Original Article

Role of Anti-Oxidant on Ciprofloxacin Induced Toxicity in Intact Bone Length of Juvinile Albino Rats

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ABSTRACT

Objective: To investigate whether ciprofloxacin induced chondrotoxicity with normal therapeutic dosage is preventable by zinc chloride if given simultaneously.

Study Design: Prospective / experimental study.

Place and Duration of Study: This study was conducted at the Department of Anatomy Basic Medical Science Institute Jinnah Postgraduate Medical centre Karachi from Jan 2014 Dec 2014.

Materials and Methods: Ciprofloxacin & ZnCl2 was administrated to juvenile albino rats. 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.) Each animal was measured their intact bone length and were compared with similar value of control animals. The results were statistically analyzed to find out the significance.

Results: Our study reveals that ciprofloxacin administered in juvenile albino rats decreased intact bone length , of Humerus right & left 9.91 ± 0.18 mm, Femur right & left 11.49 ± 0.12 mm respectively. That ciprofloxacin & ZnCl2 administration maintained the intact bone length of Humerus right & left 18.48 ± 1.25 mm, Femur right & left 14.54 ± 0.09 mm respectively. That ZnCl2 administration increased the intact bone length of Humerus right & left 14.60 ± 0.13 mm, Femur right & left 14.58 ± 0.10 mm respectively.

Conclusion: The ciprofloxacin & ZnCl2 post-natal administration in juvenile albino rats affected the mean intact bone length. ZnCl2 maintained intact bone length leading to growth of the juvenile albino rats.

Key Words: Ciprofloxacin, ZnCl2, Juvenile albino rats and Intact bone length

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INTRODUCTION

Quinolones are the fluorinated derivatives, these are ciprofloxacin, sparfloxacin, clanofloxacin, travo-floxacin, ofloxacin, and norfloxacin[1] .

Ciprofloxacin was introduced in 1987.It is on the World Health Organization's List of Essential Medicines it is an antibacterial substance with wide bacterial spectrum activity, which belongs to the chemical class 4-quinolones, and is entirely synthetic, therefore this substance is among the most commonly used antibiotics nowadays for different kinds of infections, it functions by inhibiting DNA gyrase, and a type II topoisomerase, topoisomerase IV, necessary to separate bacterial DNA, thereby inhibiting cell division [2]

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Along with its wide range of activity and common usage the drug has many side effects, i.e, degenerative changes in weight bearing joints and damage to the growing cartilage in young animals[3]. Therefore is not generally recommended for use in children, adolescents, and during pregnancy.. Some reports on hypersensitivity, chondrotoxicity and super-infection have been reported with ciprofloxacin[4] .

Zinc is the trace elements and essential for the synthesis of DNA, RNA and proteins, and physiological functions of several enzymes and fetal organ development. In addition to its role in catalysis and gene expression, zinc stabilizes the structure of proteins and nucleic acids and preserves the integrity of sub cellular organelles such as mitochondria [5]

The large number of factors are involved in skeletogenesis i.e., harmones, growth factors, receptors, signaling mediators, transcription factors, extracellular matrix components and enzymes. Factors determining the identity of skeletal cells are called differentiation factors and factors specifying the number, size and shape of skeletal elements are called patterning factors [6]

MATERIALS AND METHODS

Forty spontaneously ovulating female & 20 fertile male wistar albino rats of 16-18 weeks age were taken from animal house of basic medical science institute, Jinnah Postgraduate Medical Centre Karachi. The female rats

were mated with of same strain according to the method described by Rough[7]. Thus one male rat was mated with two female rats in a separate cage. On next day each female rats were examined for signs of mating. Such a blood strained vagina or vaginal plugh of mucoid greenish white material. Presence of both or any of these signs were considered a day zero of pregnancy, the mean gestation period of albino rat is 21 to 23 days.

Randomly selected 40 juvenile albino rats were divided into four groups, A, B,C and D. each group comprising 10 animals, group A juvenile albino rats act as control and were given normal saline in equal volume (0.1 ml) intra-peritoneally twice daily for 20 days (from day, 1 today, 20 after birth), group B were given injection ciprofloxacin at a dose of 20 mg/kg weight (0.12 mg in 1.1 ml) intra-peritoneally twice daily for 20 days (from day -1 to day -20 after birth), group C were given injection ciprofloxacin plus zinc chloride 120 µg/100 G body weight prepared in distilled water (7.4 µg in 0.1 ml) intra-peritoneally 30 minutes before administration of ciprofloxacin twice daily for 20 days (from day ,1 to day ,20 after birth), group D were given injection zinc chloride at a dose of 120 µg/ 100 G body weight prepared in distilled water (7.4 µg in 0.1 ml) twice daily for 20 days (from day ,1 to day ,20 after birth), and on day-21, the juvenile albino rats were sacrificed by giving deep anesthesia, and were operated to obtain their long bones. Skeletal specimen processed through 96% ethanol and acetone and bulk tissue stained with alizarin red "S" and alcian blue. Than material was cleared in 1% potassium hydroxide and stored in glycrine[8] . This technique demonstrate the ossified bone with Alizarin Red "S" and cartilage with Alcian blue as shown in Figs. 1 and 2. The double stained cleared specimen was observed under spenser stereomicroscope. The fore and hind limbs were disarticulated and total length of cartilaginous models of long bones of extremities were measured under stero microscope. The values from control and experimental groups were compared for statistical analysis.

RESULTS

Post-natal changes in tintact bone length (mm) of juvinile albino rats treated for 20 days: The mean value of intact bone length as determined by measuring the length of long bones of right and left fore limb (Humerus) and hind limb (Femur) respectively with the help of electronic digital vernier caliper of groups A, B, C and D is presented in Table . .

Humerus: The mean value of postnatal treated humerus (right and left) length measured in group A animals was 13.67 ± 0.93 mm. A highly significant increase in length was observed when compared with animals group B ($P < 0.001$) as shown in Table .

The mean value of postnatal humerus (right and left) measured in group B was 9.91 ± 0.18 mm. A highly significant decrease in length was observed when compared with group A, C and D ($P < 0.001$) as shown in Table.

The mean value of postnatal treated humerus (right and left) length measured in group C animals was 12.48 ± 1.25 mm. A highly significant increase in length was observed when compared with group B ($P < 0.001$) as shown in Table .

The mean value postnatal treated humerus (right and left) length measured in group D animals was 14.60 ± 0.13 mm. A highly significant increase in length was observed when compared with animals in group A and B ($P < 0.001$) as shown in Table .

Table No.1: Comparison of intact bones length (mm) of juvinile albino rats between postnatal control and treated groups

Intact Bones Length (mm)	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
Humerus				
4X - Right	13.67 ± 0.93$^{øø,◊◊}$	9.91 ± 0.18	12.48 ± 1.25^{øø}	14.60 ± 0.13 $^{**,øø,◊◊}$
4X - Left	13.67 ± 0.93 $^{øø,◊◊}$	9.91 ± 0.18	12.48 ± 1.25^{øø}	14.60±0.13 $^{**,øø,◊◊}$
Femur				
4X - Right	14.88 ± 0.04 $^{øø,◊◊,ΔΔ}$	11.49± 0.12	14.54 ± 0.09^{øø}	17.58± 0.10 ^{øø}
4X - Left	14.88 ± 0.04 $^{øø,◊◊,ΔΔ}$	11.49 ± 0.12	14.54 ± 0.09^{øø}	17.58± 0.10 ^{øø}

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

Femur: The mean value of postnatal treated femur (right and left) measured in group A was 14.88 ± 0.04 mm. A highly significant increase in length was observed when compared with animals in group B ($P < 0.001$) as shown in Table .

The mean value of postnatal treated femur (right and left) length measured in group B was 11.49 ± 0.12 mm.

A highly significant decrease in length was observed when compared with group A, C and D ($P < 0.001$) as shown Table .

The mean value postnatal treated femur (right and left) measured in group C animals was 14.54 ± 0.09 mm. A highly significant increase in length was observed when compared with group B ($P < 0.001$) as shown in Table.

The mean value of postnatal treated femur (right and left) length measured in group D animals was 17.58 ± 0.10 mm. a highly significant increase in length was observed when compared with animals in groups A and B ($P < 0.001$) as shown in Table.

Figure No.1: Photograph of fore limb right humerus bone of juvenile albino rats on 20th post natal day showing comparison of double staining technique i.e. Alizarin Red-S staining bone & Alcian blue staining cartilage, between control and treated groups-A,B,C & D used in this study.

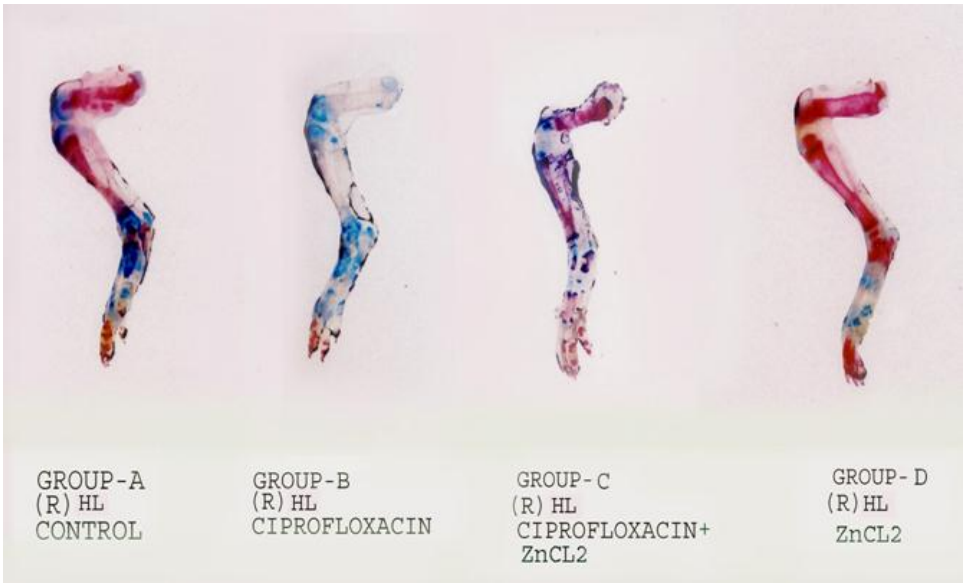

Figure No.2: Photograph of hind limb left femur bone of juvenile albino rats on 20th post- natal day showing comparison of double staining technique i.e. Alizarin Red-S staining bone & Alcian blue staining cartilage, between control and groups-A,B,C & D used in this study

DISCUSSION

Present study was designed to observe the morphological effect of ciprofloxacin and zinc chloride separately and when administered simultaneously in post-natal juvenile albino rats.

Ciprofloxacin administered in a dose of 20 mg/kg body weight to juvenile albino rats, morphology showed

highly significant decrease intact bone length, (Humerus and femur) were observed in post-natal juvenile albino rats.

Regarding juvenile albino rats A highly significant decrease in intact bone length in post-natal group B may be attributed to less food intake and degenerative changes in growing cartilage occurred following administration of ciprofloxacin. These observations are in accordance with the findings of Berkovitch[9], and Cukerski[10] . Who found that only constant findings of ciprofloxacin was decrease in weight and length post-nataly.

The post-natal groups C showed increase in their length which may be attributed to the partial protection by zinc against the unwanted effect of ciprofloxacin on bone length. These findings are in agreements with the results of MacDonald[11] . Who found that the supplementary zinc has beneficial effect on growth by increasing protein synthesis. Zinc participates in regulation of cell proliferation in several ways, it is essential to enzyme systems that influence cell division and proliferation.

Similarly, the intact bone length in post-natal group D. A highly significant increase was observed in comparison with other groups, which may be attributed to increased protein synthesis by zinc chloride. These results are in agreement with Salgueiro[12] and Jou[13] . Who found that after supplementation of zinc, the mean bone length increase was significantly greater

Our observations are in consistence with Adikwu[14] who reported the condrotoxicity of quinolones as observed in immature animals, can effect articular cartilage and the epiphyseal growth plate, depending on the development stage. Stahlmann[15] noted the juveniles are especially sensitive and in animal at an early developmental phase the epiphyseal growth is also damaged by the quinolones and these effects are associated with reversible bone damage and growth inhibition.

The non-significant change was obtained in intact bone length simultaneously given Zinc chloride animals in group C was found to be in humerous and in femur when compared with the age matched controls, these findings are attributed to protective role of zinc chloride. Our observations are in agreement with those by Hickory[16], who found that zinc help in access formation of collagen increase osteoblastic activity and increase rate of longitudinal growth and bone remodeling in experimental rats. Prassad[17] , stated that zinc directly stimulates DNA synthesize either by enzyme stimulation or by altering the binding of F1 & F3 histones to DNA, so as to effect RNA synthesize.

CONCLUSION

The ciprofloxacin & ZnCl2 post-natal administration in juvenile albino rats affected the mean intact bone length. ZnCl2 maintained intact bone length leading to growth of the juvenile albino rats. There is need for a greater focus on frequent use of antibiotics and more research should be done to help learn to affectively treat the negative side effects of ciprofloxacin with simultaneous use of anti-oxidant Zinc chloride,

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

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