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Assessment of Renal and Cardiovascular Toxicity of Pefloxacin (an Antibiotic) in Albino Rats

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Abstract

Background: Pefloxacin is a fluoroquinolone antibacterial agent which possesses broad activity against both gram-positive and gram-negative bacteria. Several reports have revealed organs/tissues toxicity of some antibiotics. Hence, the present study was designed to monitor some biomarkers of cardiovascular and renal systems in albino rats administered pefloxacin, an antibiotic drug.
Methodology: Twenty-four adult male albino rats, divided into four groups (A, B, C and D, of six rats in each group), were used in this research. Group D was the control group. Groups A, B, and C were administered (through oral intubation) 5.7, 11.4 and 17.1 mg/kg body weight of pefloxacin solution respectively for seven consecutive days.
Results: At the end of the administration, there was a decrease in feed and water intake accompanied by a decrease in physical activities and body weights in the test animals compared to the control. There was also a significant ($P<0.05$) increase in serum levels of creatinine, urea, uric acid total cholesterol, triglycerides, low density lipoprotein, cardiac troponin I, creatine kinase and lactate dehydrogenase, while high density lipoprotein decreased significantly ($P<0.05$) in the groups administered the drug compared to the control. These effects of pefloxacin were observed to be dose-dependent.
Conclusion: The findings of this research are indicative that the antibiotic may be toxic to the renal and cardiovascular systems. Thus, it should be avoided by patients having any disorder related to the renal and cardiovascular systems. .

Keywords: Antibiotics, nephrotoxicity, cardiotoxicity, Albino rats.

1.0 Introduction

Antibiotics are probably one of the most successful forms of chemotherapy in the history of medicine. They have been found effective in management and treatment of microbial infections. Antibiotics have saved many millions of lives and placed the majority of infectious diseases that plagued human history for many centuries under control [1].

.Pefloxacin, a fluorinated 4-quinolone, has recently been advocated as a first-line treatment for minimal-change nephropathy or focal segmental glomerulosclerosis with broad spectrum antibacterial activity against a wide range of Gram-negative and Gram-positive bacteria [2][3]. Pefloxacin, a preferred option to chloramphenicol in the treatment of typhoid and other bacterial infections, has proven to be quite effective. However the drug has been implicated in several toxic actions on the muscle, tendon, synovial membrane, blood etc [4]. Fluoroquinolones are generally considered well tolerated, although quinolone induced

chondropathy has been observed in young animals of several species [5].

The renal system, also called the urinary system is a group of organs in the body that filters out excess fluid and other substances from the bloodstream [6]. The renal system organs include the kidneys, ureters, bladder, and urethra. The renal system is the most powerful regulator of the body's internal environment. Metabolic wastes and excess ions are filtered out of the blood along with water, and leave the body in form of urine [7]. The renal system has many functions which are interrelated with the physiological mechanisms in the cardiovascular and respiratory systems [6]. These functions include; removal of metabolic waste products from the body (mainly creatinine urea and uric acid), regulation of electrolyte balance (e.g., sodium, potassium, and calcium), osmoregulation controls the blood volume and body water contents, blood pressure homeostasis, regulation of acid-base homeostasis and blood pH, a function shared with the respiratory system [8]. Excretion may expose the vasculature, tubules and interstitial tissues to very high concentrations of these substances. The kidneys therefore, quite commonly suffer adverse effects of drug therapy. Commonly used drugs which can affect renal functions, according to Razzaque[9], includes; diuretics, beta-blockers, vasodilators, aminoglycosides, compound analgesics, antiviral agents and lithium. Several chemical compounds have been reported to exhibit renal toxicity[10]. An examination of increase or decrease in the levels of creatinine, uric acid and urea in the body will result in the determination of toxicity of any drug[11].

The cardiovascular system comprises the heart and blood vessels. The heart is a muscular organ in both humans and other animals, which pumps blood through the blood vessels of the circulatory system [12]. Blood provides the body with oxygen and nutrients, and also assists in the removal of metabolic wastes [13].

The enzyme (EC 2.7.3.2) known as creatine kinase (CK), often referred to as creatine phosphokinase (CPK) or phosphocreatine kinase, is present in a range of tissues and cells. CK catalyzes the conversion of creatine to phosphocreatine (PCr) and adenosine diphosphate (ADP) using adenosine triphosphate (ATP). Creatine kinase is measured in blood tests as a sign of CK-rich tissue damage in conditions such as myocardial infarction (MI) (heart attack), rhabdomyolysis (severe muscle breakdown), muscular dystrophy, autoimmune myositides, and acute kidney injury [14][15]. Creatine kinase MB isoforms (CK-MB) levels can also be used to detect MI because an elevated CK-MB level is linked to myocarditis and electrical cardioversion [16]. The troponin complex is the inhibitory or contractile regulating protein complex of striated muscle. It is located periodically along the thin filament of the muscle and consists of three distinct proteins: troponin I, troponin C, and troponin T[17]. Cardiac troponin I (CTnI) has been useful in the differential diagnosis of patients presenting to emergency departments (ED) with chest pain [18]. Myocardial infarction is diagnosed when blood levels of sensitive and specific biomarkers, such as cardiac troponin, the MB isoenzyme of creatine kinase (CK-MB), and myoglobin, are increased in a clinical setting of acute ischemia [19]. Lipid profile or lipid panel of blood serves as an initial broad medicine screening tool for abnormalities in lipid such as cholesterol and triglyceride [20]. The result of this test can identify certain genetic disease and can determine approximate risk for cardiovascular disease. Lipid panel are commonly ordered as part of a physical examination along with other panel such as the complete blood count (CBC) and basic metabolic panel (BMP). The lipid profile typically includes low-density lipoprotein (LDL), High-density lipoprotein (HDL), Triglycerides and total cholesterol. Lactate dehydrogenase (LDH) is an oxidoreductase (EC 1.1.1.27) present in a wide variety of organisms. It catalyzes the inter-conversion of pyruvate and lactate with concomitant inter-conversion of NADH and NAD. When disease or injury or toxic material damages tissues, cells release LDH into the bloodstream. Lactate dehydrogenase is widely distributed in mammalian tissues, being rich in myocardium, kidney, liver and muscle. Determination of serum LDH activity is one of the most frequently performed assays as an aid

in the diagnosis of myocardial and pulmonary infarction [21].

The present research investigated the effect of pefloxacin (an antibiotic) on the cardiovascular and renal systems of albino rats by measuring the serum CTnI, CK-MB, lipid profile, creatinine, uric acid and urea levels after administration with the drug solution.

2.0 Materials and Methods

The equipment/instruments used in the research were of good working conditions. Similarly, the reagents/chemicals used were of analytical grade.

2.1 Collection of Animals.

Twenty-four adult male albino rats were purchased from the Zoology department, University of Nigeria, Nsukka (UNN) and transferred to the animal house, department of Biochemistry Ebonyi state University, Nigeria in steel cages.

2.2 Collection of Drug Sample.

One pack of pefloxacin was purchased from Clanol pharmacy, a drug store in Abakaliki metropolis, Ebonyi state, Nigeria.

2.3 Preparation of Drug Solution.

10 tablets were dissolved in 200 ml of distilled water to obtain 20mg/ml of drug solution.

2.4 Experimental Design.

The animals where group into four (4) cages A, B, C and D respectively each cage contained six (6) rats. Doses of 5.70 (underdose), 11.40 (manufacturer's recommended dose) and 17.10 mg/kg (overdose) body weight of drug solution were administered orally once a day to the rats in groups A, B and C respectively, for seven consecutive days using a 2ml syringe. Group D served as control. All the animals were allowed free access to feed and water throughout the experiment.

2.5 Collection of Blood from the rats.

After seven (7) days of drug administration, the rats where starved overnight and fresh blood was collected into plain bottles via cardiac puncture under mild anesthesia, using chloroform.

2.6 Measurement of Parameters.

Serum levels of CTnI, CK-MB and lipid profile were measured by the methods of Etievent *et al.*[22], Steen *et al.*[23] and Tietz, [24] respectively. Serum concentrations of creatinine, urea and uric acid were determined by the methods of Handy *et al.*[25], Rosenthal [26] and Trivedi *et al.*[27] respectively.

2.7 Statistical Analysis.

The generated data were presented as mean ±standard deviation. Means were compared using analysis of variance (ANOVA). $P < 0.05$ was regarded as significant.

3.0 Results

3.1 Physical Observations

During the seven days of administration, a decrease in feed and water intake, and also in physical activities was observed in the animals treated with the drug solution. There were no visible changes noticed in the control group.

3.2 Changes in Average Body Weight (g) of the Animals During Seven Days of Drug Administration

The result on changes in average body weight of the rats during seven days of drug administration is presented in table 1. There was a significant ($P < 0.05$) decrease in the weights of the test animals during the seven days of drug administration relative to the control.

3.3 Average Creatinine, Urea and Uric Acid Levels in the Serum of the Albino Rats after Seven Days of Drug Administration:

The average creatinine, urea and uric acid levels in the serum of the animals after seven days of drug administration is shown in table 2. After the seven days of administration with the drug solution, it was observed that the values of creatinine, urea and uric acid increased significantly ($P < 0.05$) in the test animals when the compared to that of the control group D. From the table, the uric acid level of group A did not differ significantly ($P > 0.05$) from the control group D. All the other values of the

measured parameters for the test group differed significantly (P< 0.05) from the control values.

Table 1: changes in the average body weight (g) of test animals during the period of administration.

DOA	GROUP A	GROUP B	GROUP C	GROUP D
1	142.27 ±5.62	154.22 ±7.23	148.68 ±7.54	126.55 ±7.32
2	136.33 ±7.28	149.64 ±5.98	139.87 ±6.91	129.47 ±4.65
3	131.32 ±4.44	141.98 ±5.86	133.78 ±7.11	135.53 ±7.23
4	129.45 ±6.46	134.25 ±6.51	123.97 ±6.44	137.54 ±3.87
5	124.88 ±6.85	128.55 ±5.33	119.88 ±4.65	141.31 ±9.12
6	121.34± 7.14	123.75 ±8.12	111.04 ±4.22	145.26 ±6.43
7	114.56 ±4.67	116.45 ±7.55	98.76 ±6.35	151.36 ±6.54

Values are mean ± SD; n = 6.
Key:
DOA = Days of administration
Group A = 5.70 mg/kg
Group B = 11.40 mg/kg
Group C = 17.10 mg/kg
Group D = Distilled water.

Table 2: Mean levels of creatinine, urea and uric acid of the albino rats after seven days of drug administration.

GROUPS	Urea (mg/dl)	Creatinine (mg/dl)	Uric Acid (mg/dl)
A	26.89±2.14^{a}	3.52±0.59^{a}	1.85±0.41^{a}
B	39.16±2.56^{b}	5.09±0.62^{b}	2.64±0.29^{a}
C	55.27±3.05^{c}	8.21±0.76^{c}	3.86±0.52^{a}
D	14.39±1.15^{d}	1.47±0.28^{d}	0.91±0.12^{b}

All values are mean ± standard deviation; n = 6.
Means in the same column with different superscripts differ significantly (P < 0.05).
Key:
Group A = 5.70 mg/kg
Group B = 11.40 mg/kg
Group C = 17.10 mg/kg
Group D = Distilled water

3.4 Mean serum lipid profile and levels of CK-MB, LDH and CTnI in the rats after seven days of drug administration:

The results on serum lipid profile and levels of CK-MB, LDH and CTnI in the rats after seven days of drug administration are presented in tables 3 & 4. There was a significant (P< 0.05) increase in concentrations of total cholesterol (TC), triacylglycerol (TAG) and low density lipoprotein cholesterol (LDL), while high density lipoprotein cholesterol (HDL) decreased significantly (P<0.05) in the groups administered the drug when compared with the control. The activity of CK-MB and LDH recorded in test groups was significantly (P< 0.05) higher than in the control. The serum concentration of CTnI in the test groups also increased significantly (P< 0.05) relative the control. These effects the cardiovascular parameters were found to increase significantly (P< 0.05) as the administered dose increased.

Table 3: Mean serum lipid profile, CK-MB, LDH and CTnI in the rats after seven days of drug administration

GROUPS	TC (mg/dl)	TAG (mg/dl)	LDL (mg/dl)	HDL (mg/dl)
A	219.25±3.63^{a}	126.57±1.56^{a}	68.75±5.87^{a}	56.88±4.10^{b}
B	261.22±1.67^{b}	154.12±4.98^{b}	86.80±7.96^{b}	47.90±2.45^{c}
C	275.38±2.32^{c}	191.65±3.78^{c}	118.75 ± 6.10^{c}	28.75±3.96^{d}
D	132.45±3.58^{d}	89.52±4.24^{d}	45.73±4.19^{d}	81.13±6.24^{a}

All values are mean ± standard deviation; n = 6. Means in the same column with different superscripts differ significantly (P < 0.05).
Key:
Group A = 5.70 mg/kg
Group B = 11.40 mg/kg
Group C = 17.10 mg/kg
Group D = Distilled water

Table 4: Serum CK-MB, LDH and CTnI levels of the rats after seven days of drug administration

GROUPS	CK-MB (U/l)	LDH (U/l)	CTnI (ng/ml)
A	197.75±11.25^{c}	271.42±12.07^{c}	1.56±0.42^{c}
B	229.42±9.36^{b}	322.62±8.09^{b}	2.15±0.55^{b}
C	272.44±10.46^{a}	396.19±11.21^{a}	3.27±0.86^{a}
D	161.21±7.89^{d}	242.11±11.25^{d}	0.82±0.20^{d}

All values are mean ± standard deviation; n = 6. Means in the same column with different superscripts differ significantly (P < 0.05).
Key:
Group A = 5.70 mg/kg
Group B = 11.40 mg/kg
Group C = 17.10 mg/kg
Group D = Distilled water

4.0 Discussion

The major biochemical events underlying the observed reduction in the physical activity, feed and water intake is not well understood at this stage of research. However, these changes may be as a result of the chemical constituents of pefloxacin, an antibiotic drug administered to the test animals. The drug may have caused a distortion in general metabolism of the animals. Loss of appetite has widely been indicated as one of the physiological effects of antibiotic and analgesic drugs [10][28][29].

Additional research is necessary to ascertain the exact biochemical mechanism responsible for the observed reduction in the mean body weight of the test animals during the period of administration. Nonetheless, this decrease in body weight may be ascribed to the reported decrease in feed and water intake caused by the administration of the drug solution. This finding is similar to the work of Agbafor *et al.*[10], where decrease in the body weight of test animals (albino rats) treated with ciprofloxacin (an antibiotic) was reported.

There was a significant (P<0.05) increase in creatinine, urea and uric acid levels. The actual reason for the elevated values cannot be stated at this stage of the research. However, it may be attributed to the failure of the renal system. Nicloau *et al.*[11] reported that when there is a high level of urea in the blood stream, a condition referred to as hyperuremia occurs which indicates the failure of the renal system. Thus, the elevated levels of urea in the test groups compared to the control group may be as a result of the drug solution administered to the test animals which maybe toxic to the renal system. An elevation in the levels of serum uric acid has also been reported by Heinig and Johnson [30] to be linked to a disorder of the renal system. Therefore, the increase in the uric acid levels of the test animals administered with the drug solution compared with the control group may be attributed to the effect of the drug on the renal system of the test animals. Maxwell [31] reported that a rise in blood serum creatinine level is associated with damage to functioning nephrons of the kidney. Therefore, the reported elevation in the creatinine levels of the test animals compared to the control group may be as a result of the effect of the drug solution administered on the renal system of the test animals. Several chemical compounds have been reported to exhibit renal toxicity. Thus, the suspected renal failure may be due to the toxicity of pefloxacin. This finding is consistent with the work of Agbafor *et al.* [10].

The exact biochemical mechanisms responsible for the reported significant (P< 0.05) elevation in serum levels of total cholesterol, triacylglycerol (TAG), low density lipoprotein-cholesterol, CTnI, activity of CK-MB and LDH, and significant (P < 0.05) reduction in level of high density lipoprotein-cholesterol are still obscure. However, the observations suggest that the drug may be toxic to the cardiovascular system. The lipid profile indices are useful in monitoring health status of the cardiovascular system. Elevated levels of total cholesterol, TAG and LDL-C may predispose to cardiovascular related disorders [32][33]. Increased level of LDL-C has been associated with higher risk of atherosclerosis while elevated level of HDL-C is linked to reduced occurrences of cardiovascular disorder [34]. Decreased serum level of LDL- cholesterol has been associated with reduced risk for cardiovascular diseases [35]. Similarly, increased serum concentration of HDL- cholesterol has been associated with reduced risk for cardiovascular diseases. Increased in the activities of LDH are associated with myocardial infarction, and the degree of elevation is of value in assessing the extent of damage and in developing a prognosis [36]. Lactate dehydrogenase activity elevations are also observed in some cases of renal disease and skeletal muscle trauma [36]. Elevation in the activity of serum CK-MB observed in the groups induced with doxorubicin could be due to injury to cardiac muscle as a result of

oxidative stress. An earlier report indicated that such elevations are noticed in cases of both myocardial infarction and other cardiovascular diseases [37]. Cardiac troponin I is not normally present in the serum unless cardiac cell necrosis has occurred. Elevated cardiac troponin I levels in the blood would have associated with an area of damaged heart muscle due to myocardial infarction [38]. Some studies have shown that cardiac troponin levels act as a specific and sensitive indicator of myocardial infarction [39]. The effect of pefloxacin on the test animals were observed to be dose dependent as the groups treated with the higher dose were observed to have more changes. This corresponds with the general principle of drug effect; the higher the dose, the higher the effect. Thus, toxicity, in general, is dependent on exposure.

5.0 Conclusion

The result from the research has shown that there was a significant negative effect on the measured renal parameters: creatinine, urea and uric acid, and cardiovascular parameters: lipid profile, CK-MB, LDH and CTnI. Based on the findings, the use of antibiotic drug, pefloxacin may lead to renal and cardiovascular toxicities especially when administered at a high doses. However, more investigations are required to establish the actual mechanism behind the effects of the drug on the renal and cardiovascular systems of the test animals. Hence, the antibiotic may not be suitable for a person having any renal and cardiovascular disorders.

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