Original Article

Anti-Oxidant Status Following Treatment with Ciprofloxacin Toxic Effects on Chondrogenic Cells in Immature Rat Liters

Haji Muhammad Aslam Channa[1], Bhojo Mal Tanwani[2], Naheed Gohar[3] and Roohi Kanwal[4]

ABSTRACT

Objective: To investigate that in reverse, supplementation of zinc chloride if given simultaneously, can diminish the typical ciprofloxacin-induced chondrotoxicity in immature rat liters.
Study Design: A prospective experimental animal study
Place and Duration of Study: This study was conducted at the Department of Anatomy. Pir Abdul Qadir Shah Jeelani Institute of Medical Sciences Gambat District Khairpur Mir`S from Jan 2014 Dec 2014.
Materials and Methods: Ciprofloxacin & ZnCl2 was administrated to immature rat liters. Ciprofloxacin with a dose of 20 mg/kg body weight & ZnCl2 120 µg/100 gm body weight two times therapeutic dose for 20 days. (From day -1 to day 20 after birth.) Their mean chondrocyte count, chondrocyte size and their nucleus size per field was measured and were compared with similar value of control animals. The results were statistically analyzed to find out the significance.
Results: It was concluded after experimentation that ciprofloxacin administered in immature rat liters decreased mean chondrocyte count, chondrocyte size and their nucleus size per field, decreased by 213.7 ± 0.41, 11.12 ± 0.06 µm and 4.37 ± 0.12 µm respectively. That ciprofloxacin & ZnCl2 administration maintained the mean chondrocyte count, chondrocyte size and their nucleus size per field maintained by 274.4 ± 0.47, 10.47 ± 0.04 µm and 5.36 ± 0.03 µm respectively. That ZnCl2 administration increased the mean chondrocyte count, chondrocyte size and their nucleus size per field by 316.0 ± 0.40 , 10.82 ± 0.10 5 µm and 5.69 ± 0.04 µm respectively.
Conclusion: Application of ciprofloxacin and ZnCl2 post-natal in immature rat liters affected the mean chondrocyte count, chondrocyte size, and their nucleus size per field. ZnCl2 maintained mean chondrocyte count, chondrocyte size and their nucleus size per field leading to growth in immature rat liters.
Key Words: Ciprofloxacin, ZnCl2, chondrocyte count per field, chondrocyte size, chondrocyte nucleus, immature rat liters

Citation of articles: Channa HMA, Tanwani BM, Gohar N, Kanwal R. Anti-Oxidant Status Following Treatment with Ciprofloxacin Toxic Effects on Chondrogenic Cells in Immature Rat Liters. Med Forum 2018;29(8):77-81.

INTRODUCTION

Ciprofloxacin antibiotic belongs to the class fluoroquinolone which is a broad spectrum antibiotic and plays an active role for the control of both Gram Positive and Gram Negative bacteria[1]. Fluoroquinones functions by the inhibition of DNA Gyrase which is an enzyme belongs the class Topoisomerase and required by bacteria to complete their metabolic activities.[2]

1. Department of Anatomy, Pir Abdul Qadir Shah Jeelani Institute of Medical Sciences Gambat.
2. Department of Physiology, Peoples University of Medical & Health Sciences for Women, Nawabshah.
3. Department of Anatomy, Sir Sayed College of Medical Sciences for Girls, Karachi
4. Department of Zoology, University of Karachi.

Correspondence: Dr. Haji Muhammad Aslam Channa, Professor & Chairman, Department of Anatomy, Pir Abdul Qadir Shah Jeelani Institute of Medical Sciences Gambat.
Contact No: 0300-3210803
Email: drmaslamchanna62@gmail.com

Received: January, 2018; Accepted: July, 2018

Quinilones are the components, which are bactericidal in nature, which denature the replication and transcription process results in the death of bacterial cells.

Large number of bacterial infections has been cured by Ciprofloxacin but many side effects have been evident from variable doses of this drug including tendon rupture, deterioration in bones, joints and disintegration to the cartilage of growing youngs[3].

Generally Physicians prescribes the quinolones to newborns, adults and even to pregnant/ nursing females without considering their side effects including the abrasions and injuries in cartilage of joints and instead of the fact that this drug is not recommended for their usage[4].

Nam et al., studied the adverse effect of continuous dosages of Quinolones and suggested that frequent dosages of this drug leads to the deterioration in bones and cartilage due to over concentration of fluoride contents in the body which results in impede growth and fragile bones.[5]

One of the essential trace element which play important role in maintenance of immune responses and initiation

of normal developmental patterns of body is Zinc, It is also responsible for the development of fetal systems, production of DNA, RNA as involved in proteins synthesis in the body.[6]

Two types of Skeletogenic cell produced during the developmental patterns of fetus, firstly Chondrocytes which are the cartilaginous cells and Osteoblasts that are bony in nature. Firstly the whole skeleton is composed of cartilaginous structure and gradually it transformed into bony skeleton in post natal period by ossification.[7]

MATERIALS AND METHODS

Effects of the different doses of Ciprofloxacin were examined in Albino rats. Through random sampling, Forty ovulating females were selected and they were mated with twenty males having age of 16 to 18 weeks. All animals were collected from Pir Abdul Qadir Shah Jeelani Institute of Medical Sciences Gambat. According to the Luck[8], the methodology of mating the members of similar strain was adopted. One male rat was mated with two different females in different cages. The signs of mating such as blood stained vagina with mucoid greenish/whitish material observed after twelve hours of mating which indicates the zero day of pregnancy.[9]

Four different groups A. B.C and D were formed by dividing 40 immature albino rats which were spontaneously selected from the whole lot. Ten members included in each group. Group A was considered as a control group, the members of which were given normal saline (0.1 ml) two time a day. The doses of normal saline were administrated intra-peritoneal for twenty days.

Group B was treated with injections of Ciprofloxacin (20mg/kg). All the doses were given intra peritoneally twice daily for twenty days.

Group C was treated with a little modification, they have given the injections of Ciprofloxacin along with the Zinc chloride (120 microgram.100 gram) prepared in distil water (7.4 µg in 0.1 ml).The injection of Zinc chloride was given thirty minutes before Ciprofloxacin administration. This whole dose was given two times a day for twenty days.

The members of Group D were treated with only Zinc chloride (120 microgram. 100 gram) for twenty days. At twenty first day immature rats were dissected for further examination. Anesthesia treatment was done before dissection. Bones were obtained from the limbs, Firstly fixation was done by putting them into 19% buffered formalin. After fixation, removal of calcium was required, the decalcification process was done by putting them in 10% Nitric acid and 10% Formic acid[10]. Pparplast was used for embedding purpose. Then sectional cutting was done by cutting the bones through rotary microtome into 4 micrometer thick longitudinal sections.

The staining was done by using Haemotoxylin and Eosin stain[11]. Measurement of forms and sizes of Tissues as well as quantitative analysis of bones were done by Histomorphometry. Statistical analysis of the results was also done by applying the 't' test mechanism on the data collected. Level of significance was also calculated[12].

RESULTS

Mean chondrocyte count per field in two weeks post-natal treated immature rat liters: Mean chondrocytes count were estimated by observing the fields in the samples from all four groups.

In group A, the mean of post natal chondocytes was estimated at 276.5 ± 1.05. Statistical analysis reveals that Group A show the high significant increase as compared to B, C and D. While B shows the non significant increase, As far as C and D are concerned, both shows the highly significant decrease (Table1).

In group B, the mean of post natal chondrocytes count was estimated as 213.7 ± 0.41. The results shows the highly significant decrease in number while observing the data from group A, C and D (Table 1).

In group C, the similar count of chondrocytes were made. It was estimated that mean count of post natal chondrocytes were 274.4 ± 0.47. Current calculation also shows a highly significant decrease by observing the data from group D ($P < 0.001$) and a non significant change ($P > 0.032$) was also observed during a comparison of present data of group C with group A (Table 1).

The results of group D shows the mean of post-natal chondrocyte count per field 316 ± 0.40 which declares a high significant increase as compared to the data from group A, B and C (Table 1).

Table No.1: Comparison of chondrocyte count per field in immature rat liters between post-natal control and treated groups

	Group A Control (n=10)	Group B Ciprofloxacin (n=10)	Group C Ciprofloxacin + Zinc Chloride (n=10)	Group D Zinc Chloride (n=10)
	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
Postnatal (Day-20)	276.5 ± 1.05^{øø}	213.7 ± 0.41	274.4 ± 0.47^{øø}	316.0 ± 0.40$^{**,øø,◊◊}$

$^{**}p<0.01$ highlysignificant as compared to Control (A),
$^{øø}p<0.01$ highly significant as compared to Ciprofloxacin (B),
$^{◊◊}p<0.01$ highlysignificant as compared to Ciprofloxacin + Zinc Chloride (C)

Mean chondrocyte size per field in two weeks post-natal treated in immature rat liters: Sizes of the chondrocytes were also observed after the treatment with normal saline, ciprofloxacin and zinc chloride.

Mean sizes of all chondrocytes per field were estimated from the samples of animals of group A, B, C and D.

In group A, the mean chondrocyte size was estimated as 10.28 ± 0.02 µm. In histological examination of samples from group B, a highly significant increase has been seen, while in the samples from group C, a non-significant change been observed. In the members of group D statistical analysis described the high significant decrease in sizes of chondrocytes (Table 2).

In the histological examination of samples from group B and their statistical analysis, the mean size of chondrocytes was estimated as 8.40± 0.06 µm. Comparative analysis showed the highly significant decrease in sizes in B from group A, C and D (Table 2).

The mean size of chondrocytes in group C was calculated as 10.47 ±0.04 µm. The comparison of mean size of group C with group D showed the highly significant decrease i.e (P<0.001), When the result has been compared with the control group A, it showed the non significant change i.e. P>0.05 (Table 2).

In group D, the mean size of chondrocytes calculated as 10.82 ± 0.10 µm. Statistic analysis showed the highly significant increase in this group i.e. (P<0.001) as compared to THE groups A, B and C (Table 2).

Mean nuclear size of chondrocyte per field in two weeks post-natal treated immature rat liters: The sizes of nucleus of chondrocytes were also estimated from the microtome cut sections of cells. In the samples of group A, the mean size of nucleus of chondrocytes was estimated at 5.46 ± 0.09 µm.. The result has been contrasted with B, C and D. The data collected from B, showed the highly significant increase, while data evaluated from C reveals the non-significant change. The data analyzed from D suggested the highly significant decrease in sizes of nucleus of chondrocytes (Table 2).

Table No.2: Comparison of chondrocyte cell size (µM) and their nucleus size (µM) in immature rat liters between postnatal control & treated groups

	Group A Control (n=10)	Group B Cipro-floxacin (n=10)	Group C Cipro-floxacin + Zinc Chloride (n=10)	Group D Zinc Chloride (n=10)
	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
Chondrocyte Size - Cell (µm)	10.28 ± 0.02	11.12 ± 0.06 **	10.47 ± 0.04	10.82 ± 0.10$^{**,◊◊}$
Chondrocyte Size - Nucleus (µm)	5.46 ± 0.09^{øø}	4.37 ± 0.12	5.36 ± 0.03	5.69 ± 0.04$^{øø,◊◊}$

**p<0.01 highlysignificant as compared to Control (A),
^{øø}p<0.01 highly significant as compared to Ciprofloxacin (B),
$^{◊◊}$p<0.01 highlysignificant as compared to Ciprofloxacin + Zinc Chloride (C)
^{ΔΔ}p<0.01 highlysignificant as compared to Zinc Chloride (D)

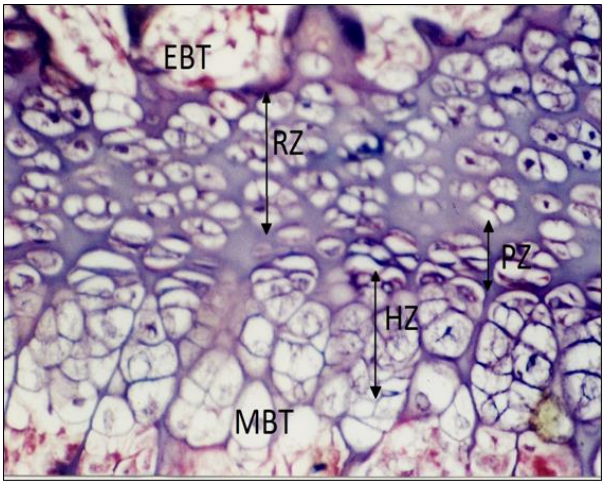

Figure No.1: H & E X 400: Photomicrograph of 5 µm thick longitudinal section of epiphyseal growth plate at proximal end of humerus from a 20th post-natal day control Group-A immature rat liters showing Metaphyseal bone trabeculae (MBT), Epiphyseal bone trabeculae (EBT),Reserve cell zone (RZ), Proliferative zone (PZ) and Hypertrophy zone (HZ)

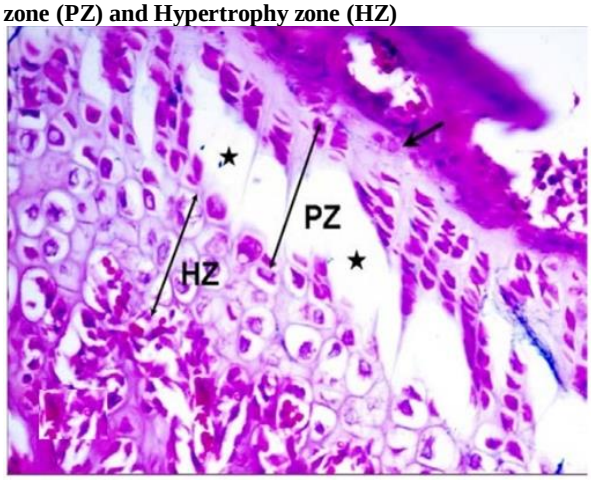

Figure No.2: H & E X 400: A photomicrograph of 5 µm thick longitudinal section in the femoral epiphyseal growth plate cartilage from a 20th post-natal day of ciprofloxacin-treated Group B immature rat liters showing few cells in the reserve zone ↑ wide clefts * with marked loss of chondrocyte columns of the proliferative zone (PZ) and diminished size of the hypertrophic zone (HZ).

The mean sizes of nucleus of chondrocytes from Group B were calculated as 4.37 ±0.12 µm. Statistic analysis revealed the high significant decrease in size of nucleus as the results were compared from the data from groups A, C and D (Table 2).

Samples from group C were also histological examined. It was found that the size of nucleus ranged as 5.36 ± 0.03 µm. Data calculated statistically and estimated that high significant decrease was found while comparing the data with group D i.e. (P< 0.001) and when it compared with the control group A, it showed the (P>0.05) i.e. a non significant change (Table 2).

In the samples from group D, mean nucleus size estimated as 5.69± 0.04 µm. The comparison of result

with other three groups i.e. A, B and C stated that high significant increase has been shown i.e. P<0.001 (Table 2).

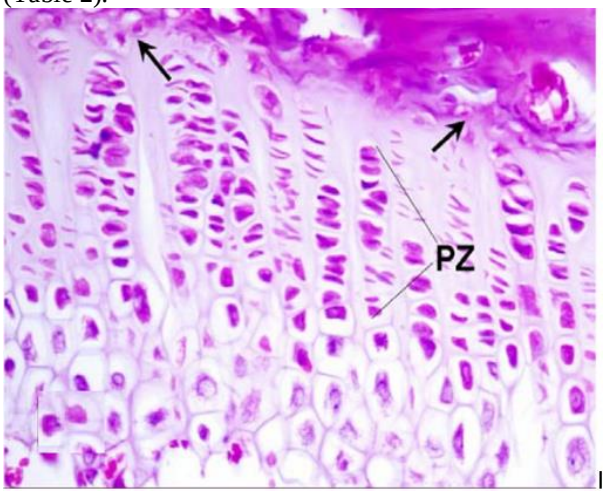

Figure No.3: H & E X 400: A photomicrograph of 5 µm thick longitudinal section of epiphyseal growth plate at proximal end of humerus from a 20th post-natal day Ciprofloxacin + Zncl2 treated Group-C immature rat liters showing preserved cellularity of reserve zone (↑) and regular cellular organization of chondrocyte columns of the proliferative zone (PZ).

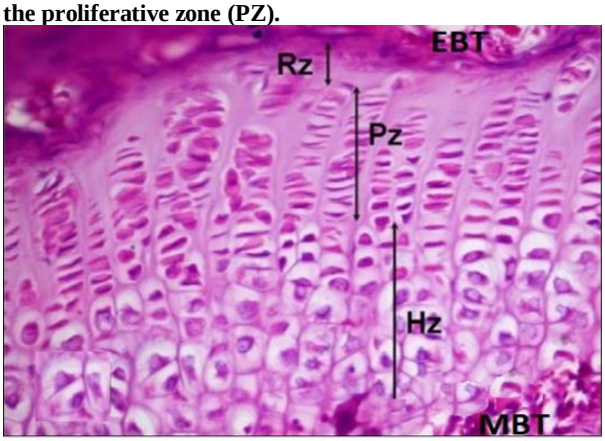

Figure No.4: H & E X 400: A photomicrograph of 5 µm thick longitudinal section of epiphyseal growth plate cartilage of humerus from a 20th post-natal day Zncl2 treated Group D immature rat liters showing junction of epiphyseal bone trabeculae (EBT),Reserve cell zone (RZ), thick Proliferative zone (PZ), Hypertrophy zone (HZ) and Metaphyseal bone trabeculae (MBT).

DISCUSSION

The effects of Ciprofloxacin in a single dose of 20mg/kg of body weight were examined in the cartilaginous cells i.e. chondrocytes of immature albino rats. The control group was treated with normal saline while zinc chloride was also added as a supplement along with ciprofloxacin to check its support during developmental period. Ciprofloxacin affected the newly developing cartilage cells and demonstrated highly significant decrease in different parameters of measurement i.e. chondrocytes count, chondrocytes sizes and the sizes of nucleus in those cells.

In group A, it was observed through microscopic histological evaluation of epiphyseal growth plate showed the abnormalities in the chondrocytes count, sizes as well as deformities in the nuclear membranes of those cartilaginous cells. During the fetal development, the main process of formation of rudimentary long bones is endochondral ossification[13].

The epiphyseal plate of the samples from group B revealed that highly significant decrease in count, sizes of cells and nuclei was found. This may be resulted in the higher ratio of cell necrosis in inner layers of epiphyseal plate.

According to Masadeh, Ciprofloxacin drugs have adverse effects on ossification, This drug accelerate the necrotic mechanism in calcification zone IV, as well as in other zones of cartilage formation. It also leads to the condensation of nuclear material in chondrocytes which results in abnormalities in morphological and cellular functions. Results of the current study line up with the results obtained by Masadeh[14].

The histological evaluation of the samples from group C resulted in the significant changes in their chondrocytes count, sizes and nuclei. The cells were normomorphic. No degradation in cellular lining or nuclear lining were showed. Addition of Zinc chloride with the doses of ciprofloxacin provided the stability to cellular development and played a supportive role during the mechanism of drug reaction. According to Nishada, Zinc provides support to the protein formation and act as a stabilizer and key integration factor during cell production. The results of current study line up with results of Nishada[15].

In the last group D, which were only treated with zinc chloride, no adverse effects has been seen in the cellular structure of chondrocytes. High significant increase has been observed. The cellular structure were normomorphic and stable.

According to Jou, Zinc components support good growth of cells and involved in quicker process of protein synthesis. Current study line up with the findings of Jou[16]

CONCLUSION

It is concluded that the application of ciprofloxacin and ZnCl2 in immature rat liters affected the mean chondrocyte count, chondrocyte size and their nucleus size per field. This study clearly shows the ability of zinc chloride to maintained the all parameters leading to growth of the immature rat liters. It is recommended that some more aspects of Ciprofloxacin and zinc chloride bonding effects should be investigated.

Acknowledgements: I am thankful to and fortunate enough to get constant encouragement, support and guidance from all staff in laboratory of Anatomy

Department Pir Abdul Qadir Shah Jilani Institute of Medical Sciences Gambat for their timely support

Author's Contribution:

Concept & Design of Study:	Haji Muhammad Aslam Channa
Drafting:	Bhojo Mal Tanwani
Data Analysis:	Naheed Gohar, Roohi Kanwal
Revisiting Critically:	Haji Muhammad Aslam Channa, Bhojo Mal Tanwani
Final Approval of version:	Haji Muhammad Aslam Channa

Conflict of Interest: The study has no conflict of interest to declare by any author.

REFERENCES

1. Masadeh MM, Alzoubi KH, Al-Azzam SI, Khabour OF, Al-Buhairan AM. Ciprofloxacin-Induced Antibacterial Activity is Attenuated by Pretreatment with Antioxidant Agents. Pathogens 2016; 5(1): 239-245.
2. Channa MA, Ashfaq M, Jokhio AL, Khan MZ, Sahito MM. Effects of ciprofloxacin and zinc chloride in adult albino rat and pre-natal conceptus J Ayub Med Coll Abbottabad 2012;24(1):55-8.
3. Pfister K, Manzur D, Vorman J, Stahlman R. Diminshed ciprofloxacin Induced chondrotoxicity by supplementation with magnisium & vitamin E on immature rats. Anti Microbe agent chemother 2007; 51(3): 1022-1027.
4. Mont MA, Mathur SK, Frondoza CG, Hungerford DS, The Effects of Ciprofloxacin on Human Chondrocytes in Cell Culture. Curr Ther Res Clin Exp 2015; 77: 14–17.
5. Nam YS, Cho SY, Yang HY, Park KS, Jang JH, Kim YT, et al. Investigation of mutation distribution in DNA gyrase and topoisomerase IV genes in ciprofloxacin-non-susceptible Enterobacteriaceae isolated from blood cultures in a tertiary care university hospital in South Korea, 2005-2010. Int J Antimicrob Agents 2013;41:126–129.
6. Salvaggio A, Marino F, Albano M, Pecoraro R, Camiolo G,Tibullo D, et al. Toxic Effects of Zinc Chloride on the Bone Development in Danio rerio (Hamilton, 1822.). Front Physiol 2016;7: 153-160.
7. Küçükoğlu M., Binokay US, Boğa PA. The effects of zinc chloride during early embryonic development in zebrafish (Brachydanio rerio). Turk J Biol 2013; 37: 158–164.
8. Luck MR, Ye J, Almislimani H, Hibberd S. Follicular fluid rheology and the duration of the ovulatory process. J Reprod Fertil 2000; 120(2):411-21.
9. Chang HH, Schwartz Z, Kaufman MH. Limb and other Postcranial skeletal defects induced by amniotic sac puncture in the mouse. J Anatl 2011; 189:37-49.
10. Channa HMA, Ashfaque M, Mastoi SM, Qureshi MA. Effects of ciprofloxacin on growing cartilage in Albino rat pups. J Ayub Med Coll Abbottabad 2006;18(3):50–4.
11. Bancroft J D; Stevens A. Theory and Practice of Histological Techniques. 3rd ed. Edinburgh: Churchill Livingston;2010.p. 88,112, 232, 503.
12. Bland M. Introduction of medical statistics. 1st ed. Oxford:Oxford University press;2013.p.165–87.
13. Wang X, Jaffer G, Fosmire, Gay CV, Leach RM . Short term zinc deficiency inhibits chondrocyte proliferation and induces cell apoptosis in epiphyseal growth plate of young chickens. The Am soc Nutr Sci J Nutr 2002;123:665-673.
14. Masadeh M, Alzoubi K, Al-Azzam S. Flouroquinolones-induced antibacterial activity atteneuation by pretreatment with vitamin b12. Int J Pharmacol 2015; 11: 67–71.
15. Nishida K, Hasegawa A, Nakae S, Oboki K, Saito H, Yamasaki S, et al. Zinc transporter Znt5/ Slc30a5 is required for the mast cell mediated delayed-type allergic reaction but not the immediate type reaction. J Exp Med 2009;206(6): 1351-1364.
16. Jou MY, Philips AF, Lonnerdal BO. Maternal zinc deficiency in rat effect growth & glucose metabolism in the offspring by inducing insulin resistance post nataly 1,2. Am society Nutr 2010; 157:172-184.