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Research Paper

Pharmacokinetics of Moxifloxacin in A 5/6th Nephrectomized Rat Model

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ABSTRACT: Moxifloxacin (MFLX) is a new 8-methoxyfluoroquinolone derivative with a broad spectrum of antibacterial activity. MFLX at doses of 200 and 400 mg was selected to conduct the pharmacokinetic study and the drug was given orally to control and nephrectomized rats. A 5/6th nephrectomized rat model was used in this study. The drug levels in the plasma were determined using a spectrofluorimetric assay. The pharmacokinetic parameters viz. peak plasma concentration (C_{max}) and area under the curve (AUC_{0-8}) of the nephrectomized and control rats were compared. The C_{max} for both 200 and 400 mg dose of MFLX in nephrectomized rats showed significant difference($P<0.001$) from the control group, which reveals the changes in the C_{max} of MFLX in renal failure. The AUC_{0-8} for both 200 and 400 mg dose of MFLX in nephrectomized rats differ significantly ($P<0.001$) from sham operated control group, which implies the variation in MFLX availability in altered renal function. The AUC_{0-8} for 400 mg dose of MFLX in nephrectomized rats differ significantly from 200 mg dose of MFLX in nephrectomized group, which reveals that in higher dose, MFLX shows an abrupt increase in the drug availability in renal failure. It is conclude that preclinical drug monitoring of moxifloxacin in laboratory animals can be performed by using 5/6th nephrectomized rat models for determining the dose of MFLX for kidney failured patients. Various pharmacokinetic parameters determined differed in nephrectomised rats when compared to the control.

KEYWORDS: Moxifloxacin, nephrectomized rat, spectrofluorimetric, AUC, $C_{max.}$

Introduction

The kidneys are dynamic organs and represent the major control system monitoring body homeostasis, i.e. water and electrolyte balance. Although, the kidneys comprise less than 1% of total body mass, they receive about 20% of resting cardiac output. The nature of renal structure and function renders the kidneys especially susceptible to toxic xenobiotics. Approximately 30% of end stage renal diseases (ESRD) result from infections (or) genetic diseases and 20% result from therapeutic agents. The majority 50% of the diagnosed cases of ESRD, however are of unknown etiology. Renal failure has significant influence on the body fluids. It also leads to alterations in the pharmacokinetics of several drugs. Standard Kidney function tests like the determination of the glomerular filtration rate (or) the reabsorption of water and electrolytes are indicative of extensive damage and measure change only when the function reserve capacity has been eroded beyond a critical value. Hence the total body clearance of a drug will be significantly decreased in renal failure and the dose level of any drug must be reduced for kidney failure patients compared with normal individuals (Guyton C and Hall EJ, 2000).

To study the influence of renal failure on pharmacokinetics of drugs, laboratory animals can be used. In these models renal failure can be induced by several methods which include 5/6th nephrectomy, ablation of renal mass, ureteral obstruction, cyclosporine-induced renal damage, chronic nitric oxide synthase (NOS) inhibition and other chemical methods such as the use of nephrotoxic agents like uranyl nitrate, mercurials, cisplatin, etc. In 5/6 Nephrectomy surgery the operation for induction of renal failure in rats is performed in two stages under anaesthesia (Ketamine 75 - 100 mg /kg). During the first operation, 2/3rd of the left kidney will be removed while protecting the adrenal glands. Three days later unilateral nephrectomy, via right flank incision will be performed. Although chemical methods are simple and reliable, it is feared that the nephrotoxic agent could interfere in the assay of MFLX. Therefore, in the present study we used a two-step 5/6 nephrectomy for inducing kidney failure in rats (Giacomini *et. al.*, 1981) and investigated the pharmacokinetics of MFLX in this animal model. In synthetic antibiotics with a wide spectrum of activity were prescribed as the drug of choice to treat various infectious diseases. New quinolone antibiotics

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including moxifloxacin (MFLX) are known to cause severe adverse reaction such as prolonged QT intervals and rhabdomyolysis. Significant amount of MFLX gets excreted via urine. In renal dysfunction, a change in pharmacokinetic parameter like decreased systemic clearance was observed. This aspect has been further investigated in our study. Our primary objective was to compare the pharmacokinetics of MFLX at 200 mg and 400 mg dose levels in sham operated and nephrectomized rat models.

Materials and Methods

Experimental Animals

Healthy adult male *Wistar* rats weighing between 200-250 g were used in the study. Animals were housed in a laboratory maintained at 12 hr light - dark cycle, at controlled room temperature (23 ± 2°C) and relative humidity (50 ± 10%). Animals were given standard diet and drinking water *ad libitum*. Animals were divided into four groups of six each. Group I and II served as sham operated control rats. Two step 5/6 nephrectomy was performed for groups III and IV by standard procedure (Fig.2 and Fig.3). The study was conducted after obtaining ethical committee clearance from the institutional animal's ethical committee in accordance with animal experimentation and care regulations.

Assessment of Renal Failure

The body weights (g) of the animals in both the control and nephrectomized groups were recorded before and 10 days after surgery. Blood haemoglobin level (g/dl) was checked for both the groups using Sahli's Adam's Haemoglobinometer before and 10 days after surgery (Ghai CL, 1999). Serum levels of alkaline phosphatase (ALP), alanine transaminase (ALT) and aspartate transaminase (AST) were estimated using standard commercial kits (Chemchek, 2006). Both sham operated and 5/6th nephrectomized groups of rats were tested for the induction of renal failure by measuring urinary and serum levels of urea, uric acid and creatinine (Chemchek, 2006).

Pharmacokinetics of Moxifloxacin

Groups I and II served as control rats (sham-operated) and received 200 and 400 mg of MFLX, respectively. Groups III and IV served as nephrectomized rats and received 200 and 400 mg of MFLX, respectively. Moxifloxacin (MFLX) tablet was powdered and dissolved in distilled water and stock solution was prepared (100 mg/ml) Groups I and III received 200 mg/kg dose of MFLX, groups II and IV received 400 mg / kg of MFLX by oral route using oral gavage needle. Blood was collected by tail tip method (Hoff J, 2000). The blood samples were collected in a heparinized tube and centrifuged immediately at 5000 rpm for 20 minutes using a micro centrifuge. The plasma samples were separated and spectrofluorimetric analysis was carried out to determine drug levels.

Spectrofluorimetric Assay

The fluorescence intensity was measured on a spectrofluorometer (Perkin - Elmer (Norwalk, USA) luminescence spectrometer equipped with a xenon lamp and a Dell model 110 L, computer working with Win Lab software. Displacing reagent was used as a micellar phase to enhance the solubility of proteins and to minimize the binding of moxifloxacin to plasma proteins. To 0.25 ml/L of aqueous sodium phosphate, sodium dodecyl sulphate was added to give a concentration of 10 m mol/L and the pH was adjusted to 3.0 with concentrated phosphoric acid. To prepare a standard curve, in a series of five test tubes, 0.2 ml of plasma was added. Moxifloxacin working reagent at concentrations of 0.2, 0.4, 0.6, 0.8 and 1.0 μg was spiked into the plasma and in each tube 1 ml of 10 mmol/L sodium dodecyl sulphate (SDS) was added and mixed well. About 2ml of distilled water was added and the fluorescent intensity was measured at excitation of 290 nm and emission at 500 nm. Standard curve was plotted using absorbance versus concentrations (Fig.1). Standard solution of moxifloxacin HCl was prepared by direct weighing of standard substance with subsequent dissolution in water. The concentration of standard solution was 10.0 mg /L (Djurdjevic *et. al.,* 2006). Working standard was prepared by diluting aliquots of 0.2 - 1.0 ml of the stock solution in a series of 10 ml volumetric flask and the volume was adjusted with distilled water to give final concentrations of 0.2, 0.4, 0.6, 0.8 and 1.0 mcg/ml (Djurdjevic *et. al.,* 2006). This solution was spiked onto blank plasma and the absorbance was estimated to construct a standard curve. To perform sample analysis, to 0.2 ml of plasma sample, 1 ml of 10 mmol/L SDS and 2 ml of distilled water were added, mixed well and the fluorescence intensity was measured at excitation of 290 nm and emission at 500 nm. The absorbance was interpolated on the standard curve to get the concentration.

Pharmacokinetic Parameters were Calculated using Following Formulae (Raghavan *et. al.,* 2006):

C_{max} = Peak plasma concentration

AUC_{0-8} = $1/2(t_2-t_1)(C_2+C_1) + 1/2(t_3-t_1)(C_3+C_2) + ...+ 1/2 (t_8-t_7)(C_8+C_7)$

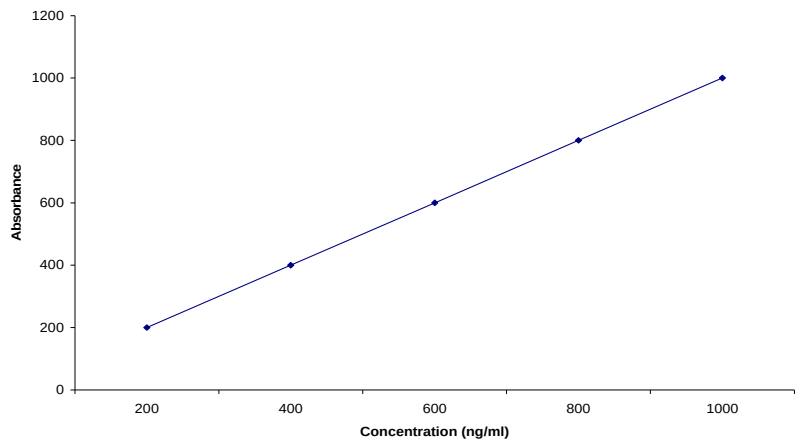

Fig 1. Calibration graph for moxifloxacin.

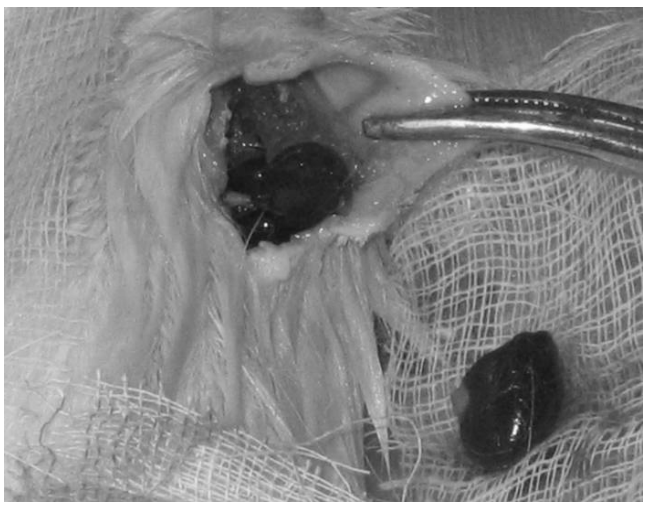

Fig 2. Removal of 2/3rd of left kidney.

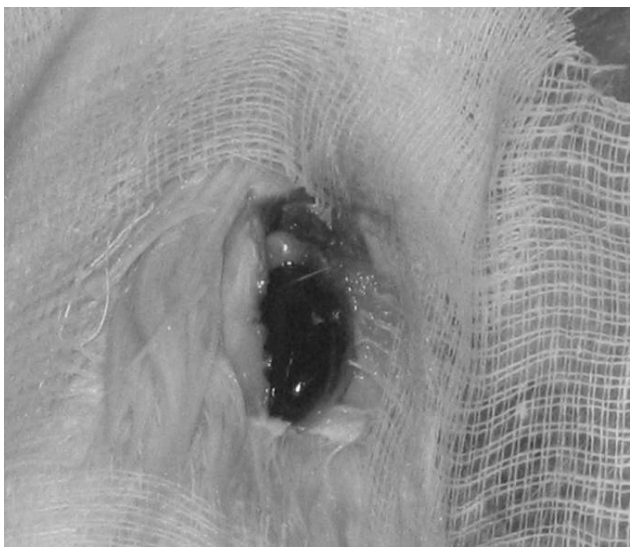

Fig.3 Removal of right kidney.

Statistical Analysis

Statistical analysis was performed using student's t test followed by one way ANOVA using statistical package for social sciences (SPSS) version 10.0.

Results

Assessment of Renal Failure

Body weight and Haemoglobin level of control and nephrectomized rats before and after surgery are shown in Table-1. Serum biochemical parameters of sham operated and nephrectomized rats 10 days after the induction of chronic renal failure are shown in Table-2. Urinary levels of creatinine, urea and uric acid in sham operated rats and nephrectomized rats 10 days after the induction of chronic renal failure are shown in Table-3.

Pharmacokinetic Analysis

Drug levels were determined using spectrofluorometric methods. The standard values are shown in Table 5 and Fig 1.

Peak Plasma Concentration (C_{max})

The C_{max} at 8 hours for 200 mg MFLX in sham operated and nephrectomized rats were 134.01 ± 1.57 and 146.25 ±1.20 ng/ml respectively and showed a significant ($P<0.001$) difference. The C_{max} at 8 hours for 400 mg MFLX, in sham operated and nephrectomized rats were 153.41±0.95 and 203.43±1.0 ng/ml respectively and showed a significant ($P<0.001$) difference.

Area under the Curve for 0-8 Hours (AUC_{0-8})

There was a significant ($P<0.001$) difference in the AUC_{0-8} for 200 mg MFLX in sham operated (558.24±3.83) and nephrectomized rats (782.60± 36.09) ng.hr/ml. There was a significant ($P<0.001$) difference in the AUC_{0-8} for 400 mg MFLX in sham operated (646.69±5.03) and nephrectomized rats (1102.03±6.04) ng.hr/ml The results are presented in Table-4.

Discussion

This study addressed the changes in pharmacokinetic profile of a new quinolone antibiotic moxifloxacin (MFLX) in renal failured rat models. Various biochemical and haematological parameters were monitored for the assessment of renal failure in laboratory animals. In the present study, renal failure was induced by two step 5/6th nephrectomy in rats. Even though chemical methods are simple and reliable, the nephrotoxins may interfere in the assay of MFLX and hence we selected 5/6th nephrectomy to induce renal failure in rats. Earlier studies showed that renal enzymuria as a marker of nephrotoxicity. The urinary levels of γ-glutamyl transferase (GGT) and alkaline phosphatase (ALP) was elevated significantly in renal failure (Melo *et. al.,* 2006). In our study, the serum levels of alkaline phosphatase (ALP), aspartate transaminase (AST) and alanine transaminase (ALT) were raised significantly ($P<0.0001$) in nephrectomized rats when compared with control rats. Serum and urinary levels of creatinine, urea and uric acid were raised significantly ($P<0.0001$) due to the negative nitrogen balance developed as a result of change in the electrolyte and acid balance in body fluids of the nephrectomized animals (Yamabe *et. al.,* 2005). Hence by assessing the above parameters, it is revealed that 5/6th nephrectomy produces chronic renal failure in rats.

Therapeutic drug monitoring (TDM) for MFLX was necessary for evaluating proper dose of the drug to patients with renal impairments. In our study, preclinical therapeutic drug monitoring has been performed for MFLX by using 5/6th nephrectomized rat models. Widespread use of moxifloxacin in clinical practice attenuates the need for rapid and reliable method of its determination in biological fluids. However, not many methods have been developed for the determination of MFLX in human body fluids. High performance liquid chromatography with fluorescence detection (λ_{ex} = 464 nm, λ_{em} = 537 nm) using column focusing was previously reported (Ulu ST, 2007). Also MFLX was determined in tablets, urine and plasma by spectrofluorimetry using micellar medium (8 mmol /L sodium dodecyl sulfate, SDS) with λ_{ex}=290 nm and λ_{em}= 500 nm (Djurdjevic *et. al.,* 2006). In this study we used a spectrofluorimetric method to analyze the drug in the plasma samples. Only limited studies were performed for MFLX in animals so far (Sorgel *et. al.,* 1989). MFLX at doses of 200 and 400 mg was selected to carry out the study and was given orally to control and nephrectomized rats. The pharmacokinetic parameters viz. peak plasma concentration (C_{max}) and area under the curve (AUC_{0-8}) of the nephrectomized and control rats were compared. The C_{max} for both 200 and 400 mg dose of MFLX in nephrectomized rats showed significant difference ($P<0.001$) from the control group, which reveals the changes in the C_{max} of MFLX in renal failure. The AUC_{0-8} for both 200 and 400 mg dose of MFLX in nephrectomized rats differ significantly ($P<0.001$) from sham operated control group, which implies the variation in MFLX availability in altered renal function. The AUC_{0-8} for 400 mg dose of MFLX in nephrectomized rats differ significantly from 200 mg dose of MFLX in

nephrectomized group, which reveals that in higher dose, MFLX shows an abrupt increase in the drug availability in renal failure. The drug profile of MFLX reveals that, majority of the drug was excreted via the kidneys (Djurdjevic *et. al.,* 2006). From the above obtained data, we have concluded that preclinical drug monitoring of moxifloxacin in laboratory animals can be performed by using 5/6th nephrectomized rat models for determining the dose of MFLX. Also pharmacokinetic parameters such as peak plasma concentration (C_{max}) and area under the curve (AUC) can be determined easily by spectrofluorimetric analysis. Further work has to be carried out for MFLX, to determine its disposition on various body fluids such as urine and saliva, in renal failure rat models.

Table 1. Body weight and Haemoglobin level of control and nephrectomized rats before and after surgery

Treatment	Body weight (g)		Haemoglobin (g/dl)	
	Before surgery	After Surgery	Before Surgery	After surgery
Sham operated	248 ± 4.0	256 ± 2.0	13.08 ± 0.32	12.13 ± 0.48
Nephrectomized	242 ± 3.0	350 ± 7.0[a]	14.12 ± 0.56	8.31 ± 1.03[a]

Values are mean ± SEM; n = 6 in each group.

[a]P<0.0001 when compared to sham operated rats; Paired 't' test.

Table 2. Serum biochemical parameters of sham operated and nephrectomized rats 10 days after the induction of chronic renal failure

Parameters	Sham Operated	Nephrectomized
ALP(U/L)	77.11 ± 3.24	128.11 ±6.81[a]
AST(U/L)	63.36 ± 4.36	85.20 ± 2.66[a]
ALT(U/L)	46.11 ± 2.25	85.20 ± 2.66 [a]
Urea (mg/dl)	38.33 ± 1.66	77.16 ± 1.97 [a]
Uric acid (mg/dl)	1.33 ± 0.26	7.83 ± 0.26[a]
Creatinine (mg /dl)	0.556 ± 0.56	1.56 ± 0.13[a]

Values are mean ± SEM; n = 6 in each group.

[a]P<0.0001 when compared to sham operated rats; Unpaired 't' test.

Table 3. Urinary levels of creatinine, urea and uric acid in sham operated rats and nephrectomized rats 10 days after the induction of chronic renal failure.

Parameters	Sham Operated	Nephrectomized
Creatinine (mg/dl)	0.505 ± 0.051	1.78 ± 0.13[a]
Urea (mg/dl)	57.40 ± 2.77	119.11 ± 7.32[a]
Uric acid (mg/dl)	4.30 ± 0.13	9.33 ± 0.47[a]

Values are mean ± SEM; n = 6 in each group.

[a]P<0.0001 when compared to sham operated rats; Unpaired 't' test.

Table 4. Pharmacokinetic parameters of MFLX in nephrectomized rats Compared with sham operated rats

Group	Treatment	C_{max} (ng/ml)	AUC_{0-8} (ng.hr/ml)
I	Sham operated (200 mg)	134.01 ± 1.57	558.24 ± 3.83
II	Sham operated (400 mg)	153.41 ± 0.95	646.69 ± 5.03
III	Nephrectomized (200 mg)	146.25 ± 1.20[a]	782.60 ±36.09[a]
IV	Nephrectomized (400 mg)	203.43 ± 1.00 [a,b]	1102.03 ± 6.04 [a,b]

[a]P<0.001 when compared to Groups I and II,

[b]P<0.001 when compared to Group III; (One way ANOVA)

Table 5. Calibration of Moxifloxacin in plasma.

Concentration (ng/ml)	Fluorescence absorption (Excitation 290 nm, emission 500 nm)
200	36
400	84
600	153
800	182
1000	212

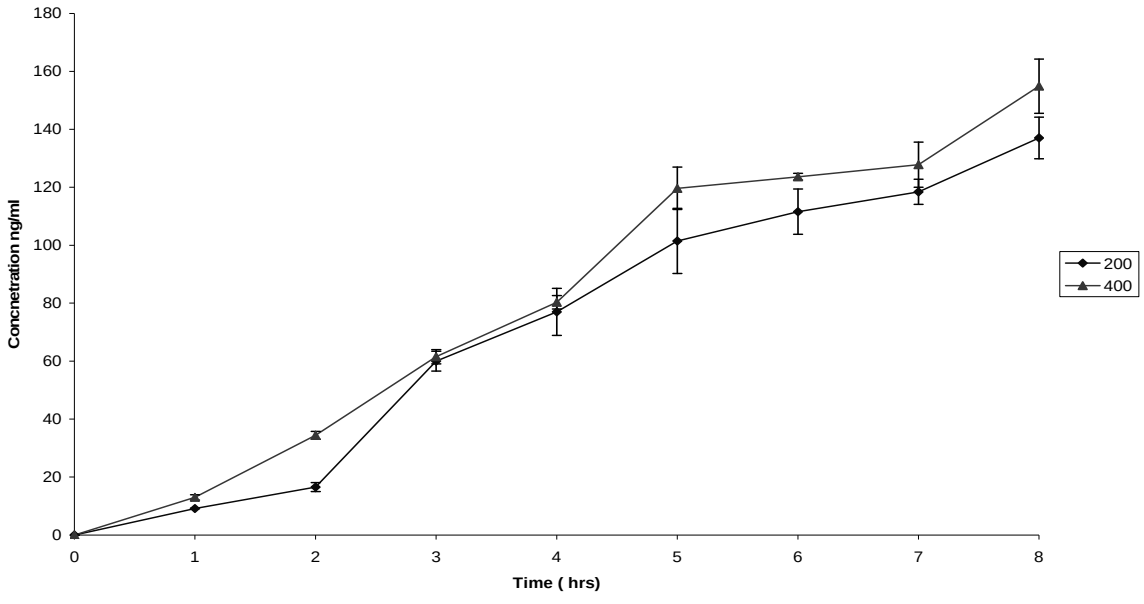

Fig 4. Cmax of MFLX at 200 and 400 mg in sham operated rats.

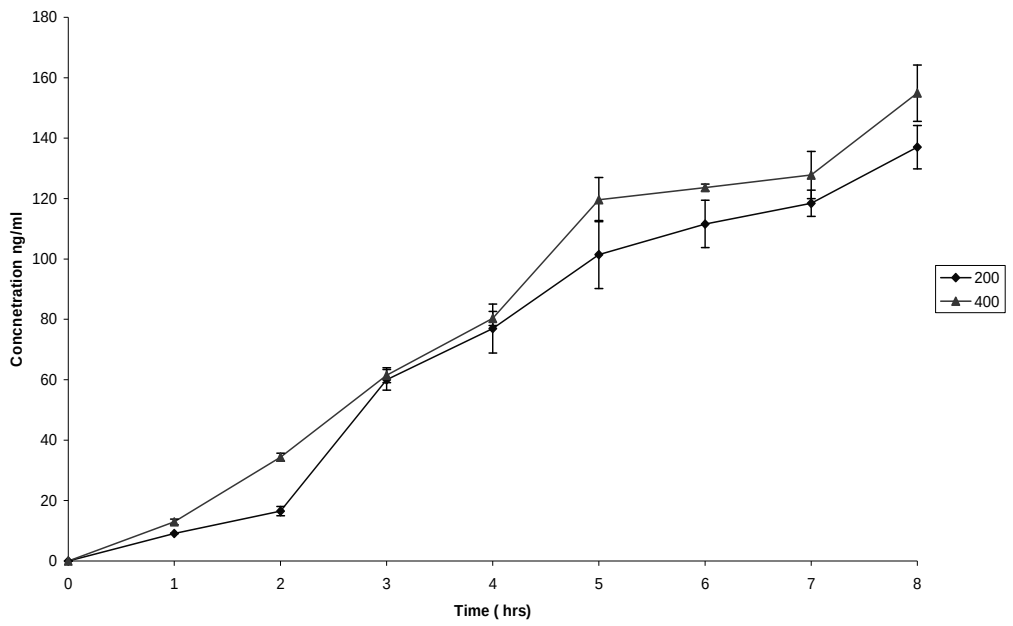

Fig 5. Cmax of MFLX at 200 and 400 mg in nephrectomized rats

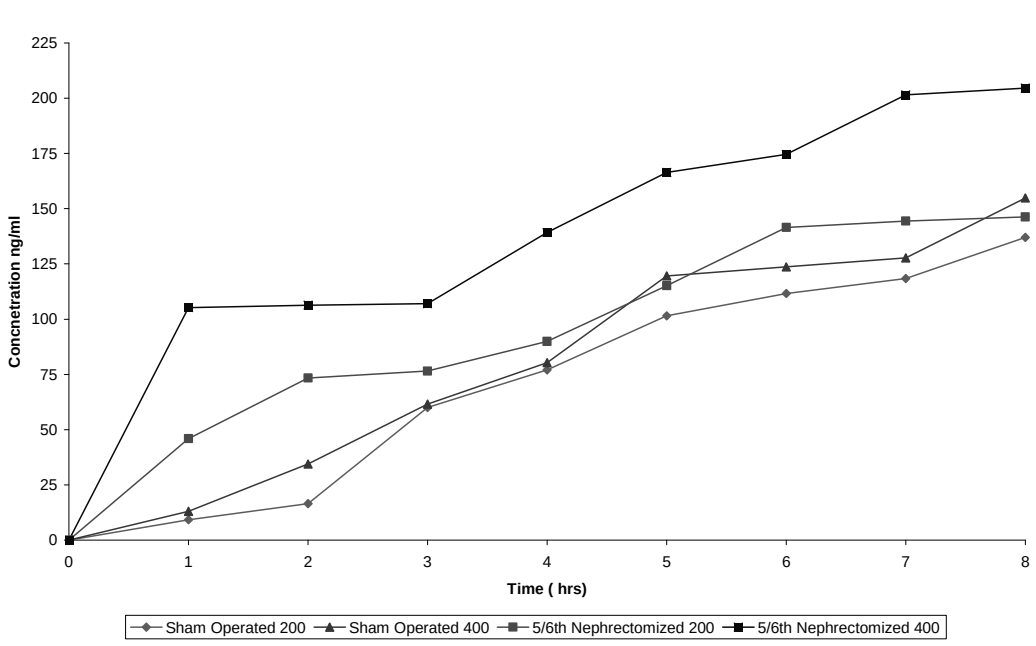

Fig 6. Cmax of MFLX at 200 and 400 mg in sham operated and nephrectomized rats

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