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Research Article

Nephroprotective Properties of Aqueous Extract of *Ficus exasperata* on Gentamicin-Induced Nephrotoxicity

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Abstract

Background: Drug induced nephrotoxicity is a major public health challenge that is increasing across the globe. *Ficus exasperata* commonly known as sand paper leaf is used in traditional medicine for treatment of many diseases; hence this study is designed to investigate the nephroprotective effect of aqueous extract of *Ficus exasperata* on Gentamicin mediated nephrotoxicity.

Materials and method: Thirty (30) adult male wistar rats, weighing 160g-200g were used for this study. The rats were divided into six (6) groups of five (5) rats each (n = 5). The rats in group (A) served as control groups and received normal saline for 14 days. Rats in Group B were administered intraperitoneally with 100mg/kg of Gentamicin for fourteen (14) days. Group C and D were administered daily with aqueous extract of *Ficus exasperata* (100mg/kg and 200mg/kg respectively) for fourteen (14) days. Group E and F were treated with extract orally for 7 days and administered with Gentamicin and extract for next seven (7) days. On day 15th, the rats were weighed and sacrificed by cervical dislocation. The kidney was harvested for histological investigation. Blood samples were collected via cardiac puncture for biochemical analysis to ascertain serum creatinine, urea level and serum protein level. Data generated from this study were analyzed using SPSS, P-value less than 0.05 was considered as statistically significant.

Results: There was significantly high level of serum creatinine and blood nitrogen urea level in group B when compared to the normal control group, this indicates a great level of kidney damage as a result of gentamicin. There was significant reduction in creatinine, urea and protein level in all the groups treated with the ficus exasperata when compared to gentamicin only treated group. Histological sections of the kidney revealed tubular necrosis in the gentamicin only treated group. However, in all the groups treated with *ficus exasperata* extract the microanatomy of the kidney showed a normal kidney histology with no sign of tubular necrosis or inflammation of the glomeruli.

Conclusion: This study indicates that aqueous extract of *Ficus exasperata* leaf extract protects the kidney against Gentamicin induced nephrotoxicity. The protective potentials of ficus exasperata extract may be attributed to high concentration of alkaloids and flavonoids

Keywords: *Ficus exasperata;* Gentamicin; Nephrotoxicity; Serum Creatinine; Blood Nitrogen Urea Level

Introduction

The kidney is an essential organ required by the body to perform several important functions including the maintenance of homeostasis, regulation of the extracellular environment, such as detoxification and excretion of toxic metabolites and drugs. Therefore, the kidney can be regarded as a major target organ for exogenous toxicants [1]. Nephrotoxicity is the most common kidney problem and it occurs when the kidney is exposed to toxins and drugs. One common adverse event that raises the risk of morbidity and leads to higher healthcare costs is drug-induced kidney injury. It is becoming more widely accepted that drug-induced nephrotoxicity plays a significant role in kidney disease, including acute kidney injury (AKI) and chronic kidney disease (CKD) [2]. The wide spectrum of nephrotoxicity reflects harm to various nephron segments depending on the specific drug mechanisms. Both glomerular and tubular injuries are acknowledged drug toxicity targets and may lead to short-term or long-term functional changes [2].

A number of therapeutics drugs can adversely affect the kidney resulting in acute and chronic kidney failure, some known therapeutics drugs that can induce nephrotoxicity are aminoglycosides, antibiotics, NSAIDs and some chemotherapeutics agents. some chemotherapeutics agents. Exposure to toxins and heavy metals like mercury, cadmium, aluminum chloride and arsenic have also been reported to cause nephrotoxicity [3-5].

The development of drugs with therapeutic benefits and fewer side effects can benefit from knowledge of the toxic mechanisms underlying nephrotoxicity. Modifications in glomerular hemodynamics, tubular cell toxicity, inflammation, crystal nephropathy, rhabdomyolysis, and thrombotic microangiopathy are a few of the mechanisms for drug-induced nephrotoxicity [6].

Gentamicin was discovered in 1963 [7] it is an aminoglycosides antibiotic that is widely used for gram- negative bacterial infections, which includes bone infection, endocarditis, pelvic inflammatory diseases, meningitis, pneumonia and urinary tract infection. Gentamicin has been reported to have serious side effects such as hearing impairment and kidney damage. Gentamicin induced nephrotoxicity characterized by tubular necrosis, basal membrane disruption, proliferation and apoptosis indicated by glomerular filtration and alteration in intra glomerular dynamics [8].

Histopathological evidence has demonstrated that administration of aminoglycosides can cause apoptosis, intracellular edema, basal membrane interruption, glomerular narrowing of the bowman capsule and tubule necrosis [9]. Gentamicin nephrotoxicity occurs in about 15-30% of treated subjects with a slow rise in serum creatinine and hypo-osmolar urinary output, developing after several days of treatment [10]. Increased oxidative damage is linked to acute kidney injury and animal studies have shown the value of various endogenous and synthetic antioxidants that reduce oxidants from both their source and their oxidation products.

The use of medicinal plants and herbs is gaining more patronage in scientific research, owing to the fact that they are readily and cheaply available in our environment. Medicinal plants are a major source of biodynamic compounds of therapeutic values, which are cheaply available and can serve as an alternative allopathic medicine. *Ficus exasperata*, also known as sandpaper leaf, has been implicated in treating a number of illnesses. The plant contains anti-oxidative, anti-inflammatory, anxiolytic, anti-diabetic and neuroprotective properties according to reported findings [11,12]. This study is designed to evaluate the nephroprotective properties of *Ficus exasperata* on gentamicin induced kidney toxicity.

Materials and Methodology

Plant collection

Fresh leaves of *Ficus exasperata* were harvested from a garden at the University of Nigeria, Enugu Campus (UNEC). The leaves were identified and authenticated at the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka with plant identification no- UNH No 202.

Preparation of plant extract

Fresh leaves *of Ficus exasperata* were washed in fresh water and air dried in the laboratory at room temperature, The air-dried leaves were milled into fine powder in an electric blender and the fine powder soaked in 2 liters of distilled water for 24 hours, filtered with Whatman No.1 filter paper (150mm), and evaporated with water bath/rotatory evaporator at 50 ◦ c to crude extract of *Ficus exasperata*. Crude extract residue was weighed and dissolved in distilled water for use on each day of the experiment; 10g of crude extract was dissolved in 100ml of normal saline to get the stock solution of extract used for daily administration.

Phytochemical analysis

Phytochemical analysis of *Ficus exasperata* leaves was done at Project Development institute (PRODA), Enugu to determine the active biochemical properties of *Ficus exasperata* leaves. Phy-

tochemical composition of the leaves was determined using the methods described by [13].

Drugs and chemicals

Gentamicin, routine histological reagent and chemicals were purchased from a pharmacy and chemical store in Enugu state, Nigeria. All chemicals were of analytic standard grade.

Experimental animals

Thirty (30) male Wistar rats weighing 160g-200g were obtained from the animal house section university of Nigeria, Nsukka. The rats were housed in cages at the animal house of the Department of Anatomy university of Nigeria, Enugu campus. The rats were acclimatized for two weeks allowed free access to standard feeds and clean water ad libitium. The environmental condition was maintained with proper ventilation and a good source of light (12h light -12h dark and 24ºC ± 30ºC).

Animal grouping/administration

The rats were randomly assigned into 6 groups of 5 rats each. The rats in group (A) served as control groups and received food pellet and water for fourteen (14) days. Rats in Group B were administered intraperitoneally with 100mg/kg of Gentamicin for fourteen (14) days. Group C and D were administered daily with aqueous extract of *Ficus exasperata* (100mg/kg and 200mg/kg respectively) for fourteen (14) days. Group E and F were treated with *Ficus exasperata* extract orally for 7 days and administered with Gentamicin and extract for next seven (7) day.

Group	Treatment
A	Normal saline (14days)
B	Gentamicin 100 mg/kg/day for 14days
C	100mg/Kg of *F.E.E* for 14days
D	200mg/Kg of *F.E.E* for 14days
E	100mg/Kg of *FEE* for 7 days + Gentamicin 100 mg/kg/day and 100mg/kg of F.E.E for the next 7days
F	200mg/Kg of *F.E.E* for 7 days + Gentamicin 100 mg/kg/day and 100mg/kg of F.E.E for the next 7days

Table 1: Showing experimental grouping and administration.

*F.E.E= *Ficus exasperate.*

Sacrifice of experimental animals

Twenty- four hours after last administration, the rats were weighed and sacrificed by cervical dislocation and incision was made through the abdominal wall. The kidney was isolated from surrounding organs, and fixed in 10% formaldehyde for histological investigations. Blood was collected via cardiac puncture using sterile syringes and needle and taken to the laboratory for biochemical analysis.

Biochemical analysis (serum creatinine, urea and serum protein level)

Blood samples were emptied into tubes and allow to clot for about 2 hours. The clotted blood was thereafter centrifuge for 10mins to recover serum. The Serum was analyzed for serum creatinine, urea level and total protein count using diagnostic kit and auto analyzer. The measurements were recorded in mg/ dl according to previously reported procedure [14].

Statistical analysis

All data obtained from this study were analyzed using statistical package for social sciences (SPSS), and result expressed as mean ± Standard deviation. Statistical difference between groups was analyzed using one way ANOVA (Analysis of variance), P-value less than 0.05 was considered as statistically significant.

Result

Phytochemical analysis

The phytochemical analysis of *Ficus exasperata* showed the different phytochemical component.

1	Alkaloids	+++
2	Anthraquinones	-
3	Flavonoids	++
4	Cardiac Glycosides	++
5	Saponins	++
6	Steroids	+
7	Tannins	+

Table 2: Showing Result of Phytochemical analysis of *Ficus exasperate.*

- +++ Very high concentration
- ++ High concentration
- + Low concentration
- - Absence.

Result of morphological/physical observation

Some morphological and physical changes were noted in Wistar rats administered with 100mg/kg of gentamicin; the following were noted:

- Weakness/Reduced activity
- Loss of body weight
- Increased volume of urine
- Difference in kidney coloration and texture – after isolating the kidney during sacrifice on day 15, our observation showed that the group administered with gentamicin throughout the period of this research showed whitish coloration which is different from the normal reddish-brown coloration. Rats treated with just extracts group throughout the period of study showed normal morphological characteristics just as those in the normal control group(A).

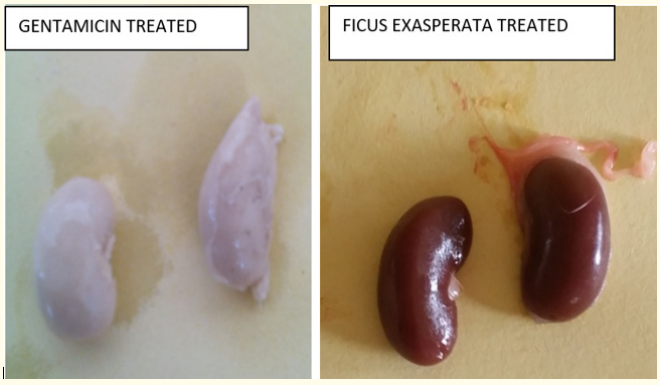

Figure 1: Showing morphological changes of kidney exposed to gentamicin vs normal kidney.

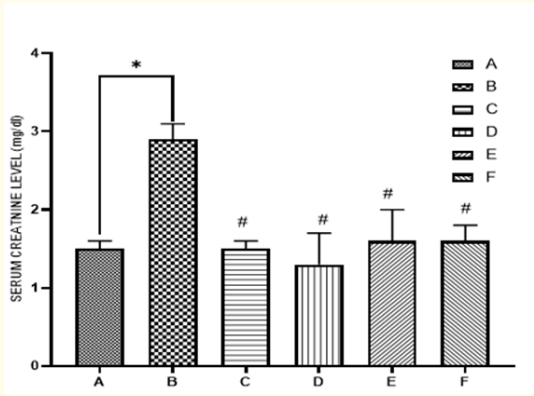

Figure 2: Showing serum creatinine level.

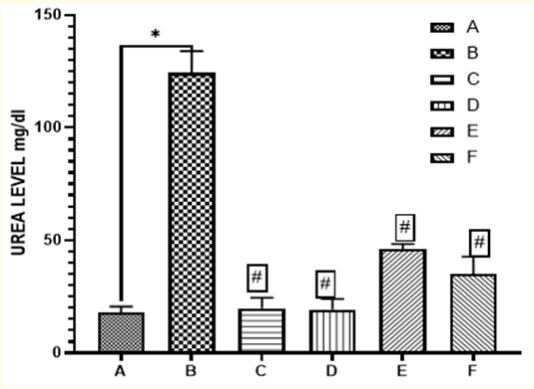

Figure 3: Showing Urea level.

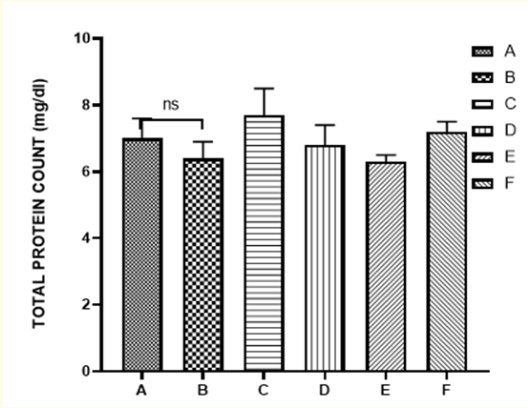

Figure 4: Serum Protein level

Figure 2-4: Role of ficus exasperate on serum creatinine, urea, and total protein level.

Values expressed as mean ±SD, P value ≤ 0.05.

*: Statistically significant when compared with normal control.

#: Statistically significant when compared with group B

ns: not statistically significant.

Figure 5 Photomicrograph of the microanatomy of the kidney of wistar rats in group A-F. Mg × 100. Stain: H and E. A: showed a normal histology with normal glomeruli (arrows) surrounded by renal tubules and interstitium (stars), B: showing extensive tubulointerstitial lymphocytic inflammation (stars), focal areas of tubular necrosis and inflammation in the glomeruli. C-F: normal histology of the normal glomeruli (arrows) surrounded by renal tubules and interstitial and a well distinct basal membrane.

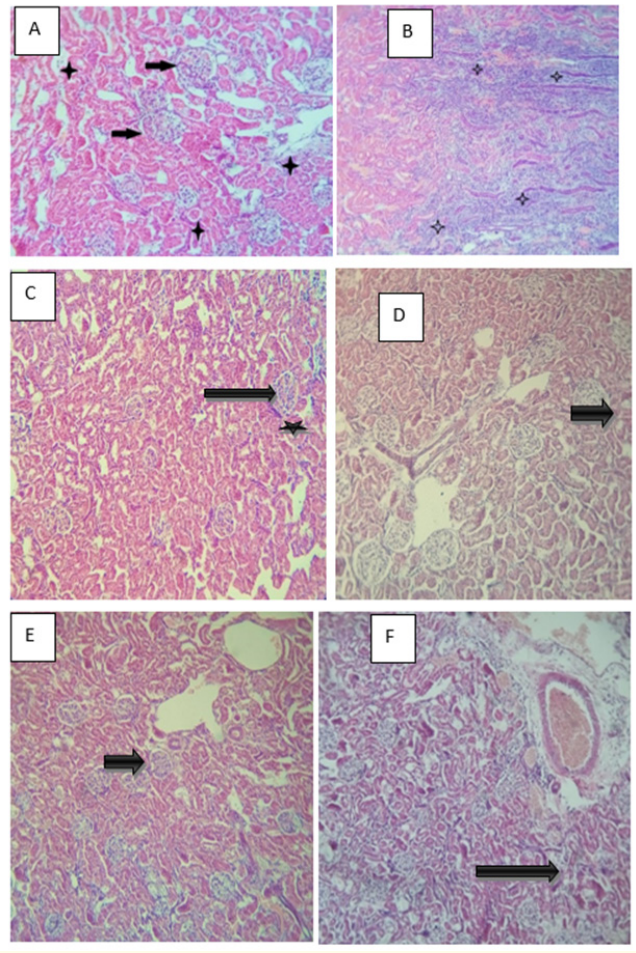

Figure 5: Effect of ficus exasperata on the histology of the kidney.

Discussion

The phytochemical analysis carried out in this study showed phytochemical result from this study showed the presence of alkaloids, saponins, flavanoids, cardiac glycosides, steroids and tannins. Alkaloids were higher than the other phytochemicals components, Saponin flavonoids and cardiac glycosides showed moderate amount while steroids and tannin showed very low amount. According to reports, the roots, stem bark, and leaves of *ficus exasperata* all contain steroids, alkaloids flavonoids, phlobatannins, tannins, and saponins [15]. This result is similar to the studies [16-19]. The high concentration of alkaloid and flavonoids in *Ficus exasperata* can be attributed to it's anti-oxidative and nephroprotective properties.

There was an elevated value of serum creatinine and urea level in the group that was administered with gentamicin only (group B) when compared to the normal control group A. The elevated level for serum creatinine and urea level observed in the gentamicin only group indicates an impairment in renal function either due to reduction of glomerular filtration rate or obstruction that interferes with urinary excretion. Serum protein level shows a decreased when compared to the normal control, however this was not statistically significant. Alteration in kidney function markers seen in the group exposed to only gentamicin has also been previously reported by previous findings [20,21]. In gentamicin-induced nephropathies, morphological changes like proximal tubular edema and tubular necrosis are seen alongside functional changes like elevated serum creatinine and urea level [22]. The decline in glomerular filtration rate and elevation of serum levels of urea and creatinine above normal levels, as well as the loss of albumin in urine, are all symptoms of gentamicin-induced renal function deterioration [22]. Administration of *ficus exasperata* only and in the groups co-treatment with *ficus exasperata* and gentamicin, results showed that there was significant decrease in the level of serum creatinine and urea. This finding correlates with previous studies [19,23].

Histological findings showed that there was tubular necrosis, inflammation of the glomeruli, glomerular atrophy, basal membrane disruption, tubulointerstitial lymphatic in the kidney of Wistar rats administered with gentamicin only, this finding is in agreement with reports which showed that gentamicin adversely alter the microanatomy of the kidney resulting in toxicity [23-25]. However, in the normal control group A, the groups (C and D) that received *Ficus exasperata* aqueous extract only (100mg/kg and 200mg/kg respectively) and the groups co-treated with *ficus exasperata* (100mg/kg and 200mg/kg respectively) and gentamicin (E and F), microanatomical investigation of the kidney showed a normal kidney with normal glomeruli and a distinct basal membrane, this implies that aqueous extract of *Ficus exasperata* is not toxic to the kidney of wistar rats.

Conclusion

In conclusion, findings from this study suggest that *Ficus exasperata* has demonstrated protective potentials against gentamicin induced renal damage, this was evident by the attenuation of serum creatinine, urea level and improvement of the microanatomy of the

kidney. The protective potentials of *ficus exasperata* extract may be attributed to high concentration of alkaloids and flavonoids.

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