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HISTOLOGICAL EFFECT OF CIPROFLOXACIN ON THE KIDNEY HISTOLOGY OF ADULT RATS

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Abstract: **The antibiotic Ciprofloxacin is routinely used by urologists, andrologist and fertility specialist to treat such bacterial infections occurring prior to in vitro fertilization treatment, or when high concentration of leukocytes are present in the semen of these patients, irrespective of microbial evidence of infection. In this study, a total of 30 rats were used. The rats were divided into control group and experimental group, the experimental group was divided into two major groups of 12 rats each i.e. therapeutic dose and double therapeutic dose groups respectively, the experimental group (group B and C) received 12.5mg/kg oral ciprofloxacin (Sigma 33433, USA) via gavage, while the double experimental group (group D and E) received 25mg/kg oral ciprofloxacin, the two experimental groups were further divided into two groups of 6 rats each i.e acute and chronic groups. The acute received corresponding dose of ciprofloxacin for 4 four weeks (28 days). The effect of ciprofloxacin was dosage dependent on the kidney. Although there was no histological changes in the renal corpuscles and renal tubules in all the groups, but the study revealed interstitial congestions which were noticed in group D and E due to the use of overdose. It is thereby recommended that prolonged usage of Ciprofloxacin should be avoided and also drug abuse and over dosage with ciprofloxacin should be avoided.**

1. INTRODUCTION

Fluoroquinolones (FQS), such as ciprofloxacin (CPFX), represent an important class of antimicrobial agents used in treatment of a wide range of infectious diseases in different organs such as urinary tract, bone and joint, lower respiratory tract and skin (Lietman, 1995). Ciprofloxacin is very active against wide variety of pathogenic bacteria including some gram-positive and most gram- negative organisms (Hooper and Wolfson, 1985). Little is known about the toxic effects of ciprofloxacin on pregnant female rats. There has been a number of developmental toxicity studies showing maternal toxicity by fluoroquinolones, e.g. decreased body weight and reduced food intake in rats and rabbits (Kim *et al*., 2000; Guzman *et al*., 2003). Chann and Janjua (2003) reported that ciprofloxacin administration during gestation caused severe liver damage; pyknotic nuclei within hepatocytes and few distinctly visible nucleoli in hepatocytes of albino rats. Several cases of ciprofloxacin associated severe liver damage were reported. In such cases liver biopsy revealed extensive hepatocellular necrosis and a mixed inflammatory infiltrate with abundant eosinophils in livers of patients (Contreras *et al*., 2001; Batailte *et al*., 2002; Goetz *et al*., 2003; Xie *et al*., 2003 and Zimpfer *et al*., 2004). Hussy *et al*. (1986) suggested that fluoroquinolones may exert an inhibitory effect on eucaryotic DNA topoisomerase III resulting in the suppression of DNA synthesis. Several quinolone antibiotics, including ciproflaxacin were assayed in the in vitro hepatocyte primary culture/DNA repair test. MeQueen and Williams (1987) reported that these compounds yielded positive results in the in vitro assays, but ciprofloxacin had negative results in the in vivo assays. In addition mammalian DNA synthesis by the polymerase- primase complex was inhibited by high concentrations of quinolones (>100mg /L), but to a greater extent by

ciprofloxacin and norfloxacin than by ofloxacin. Pino *et al*. (1991) have investigated that norfloxacin for DNA damage in rat livers and kidneys after oral administration. Earlier studies by Maura and Pino (1988) indicated that, after oral administration of quinolones, they are susceptible to be activated, presumably in the liver, to stable intermediates, which may be transformed in other organs into final reactive species interacting with DNA. Minuk *et al*. (1997) found that the quinolone antibiotics inhibit eukaryotic as well as prokaryotic cell growth and protein synthesis by interfering with DNA and RNA replication. Positive results were also observed in cytogenetic studies in vitro and in vivo, unscheduled DNA synthesis and alkaline elution tests (Goral *et al*., 1999). Abd-Allah *et al*. (2000), Abdo llahi and Isazadeh (2001) and Kashida *et al*. (2002) mentioned that ofloxacin induced its antibacterial action mainly by inhibition of DNA gyrase in rat and mice, which is equivalent to topoisomerase II in mammalin cells.

The antibiotic ciprofloxacin is routinely used by urologists, andrologist and fertility specialists to treat such bacterial infections occurring prior to in vitro fertilization treatment, or when high concentration of leukocytes are present in the semen of these patients, irrespective of microbial evidence of infection (Herbold *et al*., 2001).Ciprofloxacin is a synthetic antibacterial agent belonging to the family of fluoroquinolones with a very broad spectrum against of microbial pathogens, especially Gram- negative infectious diseases, that has been approved in more than 100 countries world-wide (Wolfson and Hooper, 1985). Ciprofloxacin is well absorbed orally and induced its antibacterial action mainly by inhibition of DNA gyras, which is equivalent to topoisomerase II in mammalian cell (Akasaka *et al*., 1998; Liu and Wang, 1999).

The pathology of ciprofloxacin on the kidney of rats have not been clear in time past as there have been different views by researchers with some researchers reporting that it is non-toxic while others have reported that it has various toxic effects on the kidney (Baykal *et al*., 2005).

Nada (2002) reported that the kidneys of juvenile rats showed cells swelling of renal tubules and coagulative necrosis of the kidneys of rats administered with ciprofloxacin.

Ciprofloxacin is an antibacterial drug which is used by a large percentage of the populations in treatment of different health conditions and in most cases ciprofloxacin have also been reported to be abused by users (Goetz *et al*., 2003).

Although ciprofloxacin was generally considered non-toxic by several authors (Baykal *et al*., 2005) but researchers such as Ismail (2006) and Nada (2012), have shown that the drug is capable of causing histo-pathological alterations in different body organs including the kidneys of rats. Ciprofloxacin have been implicated in causing various alterations in the histology of different organs of rats. Researches such as that of Betuile *et al*., (2002), Goetz *et al*., (2003) and Zimpfer *et al*., (2004) have shown severe hepato-cellular necrosis as well as testicular alterations following ciprofloxacin administration.

2. MATERIALS AND METHODS

GEOGRAPHICAL DESCRIPTION OF THE STUDY AREA

This research was carried out in Diagnosistic Laboratory, College of Medicine, Ambrose Alli University Ekpoma, Edo state, Nigeria. Edo state lies longitudinally at 04ºE and 43ºE and Latitude 05º44ºN and 07º34ºN. Its geopolitical location is the South South and it has a population of 3.1 million people (World Gazzetter, 2007)

RESEARCH DESIGN

A total of 30 albino rats weighing 350-500g were used in this study. All animals were treated in accordance to the Principles of Laboratory Animal Care. All rats were fed a standard diet and water. The daily intake of animal water was monitored at least one week prior to start of ciprofloxacin treatment in order to determine the amount of water needed per experimental animal. The rats were divided into control group and experimental group, the experimental group was divided into two major groups of 12 rats each i.e therapeutic dose and double therapeutic dose groups respectively, the experimental group (group B) received 12.5 mg/kg oral ciprofloxacin (Sigma 33433, USA) via gavage, while the double experimental group received 25 mg/kg oral ciprofloxacin, the two experimental groups were further divided into two groups of 6 rats each i.e acute and chronic groups. The acute received corresponding dose of ciprofloxacin for two weeks (14 days) and the chronic groups received corresponding dose of ciprofloxacin for 4 four weeks (28 days). The dosages of ciprofloxacin were similar to those used in human therapy. The control group received only water and food.

SAMPLE SIZE

The reasonable estimate of the key proportion to be studied will show no pilot proportion prevalence study published in the location of research. The number of albino rats sample required in this research will be guided by upper limit to give 95% level of confidence at an expected prevalence of about 2% (No retrospective study) using the precise formula:

The number of sample studied was guided by the upper limit required, and gave 95% level of confidence at an expected prevalence of about 55% pilot study, after using the precise prevalence formula.

$$N = \frac{z^2pq}{D^2}$$ (Araoye, 2004)

Where N= the desired sample size (when population is greater than 10,000)

z= is a constant given as 1.96 (or more simply at 2.0) which corresponds to the 95% confidence level.

P= expected prevalence

q= 1.0-p

d= acceptable error 5%.

$$N = \frac{(1.96)^2 \text{ X } 0.02 \text{ X } (1 - 0.02)}{(0.05)^2}$$

$$N = \frac{(1.96)^2 \text{ X } 0.02 \text{ X } 0.98}{(0.05)^2}$$

$$N = \frac{3.8416 \text{ X } 0.02 \text{ X } 0.98}{0.0025}$$

N= 30

A total of thirty (30) rats were therefore purchased for the study.

FUNDAMENTAL ANIMAL HOUSING CONDITION

Thirty (30) Adult Albino rats of comparable sizes and weights ranging from 350-500g were procured from the Animal Farm, College of Medicine, Ambrose Alli University Ekpoma, Edo State and transferred to the Histopathology Laboratory where they were allowed two (2) week of acclimatization. The rats were kept in wooden cages with tripod that separates the animal from its faeces to prevent contamination. During this period, the rats were maintained in accordance with the standard guide for the care and use of Laboratory animals.

ANIMAL GROUPING

The rats were divided accordingly into five groups withsixt rats each, as follows:

Group A: Consists of six (6) male rats (Control)

Group B: Consists of six (6) male rats (Acute therapeutic dose)

Group C: Consists of six (6) male rats (Chronic Therapeutic)

Group D: Consists of six (6) male rats (Acute overdose)

Group D: Consists of six (6) male rats (Chronic overdose)

STUDY DURATION

The preliminary studies, animal acclimatization, substance (ciprofloxacin) preparation, actual animal experiment and evaluation of results lasted for a period of three months (from, August, 2017 – October, 2017). However, the actual administration of the animals lasted for four (4) weeks.

SUBSTANCE PREPARATION

Ciprotab 500mg by was procured from Rehoboth Pharmacy, Ekpoma, Edo state. Ciprofloxacin solution was made by dissolving 500mg (1 tablet) of ciprotab in 100mls of distilled water. Appropriate volume of this solution was then administered to the various groups for the stipulated period of time.

SUBSTANCE ADMINISTRATION

The administration of ciprofloxacin was primarily by the following:

Group A: Was fed with normal diet and water as control

Group B: Was fed with normal diet and 12.5 mg/kg of ciprofloxacin for 14 days.

Group C: Was fed with normal diet and 12.5 mg/kg of ciprofloxacin for 28 days.

Group D: Was fed with normal diet and 25 mg/kg of ciprofloxacin for 14 days.

Group E: Was fed with normal diet and 25 mg/kg of ciprofloxacin for 28 days.

METHOD AND SAMPLE COLLECTION:

SAMPLE COLLECTION

Tissue Preparation: Twenty-four hours after the end of each experimental period, the animals were euthanized by cervical dislocation. The kidney of the entire rats were quickly removed, rinsed with saline solution (0.9%), and fixed in formalin 10%. The longitudinal section of the kidney were obtained and processed in Histopathology laboratory of Medical Laboratory Science department, Ambrose Alli University, Ekpoma. The processed tissues were embedded in paraffin, sectioned at 4 μm thickness, and placed on frosted glass slides for further evaluation. The tissue macroscopic alterations were also analyzed. The samples were stained using hematoxylin and eosin (H&E) stains.

METHOD OF STAINING:

HAEMATOXYLIN AND EOSIN

PRINCIPLE

Alum acts as mordant and hematoxylin containing alum stains the nucleus light blue. This turns red in presence of acid, as differentiation is achieved by treating the tissue with acid solution. Bluing step converts the initial soluble red color within the nucleus to an insoluble blue color. The counterstaining is done by using eosin which imparts pink color to the cytoplasm.

REAGENTS

- Harri's Hematoxylin stain

  A = 1 gm hematoxylin in 10 ml ethanol

  B = 20 gm ammonium alum in hot distilled water

  MixA & B, boil and add 0.5 gm of mercuric oxide and filter.

- Eosin solution

  Yellow eosin = 1 gm
  Distilled water = 80 ml
  Ethanol = 320 ml
  Glacial Acetic Acid = 2 drops

- 0.5% HCl

- Dilute ammonia water

PROCEDURE

1.	Dewax in two changes of xylene	2 minutes each
2.	Hydrate in descending grades of alcohol (absolute, 95%, 80% and 70%)	
3.	Rinse in water	
4.	Stain in harris haematoxylin	5 minutes
5.	Rinse in water	
6.	Differentiate in 1% acid alcohol	3 seconds
7.	Blue in running tap water	10 minutes
8.	Counterstain in 1% eosin	3 minutes
9.	Rinse in water	
10.	Dehydrate in descending grades of alcohol (70%, 80%, 95% and absolute)	
11.	Clear in xylene	
12.	Mount in dibuthylphthalate (DPX)	

3. RESULTS

Table 1 below shows the mean ± standard deviation (SD) of body weights of albino rats administered with varying doses of ciprofloxacin. The mean ± standard deviation (SD) of body weights before the administration of ciprofloxacin for control group, acute therapeutic, chronic therapeutic, acute overdose and chronic overdose were recorded as 350.00±0.00, 420.00±27.38, 430.00±27.38, 440.00±22.36 and 510.00±55.90 respectively.

The mean weights and standard deviation of body weights after the administration of ciprofloxacin for control group, acute therapeutic, chronic therapeutic, acute overdose and chronic overdose were recorded as 414.00±21.90, 330.00±27.90, 304.00±36.46, 366.00±23.90 and 340.00±52.67 respectively.

Statistical analysis of the weights before ciprofloxacin administration when compared with control showed a significant increase which is indicated with 'x'. The weights after ciprofloxacin administration when compared with control showed a significant decrease which is indicated with 'y'. Comparison of the different groups before and after ciprofloxacin administration showed a significant decrease.

TABLE 1: BODY WEIGHTS OF ALBINO RATS ADMINISTERED WITH VARYING DOSES OF CIPROFLOXACIN

	CONTROL	ACUTE THERAPEUTIC (12.5Kg/BW FOR 2WEEKS)	CHRONIC THERAPEUTIC (12.5Kg/BW FOR 4WEEKS)	ACUTE OVER DOSE (25Kg/BW FOR 2WEEKS)	CHRONIC OVER DOSE (25Kg/BW FOR 4WEEKS)
WBCA	350.00±0.0	x 420.00±27.38	x 430.00±27.38	x 440.00±22.36	x 510.00±55.90
WACA	414.0±21.90	y 330.00±27.90	y 304.00±36.46	366.00±23.90	y 340.00±52.67
T-VALUE	-6.532	5. 196	6. 178	5. 156	16.000
P-VALUE	0.003 (S)	0.001 (S)	0.000 (S)	0.001 (S)	0.000 (S)

KEY:
x - Significant when compared with control before ciprofloxacin administration
y - Significant when compared with control after ciprofloxacin administration

S - Significant

WBCA – Weight before ciprofloxacin administration

WACA – Weight after ciprofloxacin administration

Kg/BW – Kilogram per body weight

Table 2 below shows the histological effects of ciprofloxacin on the kidney of albino rats. From the photo micrographs, there was no histological change observed in any group for the renal corpuscles C and the renal tubule T. There was interstitial congestion G in group D and E which was not seen in group B and C.

TABLE 2: HISTOLOGICAL OBSERVATIONS OF KIDNEY SECTIONS OF RATS ADMINISTERED WITH VARYING DOSES OF CIPROFLOXACIN.

HISTOLOGICAL OBSERVATION	CONTROL	ACUTE THERAPEUTIC (12.5Kg/BW FOR 2WEEKS)	CHRONIC THERAPEUTIC (12.5Kg/BW FOR 4WEEKS)	ACUTE OVER DOSE (25Kg/BW FOR 2WEEKS)	CHRONIC OVER DOSE (25Kg/BW FOR 4WEEKS)
Renal Corpuscles C	+	+	+	+	+
Renal Tubule T	+	+	+	+	+
Interstitial Congestion G	-	-	-	+	+

KEY:
- = Absent
+ = Present

HISTOLOGICAL FINDINGS

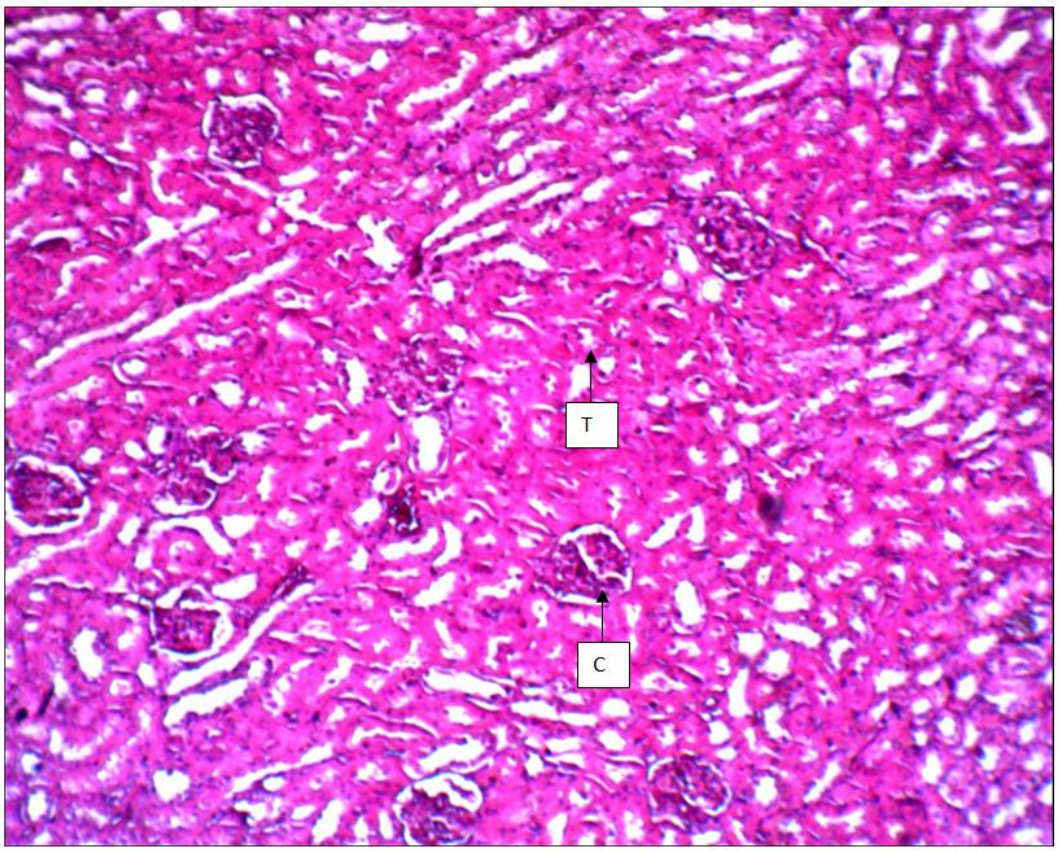

Plate 1: GROUP A; Rat kidney section showing a normal histology composed of mainly renal corpuscles C and tubules T

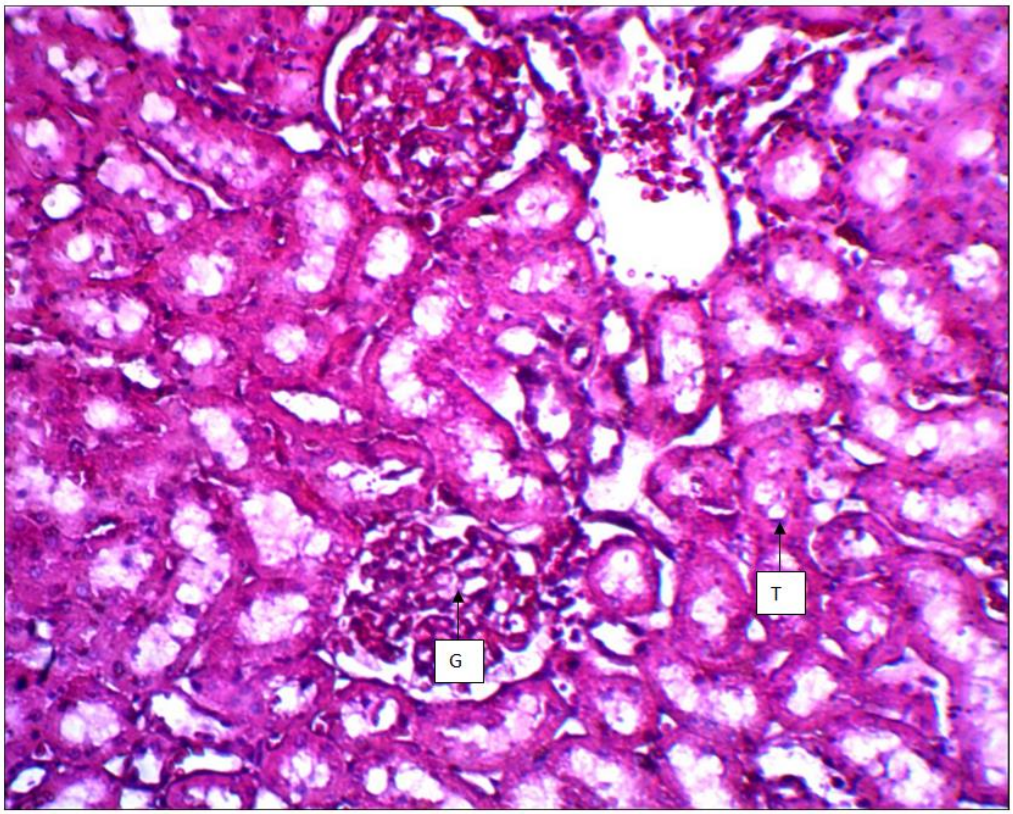

Plate 2: GROUP B; Rat kidney section showing a normal histology composed of mainly renal corpuscles C and tubules T after the administration of 12.5kg/body weight of Ciprofloxacin for 2 weeks (H&E x 100)

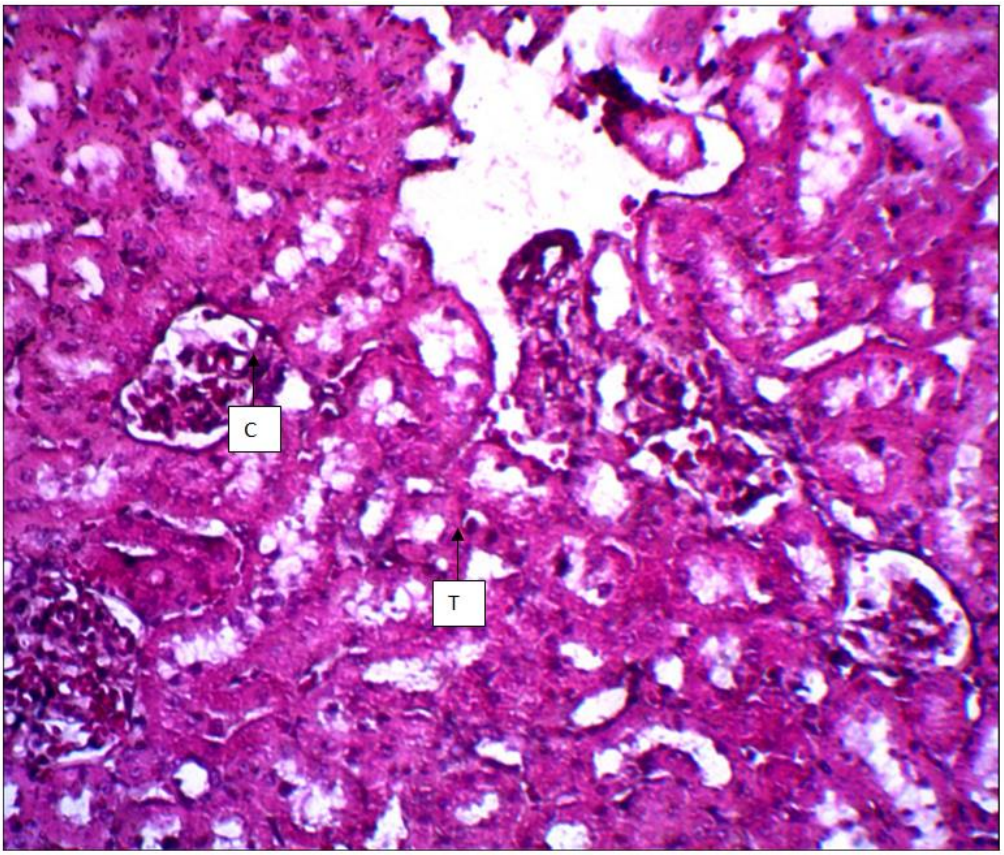

Plate 3: GROUP C; Rat kidney section showing a normal histology composed of mainly renal corpuscles C and tubules T after the administration of 12.5kg/body weight of Ciprofloxacin for 4 weeks (H&E x 100)

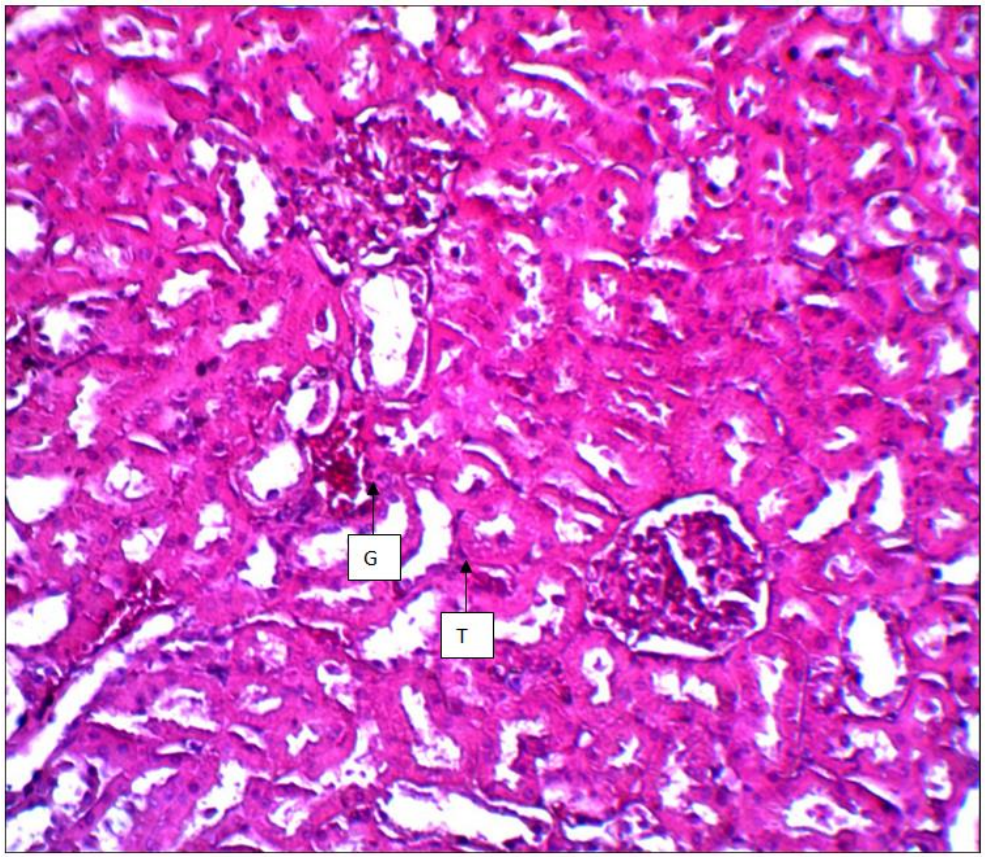

Plate 4: GROUP D: Rat kidney section showing mild interstitial congestion G after the administration of 25kg/body weight of Ciprofloxacin for 2 weeks (H&E x 400)

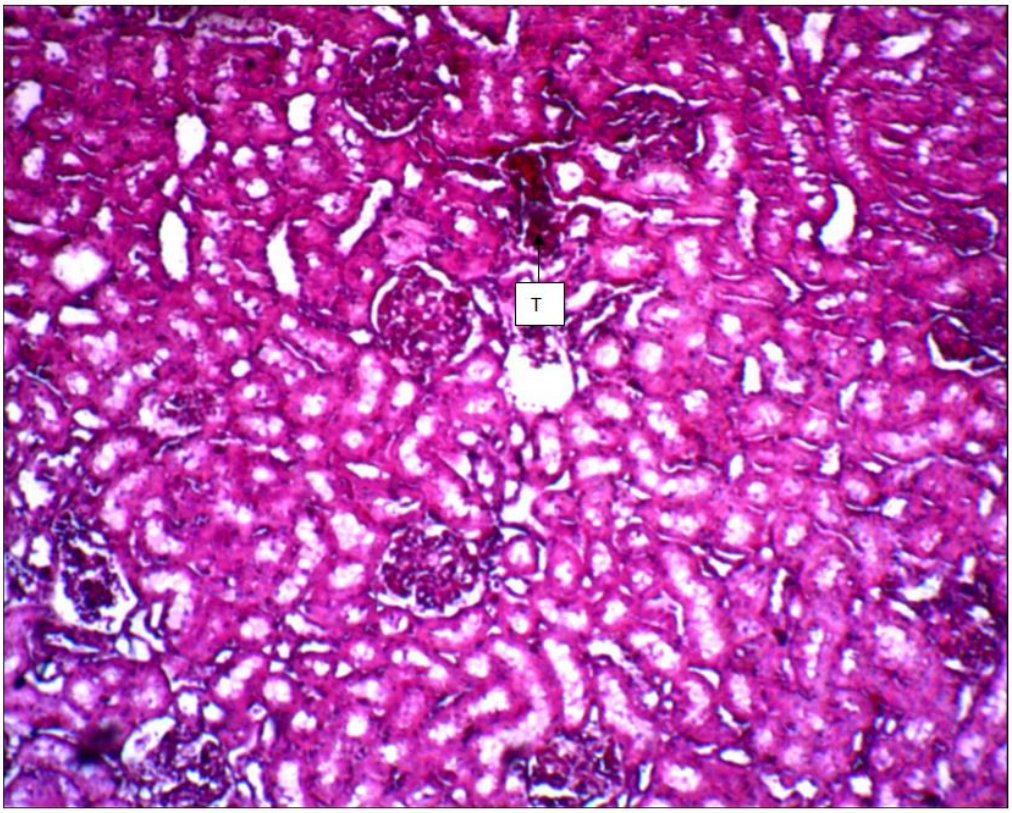

Plate 5: GROUP E: Rat kidney section showing mild interstitial congestion G after the administration of 25kg/body weight of Ciprofloxacin for 4 weeks (H&E x 400)

4. DISCUSSION

The kidney participates in whole-body homeostasis, regulating acid-base balance, electrolyte concentrations, extracellular fluid volume, and blood pressure. The kidney accomplishes these homeostatic functions both independently and in concert with other organs, particularly those of the endocrine system. Various endocrine hormones coordinate these endocrine functions; these include renin, angiotensin II, aldosterone, antidiuretic hormone, and atrial natriuretic peptide, among others. Many of the kidney's functions are accomplished by relatively simple mechanisms of filtration, reabsorption, and secretion, which take place in the nephron (Bruce, 2004).

In this study, the body weight of the experimental animals after the administration of ciprofloxacin reveals a significant decrease on the weight when compared with those before the administration of ciprofloxacin. This weight reduction occurred in all the groups, indicating that ciprofloxacin reduces the weight of experimental animals.

The study also reveal the histological changes and it was recorded that in all the groups (acute therapeutic, chronic therapeutic, acute overdose and chronic overdose which received 12.5kg/body weight for 2 weeks, 12.5kg/body weight for 4 weeks, 25kg/body weight for 2 weeks and 25kg/body weight for 4 weeks respectively) had normal renal corpuscles and normal renal tubules. It was also noticed that there was interstitial congestion for group 3 and 4 (acute overdose and chronic overdose). This indicates that ciprofloxacin cannot cause any abnormality when taken in the normal therapeutic dose but over long use (overdose) of it can cause damages to the kidney. Ciprofloxacin is dosage dependent. The reasons for the discrepancy may be due to differences between invitro and invivo studies. Thus, up to 20% of each ciprofloxacin dose is metabolized in vivo, and ciprofloxacin metabolites may exert some biological activities that are not detectable by in vitro assays (Yao and Moellering, 1991). On the other hand, the possible release of bacterial factors, from indigenous bacteria killed by the drug, should not be neglected.

5. CONCLUSION

The effect of ciprofloxacin on the kidney was detected in this study where it was noticed that the effect of ciprofloxacin was dosage dependent on the kidney. Although there was no histological change in the renal corpuscles and renal tubules in all the groups, but the study revealed interstitial congestions which were noticed in group D and E due to the use of overdose.

6. RECOMMENDATIONS

- Prolonged usage of Ciprofloxacin should be avoided and also drug abuse and over dosage with ciprofloxacin should be avoided.
- Further research should be carried out on the effect of ciprofloxacin on the kidney of albino rats with more prolonged duration and higher doses.
- Alternative drugs from fluoroquinolones class should be used with the same dosage as in this study to assess the kidney histology.
- The weight of individuals administered with Ciprofloxacin should be checked at regular interval since it reduces body weight.
- More research should be carried out on other organs with the same dosage as in this study to ascertain if ciprofloxacin is dosage dependent.

REFERENCES

[1] Abd Allah, A.R.; Aly. A.M; Gannam, B.B. and Hamada, F.M. (2000): The impact of ofloxacin on rat testicular DNA: application of image analysis. *Pharmacol. Res.*, 42(2): 145-150.

[2] Abdollahi, M. and Isazadeh, Z. (2001): Inhibition of rat parotid and submandibular gland functions by ofloxacin, afluoroquinolone antibiotic. *Fundam. Clin. Pharmacol*.15 (5):307-11.

[3] Batalle, L., Rahier, J. and Guebel., A. (2002): Delayed and prolonged cholestatic with ductopenia after long term ciprofloxacin therapy for crohn's disease. *J. Hepatol*.; 37:696-699.

[4] Baykal, A., Arigul., F.S., Suleymanlar, P.I., Moreiea, G., Smith, M.A. and Aliciguzel, Y. (2005): Ciprofloxacin does not exert nephro-toxicity in rats; *Arn.J. Infect. Dis*.; 1:145-148

[5] Channa, M.A. and Janjua, M.Z. (2003): Effects of ciprofloxacin on foetal hepatocytes. *J. Pak. Med. Assoc*. 53 (10) 448-450.

[6] Contreras, M.A.; Luna, R.; Mulero, J. and Andreu, J.L. (2001): Severe ciprofloxacin-induced acute hepatitis. *Eur J Clin Microbiol Infect*. Dis. 20: 434-435.

[7] Goetz, M., Galle, P.r. and Schwarting, S. (2003): Non-fetal acute liver injury possible related to high dose Ciprofloxacin. *Eur. J. Microbiol. Infect.Dis.*; 22:294-299.

[8] Guzman, A.; Garcia, C.; Marin, A.P.; Willoughby, C. and Demestre, I. (2003): Developmental toxicity studies of the quinolone antibacterial agent irloxacin in rats and rabbits. *Arzneimittelforschung*, 53(2): 121-125.

[9] Hooper, D.C. and Wolfson, J.S. (1985): The fluoro- quinolones: pharmacology, clinical uses, and toxicities in human. Antimicrob. *Agents Chemother*. 28: 716-721.

[10] Hussy, P., Maass, G., Tummler, B., Grasse, F., Schomburg, U. (1986): Effect of 4-quinolones and novabiocin of calf thymus DNA polymerase a complex, topoisomerase I and II and and growth of mammalian tymphoblasts. *Antimicrob. Agents Chemother.*, 29: 1073-1078.

[11] Ismail, N.H. (2006): assessment of histopathological and histo-chemical changes in liver of pregnant female rats and their fetuses following ciprofloxacin administration. J. Egypt. *Soc. Toxicol.*; 35:7-17.

[12] Kashida, Y.; Sasaki, Y.; Ohsawa, K.; Yokohama, N.; Takahashi, A.; Watanabe, T. and Mitsumori, K. (2002): Mechanistic study on fiumequine hepatocarcinogenicity focusing on DNA damage in mice. *Toxicol. Sci*., 69(2): 317-321.

[13] Kim, J.C.; Yun, H.I.; Shin, H.C.; Han, S.S. and Chung, M.K. (2000): Embryo lethality and teratogenicity of a new fluoroquinolone antibacterial DW.-116 in rats. *Arch. Toxicol.*, 74(2): 120-124.

[14] Lietman, P. S. (1995): Fluoroquinolones toxicities: an update. Drugs. 49 (3): 156-163.

[15] McQueen, C.A. and Williams, G.M. (1987): Effects of quinolone antibiotics in tests for genotoxicity. *Am. J. Med.* 82(4A): 94-96.

[16] Minuk, G.Y.; Assy, N. and Ding, L.X. (1997): Effects of quinolone antibiotics on hepatic growth and protein synthesis following partial hepatectomy in rats. *J. Gastroenterol. Hepatol.*, 12:5-7.

[17] Nada,N.A. (2012): possible histopathological changes induced by therapeutic doses of ciprofloxacin in liver and kidney of juvenile rats; *phamacologia*; 3(9): 477-480.

[18] Pino, A.A.; Maura, A.V. and Masciangelo, L. (1991): Evaluation of DNA damage induced by norfloxacin in liver and kidney of adult rats and fetal tissues after transplacental. *Exposure Mutation Res*., 264: 81-85.

[19] Xie, H.J.; Broberg, U.G.L.; Lundgren, S.C.S.; Meurling, P.C. and Rane, A. H.M. (2003): Alteration of pharmacokinetics of cyclophosphamide and suppression of the cytochrome P450 genes by ciprofloxacin. *Bone Marrow Transplant*. 31: 197-203.

[20] Zimpfer, A.; Propst, A.; Mikuz, G.; Vogel, W.; Terracciano, L. and Stadlmann, S. (2004): Ciprofloxacin-induced acute liver injury: case report and review of literature. *Virchows Arch*, 444(1): 87-89.