Original Research Article

HYDROETHANOLIC EXTRACTS OF *FICUS PUMILA* LINN. IS PROTECTIVE AGAINST GENATAMICIN-INDUCED KIDNEY DAMAGE IN RATS

Abstract

Ficus pumila Linn. has been reported to be rich in phenols, hepatoprotective and antiproliferative on leukemic cancer cells. The aim of this study was to evaluate the nephroprotective effect of hydroethanolic leaves extracts of *F. pumila* on gentamicin-induced kidney damage in rats. Twenty seven female Wistar albino rats were divided into 9 groups (n=3). Group 1 being normal; group 2 was the gentamicin (GM) induced only (80 mg/kg b/w ip for 5 days); groups 3, 4, & 5 rats were treated with gentamicin (80mg/kg b/w ip for 5 days) and *F. pumila* extract at 100, 250, and 500mg/kg b/w orally respectively; groups 6, 7 & 8 rats received the extract only (100, 250, and 500mg/kg b/w orally) respectively and group 9 being gentamicin and silymarin (100 mg/kg b/w orally) for 21 days. Blood samples were taken 24 hrs after the experimented period and biochemical and haematological parameters were analyzed. GM nephrotoxicity was characterized by a significantly increased levels of serum creatinine, urea, sodium, potassium and WBC, while reduced RBC, HGB, MCH and MCV levels compared with normal group. Rats treated with gentamicin and the extract showed a significant reduction in the levels of these markers. The results suggest that hydro-ethanolic extract of *Ficus pumila* leaves protect against gentamicin-induced nephrotoxicity in female Wistar albino rats.

Keywords: Nephrotoxicity, *Ficus pumila* Linn. Creatinine, Urea, electrolytes

Introduction

Nephrotoxicity is known to be one of the most common kidney problems worldwide. It occurs when the body is exposed to high dosages of a drug or a toxin. Kidney damage is characterized by increased levels of serum urea and creatinine and imbalance of blood electrolytes such as potassium and magnesium [1]. Aminoglycoside antibiotics are commonly used in the treatment of bacterial infections. They have potent antibacterial activity against infections produced by gram negative bacteria [2]. Gentamicin is an aminoglycoside antibiotic isolated from the bacterium *Micromonospora purpurea*. It has a hexose ring to which various amino sugars are attached by glycosidic linkages [3]. Despite its clinical benefits, it is known to be the most nephrotoxic of all the aminoglycosides [4]. Gentamicin-induced nephrotoxicity is indicated by elevated levels of plasma creatinine and urea with severe necrosis of the renal proximal convoluted tubules followed by failure of renal functions [5]. According to Al-Majed *et al.*[6], its nephrotoxicity is as a result of the selective accumulation of reactive oxygen species in renal cortical areas leading to damage of membranes.

Some species of the Moraceae have been shown to possess significant nephroprotective activity. They include *F. religiosa* latex on cisplatin [7], *F. dalhousiae* leaf methanolic

extracts on gentamicin and acetaminophen [8], *F. carica* leaf extract on gentamicin [9], *F. racemosa* aqueous bark extract on gentamicin [10] and *F. benghalensis* latex on cisplatin [11]. *Ficus pumila* Linn. is a creeping vine-like fig plant which also belongs to the family *Moraceae*. It is native to South and east China, Malaysia, Vietnam and Africa [12]. *F. pumila* is ingested to treat conditions such as diabetes, dizziness, skin diseases and high blood pressure [13]. The hydroethanolic extract of *Ficus pumila L.* is a rich source of tannins, saponins, general glycosides, alkaloids, flavonoids, triterpenes, and sterols and has been demonstrated to be hepatoprotective in animals [14,15], and it is a potent anticancer agent. The leaves of this plant have been shown to have antioxidant, antimicrobial, anti-mutagenic, anti-inflammatory and analgesic activities [14, 16].

The aim of this study was to determine the nephroprotective effect of the 50% aqueous-ethanolic leaves extract of *Ficus pumila* Linn. in gentamicin-induced kidney damage in female Wistar albino rats.

Materials and Methods

Plant collection and authentication

The leaves of *Ficus pumila Linn*. were collected in October, 2015 from the Republic Hall, Kwame Nkrumah University of Science and Technology (KNUST) Campus. They were identified based on voucher specimen deposited at the herbarium of the Department of Herbal Medicine (KNUST, Kumasi; voucher number KNUST/HM1/2014/L093).

Extract Preparation

The plants were washed, shade-dried for a month, and milled. 50% ethanol extraction of the plants were carried out by suspending 100 grams of the powder in 1000 ml of 50% ethanol (50: 50 ethanol, water, v/v). The leaves-solvent mixtures were allowed to stand for 24 hours at room temperature on a shaker. The extracts were filtered through cotton wool and concentrated using a rotary evaporator under reduced pressure. They were transferred into sterile bottles and freeze dried to obtain the *Ficus pumila* ethanolic leaf extract (FPE). The extract was dissolved in distilled water at respective doses and used for the study.

Animal Model

The study was performed on twenty-seven female Wistar albino rats (150 – 200g). They were obtained from the SMS-UG, Accra and kept at the animal holding facility at the Department of Biochemistry and Biotechnology, KNUST-Kumasi. The animals were labeled, housed in a clean standard metal cage and had free water and standard rodent feed (Agricare, Kumasi, Ghana) *ad libitum* at room temperature. Food intake by animals was monitored daily. All animal experiments were conducted in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiment on Animals (CPCSEA, New Delhi, India) and guide for care and use of laboratory animals (Washington, US).

Experimental Drug

Gentamicin injection (Letap Pharmaceuticals, Ghana) at 80 mg/kg body weight was administered to the rats intraperitoneally (ip) from the 16^{th} -20^{th} day of treatment to induce kidney damage.

Experimental Design

The rats were divided into 9 groups with 3 animals in each group. The groups were divided as follows: Group I rats served as normal control and received 1 ml/kg b/w distilled water

throughout the duration of the experiment, Group II were injected with gentamicin, Group III, IV and V rats were treated with gentamicin and FPE (100, 250 and 500 mg/kg body weight respectively). Groups VI, VII and VIII rats were also treated with FPE only at a dose 100, 250 and 500 mg/kg body weight respectively. Group IX were treated with gentamicin and silymarin (100 mg/kg body weight). The experiment was terminated with an overnight fast at the end of 21 days. The rats were sacrificed after mild ether anesthesia. Incisions were made in the cervical region of the animals and blood samples were taken for biochemical and haematological analysis.

Effect of Treatment on Body Weight

Body weight of the rats were taken every two days and percent change in body weight calculated with the following formula:

$$Percent\ Chnage\ in\ Body\ Weight = \frac{Weight_n - Weight_{initial}}{Weight_{initial}} \times 100$$

where $Weight_n$ is the body weight on Day 4, D8 …. D21 and $Weight_{initial}$ is the body weight on D0

Effect of Treatment on Kidney Weight

The kidneys of sacrificed animals were excised, washed in buffered saline and blotted with paper tissue. They were weighed to obtain the absolute organ weight (AOW). The Relative Organ Weight (ROW) was calculated with the following formula:

$$Relative\ Organ\ Weight\ (\%) = \frac{Absolute\ Organ\ Weight}{Body\ Weight\ at\ Sacrifice} \times 100$$

Assessment of Kidney Function

The blood samples were collected into clean sterile tube and left to stand for an hour and centrifuged at 3000g for 15 minutes at 5^0C to separate the serum for biochemical analyses which included urea, creatinine, electrolytes, cholesterol, fasting blood glucose, alanine aminotransferase (ALT) and total protein using the Cobas Integra Autoanalyser and kits (Fortress Diagnostics, UK).

Haematological Analyses

Part of the blood sample was placed in EDTA tubes for haematological analyses which included red blood cell (RBC), hemoglobin (HGB), hematocrit (HCT), mean cell hemoglobin concentration (MCHC), mean cell volume (MCV) and platelets (PLT) count using the Sysmex KX21N autoanalyzer to run a full blood count in the whole blood mode.

Statistical Analysis

Data was analysed using GraphPad Prism 5 for windows. The results were expressed as the Mean ± Standard error mean (SEM). One – way Analysis of variance followed by Newman-Keuls multiple comparison test was used for comparison between groups (i.e. control and treated groups). All statistical tests were run at a 95% confidence interval and values of $P< 0.05$ were considered statistically significant. Percentage protection was calculated with following formula based on significant indicators of nephroprotection including urea, creatinine,

$$\text{Percent Protection} = 100 * \frac{\text{Values of Toxin Contol} - \text{Values of Test sample}}{(\text{Values of Toxin Control} - \text{Values of Normal Control})}$$

Results

Effect of treatment on body weight

Table 1 shows the effect of treatment on the body weight of the rats. There was a reduction in the body weight of rats treated with gentamicin only compared with the normal. However, the body weight of groups treated with plant extract only was almost the same as the normal but comparing the body weights of groups treated with gentamicin and plant extract at varying concentration to the gentamicin only group, a decrease was observed.

Effect of treatment on relative kidney weight

Figure 1 shows the effect of the treatment of FPE on relative weight of the kidneys. Administration of FPE and GM to the animals did not provoke any significant increase in the relative kidney weights.

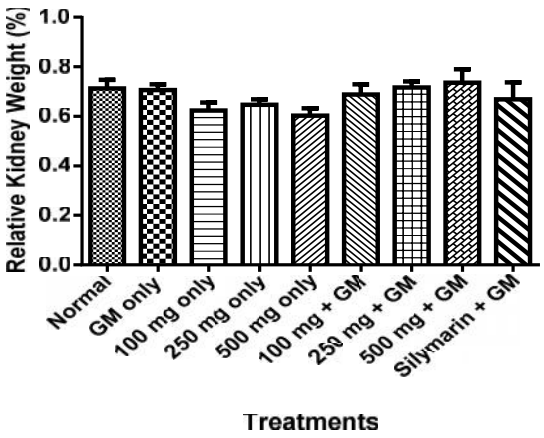

Fig. 1: Effect of treatment on kidney weight. Each column represents a mean ± SEM.

Effect of treatment on some biochemical parameters

Table 2 shows the biochemical data obtained for the normal and treated rats. The rats to which GM only was administered showed a significant increase in the blood urea, serum creatinine, total protein and fasting blood sugar levels and a decrease in ALT levels compared to the normal. Those parameters however, had reduced levels in the groups that were treated with FPE and GM suggesting nephroprotection, while GM significantly reduced the serum potassium, sodium and chloride levels as compared to normal. The electrolyte levels were however significantly increased in the treated groups.

Effect of treatment on haematological parameters

Table 3 shows the effect of treatment on some hematological parameters. There were no significant changes in the haematological parameters assayed excepted a significant increase in animals treated with both GM and extract.

Percentage Protection

Fig. 2 shows the percent protection of extract alone and with GM on the kidney. The extract at all doses protected the kidney (94-99%). With GM, only the 250 mg/kg showed a good protection of 58%.

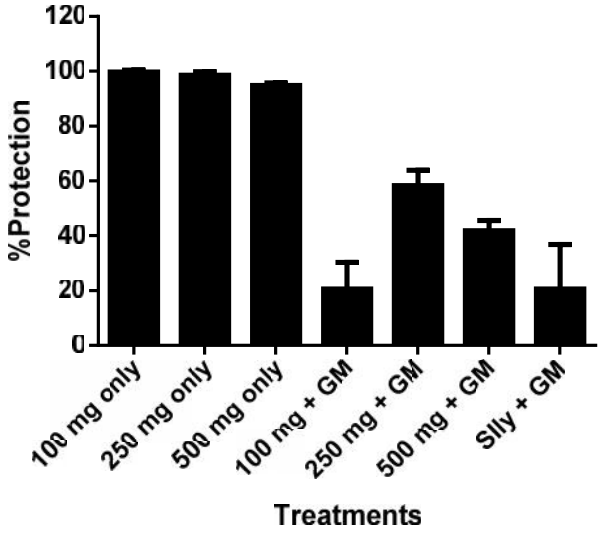

Fig. 2: Effect of treatment on percent liver protection

DISCUSSION

Owing to the increasing kidney disease burden annually and the high cost of treatment, there is the need to develop new therapies to overcome these challenges. Therefore, in this study, the nephroprotective effect of the aqueous-ethanolic leaves extract of *F. pumila* Linn. was investigated. Administration of gentamicin (80 mg/kg b/w ip) for 5 consecutive days caused marked nephrotoxicity as is evident from Table 2, showing significant increase in serum creatinine (332.80 mg/dL ±12.96 mg/dL at $p< 0.0001$) and serum urea (261.50 mg/dL ± 26.32 mg/dL at $p<0.0001$) compared with normal serum creatinine (34.60 mg/dL ± 2.428 mg/dL) and urea (59.12 mg/dL ± 2.43 mg/dL). The elevation of the serum creatinine is produced by kidney damage, which lead to a decreasing glomerular filtration rate (GFR) and serum creatinine filtration. The increase in the serum creatinine levels in the gentamicin (GM) treated group is due to decreased GFR caused by the gentamicin [17]. The gentamicin nephrotoxicity was significantly protected in groups treated with gentamicin and the FPE and the 250mg + gentamicin group reduced the urea and creatinine levels even better than the Silymarin (test drug used). The results thus indicated that FPE is effective in reducing serum creatinine and urea level in gentamicin toxicity. According to Larbie *et al*. [14], the hydroethanolic extract of FPE had significant antioxidant activity and contains tannins, saponins, general glycosides, alkaloids, flavonoids and triterpenes. The nephroprotective effects of FPE in GM-induced nephrotoxicity may be due to flavonoids and tannins present in the extract. These findings are in accordance with those reported earlier in which *Ficus carica* fruit extract caused marked reduction in serum urea and creatinine levels in GM-induced nephrotoxicity [18]. Serum potassium, chloride and sodium were significantly reduced in groups treated with gentamicin only compared with normal which indicated kidney damage since the kidneys are involved in osmotic and ion balance in the body, therefore an imbalance in serum electrolytes was indicative of kidney damage [19]. The effects induced by GM were

significantly prevented by FPE which further buttress the fact that this plant has the potential to be used to ameliorate gentamicin nephrotoxicity. Again FBG and total protein increased while ALT decreased in groups treated with gentamicin only compared with normal. This can also be attributed to the fact that gentamicin is known to be nephrotoxic rather than hepatotoxic.

There was observed decreases in RBC indices (HCT, MCH, MCHC, PLT and HGB) in rats treated with GM only as compared to the normal, possibly indicating an impairment of kidneys because at normal conditions the kidneys produce enough of erythropoietin for the production of red blood cell [19]. On the other hand, the aqueous ethanolic extract of the leaves of *Ficus pumila* was able to increase the levels of these parameters upon treatment. This protection may be because the plant extract was able to increase the production of erythropoietin to enhance the production of red blood cells in the bone marrow.

Balakumar *et al*. [20] revealed that gentamicin in the cytosol acts on mitochondria directly and indirectly to activate the intrinsic pathway of apoptosis, interrupts the respiratory chain, impairs ATP production and causes oxidative stress by increasing superoxide anions and hydroxyl radicals which further contribute to cell death. This means that gentamicin administration enhances the production of free radicals indicating oxidative damage at the cellular level of the renal cortex. Other manifestations of gentamicin nephrotoxicity include electrolyte imbalance and water and non-electrolyte transport in a variety of cells and tissues, the principal target organ being the kidneys. Flavonoids, one of the phytochemical constituents of the leaves of *Ficus pumila* Linn. has been reported to show strong antioxidant activity [14]. This may account for the mechanism of the nephroprotective effect of *Ficus pumila*. In addition, the extract was observed to restore electrolytes to near normal levels in treatment group. Summarizing all these facts, it can be said that these phytoconstituents are responsible for the observed biological protective effect in this study.

CONCLUSION

In conclusion, this study gives the experimental evidence that the aqueous ethanolic extract of the leaves of *Ficus pumila* Linn. was able to produce considerable protection from the nephrotoxic action of gentamicin in female Wistar rats. Further studies will be required to understand the mechanism of protection and also its protective effect against other nephrotoxic agents.

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Table 1: Effect of treatment on body weight of the rats. Each point represents a mean ± SEM of 3 animals

Days	*Normal*	*GM*	*100 mg FPE*	*250 mg FPE*	*500 mg FPE*	*GM+100 mg FPE*	*GM+250 mg FPE*	*GM+500 mg FPE*	*GM + Sily*
D0	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
D2	6.75±1.40	2.94±1.16	4.18±0.32	3.10±1.57	3.81±0.74	-0.25±0.87	-0.28±1.62	0.68±1.18	1.52±0.43
D4	9.48±1.32	7.01±0.40	4.20±1.33	3.70±1.07	4.36±0.58	-0.18±2.80b	0.91±0.60b	1.61±0.47b	3.48±0.60
D6	13.23±0.64	7.63±0.64	11.64±0.46	6.17±1.52	5.73±1.01	2.96±1.13	0.19±1.41	3.44±0.69	8.23±0.90
D8	12.87±1.14	8.32±1.58	11.03±1.52	5.90±2.59	8.15±1.62	3.72±1.35	3.20±1.18	4.82±1.05	8.02±0.51
D10	17.98±1.43	10.42±0.85	15.22±0.45	8.44±4.01b	8.45±1.43b	5.67±1.78b	3.65±1.76b	5.04±0.88b	9.97±0.75b
D12	20.69±1.25	10.45±1.80b	15.52±0.30	8.70±3.71b	9.24±1.51b	3.73±1.89b	1.36±1.04b	3.22±0.61b	8.90±0.28b
D14	24.12±2.88	11.48±0.49b	19.41±0.88	10.90±1.02b	7.92±2.37b	7.62±1.72b	3.88±1.84b	6.42±0.57b	10.40±1.67b
D16	26.47±1.44	12.35±1.27b	23.01±1.48	14.27±2.47b	10.40±3.60b	10.12±3.27b	6.90±1.36b	8.48±1.39b	11.51±0.86b
D18	30.21±2.34	11.69±0.70b	25.39±1.19	13.71±1.91b	13.89±1.78b	7.68±3.48b	3.41±1.27b	6.86±1.94b	10.83±1.26b
D20	36.31±2.71	16.35±1.37b	28.96±1.66	17.91±1.95b	15.75±3.10b	10.40±2.96b	4.44±2.08b	12.39±1.06b	11.94±1.73b
D21	37.33±2.41	16.96±1.51b	28.96±1.36b	16.80±2.81b	16.84±3.37b	11.13±2.98b	9.93±2.38b	13.29±1.46b	12.81±1.94b

b-Significant difference from Normal at $p<0.05 - 0.001$

Table 2: Effect of FPE on biochemical parameters in gentamicin induced nephrotoxicity.

	TREATMENT								
PARAMETERS	Normal	GM only	100 mg only	250 mg only	500 mg only	GM+ 100 mg	GM+ 250 mg	GM+ 500 mg	GM+Sily
Creatinine µmol/L	34.60±2.428	332.80±12.96a	37.38±0.72	42.79±1.93	47.40±2.00	300.20±27.89ab	175.40±38.21ab	218.80±33.87ab	319.40±22.82ab
Urea mg/dL	59.12±2.43	261.50±26.32a	58.28±3.28	59.33±3.42	71.27±7.73	200.00±4.00ab	132.30±16.65ab	169.40±187.00ab	187.00±5.87ab
ALT U/L	70.87±5.09	47.03±3.65	61.83±8.09	53.13±4.77	48.50±3.96	41.93±4.66	47.97±2.41	35.33±6.59	52.50 ±5.47
FBG mg/dL	84.57±2.18	117.50±15.06	93.60±98.10	98.10±1.89	102.1±4.24	95.60±9.00	97.93±6.20	84.10±8.13	108.00±14.35
Chloride g/dL	129.00±25.51	122.70±35.05	99.33±5.67	105.80±2.21	127.70±11.20	100.00±1.16	122.70±13.62	194.70±22.67	96.00±5.03
Potassium g/dL	6.23±1.11	4.77±0.79	2.83±0.68	7.10±0.83	4.80±0.85	6.30±0.10	9.33±1.27b	3.32±0.52	8.17±0.67
Sodium g/dL	200.80±2.91	97.33±8.09	109.40±7.54	127.30±13.12	118.70±4.43	129.00±5.56	150.70±7.96	76.33±6.56a	80.33±9.28

a Significantly different from Normal ($p<0.05 – 0.001$); b Significantly different from GM only ($p<0.05 – 0.001$)

Table 3: Effect of treatment on some haematological parameters

	TREATMENTS								
PARAMETERS	Normal	GM only	GM +100 mg	GM + 250 mg	GM + 500 mg	100mg	250mg	500mg	GM + Sily
WBC*10^3/µL	6.30±2.16	7.70±0.55	7.50±2.20	10.57±0.62	5.800±1.18	5.13±0.17	5.67±0.96	7.23±132	6.73±0.47
RBC*10^6/µL	6.76±0.30	6.79±0.19	6.80±0.27	6.59±0.21	6.79±0.33	7.25±0.15	7.25±0.06	7.39±0.27	6.29±0.36
HGB g/dL	10.83±2.42	9.67±0.22	12.77±0.38	12.37±0.28	12.93±0.54	13.80±0.06b	13.53±0.28b	13.67±0.38b	12.50±0.55
HCT %	38.60±1.10	37.57±0.67	37.23±1.07	35.90±1.11	38.13±1.92	41.63±0.59	40.23±0.18	40.50±1.16	35.40±1.89
MCH pg	57.20±0.95	55.37±0.77	54.83±0.62	54.53±0.09	56.17±1.92	57.40±0.49	55.47±0.73	54.80±0.49	55.36±0.56
MCV /fL	18.77±3.03	15.63±0.28	18.80±0.20	18.800±0.23	19.07±0.20	19.03±0.48	18.67±0.54	18.47±0.24	19.57±0.35
MCHC g/dL	33.73±5.62	27.73±0.03	34.30±0.06	34.47±0.35	33.97±0.43	33.03±0.64	33.63±0.58	33.77±0.20	35.37±0.86

PLT* 10^3/µL	900.00±2221.99	859.33±253.92	1295.67±141.14	1240.33±187.15	1181.67±52.32	1220.33±264.71	1331.67±190.19	1290.00±47.82	1331.00±87.32

b Significantly different from GM only ($p<0.05 – 0.001$)