

EVALUATION OF NEPHROPROTECTIVE PROPERTIES OF AQUEOUS AND ETHANOLIC EXTRACTS OF GOMPHRENA CELOSIODES, COLA NITIDA AND ENTANDROPHRAGMA ANGOLENSE AGAINST GENTAMICIN INDUCED RENAL DYSFUNCTION IN THE ALBINO RATS.**Bamba Abou*¹, Yapi Houphouet Felix¹, Aka Kouadio Ayebe Edwige² and Djyh Bernard Nazaire¹**¹Laboratory of Biochemical Pharmacodynamics, UFR Biosciences, FELIX Houphouet Boigny University of Abidjan-Cocody (Côte d'Ivoire), 22 BP 582 Abidjan.²Institut Pasteur de Côte d'Ivoire département de Biochimie médicale et fondamentale, 01 BP 490 Abidjan 01.**Corresponding Author: Dr. Bamba Abou**

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Article Received on 30/08/2016

Article Revised on 20/09/2016

Article Accepted on 11/10/2016

ABSTRACT

Nephrotoxicity is one of the important side effects of certain antibiotics such as gentamicin. The purpose of this study was to determine the protective effect of aqueous and ethanolic extracts of entandrophragma angolense, cola nitida and gomphrena celosiodes on gentamicin induced nephrotoxicity in rats; using biochemical approaches as a guide. The extractions were performed by macerating 100 g of plant dry powder in 2 liters 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 (200 and 500mg / kg) to that of vitamin E (250 mg / kg) against gentamicin-induced renal toxicity by (80 mg / kg). Technically, gentamicin is administered to the animals one hour after treatment with the extracts for seven days. The results showed that intraperitoneal injection of gentamicin causes a significant decrease ($p < 0.05$) of total serum rate of protein and a significant raise ($p < 0.05$) in serum rate of creatinine and urea. The results also show a significant increase ($p < 0.05$) in total protein and albumin in the urine of animals. However, changes in biochemical parameters measured were significantly ($p < 0.05$) attenuated in co-treated rats with the extracts. In conclusion, our studies have shown that treatment with the extracts significantly attenuate ($p < 0.05$) levels of serum creatinine, serum urea, serum protein, proteinuria and albuminuria.

KEYWORDS: nephrotoxicity, gentamicin, extracts, Entandrophragma, Cola, gomphrena.**INTRODUCTION**

The kidneys are the final common pathway for excretion of many drugs and their metabolites, and thus are often subjected to high concentrations of potentially toxic substances. Consequently, direct toxic damages occur, usually affecting renal tubular cells and renal papillae. Many classes of drugs can cause kidney damages.^[1] The kidney also ensures the excretion of metabolic waste and exogenous products, while maintaining the essential elements to the body.^[2] In addition, the kidney is particularly vulnerable to several toxic substances that would cause damage to various sites and thus disrupt some of its functions.^[3] then It is understandable why Renal toxicity is one of the most frequently encountered in toxicology studies often causing the arrest of development of certain compounds.^[4] The medicinal plants are widely used for the prevention and treatment of various diseases in Africa and in developing countries.^[5] Because they are believed to be very effective, available at low cost, less or no side effects and are used as an alternative to allopathic medicines.^[6]

Cola nitida, Gomphrena celosiodes and Entandrophragma angolense are three plants of the Ivorian pharmacopoeia for the conventional treatment of various pathologies. Among these pathologies we can note malaria, jaundice, cough, fontanelle, fever, stomach pains, gastric ulcers, earache and also kidney pain.^[7,8,9] Scientifically, several studies were carried out on phytochemistry and biological properties of various extracts of these plants. Among many, we note analgesic, antioxidant, anti-inflammatory and antianemic properties.^[10,11,12,13,14]

Based on the traditional use of these plants in the treatment of many diseases and because of their richness in biodynamic compounds with therapeutic values, this study was designed to evaluate the effect of aqueous and ethanol extracts of these plants on the gentamicin-induced nephrotoxicity in albino rats.

MATERIALS AND METHODS

Plant material

The plant material consists of *Gomphrena celosioides* (stems, leaves, flowers) of the *Amaranthaceae* species, fruit of *Cola nitida* (*Sterculiaceae*) and the bark of *Entandrophra gmaangolense*. (*Meliaceae*). A sample of each 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.

Animal Material

The animal species chosen for this study was the albino rat (*Rattus norvegicus*) of Wistar strain. 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. Ninety rats of both sexes, aged about four months of average weight of 134.98 g were used for the experiments. Selected rats were nulliparous, non-pregnant and had not been subject to previous studies. All the animals experimental procedures were conducted after the approval of the Ethical Guidelines of University (Côte d'Ivoire) Committee on Animal Resources. They 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).^[15,16]

Preparation of aqueous and ethanol extracts

The plant was dried in the open air, protected from light, at room temperature (25° c) for two weeks and then ground using an electric grinder. The aqueous extraction was performed according to the method described by Guédé-Guina.^[17] Hundred (100) grams of plant dry powder were soaked in 2 liters of distilled water using a magnetic stirrer for 24 hours. The homogenate obtained is sieved, filtered 3 times on cotton wool and once on Whatman N°1. The filtrate is thereafter brought to evaporation *in vacuo* at 50 ° C in an oven. A dry powder is thus obtained which constitutes the aqueous extract. The ethanol extract has been prepared according to the same principle using 2 liters of water / ethanol mixture (30/70 V / V).

Treatment of Animals

The evaluation of the nephroprotective activity of the aqueous and ethanol extracts of *Cola nitida*, *Gomphrena celosoides* and *Entandrophragma angolense* was conducted using the method described by Paoulomi^[18] with some modifications. The principle of this method is based on a sequential process of first treating animals with vitamin E (reference nephroprotective referencial) or with extracts .then an hour after, administration of gentamicin (nephrotoxic referencial). Intoxications are conducted with doses which induce a significant

nephrotoxicity in animals without causing their death during the experiment. This test aims to highlight nephroprotective extract in comparison with that of vitamin E. Treatments are performed every day at the same hour for seven days and the animals were sacrificed on the eighth day. The solutions are prepared extemporaneously prior to treatments. Extracts and vitamin E are administered by gavage, gentamicin is by intraperitoneal route. The animals were deprived of food for 12 hours and of water only one hour before manipulation. They are fed an hour after manipulation and weighed daily during the experimental period. Conveniently, ninety (90) rats weighing between 120g and 150g were divided into 15 batches of six animals.

Batch C: distilled water and 0.9% NaCl.

Batch N C: Distilled Water and gentamicin (80 mg/kg).

Batch PC: Vitamin E (250 mg / kg) and Gentamicin (80 mg/kg).

Batch NEG1: ethanolic extract of *Gomphrena celosoides* at (200 mg/kg) and gentamicin (80 mg / kg).

Batch NEG2: ethanolic extract of *Gomphrena celosoides* at (500 mg/kg) and gentamicin (80 mg/kg).

Batch NAG1: aqueous extract of *Gomphrena celosoides* (200 mg/kg) and gentamicin (80 mg/kg).

Batch NAG2: aqueous extract of *Gomphrena celosoides* (500 mg/kg) and gentamicin (80 mg/ kg).

Batch NEE1: ethanolic extract of *Cola nitida* (200 mg/kg) and gentamicin (80 mg/kg).

Batch NEE2: ethanolic extract of *Cola nitida* (500 mg/kg) and gentamicin (80 mg/kg).

Batch NAE1: Aqueous extract of *Cola nitida* (200 mg/kg) and gentamicin (80 mg/kg).

Batch NAE2: Aqueous extract of *Cola nitida* (500 mg/kg) and gentamicin (80 mg/kg).

Batch NEY1: ethanolic extract *Entandrophragma angolense* (200 mg / kg) and gentamicin (80 mg/kg).

Batch NEY2: ethanolic extract *Entandrophragma angolense* (500 mg/kg) and gentamicin (80 mg/kg).

Batch NAY1: Aqueous extract of *Entandrophragma angolense* (200 mg/kg) and gentamicin (80 mg/kg).

Batch NAY2: Aqueous extract of *Entandrophragma angolense* (200 mg/kg) and gentamicin (80 mg/kg).

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

Samples

All test animals were weighed and euthanized 24 hours after the last treatment. Their kidneys were removed, rinsed with normal saline, weighed and then fixed in Bouin. The blood of each animal was taken (puncture of the orbital sinus) in a tube without anticoagulant, for metering the biochemical parameters. The collected urines were quantified and a sample of each urine was stored in eppindoff tubes for the determination of certain biochemical parameters.

Biochemical study

Blood samples collected in the tubes without anticoagulant were centrifuged at 3000 revolutions/min for 15 minutes. The collected sera were used to assay the biochemical parameters that is,; The creatinine, total proteins and urea. Also, the collected urines were used to assess the levels of albumin and total proteins in the urine of animals with a kind of automated Cobas type C311 HITACHI ROCK (France).

Processing and analysis of data

Data entry is performed using Excel 2010 software. The relative weight (RW) of the liver was determined relatively to body weight with the following formula:

$$RW = \frac{\text{Absolute Kidneys Weight}}{\text{Body Weight (PC)}} \times 100. [5]$$

$$WG = \frac{P_n - P_{n-1}}{P_{n-1}} \times 100. [19]$$

Weight: Gain, P_n: n measuring of body weight, P_{n-1}: (n-1) measurement of body weight, n: entire value 1 ≤ n ≤ 8.

The statistical analysis was performed using analysis of variance (ANOVA ONE WAY) of Graph Pad Prism 5.0 software (Microsoft USA). The differences between averages were determined according to the multiple comparison tests of Tukey. These differences are considered significant when p was less than 0.05 (p<0.05)

RESULTS

Effect of different treatments on serum concentration of urea.

These results (Figure 1) show the urea concentration of NC (1.3 ± 0.1 g / L) is higher (p < 0.001) than that of C (0.3 ± 0.03 g / L) and PC (0.42 ± 0.09 g / L). Administration of the extracts results in lower concentrations of urea (p < 0.001) than the one of NC. It is noted that NEE2 has a serum concentration of urea (0.5 ± 0.05 g / L) identical (p > 0.05) to that of C and PC. On the other side, urea concentrations of batches of NEY2 and NEG2 are higher (p < 0.05) to those of C and PC.

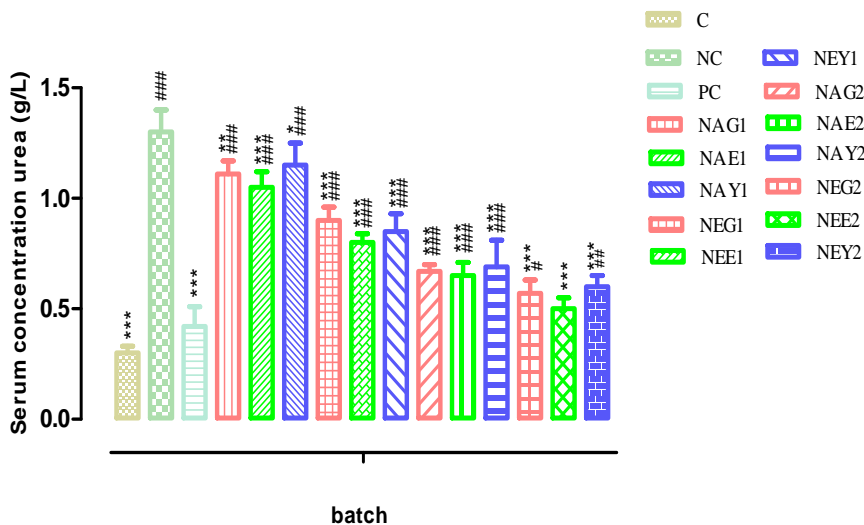


Figure 1: Effect of the extracts and the vitamin E on serum concentration urea

Values are Average ± S.E.A (Standard Error of the Average) with n = 6. * P <0.05; ** P <0.01; *** P <0.001: significantly different from the batch NP. # P <0.05; ## P <0.01; ### P <0.001: significantly different from the batch PC

C: NaCl, NC: NaCl + gentamicin, PC: Vitamin E + gentamicin NAG1: aqueous Extract of G.celosioides 200 mg / kg + gentamicin NAE1: Aqueous extract of C.nitida 200 mg / kg + gentamicin NAY1: Aqueous extract of E.angolense of 200 mg / kg + gentamicin NEG1: ethanol extract of G celosioides 200 mg / kg + gentamicin NEE1: ethanol extract C.nitida 200 mg / kg + gentamicin NEY1: ethanol extract of E.angolense 200 mg / kg + gentamicin nag2: aqueous extract of G

celosioides 500 mg / kg + gentamicin NAE2: aqueous extract of C.nitida 500 mg / kg + gentamicin NAY2: aqueous extract of E.angolense 500 mg / kg + gentamicin NEG2 : ehanolic Extract of G celosioides 500 mg / kg + gentamicin, NEE2: ethanolic Extract of C.nitida 500 mg / kg + gentamicin, NEY2: ethanolic Extract of E.angolense of 500 mg / kg + gentamicin.

Effect of different treatments on serum concentration of creatinine

The assay results of serum creatinine show that NC creatinine serum concentration (11.83 ± 0.27 g / L) was higher (p < 0.001) than the one of C (3.66 ± 0.44 g / L) and PC (4.33 ± 0.44 g / L). The administration of the extracts gave serum concentrations of creatinine lower (p

< 0.001) than the one of NC. These batches also have concentrations of creatinine greater than C and PC. Batch

NEE2 has a serum concentration of creatinine identical ($p > 0.05$) to that of C and PC (**Figure 2**).

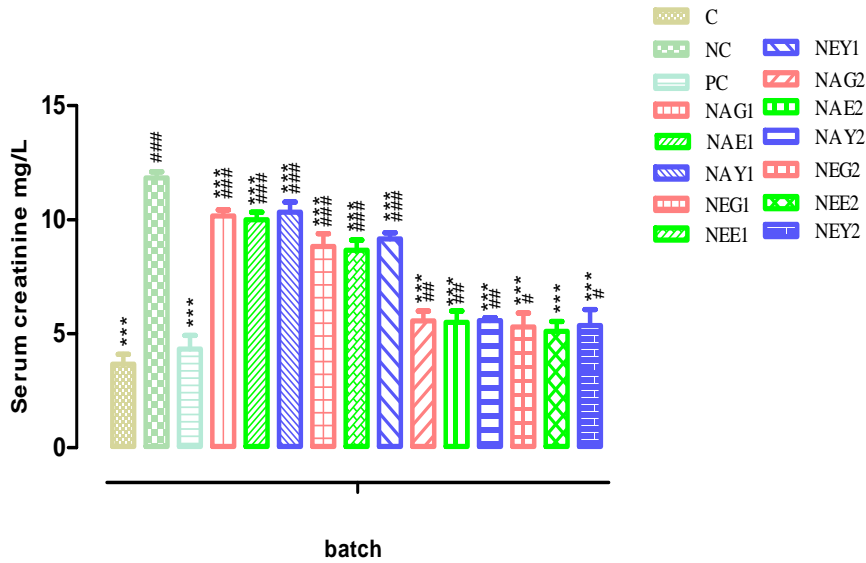


Figure 2: Effect of the extracts and vitamin E on serum creatinine.

Effect of treatments on serum concentrations of total protein.

The protein assay (Figure 3) shows that the concentration of total protein of NC (51.90 ± 1.36 g / L) suffered a significant decrease ($p < 0.001$) compared to that of C ($71.4 \pm 1,8$ g / L) and PC (68.88 ± 1.2 g / L). Batches of

intoxicated animals treated with the extracts yielded higher concentrations of total proteins ($p < 0.001$) to the one of PC. These results also show that the batch NEE2 has a serum concentration of total proteins statistically identical ($p > 0.05$) to that of C and PC.

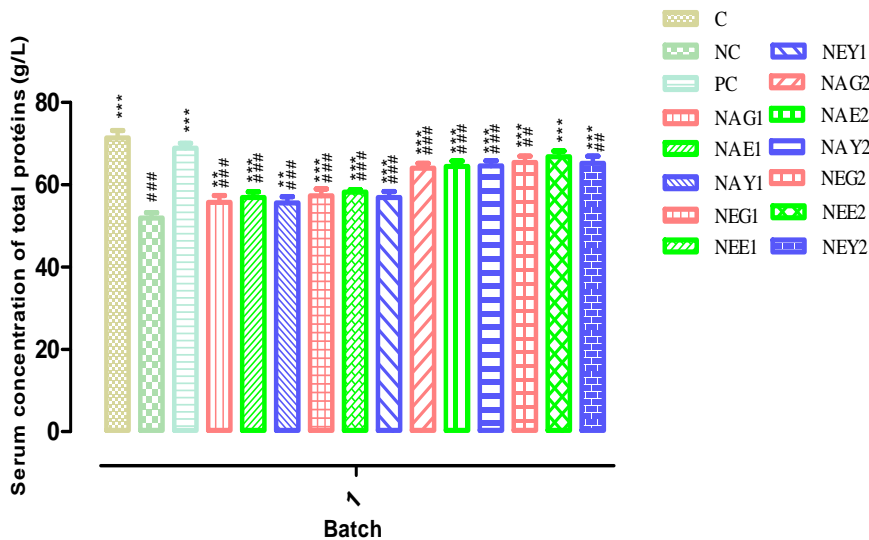


Figure 3: Effect of the extracts and vitamin E in serum concentration of total proteins.

Effect of different treatments on the concentration of total protein of urine

The assay results of the concentration of urine total proteins showed that all batches of animals treated with the extracts have concentrations of total protein in urine greater than PC and C ($p < 0.001$; $p < 0.01$ and $p < 0.05$)

and lower than that of NP ($p < 0.001$) (**Figure 4**). These results also show that the total protein concentration of urine from the NC (17.33 ± 0.44 g / L) is higher ($p < 0.001$) to the one of C (8.33 ± 0.66 g / L) and NC (9.83 ± 0.55 g / L).

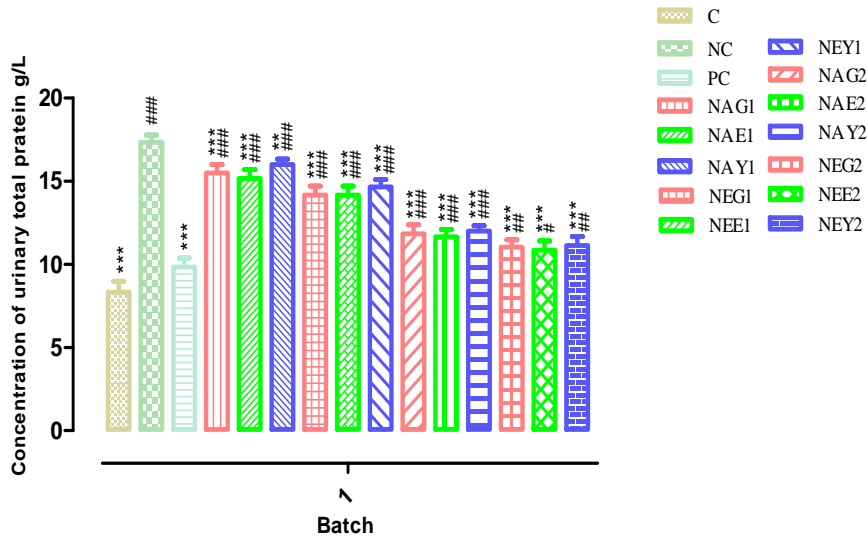


Figure 4: Effect of the extracts and vitamin E on the concentration of urinary total protein.

Effect of different treatments on the concentration of urinary albumin

The assay of urinary albumin indicate that preventive treatment by the extracts favored a decrease ($p < 0.001$) in concentrations of urinary albumin compared to those ones of NC (3.09 ± 0.09 g / L). These results also show

that the concentration of urinary albumin of NC (3.09 ± 0.09 g / L) is higher ($p < 0.001$) than that of C (0.99 ± 0.12 g / L) and PC (1.16 ± 0.16 g / L) (Figure 5). Batch NEE2 has an identical albumin level ($p > 0.05$) to that of PC and C.

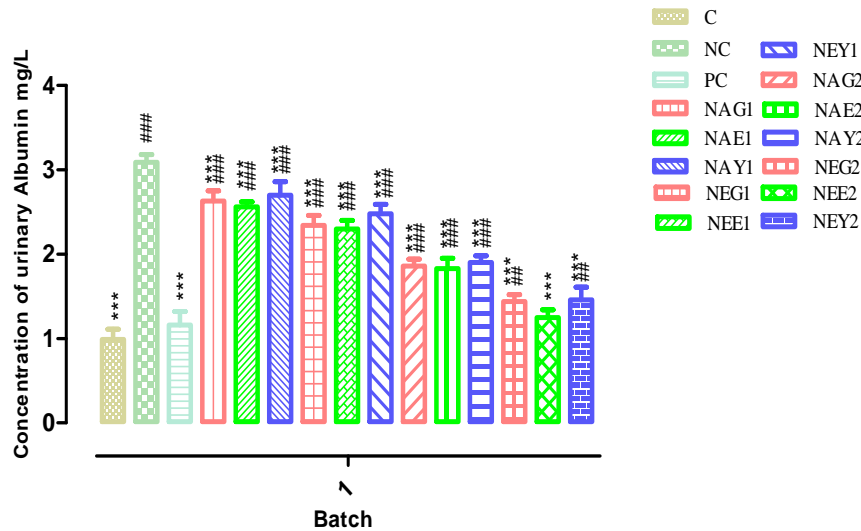


Figure 5: Effect of the extracts and the vitamin E on the concentration of urinary albumin.

Effect of different treatments on body weight of the animals

The change in body weight of animals according to treatments is summarized in Figure 6. In this figure, we see that the C batches ($5.09 \pm 0.65\%$) and PC ($4.86 \pm 0.40\%$) have higher weight gains ($p < 0.001$) than those of NC ($0.6 \pm 0.6\%$). Also the weight gain of the batch NC is less ($p < 0.001$) than the set of batches having received preventive treatments with extracts. The figure also suggests that the batches treated with the extracts

have lower weight gains ($p < 0, 05$) than in control C and PC.

Effect of different treatments on the relative weight of the kidneys

The change in body weight of animals per treatments is summarized in Figure 7. The average values of the relative weights indicate that all animals studied suffered no variation in kidney weights during treatment ($p > 0.05$).

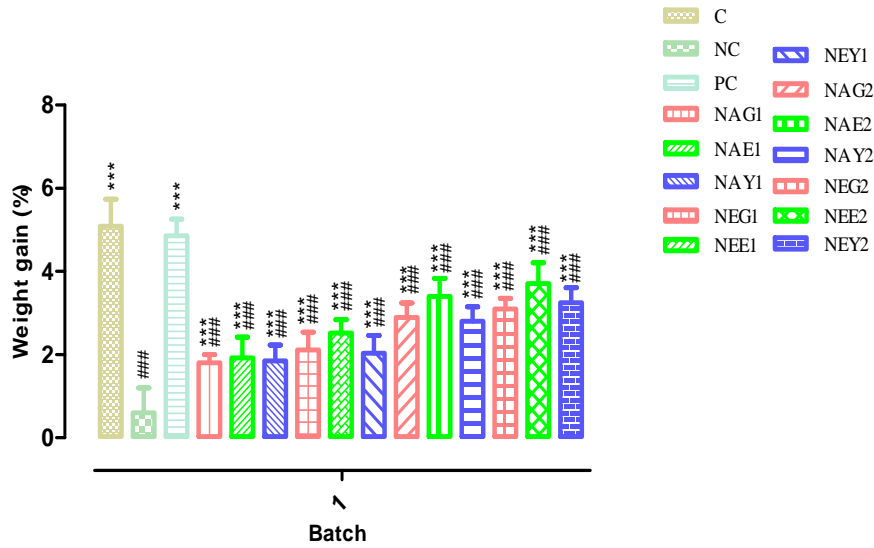


Figure 6: Effect of the extracts and vitamin E on the weight gain of animals.

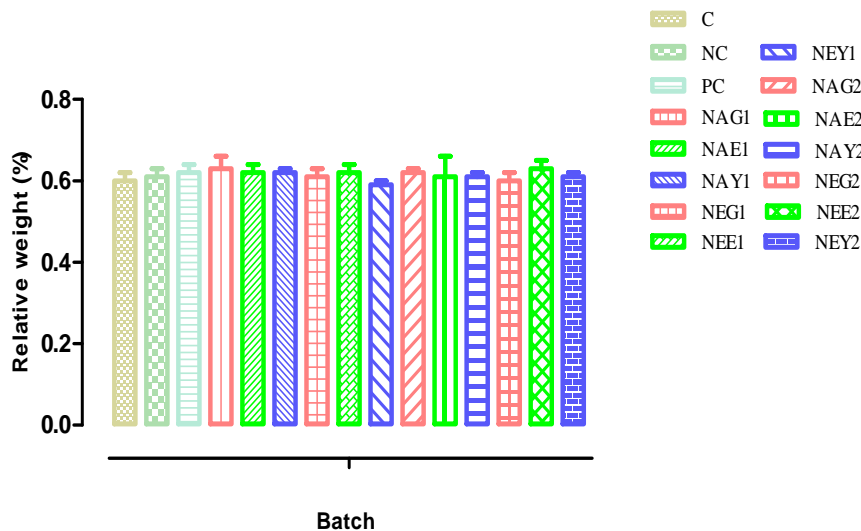


Figure 7: Effect of the extracts and vitamin E on the relative weight of animals.

DISCUSSION

Humans are exposed intentionally or unintentionally to a variety of harmful chemicals that can harm the kidneys. Causes of nephrotoxicity are numerous. Among others include medicines, natural products, industrial chemicals and environmental pollutants. These nephrotoxic can produce a variety of clinical syndromes such as acute renal failure, chronic renal failure, nephrotic syndrome and hypertension.^[20] In the light of these continual attacks the kidney we have done this work in order to assess the nephroprotective activity of aqueous and ethanol extracts of *G.celosioides*, *C.nitida*, and *E.angolense* against gentamicin induced nephrotoxicity. Gentamicin is an aminoglycoside antibiotic widely used, recognized as having an important nephrotoxic potential in humans and experimental animals.^[21] The results of our study show that the relative kidney weight was not significantly influenced by the different

treatments administered to the animals. This indicates that vitamin E and Gentamicin would have no effect on kidney weight, at least at the doses used for one week of treatment. Analysis of weight gain in animals indicates a weight loss of the batches of intoxicated and untreated controls. This weight loss is almost nonexistent in the batches of non intoxicated controls and treated controls with Vitamin E. The treatment with aqueous and ethanolic extracts of *G.celosioides*, *C.nitida*, and *E.angolense* corrects this weight loss but the affected batches still have lower weight gains ($P < 0.05$) than the control treated with vitamin E. administration of extracts thus mitigate the effects of gentamicin on the weight of the animals without so neutralizing them. This attenuating activity is greater at a dose of 500 mg / kg as shown by studies Prusty.^[20]

As a measure of the condition of renal function, urea and serum creatinine are often considered reliable markers.^[22] Thus, increases in serum levels of these markers are indicative of renal injury.^[22] 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 micro albumin) of animals. It is still important to remember that Urea is a product of the final protein catabolism. It is freely filtered by the glomerulus, excreted in high concentrations in the urine. The serum level of urea is used as an index of renal function.^[23] Creatinine is a product of muscle catabolism, which is removed at a constant speed 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.^[23]

The serum examination revealed a significant increase in ($p < 0.05$) concentrations of urea and creatinine for intoxicated batch with gentamicin and not treated NC (Figures 12,13). Also, serum concentration of total proteins is significantly reduced in the same batch as compared to other batches. This induces that gentamicin would be the basis of the significant variation in these biochemical parameters, symbol of a renal dysfunction. These results are corroborated by those of Mason and Lesely.^[24,23] Pretreatment with the vitamin E or by aqueous and ethanolic extracts of *G.celosioides*, *C.nitida*, and *E.angolense* at both doses (200 mg/kg and 500 mg/kg of PC) promotes a significant decrease in concentrations of urea and creatinine with an increase in serum concentration of total protein. These good significant changes (increase in serum total protein, decrease in urea and creatinine concentrations) of the studied biochemical parameters indicate a significant mitigation of gentamicin induced renal toxicity. This is also suggested by the results of the work of Kotnis.^[25]

Proteinuria is the most common urinary abnormalities. It may have a glomerular origin, tubular, pre-renal, or postrenal (cystitis).^[26] Proteinuria, usually reflecting the loss of normal glomerular filtration impermeability to plasma proteins is an early sign of kidney disease.^[6] Thus, detection of proteinuria is necessary for the recognition of most kidney disease.^[27]

The exploration of animal urine noted significant increase in the values of the studied parameters (total protein and micro albumin) for batch intoxicated with gentamicin and untreated, compared to the batch not intoxicated control. Gentamicin would be the basis of the abnormal increase of these parameters in urine. We also note that the values of these parameters were statistically reduced by treatment with vitamin E and extracts studied in comparison to the batch NC. The concentrations of these urine parameters were statistically lower when the animals were treated with the extracts in a dose of 500

mg / kg compared to the dose of 200 mg/kg body weight (bw).

These observations suggest that aqueous and ethanol extracts of *G.celosioides*, *C.nitida*, and *E.angolense* at 500 mg/kg more effectively mitigate the effects of gentamicin on proteinuria and albuminuria. These results are corroborated by those of Kotnis^[25] and those of Suji^[6] in relation to work respectively on the de *Hemidesmusindicus* and *Aristolochia indica* extracts. Note that oxidative stress is the main factor of the gentamicin-induced nephrotoxicity.^[6] Moreover, the literature has shown that the medicinal plants have nephroprotective properties via antioxidants because flavonoids and alkaloids they contain.^[28,29] Indeed, characterization of the chemical constituents of the aqueous and ethanolic extracts of *G.celosioides*, *C.nitida*, and *E.angolense* revealed the presence of sterols and triterpenes, flavonoids, saponins, tannins and coumarins.^[30] In light of these findings it is likely that these chemicals are responsible for the observed biological effects.

CONCLUSION

In conclusion, it appears from our study that the aqueous and ethanol extracts of *G.celosioides*, *C.nitida*, and *E.angolense* mitigate the effects of gentamicin-induced nephrotoxicity. This attenuating activity has resulted in a significant decrease in urea and serum creatinine, a significant increase in serum total protein, a significant decrease in total protein and urinary micro albuminuria and finally by a significant increase in weight gain. The nephroprotective activity of our extracts, although acceptable at the dose of 200 mg / kg of bw is even better at a dose of 500 mg/kg of bw. And the ethanol extract of *Cola nitida* gives better results than other studied extracts.

ACKNOWLEDGEMENT

We express our gratitude to the Management staff of pharmacodynamics-Biochemical Laboratory of the Félix Houphouët Boigny University. So should we thank all the officials of the ENS and Institut Pasteur of Ivory Coast.

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