**ORIGINAL** **PROF-718**

# PROTECTIVE ROLE OF ANTI-OXIDANT
## AGAINST HEPATOTOXICITY BY CIPROFLOXACIN IN WISTAR ALBINO RATS

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## ABSTRACT
**Objectives:** To evaluate the preventive role of zinc chloride on hepatotoxicity of Ciprofloxacin administration in Wistar albino rat pups. **Design of Study:** Prospective and Comparative study. **Setting:** The study was carried out in the department of Anatomy, Basic Medical Sciences Institute, Jinnah Postgraduate Medical Centre, Karachi. **Period:** From 2001 to 2002. **Material & Method:** Ciprofloxacin and zinc chloride were administered to newly born albino rat pups separately and simultaneously at a dose of 20 mg/kg body weight and 120 ug/100 gm body weight respectively intraperitoneally twice daily from day-1 to day-14 after birth. Pups weighed on postnatal day-1 and postnatal day-14, on day-15 after birth these animals were killed by deep ether anaesthesia, their liver removed, weighed, and fixed in 10% buffered formalin, embedded in paraplast, 3 um thick sections were cut on rotary microtome and stained with H&E. The histomorphological features of livers were compared with those of control animals and analyzed statistically. **Results:** The study revealed that Ciprofloxacin administration in new born albino rat pups produced necrotic changes in liver and simultaneously zinc chloride partially prevented hepatotoxicity. **Conclusion:** These results strongly suggest that Ciprofloxacin causes severe liver damage in pups. However Ciprofloxacin induced hepatotoxicity could be partially prevented by simultaneous administration of zinc chloride in Wistar albino rat pups.

**Key Words:** Ciprofloxacin, Hepatotoxicity, Zinc chloride

## INTRODUCTION

In 1980s, a new drug application for intravenous Ciprofloxacin was submitted to United States Food and Drug Administration[1]. Ciprofloxacin is a fluorinated derivative of quinolones. It is an anti-bacterial substance with wide bacterial spectrum of activity and is entirely synthetic[2]. The Ciprofloxacin is among the most commonly used antibiotics nowadays for different kinds of infection along its wide range of activity and common usage of drug has many side-effects, i.e. hepatotoxic[3], nephrotoxic[4], and damage to the growing cartilage in young animals[5].

Ciprofloxacin is described as gyrase inhibitor because of its mode of action, gyrase enzyme is important for metabolic activity of bacteria[6]. Zinc is one of the trace elements and is known to be essential for synthesis of DMA, RNA, proteins, and physiological functions of several enzymes. Zinc stabilizes the structure of proteins and nucleic acids and preserves the integrity of subcellular organelles such as mitochondria[7].

This study was done to evaluate the effects of ciprofloxacin and zinc chloride administration during infancy separately and simultaneously on morphology of liver parenchyma of laboratory animals.

## MATERIALS & METHODS

One hundred and twenty pups were used in this study. They were obtained from 30 pregnant female Wistar albino rats, 16-18 weeks of age, weighing 140-200 gm, looking active and healthy, taken from Animal House of BMSI, JPMC, Karachi, and approval taken from Animal Care Ethics Committee of JPMC, Karachi. These female rats were mated with fertile males of same strain allowing one male rat with two female rats in one cage[8]. On next morning the female rats were examined for sign of mating in the form of blood stained vagina or a vaginal plug (a mucoid greenish white material) or presence of any one of these signs was considered as day-1 of pregnancy[9]. The normal gestational duration in albino rat ranged between 21 and 23 days[10]. Thirty pregnant albino rats were allowed to deliver their pups. Randomly selected 120 pups were divided into three groups; A, B and C, each comprising 40 animals. Sex of these offspring was omitted.

Group-A (n=40) pups were given injection Ciprofloxacin (developed in Bayer Research Laboratories, AG Germany) at a dose of 20 mg/kg/ body weight[11] (0.12 mg in 0.1 ml) intraperitoneally twice daily for 14 days (from day-1 to day-14 after birth). Group-B (n=40) pups were given simultaneously zinc chloride (developed in Laboratory Chemical, West Germany) at a dose of 120 µg/100 gm body weight[12] (7.4 µg in 0.1 ml) intraperitoneally 30 minutes before administration of ciprofloxacin twice daily for 14 days (from day-1 to day-14 after birth).

Group-C (n=40) pups acted as control and were given normal saline in equal volume (0.1 ml)[13] intraperitoneally twice daily for 14 days (from day-1 to day-14 after birth).

After weighing pups all the three groups were sacrificed on 15th post-natal day by giving deep anaesthesia and were operated to obtain their livers which were weighed, fixed in 10% buffered formalin, embedded in paraplast and 3 (µm thick sections were cut on rotary microtome. These sections were stained with haematoxylin and eosin (H&E).[14]

## STATISTICAL ANALYSIS

All results are given as mean ± SEM. Individual differences between control and experimental groups were significantly determined by students 't' test[16]. The values were determined with the help of reticule per unit area (0.00324 $mm^2$/Field).

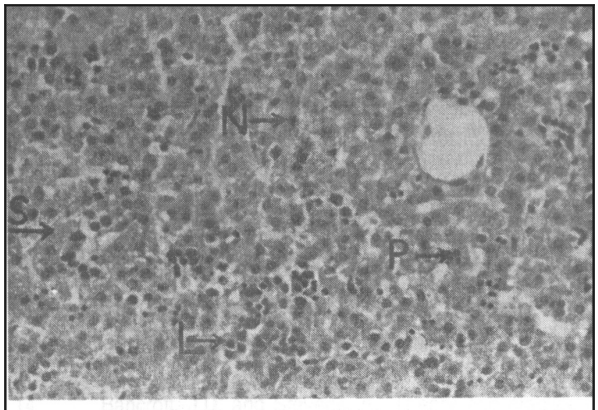
## RESULTS

The greatest changes were observed in body weight (gm), liver weight (gm), hepatocyte count per unit area, hepatocyte size (µm), and their nuclear size (µm) in treated group-A and protected group-B are shown in Table.

Table-I. Comparison of Body weight and Liver weight (gm), Hepatocyte count per unit area, Hepatocyte size and Teir nuclear size (µm) Between Experimental and Control Pups of Wistar Albino Rats.

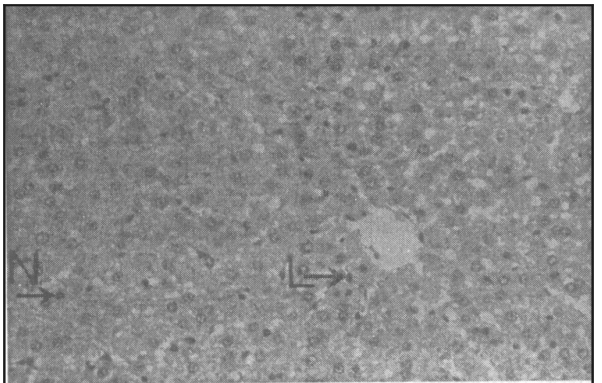
| Groups | Body Weight | | | Liver Weight | Hepatocycte count | | Hepatocyte Size | | | Nuclear size |
|---|---|---|---|---|---|---|---|---|---|---|
| | **PND1(I)** | **PND14(F)** | **WT (G/L)** | | **NFO** | | **NFO** | **NCO** | | |
| A (n=40) | 5.83± 0.11 | 19.15± 0.6 | 13.32± 0.6* | 1.14± 0.02* | 10 | 2113.71 ± 0.40* | 10 | 125 | 11.12 ± 0.08 ±** | 4.375 ± 0.12 ±* |
| B (n=40) | 5.50 ± 0.10 | 23.23 ± 0.19 | 17.73 ± 0.16 *** | 1.84 ± 0.05*** | 10 | 274.39 ± 0.47*** | 10 | 125 | 10.47 ± 0.04 ±*** | 5.359 ± 0.03 ±*** |
| C (n=40) | 55.55 ± 0.11 | 23.19 ± 0.06 | 17.64 ± 0.07 | 1.88 ± 0.03 | 10 | 276.54 ± 1.05 | 10 | 125 | 10.082 ± 0.017 ± | 5.453 ± 0.094 ± |

PND = Postnatal day; I = Initial; F= Final' Wt = Weight; G/L = Gain/Loss; NFO = NO. of Fields Observed (0.00324$mm^2$/field);
NCO = No. of Cells Observed; µ = Micron
*P<0.001 highly significant decrease**P<0.001 highly significant increase**P>0.05 non-significant



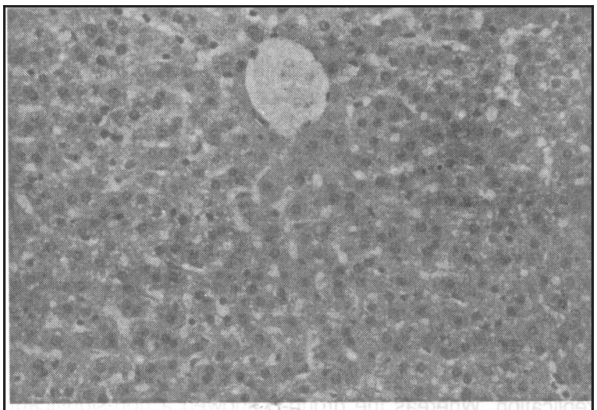

Figure–1. Photomicrograph of 3 µm thick paraplast section of liver stained with H&E. Ciprofloxacin treated group-A Wistar albino rat pups showing hepatic cell degenerating (N→) and marked lymphocytic infiltration at midzonal area of hepatic lobule (L→) and congestion of sinusoids (S→) and pyknotic nuclei (P→). Under high power objective x 416

These results showed mean reduction in body weight (13.32±0.6 gm), liver weight (1.14±0.02 gm), and hepatic nuclear size (4.375 ± 0.12 µm) a highly significant decrease when compared with control group-C ($P<0.001$) while the hepatic cell size showed mean increase (11.2 ± 0.08 µm) when compared with control group-C ($P<0.001$). Whereas group-B animals showed non-significant changes ($P>0.05$) are shown in Table.




Figure–2. Photomicrograph of 3 µm thick paraplast section of liver stained with H&E in ciprofloxacin and zinc chloride treated group-B Wistar albino rat pups showing mild hepatic cell degenerating (N→) and lymphocytic infiltration (L→) are comparable to the group-C under high power objective. x 416

As far as histological changes are concerned, in group-A hepato-cytes were found to be closely packed with intact bile canaliculi. Sinusoids were moderately dilated, there was lymphocytic infiltration and some degenerating cells were also observed as shown in Figure-1. The group-B animals showed decreased lymphocytic infiltration and degenerating cells are shown in Figure-2.



Figure–3. Photomicrograph of 3 µm thick paraplast section of liver stained with H&E in control (group-C) Wistar albino rat pups showing normal hepatic parenchyma. Hepatocytes are arranged in the form of plates with intervening sinusoids under high power objective x 416

## DISCUSSION

The present study was aimed to know the effects of ciprofloxacin and preventive effects of zinc chloride when administered separately and simultaneously on morphology of liver parenchyma in albino rat pups.

In the present study the body weight and liver weight decreased and microscopic examination revealed the decreased hepatic cell count per unit area, the increased hepatic cell size with decreased their nuclear size.

The results of present study are inconsistent with the studies by Hooper et al[16] and George et al[17]. In those studies the investigators found the common lesion in the liver, i.e. hepatic cellular necrosis on the basis of laboratory analysis. However none of these workers has counted the cells per unit area, size of hepatocytes, and their nuclei. Therefore our observations could not be compared with previous studies.

A highly significant decrease in body weight (gm) in group-A, which may be attributed to less food intake occurred following administration of ciprofloxacin. These observes are in accordance with findings of Berkovitch et al[18] and Cukerski et al[19] who found that constant findings of ciprofloxacin were decrease in body weight and length both in pre- and post-natally. While the group-B animals showed non-significant result which may be attributed to the partial protection by zinc against unwanted effect of ciprofloxacin on body weight. These findings are in agreement with the results of Ekhert and Hurley[20] who found that zinc is reported to be integral part of several metallo-enzymes and is essential for metabolism of nucleic acids and for synthesis of proteins.

The absolute liver weight in group-A showed a highly significant decrease which could be attributed to the necrosis of cells and resorption of necrosed cells. Our results are in agreement with Minuk et al[21], who found that the quinolones antibiotics inhibit eukaryotic as well as prokaryotic cell growth and protein synthesis by interfering with DMA and RNA replication. Whereas the group-B showed a non-significant result, which may be attributed to protective role of zinc against the ciprofloxacin toxicity. Similar observations were noted by Keppen et al[22], who found that supplementary zinc has beneficial effect on foetal growth by increase protein synthesis.

The number of hepatocytes in group-A animals showed decrease in cells per unit area while size of the hepatic cells increased with decreased nuclear size which may be attributed to fat deposition and interference with RNA, DNA and protein synthesis in response to toxic effects of ciprofloxacin. The necrosed cells were found in zone-II and III accompanying marked infiltration of inflammatory cells. Our observations correlate with findings of George et al[17], who found that in ciprofloxacin treated liver there was marked congestion of sinusoids with some midzonal and centrilobular necrosis and leukocytic infiltration. The animals in group-B showed a non-significant change as compared to group-C, which may be attributed to protective role of zinc chloride. Our observations are in agreement with those by Ames et al[7], who found that zinc stabilizes the structure of proteins and nucleic acid which preserves the integrity of sub-cellular organelles such as mitochondria, rough endoplasmic reticulum, participates in transport process and has important role in viral and immune phenomena.

## CONCLUSION

These results strongly suggest that ciprofloxacin causes severe liver damage in pups. However, the ciprofloxacin-induced hepatotoxicity could be partially prevented by simultaneous administration of zinc chloride in albino rat pups postnatally.

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