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Study of the nephroprotective potential of aqueous and hydro-ethanol extracts of the leaves of *Erythrococca anomala* (Juss. Ex Poir.) Prain (Euphorbiaceae)

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Abstract

As part of efforts to promote traditional medicine, a pharmacological study was conducted on aqueous and hydroethanolic extracts of the leaves of *Erythrococca anomala*, a plant used in traditional medicine. The study focused on the nephroprotective potential of these extracts. Rats whose kidneys had been poisoned by gentamicin (80 mg/kg) for seven days were treated with aqueous and hydroethanolic extracts of Erythrococca anomala leaves at a dose of 200 mg/kg, and vitamin E (250 mg/kg). The results showed that the aqueous extract and vitamin E significantly reduced ($p < 0.05$) the nephrotoxicity of the rats compared to the hydroethanolic extract, with respective creatinine concentrations of 0.37 ± 0.18 mg/dL. 0.38 mg/dL and 0.43 ± 0.15 mg/dL. Urea concentrations were 0.40 ± 0.17 g/L (hydroethanolic extract), 0.30 ± 0.16 g/L (aqueous extract), and 0.30 ± 0.15 g/L (vitamin E). Total protein concentrations were 78.33 ± 0.20 g/L (hydroethanolic extract), 82.33 ± 0.19 g/L (aqueous extract), and 83 ± 0.23 g/L (vitamin E). This study showed that the aqueous extract has a greater efficacy than the hydroethanolic extract in reducing nephrotoxicity. This greater efficacy of the aqueous extract could be explained by the higher presence of secondary metabolites in this extract compared to the hydroethanolic extract. The efficacy of the aqueous extract of *Erythrococca anomala* leaves, similar to that of reference medications for treating nephrotoxicity, could offer hope for traditional medicine.

Keywords: Aqueous Extract; Hydroethanolic Extract; *Erythrococca anomala*; Nephrotoxicity.

1. Introduction

The kidneys are the primary organs of urinary excretion, one of whose fundamental functions is the maintenance of homeostasis. They regulate acid-base balance and purify the body by eliminating terminal metabolic waste products, such as urea and uric acid, as well as exogenous substances like drugs and toxins **(Vaubourdolle, 2007).** Due to this elimination function, primarily through glomerular filtration, the renal parenchyma is particularly vulnerable to drug nephrotoxicity **(Ali, 2003).** Therefore, it is essential to protect this vital organ. The aim of this study is to demonstrate the nephroprotective potential of aqueous and hydroethanolic extracts of *Erythrococca anomala* leaves. Indeed, *Erythrococca anomala* is a sub-Saharan plant used in traditional medicine to treat certain conditions such as bacterial infections, meningitis, inflammation, arthritis, and dental pain **(Adjanohoun *et al.,* 1996).** Previous studies have demonstrated the anti-inflammatory, antibacterial, and hepatoprotective potential **(Patrice *et al.,* 2016; Miezan *et al.,* 2017)** of aqueous and hydroethanolic extracts of this plant.

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2. Materials and Methods

2.1. Materials

2.1.1. Plant Material

For the evaluation of the nephroprotective potential of *Erythrococca anomala*, the plant material consisted of leaves harvested in the Aboisso region and identified at the National Floristic Center of Côte d'Ivoire. The leaves were dried and then ground into a powder from which aqueous and hydroethanolic extracts were prepared.

2.1.2. Animal Material

The animal material consisted of 20 four-month-old albino rats (Rattus norvegicus) of the Wistar variety, with an average weight of 118.9 ± 0.076 g, which were used in the in vivo study. These animals came from the animal facility of the Faculty of Biological and Pharmaceutical Sciences at Felix Houphouët Boigny University. The animals were kept in favorable breeding and ethical conditions, in an air-conditioned room for 5 weeks.

2.2. Methods

2.2.1. Leaf Collection and Drying

For this study, the plant material consisted of 5 kg of *Erythrococca anomala* leaves. The leaves were stored in biodegradable bags and then transported in a van. The plant material was kept in the laboratory, protected from light, for three weeks before being ground using a mechanical grinder (IKAMAG, Japan) to produce a powder from which aqueous and hydroethanolic extracts were prepared.

2.2.2. Extraction

Aqueous Extraction

The leaves of the *Erythrococca anomala* plant were used to determine their nephroprotective potential. The leaves were dried and then ground into a powder. One hundred (100) grams of leaf powder were placed in one liter of water and boiled for 15 minutes before being filtered through 3 mm diameter Whatman filter paper. The resulting filtrate was oven-dried at 40°C for one week to obtain the aqueous extract of *Erythrococca anomala* leaves.

Hydroethanolic Extraction

The method of Guédé-Guina et al. (1990) was used to prepare the hydroethanolic extract of *Erythrococca anomala* leaves. A 70% hydroethanolic solution (ethanol/water: 70/30) was used to prepare the hydroethanolic extract of *Erythrococca anomala* in flasks. One liter of the hydroethanolic solution and 100 g of leaf powder were used. The resulting mixture was homogenized using a magnetic stirrer for 24 hours. The homogenate was then filtered through 3 mm Whatman filter paper and dried under vacuum for 1 hour. The filtrate was concentrated in a rotary evaporator and then dried in an oven at 40°C for one week. This yielded the powdered hydroethanolic extract.

2.2.3. Treatment of rats for the evaluation of nephroprotective activity

The experiment was conducted on five groups of four rats, composed of healthy adult rats with an average weight of 123.64 ± 1.1 g. The determination of the nephroprotective potential of the two types of extracts was carried out according to the method described by **Paoulomi et al., (2012)** and **Bamba et al., (2016).**

This method makes it possible to highlight the preventive properties of aqueous and hydroethanolic extracts of *Erythrococca anomala* leaves against gentamicin poisoning. Indeed, gentamicin is an antibiotic that causes renal toxicity following prolonged treatment of at least seven days **(Suji and Vimalastalin, 2014).**

Thus, the animals received the following treatments:

- Group 1: the normal control group received distilled water orally for seven days;
- Group 2: The negative control was treated with distilled water orally daily and, one hour later, with gentamicin (80 mg/kg body weight) intraperitoneally for seven days;
- Group 3: Rats in this group were treated daily for seven days by gavage with aqueous extract of *Erythrococca anomala* (200 mg/kg), followed one hour later by an intraperitoneal injection of gentamicin (80 mg/kg);

- Group 4: Rats in this group were treated daily for seven days by gavage with the hydroethanolic extract of *Erythrococca anomala* (200 mg/kg), followed one hour later by an intraperitoneal injection of gentamicin (80 mg/kg);
- Group 5: Rats in this group received oral vitamin E (250 mg/kg body weight), the reference nephroprotective drug, daily and one hour later with intraperitoneal gentamicin (80 mg/kg body weight) for seven days.

2.2.4. Biochemical analysis for the evaluation of nephroprotective activity

All animals were anesthetized with ether, and blood was collected by tail puncture, before and after treatment, into dry tubes. The blood was centrifuged at 3000 rpm for 10 minutes. The serum was collected in an Eppendorf tube protected by aluminum foil and stored at -20°C until analysis.

Creatinine Assay

The determination of serum creatinine is based on the Jaffe reaction **(Larsen, 1972).** The general principle of this method consists of measuring, at 520 nm, the intensity of the red-orange complex formed by creatinine and picric acid in an alkaline medium.

- Procedure

Ten (10) µL of the sample diluted in 30 µL of distilled water is added to 13 µL of buffer (80 mmol/L potassium hydroxyl and 12 µL phosphate, diluted in 41 µL of distilled water) at pH 13. To this mixture, an equal volume of the other reagents and 4.4 mmol/L picric acid at pH 6.5 are added, and the mixture is incubated for 30 s. The absorbance is measured at 520 nm, and the creatinine concentration in the sample is determined as follows:

$$\text{Creatinine concentration (mg/L)} = \frac{\Delta OD\ \text{sample}}{\Delta OD\ \text{standard}} \times \text{Standard concentration}$$

ΔOD sample: change in optical density of the sample;

ΔOD standard: change in optical density of the standard, and the standard concentration of creatinine is 20 mg/L.

Serum Urea Assay

The urease kinetic method is used to measure urea after 24 hours of serum storage (**Talke and Schubert, 1965)**. In the presence of urease, urea is hydroxylated to carbonate and ammonia. The ammonia reacts with 2-oxoglutarate in the presence of glutamate dehydrogenase (GLDH) and the coenzyme NADH to form L-glutamate. The decrease in NADH is directly proportional to the urea concentration in the sample. This concentration is measured at a wavelength of 340 nm.

-Procedure

The working reagent is prepared in TRIS buffer (45 mmol/L) adjusted to a pH of 8.6. It contains 2-oxoglutarate (15 mmol/L), NADH (0.5 mmol/L), ADP (1.1 mmol/L), and sodium azide (0.02%). The enzymes urease and GLDH are present at concentrations of ≥ 3.7 kU/L and ≥ 1 kU/L, respectively. A 50 µL volume of this reagent is first diluted in 95 µL of distilled water. Simultaneously, 2 µL of the sample are diluted in 98 µL of distilled water and then added to the previous mixture. The entire mixture is incubated for 10 minutes at 37 °C. The absorbance is then read at a wavelength of 340 nm. Finally, the urea concentration is calculated using the following formula:

$$\text{Urea concentration(g/L)} = \frac{\text{ABS sample}}{\text{Standard ABS}} \times \text{Standard concentration}$$

Standard ABS: absorbance of the standard.

The standard concentration of urea was 0.5 g/L.

Total Protein Assay

The total protein concentration was determined in serum using the biuret method (Lowry et al., 1951). In an alkaline medium, proteins form a blue-violet complex with copper (II) ions. The intensity of this color change, proportional to the protein concentration, can be measured using a spectrophotometer at 550 nm.

To perform this procedure, 1 mL of the reaction medium containing sodium chloride (0.15 mol/L), sodium hydroxide (0.75 mol/L), sodium potassium tartrate (6 mmol/L), potassium iodide (6 mmol/L), and copper sulfate (6 mmol/L) was added to 0.02 mL of serum. After automatic stirring and incubation at room temperature for 10 min, the absorbance was read using a spectrophotometer at 550 nm. A protein solution of known concentration (53.33 g/L) is used as a standard solution. The protein concentration is determined according to the formula:

$$\text{Protein concentration} = \frac{\text{Standard ABS}}{\text{ABS sample}} \times \text{Standard concentration}$$

ABS sample is the absorbance of the sample and Abs standard is the absorbance of the standard.

The standard protein concentration is 60 mg/L.

2.3. Statistical Analyses

Data representation and analysis were performed using GraphPad Prism 5.0 software (Microsoft USA). Differences between means were determined by ANOVA followed by Dunnett's test, $p < 0.01$ (highly significant difference) and $p < 0.05$ (significant difference).

3. Results

3.1. Effect of aqueous and hydroethanolic extracts of *Erythrococca anomala* leaves, gentamicin, and vitamin E on serum creatinine concentration

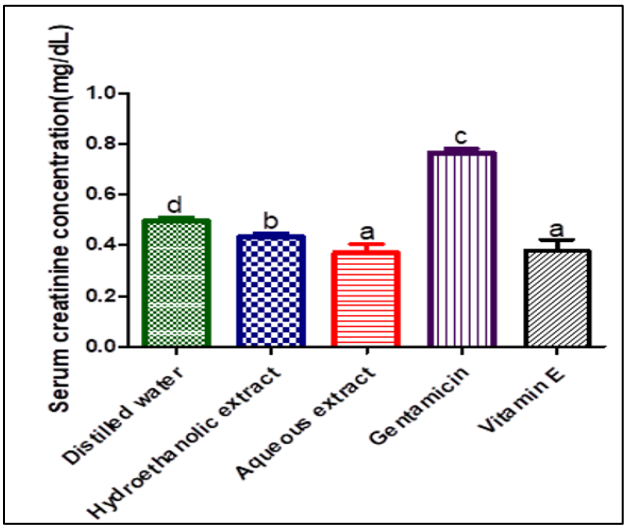
Intraperitoneal injection of gentamicin for seven days resulted in a significant increase ($p < 0.001$) in creatinine concentration in untreated rats (0.76 ± 0.17 mg/dL), compared to controls receiving distilled water (0.43 ± 0.13 mg/dL). In contrast, concomitant administration of aqueous and hydroethanolic extracts of *Erythrococca anomala*, or vitamin E, significantly reduced this concentration ($p < 0.05$ and $p < 0.01$, respectively). The recorded creatinine concentrations were 0.43 ± 0.15 mg/dL for the hydroethanolic extract, 0.37 ± 0.18 mg/dL for the aqueous extract, and 0.38 mg/dL for vitamin E. Statistical analysis shows that the effect of the aqueous extract is comparable to that of vitamin E ($p > 0.05$). However, the aqueous extract and vitamin E exhibit greater efficacy than the hydroethanolic extract in reducing gentamicin-induced toxicity ($p < 0.01$) (Figure 1).

3.2. Effect of aqueous and hydroethanolic extracts of *Erythrococca anomala* leaves, gentamicin, and vitamin E on serum urea concentration

Intraperitoneal administration of gentamicin for seven days resulted in a significant increase ($p < 0.001$) in urea concentration in untreated rats (0.69 ± 0.18 g/L), compared to the control group receiving distilled water (0.46 ± 0.12 g/L). In contrast, administration of aqueous and hydroethanolic extracts of *Erythrococca anomala*, as well as vitamin E, significantly reduced this concentration ($p < 0.05$ and $p < 0.01$, respectively). The recorded urea concentrations were 0.40 ± 0.17 g/L for the hydroethanolic extract, 0.30 ± 0.16 g/L for the aqueous extract, and 0.30 ± 0.15 g/L for vitamin E. Statistical analysis revealed that the effect of the aqueous extract was comparable to that of vitamin E ($p > 0.05$). However, a significant difference was observed between the efficacy of the hydroethanolic extract and that of the other two substances (aqueous extract and vitamin E), the latter showing a more pronounced reduction in concentration ($p < 0.01$) (Figure 2).

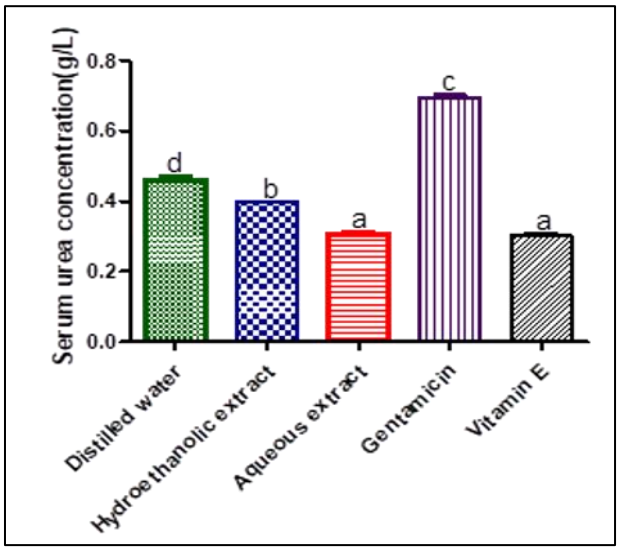
3.3. Effect of aqueous and hydroethanolic extracts of *Erythrococca anomala* leaves, gentamicin, and vitamin E on total protein concentration

Intraperitoneal injection of gentamicin for seven days caused a significant decrease ($p < 0.001$) in total protein concentration in untreated rats (48.33 ± 0.19 g/L), compared to the control group (77 ± 0.15 g/L). Conversely, administration of aqueous and hydroethanolic extracts of Erythrococca anomala, as well as vitamin E, limited this decrease by significantly increasing total protein concentration ($p < 0.05$ and $p < 0.01$, respectively). The total protein concentrations recorded were 78.33 ± 0.20 g/L for the hydroethanolic extract, 82.33 ± 0.19 g/L for the aqueous extract, and 83 ± 0.23 g/L for vitamin E. Statistical analysis indicates that the effect of the hydroethanolic extract is comparable to that of vitamin E ($P > 0.05$). However, the increase in total protein induced by these two substances is significantly greater than that observed with the hydroethanolic extract ($P < 0.01$) **(Figure 3).**



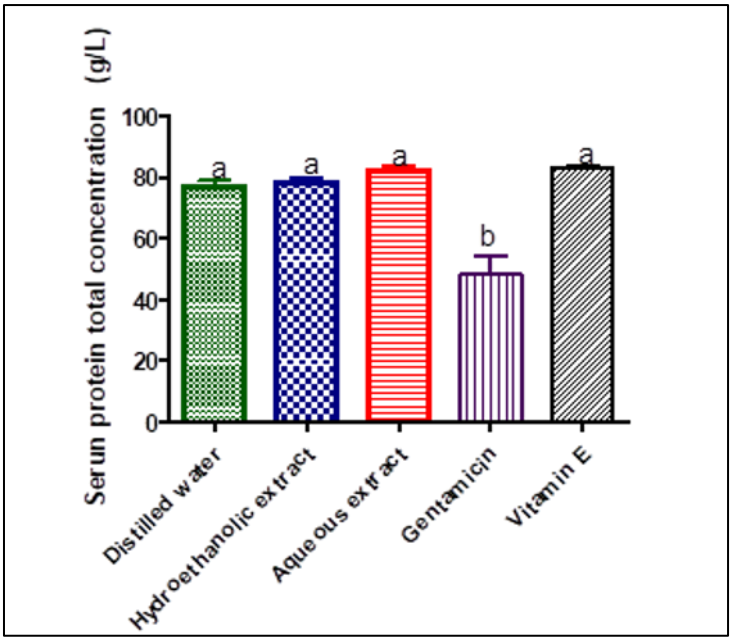
Each value is the mean ± standard deviation. Histograms with the same letter are not significantly different at the $p > 0.05$ level. However, there is a significant difference at the $p < 0.05$ and $p < 0.01$ levels between histograms with different letters. The test was performed over seven days.

Figure 1 Effect of different substances on serum urea concentration in rats intoxicated with gentamicin



Each value is the mean ± standard deviation. Histograms with the same letter are not significantly different at the $p > 0.05$ level. However, there is a significant difference at the $p < 0.05$ and $p < 0.01$ levels between histograms with different letters. The test was performed over seven days.

Figure 2 Effect of different substances on serum urea concentration in rats intoxicated with gentamicin



Each value is the mean ± standard deviation. Histograms with the same letter are not significantly different at the $p > 0.05$ level. However, there is a significant difference at the $p < 0.05$ and $p < 0.01$ levels between histograms with different letters. The test was performed over seven days.

Figure 3 Effect of substances on total serum protein concentration in rats intoxicated with gentamicin

4. Discussion

Gentamicin injection caused acute renal dysfunction characterized by a drastic increase in serum urea and creatinine levels. Since creatinine is a muscle breakdown product exclusively eliminated by glomerular filtration, its accumulation in the blood is a direct marker of a decrease in glomerular filtration rate. This nephrotoxicity is explained by the selective accumulation of gentamicin in the cells of the proximal convoluted tubule, triggering intense oxidative stress. This process leads to acute tubular necrosis and glomerular atrophy (Shahat *et al.,* 2002; Steyger *et al.,* 2003), preventing the normal reabsorption of the small amounts of protein that cross the glomerular barrier. Our results confirm those of Yaman and Balikci (2010), highlighting that the alteration of the glomerular filtration barrier is responsible for protein leakage or defective synthesis, explaining the decrease in total protein observed in our intoxicated rats. Treatment with an aqueous extract of *Erythrococca anomala* significantly restored biochemical parameters ($p < 0.05$), with efficacy comparable to that of Vitamin E (positive control). This protection is likely due to the extract's richness in antioxidant compounds, particularly polyphenols and saponins, which significantly reduce the oxidative stress generated by gentamicin. By restoring total protein levels and lowering urea and creatinine, the aqueous extract demonstrates a direct cytoprotective effect. The polyphenols in the extract act as free radical scavengers by donating electrons to the reactive oxygen species generated by gentamicin. This halts the lipid peroxidation chain reaction that destroys the mitochondrial and lysosomal membranes of nephrons (Stevens and Levey, 2005). This action is entirely comparable to that of Vitamin E, a reference lipophilic antioxidant that acts by stabilizing these membranes and improving renal perfusion (Launay, 2017). The protective effect of *Erythrococca anomala* is therefore part of a dynamic of cellular protection against toxin-induced apoptosis, aligning with the conclusions of Raju *et al*. (2011) on other plants with antioxidant potential. This ability to maintain protein homeostasis and reduce uremia positions this plant as a promising alternative for the prevention of acute kidney injury of toxic origin.

5. Conclusion

The results obtained in this study showed that the aqueous extract of *Erythrococca anomala* leaves possesses pronounced nephroprotective properties compared to the hydroethanolic extract of *Erythrococca anomala* leaves. Its nephroprotective effect was clearly demonstrated by a remarkable reduction in plasma urea and creatinine levels and an increase in total protein levels in the tested models. These results provide a scientific basis that justifies the traditional use of Erythrococca anomala leaves in the management of kidney diseases.

Compliance with ethical standards

Acknowledgments

The authors thank Mr. Ouoplé Clément (UFR of Pharmaceutical and Biological Sciences, Félix Houphouët Boigny University) for his help with the experiments.

Disclosure of conflict of interest

The authors declared no conflict of interest

Statement of ethical approval

The experimental procedures and protocols used in this study were approved by the ethics committee, Health Sciences Committee, Félix Houphouët-Boigny University. These guidelines were in accordance with those of the European Council Legislation 87/607/EEC for the protection of experimental animals. Every effort has been made to minimize animal suffering and reduce the number of animals used.

Author's contribution

All authors contributed to the conception, execution, financing of this project and its publication.

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