an index 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.

In our experience we observed that gentamicin at a dose of 80mg/kg produces significant renal damage when compared to normal group which is demonstrated by increase in serum urea and creatinine with low total protein in serum. The results of this investigation is in conformity with

previous reports attributing these changes to nephrotoxicity induced by gentamicin [29,30,31]. Pretreatment with the vitamin E or by aqueous and hydroethanolic extracts of Crinum scilifolium at doses 200 mg/kg of PC restored significantly ($P < 0.001$ and $P < 0.01$) creatinine and urea levels (Figs. 1 and 2) compared to the negative group. This result is supported by Cyril et al. [32] who indicated that supplementation of Trema guineensis leaves restored significant gentamicin-induced perturbation rates on biochemical parameters such as urea, creatinine and total protein in serum. However, treatment with Crinum scilifolium aqueous and hydroethanolic extracts resulted in increased serum total protein levels significantly ($P < 0.001$ and $P < 0.01$) in comparison with negative control (Fig. 3); this suggests that Crinum scilifolium contents protected the kidney tissue integrity. This result is reinforced by Bamba et al. Studies [29].

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 [33]. Proteinuria, usually reflecting the loss of normal glomerular filtration impermeability to plasma proteins is an early sign of kidney disease. Thus, detection of proteinuria is necessary for the recognition of most kidney disease. [34].

The exploration of animal urine noted significant increase ($P<0.001$ and $P<0.01$) in the values of the studied parameters such sus total protein and albumin in gentamicin induced rats and aqueous extracts and hydroethanolic-treated groups (EA 100 and EE 100) when compared to the normal rats (Figs. 4 and 5). Gentamicin which generate free radical would be the basis of the abnormal increase of these parameters in urine.

There was no statistical difference between the animals groups treated with aqueous and hydroethanolic extracts (200 mg/kg body weight) and the group of normal rats; that proves that aqueous and hydroethanolic extracts at the dose of 200 mg/kg body weight would have reduced the nephrotoxicity induced by gentamicin in rats, so that the urinary total protein and albumin concentrations would be similar at those of untreated rats. Treatment by Crinum scilifolium normalized the levels of urinary total protein and albumin in gentamicin treated rats. These results are corroborated by those of Bamba et al. [29] and those of Cyril et al. [32]. Bamba and his collaborators showed that aqueous and ethanolic extracts of Gomphrena celosioides, Cola nitida, and Entendrophragma angolense effectively mitigated the effects of gentamicin on proteinuria and albuminuria. Cyril and his colleagues showed that the aqueous and hydroethanolic extracts of Trema guineensis leaves restored significant gentamicin-induced perturbation rates on biochemical parameters such as urinary total protein and albuminin.

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Natural antioxidants have a variety of biochemical actions such as inhibition of reactive oxygen species production, scavenging of free radicals [35]. Many studies showed that the presence of antioxidant compounds in plants conferred them a nephroprotective activity [36,37,38]. The phytochemical investigation of Crinum scilifolium revealed the presence of antioxidant compounds such as polyphenols, flavonoids, and sterols [39]. The nephroprotective activity of Crinum scilifolium may be due to these phytochemical constituents present in it.

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4. CONCLUSION

The nephroprotective effect of Crinum scilifolium leaves extracts was evaluated. This study indicated that the aqueous and hydroethanolic extracts of Crinum scilifolium leaves restored significant gentamicin-induced perturbation rates on biochemical parameters such as urea, creatinine and total protein in serum thus that total protein and albuminin in urine. The aqueous and hydroethanolic extract at dose 200 mg/kg body weight possessed nephroprotective activity. The present study revealed that the aqueous extract at dose 200 mg/kg body weight possessed profound nephroprotective activity which can be utilized for preventives purposes.

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).

COMPETING INTERESTS

We declare that we have no conflict of interest.

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