PROTECTIVE ROLE OF ZINC CHLORIDE AGAINST TOXICITY OF CIPROFLOXACIN ON FOETAL HEPATOCYTES OF ADULT WISTAR ALBINO RATS.

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ABSTRACT

Objective: This investigation was aimed to evaluate the unknown defensive function of zincin mitigating the toxicity levelstimulated by ciprofloxacin in the liver tissues of Wistar albino rats. This research was carried out as a prospective study. **Setting/Design:** The study has been done in the Department of Anatomy, Pir Abdul Qadir Shah Jeelani Institute of Medical Sciences Gambat, and District Khairpur Mir's. Pakistan. **Time Duratio**n: One year of studyhas been completed from March 2019 to February 2020. **Method:** Separate doses of Ciprofloxacin and Zinc chloride has been given to female Wistar albino rats during their gestation period. Different concentrations of 20 mg/kg of body weight and 1200 g/kg of body weight have been used. Doses of Ciprofloxacin and Zinc chloride have been injected intraperitoneally two times daily. Doses have been given from 8th day of gestation to 18th day of gestation. Albino rats were divided into three groups A, B and C. Each group contains 40 specimens; all the selected animals were treated by deep ether anesthesia and then dissected to remove the attached fetuses from the body of the females. Sexes of those fetuses were not considered. All the fetuses were dissected on the 18th day of gestation to get their liver. Weighing and fixation of liver were done by using 10% non-buffered formalin, Implantation has been done by using paraplast and thick sections of about 3 micrometers were cut by using rotary microtomy techniques. Staining of microtomic sections was done with the help of hematoxylin and eosin stains. The morphology histopathology of the liver tissues has been observed. Their structural differences have been compared with the histological patterns of liver tissues of controlled animals. The data has been compiled and evaluated statistically. **Result:**This study resulted the adverse effects of ciprofloxacin on the liver tissues as it was found that weight of liver from treated animals were remarkably decreased as equated to control animals. Histological evaluation has also been done.Hepatocytes was found effected by the doses of ciprofloxacin as decrease in sizes and number has been noted. Sizes of nuclei were also observed lesser as compared to controlled samples. The use of Zinc chloride along with ciprofloxacin has been shown to control the modified state of histological properties into the average conditions.

Conclusion: Progressive changes have been observed in morphological conditions of hepatocytes of Wistar albino rats while they were treated with ciprofloxacin along with the supplements of Zinc chloride. This declared the role of zinc chloride in rebuilding the damaged structure of hepatocytes.

Keywords: Ciprofloxacin, Zinc chloride, Liver weight, hepatotoxicity, Hepatocyte. Hepatic Nuclei.

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INTRODUCTION

As the Ciprofloxacin has been discovered in 1982 in Germany, the clinical experiments were started by researchers. The effects of oral dosages, parenteral and intravenous dosages have been evaluated. In 1985 researches were done by injecting the ciprofloxacin intravenously. This work was continued up till April 1988[1]. As US Food and Drug administration[2] had acquiesced a new method of intravenous injectable ciprofloxacin. Ciprofloxacin is the artificially formulated broad spectrum antibiotic which effectively functions against a variety of microbes. It is included in the separate category of drugs termed as fluoroquinolone. It is usually active against an extensive variety of microbes including gram positive and gram negative bacteria [3].

The mechanism of action of Ciprofloxacin is different from other antibiotics as it acts on the bacterial DNA by altering their functions. The obstruct and seized the enzymatic activity i.e. DNA Gyrase and topoisomerase enzymes have been inhibited by the effects of ciprofloxacin and ultimately it leads to the decline and detained the reproductive activity of microbes by terminating the DNA replication and transcription. The process of repair and recombination have also

been deteriorated by the interference of ciprofloxacin.World Health Organization included the Ciprofloxacin in the category of "Essential Medicines for Children"[4]. The use of Ciprofloxacin has been found effective in the treatment of various different disorders and infections included the Urinary tract infections, Respiratory tract infections, and Integumentary infections, infections in bones, muscles and joints, as well as digestive tract infections[5].

The use of Ciprofloxacin is not recommended in pregnancy but it was found that thatthis medicine has been used in an unrestrained manner that should be customized by Governmental legislations [6]. The role of Zinc has been monitored in this research as it is vital trace element. It is specifically incorporate in many different physiological and cellular metabolic activities like it involves in DNA synthesis, production of new protein in RNA and it also accelerates the chemical reactions and works as catalyst for completing various enzymatic reactions. It also alleviates the protein patterns in nucleic acid and gives the stability. It is also helpful to defend the intracellular framework of many different organelles like Mitochondria [7]. Zinc also deals with mechanism of cellular production and proliferation as it involves boosting up the enzymatic activity which leads to the cellular division [8]. Physiological studies revealed that Zinc plays an important role in humans and other vertebrates as they regulate the self-defensing mechanism and antioxidant formation which leads to the better growth and smooth developmental processes [9]. It is required that further investigations should be done to analyze the limiting role of zinc in cell proliferation [10].

The present research work has been done to evaluate the protective effects of zinc chloride on morphological features of parenchymal calls of liver which have been extracted from the prenatal animals reared in laboratory conditions.

MATERIAL AND METHODS

Experiments were performed on one hundred and twenty rat's fetuses which were collected from 30 pregnant female Wistar albino rats having age of about 16 to 18 weeks. Their weights were ranging from 140 to 200 grams. They were collected from Animal House of Pir Abdul Qadir Shah Jeelani Institute of Medical Sciences Gambat, District Khairpur Mir`s. All fit and lively animals were selected for the experiments. Two female rats were placed in a single cage accompanied with one fertile male rat of same species. They were facilitated for mating [11]. Female rats were clinically examined next day for the marks of copulation. Many clinical signs were noted as blood stained vagina or vaginal plug. If any of these signs were found that was measured as the occurrence of pregnancy [13]. Then all thirty pregnant animals were collected and they have been distributed into three groups of ten individuals in each group.

Group A: Doses of Ciprofloxacin has been insertedby intra peritoneal injections to experimental animals containing 20 mg/kg of body weight[14]. Formulation of has been done by Bayer Research Laboratories AG, Germany. The injections were given to rats on 8th day of their gestation to 18th day. Two dosages of injections were given in a single day with similar time durations. This practice has been repeated in all ten days.8th day of gestation was selected as it contains a much more importance because the process of organ formation in Albino rats usually starts from the 8th day of gestation period.

Group B: In this group of rats altered treatment has been used as they have been injected by Zinc chloride salts which were given to the albino rats as supplements. The formulations of Zinc chloride as laboratory chemical has been done by research laboratory in West Germany. Selected dosage of 1200 gm/kg of weight of laboratory animal has been injected[15]. The formulation has been prepared by dissolving 7.4 gm of salt in 0.1ml of solvent. Intraperitoneal infusions have been made. The dosages have been inserted before half an hour from the dosage of Ciprofloxacin regularly two times in a day. Similarly,as the members of Group A, the treatments were started on the 8th day of gestation because of its importance due to organ formation processes.

Group C: In the third group no treatments by Ciprofloxacin and Zinc chloride salts have been done. The experimental animals in this category were treated with normal saline according to the measurements of body weight of laboratory animals i.e. 3ml per kg of body weight. Again, intra peritoneal an injection has been applied started on the 8th day of gestation period. Infusion was made two times a day [16].

On the 18th day of development, pregnant mothers have been sacrificed by the process of cervical displacement and attached fetuses have been removed from their bodies. Arbitrary choices of 120 samples have been done from all groups as 40 specimens have been selected from each group respectively. The genders of all the samples have been ignored. Dissection of all those fetuses was made on the 18th day of gestation. They all were treated with deep ether anesthesia. Liver has been removed and collected from the entire specimen to make the histological studies of hepatocytes.All liver samples were measured by digital weighing scales and then fixation of samples was done by using 10 percent of non-buffered formalin. After fixation all the liver samples were implanted in paraplast. After the infiltration, sectioning has been done. Thick slices of about 3 micro meters were prepared by using rotary microtome. Staining of tissue samples have been done by using Hematoxylin and Eosin stain. After staining procedure histopathological studies has been done in hepatocytes of albino rats' fetuses. Micrometer device has been used to observe the structure ofhepatocytes in different dimensions. Statistics have been incorporated on the data and data has been interpreted in the tabulated form. Level of significance by "t" test method has been found [17].

RESULTS

In this current research, investigations have been done about the effect of ciprofloxacin and zinc chloride on the hepatocytes of fetuses of Wistar albino rats. Three groups of rats named as A, B and C were treated with Ciprofloxacin, Ciprofloxacin and Zinc chloride and Normal saline respectively. Group C was termed as control group. Animals were selected on the basis of 8th day of gestation period. Treatment continued up till 18th day of gestation. Pregnancy was considered established by observing the vaginal smear stained with Hematoxylin and Eosin stain. Occurrence and concentration of spermatozoa were also observed (Figure 1). Histopathological studies have been done on hepatocytes of 120 selected fetuses. Observations were matched and compared with the histological parameters of controlled group i.e. Group C.

Observations revealed that the specimen collected from animals of Group A displayed altered structures of hepatocytes. Hepatocytes were found concentrated with integral bile canaliculi. Dilations were seen in sinusoids. Hepatic lobules were found hexagonal in structure. The assemblage of hepatocytes was also transformed in to polyhedral shapes with moderate to immense bulky structural formations. Small rounded nuclei were observed in the hepatocytes. Pyknosis were found in nuclei of many hepatocytes as condensation in chromatin was observed while some of the cells contained vibrant nuclei. In zone II and zone III of the slides of samples from Group A, lymphocytic permeation was observed. Cells were found deteriorating in condition (Figure 2). While in the slides prepared from samples of liver tissues from Group B and C, it was apparent that no significant micromorphological changes in parenchymal cells were noticed. Disintegration in hepatocytes was not evident. Lymphocytic permeation was detected in the treated samples (Figure 3, Figure 4).

In Table 1, comparison of liver weight of pre-natal Albino rat fetuses between treated and control group has been done. The mean value of liver weight of fetuses in groups, groups-B and C was recorded 0.189 ± 0.003 G, 0.27 ± 0.005 G and0.281 ± 0.002 G respectively as shown in Table -1. A highly significant decrease in liver weight in group-A was observed when difference of mean was compared with group-B and control group-C ($P<0.001$).

The mean value of viable hepatocyte count per field in groups-A, groups-B and group C was recorded as 286.75±1.2, 425.85 ± 1.4and 423.95±1.2 respectively, as shown in Table - 2. A highly significant decrease in hepatocyte number was observed when difference of mean was compared with group-B and control group-C ($P<0.001$).

In Table 3, comparison of hepatocytes cell sizes, and their nuclei sizes in pre- natal albino rats' fetuses have been observed. The mean value of hepatocyte size per field in groups A, group-B and group C was recorded as 8.60±0.17 µm, 9.229 ± 0.06 µm and 9.012±0.1µm respectively. The magnitude of the nucleus was evidently observed reduced when contrast has been done between mean values of group B and Controlled group C ($P<0.001$).

The mean value of hepatic nuclear size per field in groups A, groups B and groups C was recorded as 4.223±0.1 µm, 5.041 ± 0.04 µmand 5.068±0.12 µm respectively, as shown in Table - 3.The size of the nucleus was clearly found decreased when comparison has been done between mean values of group B and Controlled group C ($P<0.001$). The instantaneous cure with the help of zinc chloride leads to healthy conditions as well as exceeded the conditions of the animals from group C.

TABLE – 1COMPARISON OF LIVER WEIGHT OF PRE-NATAL ALBINO RAT FETUSES (Gestational Day -18) **BETWEEN TREATED AND CONTOL GROUPS**

	Group A CIPROFLOXACIN (n=40)	Group B **CIPROFLOXACIN+ZINC CHLORIDE** (n=40)	Group C **CONTROL** (n=40)
	Mean ± SEM	Mean ± SEM	Mean ± SEM
Pre-natal (Day-18)	0.189 ± 0.003 G	0.27 ± 0.005 G^{øø}	0.281 ± 0.002 G ^{øø}

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

TABLE – 2COMPARISON OF HPATOCYTE COUNT PER FIELD IN PRE-NATAL ALBINO RAT FETUSES (Gestational Day -18) **BETWEEN TREATED AND CONTROL GROUPS**

	Group A CIPROFLOXACIN (n=40)	GroupB**CIPROFLOXACIN+ ZINC CHLORIDE**(n=40)	Group C **CONTROL** (n=40)
	Mean ± SEM	Mean ± SEM	Mean ± SEM
No. Of Fields Observed (0.0324 Mm^2/Field)	10	10	10
Pre-natal (Day-18)	286.75 ± 1.2	425.85 ± 1.4^{øø}	423.95 ± 1.2 ^{øø}

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

TABLE -3 COMPARISON OF HEPATOCYTE CELL SIZE (µm) AND THEIR NUCLEUS SIZE (µm) IN PRE-NATAL ALBINO RAT FETUSES (Gestational Day -18) **BETWEEN TREATED AND CONTROL GROUPS**

	Group A **CIPROFLOXACIN** (n=40)	Group B **CIPROFLOXACIN + ZINC CHLORIDE** (n=40)	Group C **CONTROL** (n=40)
	Mean ± SEM	Mean ± SEM	Mean ± SEM
No. of Fields Observed (0.0324 mm^2/Field)	10	10	10
No. of Cells Observed µm = Micron	125	125	125
Hepatocyte Size - Cell (µm)	08.60 ± 0.17 µm**	9.229 ± 0.06 µm	9.012 ± 0.31 µm$^{**,◊◊}$
Hepatocyte Size - Nucleus (µm)	4.223 ± 0.10 µm	5.041 ± 0.04 µm	5.068 ± 0.12 µm$^{øø,◊◊}$

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

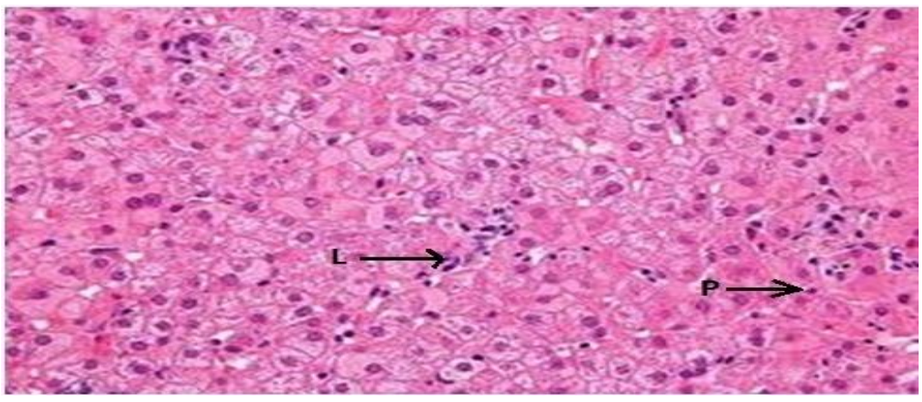

FIG. 1 : H & E X 400.

A photomicrograph of 3 µm thick paraplast section of the liver in Pre-Natal Albino Rat Foetuses (Gestational Day -18) of ciprofloxacin-treated Group A showing hepatocytes closely packed with intact bile canaliculi, pyknotic nucleus and The lymphocytic infiltration and degenerating cells markedly increased in zone- II and III .

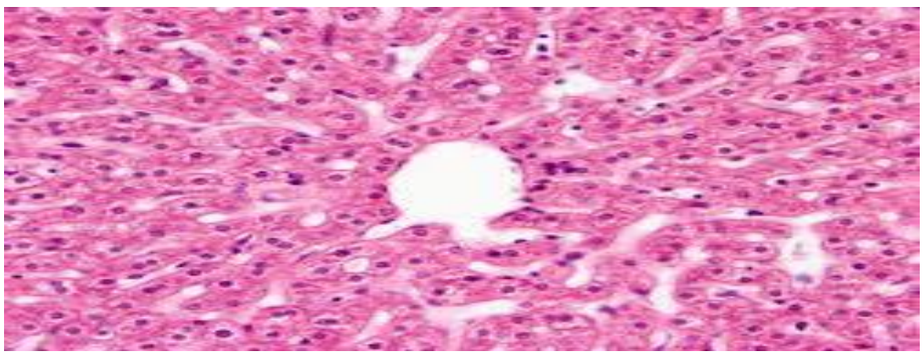

FIG- 2 : H & E X 400.

A photomicrograph of 3 µm thick paraplast of the liver in Pre-Natal Albino Rat Foetuses (Gestational Day -18) of Ciprofloxacin + $Zncl_2$ treated Group-B showing preserved cellularity of zones and regular cellular organization of hepatocytes, The lymphocytic infiltration and abscent of degenerating cells were observed

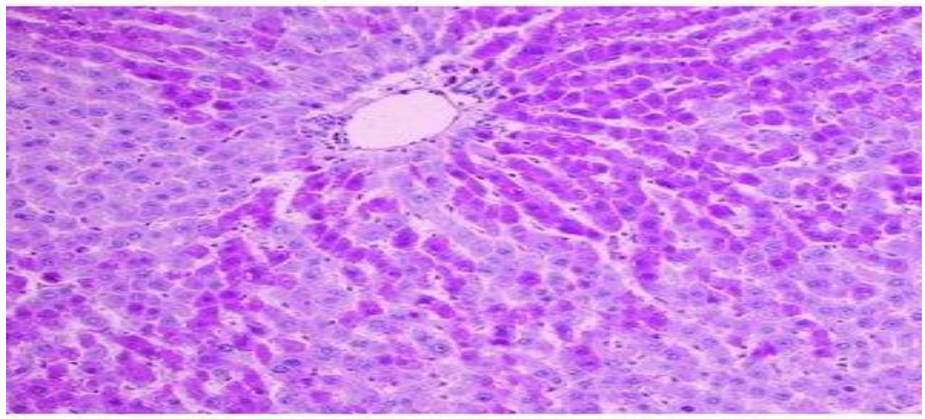

FIG-3 : H & E X 400.

A photomicrograph of 3 µm thick paraplast of the liver in Pre-Natal Albino Rat Foetuses (Gestational Day -18) of Control Group-C showing No change in liver parenchyma . preserved cellularity of zones and regular cellular organization of hepatoarchitecture .

DISCUSSION

Studies revealed the effects of fluroquinolones on the cytological structures of treated animals as fluroquinolones are considered as robust DNA inhibitors, they obstruct the DNA synthesis by blocking the activity of essential enzyme DNA complex. They have the tendency to alleviate the broken strands of DNA by catalytic activity of DNA gyrase and Topoisomerase IV. They constrain the action of enzymes leads to the formation of single stranded and double stranded DNA parts. This inclination by ciprofloxacin increased its ability to block and stops the DNA supercoiling by certain enzymatic activities [18].

In the current research the effects of ciprofloxacin and zinc chloride has been analyzed on the histology of hepatocytes of albino rat`s fetus. Structural changes in liver parenchymal cells have also been noted.

Morphological and histological studies of hepatic parenchymal cells have been done to investigate the alteration in tissue patterns, sizes and formations. Cell count per unit area has also been done in the current research. It was clearly observed that decline in the size ratio has been found. The cell structures were also found modified, their sizes were decreased from the normal ranges and their nuclei were also seems decreased in sizes. Many different researchers have analyzed the above-mentioned parameters in their researches and it is found that results of current research also relate with them. These researchers included Hooper. *et al*[19], Eric, S. *et al*[20] and Jain, S. *et al*[21]. They have observed the changes in morphometrical conditions in hepatic cells included the cell necrosis and presence of lesions on the liver tissues. But the cell count technique, cell size analysis and nuclei size analysis have not been done before. So, these parameters cannot relate with the prior investigations.

In the members of group B, it was found that zinc chloride doses have played a significant role in tissue repair and recovery as liver tissues were found in normal sizes and in controlled parameters as compared to the members of group C. Liver weight in the members of group B were found 0.27 ± 0.005 G while in the members of group C, it was 0.281 ± 0.002 G. It shows the defensive abilities of zinc chloride. Kumar, S.D. *et al*[22] stated that zinc facilitates in the production of collagen inside the cells which enhance the metabolic activities inside the cells. The current researches relate the work of Kumar, S. D. *et al*. Another researcher Kloubert, V. has also checked the effects of zinc on DNA synthesis and stated that zinc is capable to accelerate the process of DNA synthesis by initiating the enzymatic activity or by modifying the attachments of F1 and F3 histone proteins to the strands of DNA which ultimately leads to the synthesis of RNAs [23].

Cellular structures were also altered as per unit cells were also found declined from the normal ranges, their nuclear sizes were also declined from their normal limits. These all consequences may be due to the reactivity of RNA, DNA and protein synthesis mechanisms resulted by the toxic effects of dosages of ciprofloxacin. Inflammation in the parenchymal cells of liver was also been observed in zone II and zone III of the prepared slides. Cell necrosis and cellular infiltration have been seen in the prepared slides. Einar, B. *et al*[24] stated that in the hepatocytes treated with ciprofloxacin concentration of sinusoids were found with centro-lobular necrosis and leucocytic infiltration. Current research work relates with the research of Einar, B. *et al*.

Zinc chloride supplements given to animals of group B resulted in recovery of tissue structures included the sizes of hepatic parenchymal cells, sizes of their nuclei and their cell count, all parameters were found near to normal when compared with the animals of group C i.e. the control group. Current research confirmed the significant role of zinc chloride to regulate the adverse effects of ciprofloxacin on hepatic cells. This research correlates with the previous researches of Ergul, B. *et al*. [25] and WHO contributors [26].They stated that zinc addition along with dosages of ciprofloxacin contributes to regulate the cellular metabolism. Zinc also

play important role in enhance the growth of children which do not show normal growth patterns.

Current research revealed that synchronized dosages of zinc chloride have the tendency to reduce the toxic effects of ciprofloxacin on the liver tissues. It is recommended that further investigations should be made to investigate the consequences of ciprofloxacin administration and their effects on the cellular micro histological patterns.

CONCLUSION

Administration of zinc chloride to ciprofloxacin treated Wistar albino rats resulted in marked improvement in the structure of hepatocytes, thus emphasizing the protective potential of zinc chloride in restoring the altered hepatic histoarchitecture.

RECOMMENDATIONS

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,

ETHICS APPROVAL: The ERC gave ethical review approval

CONSENT TO PARTICIPATE: written and verbal consent was taken from subjects and next of kin

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