

"CIPROFLOXACIN-INDUCED HEPATOTOXICITY IS ATTENUATED BY PRETREATMENT WITH ANTIOXIDANT AGENT IN IMMATURE ALBINO- RAT LITERS"

Haji Muhammad Aslam Channa¹ Bhojo Mal Tanwani² Wazir Ahmed Baloch³. Roohi Kawal⁴ Muhammad Adnan Channa⁵

ABSTRACT

OBJECTIVE: Current research was based on the detection of the effects of the supplementation of Zinc chloride along with the ciprofloxacin on hepatic tissues of immature albino rat litters. Immature Albino rats have been used for the post natal studies. **SETTING / DESIGN:** Department of Anatomy. Gambat Medical College Gambat / Pir Abdul Qadir Shah Jeelani Institute of Medical Sciences Gambat District Khairpur Mirs. **TIME DURATION:** One year from Jan 2020 Dec 2020. **MATERIAL & METHODS:** Different doses of Ciprofloxacin and Zinc chloride has been given to immature albino rat litters. Ciprofloxacin was used as a curative dose two times for 14 days. Days have been counted from the day of the birth of Albino rats. Dose of 20mg/ kg of Ciprofloxacin has been given to the rat litters while Zinc chloride has been given in 120 µg/100 gm of body weight. The parameters which were evaluated included hepatocyte count, hepatocyte size and variations in the nucleus. All the estimated values have been contrasted with the values estimated from the control group. Statistical assessment have been done to achieve the significant values. **RESULT:** This study shows that variations has been found in total count of liver cells after the administration of ciprofloxacin in post natal albino rats. Decline in mean hepatocyte count has been noted. A major variation has been seen in the hepatocyte count which also included reduction in hepatocyte size, and nucleus size per field as they have been declined by 203.7 ± 0.41 , 08.12 ± 0.06 µm and 4.37 ± 0.12 µm respectively. While the supplementation of Zinc chloride along with the ciprofloxacin retained the mean hepatocyte count, size and their nucleus size by 212.4 ± 0.47 µm, 10.47 ± 0.04 and 5.36 ± 0.03 µm correspondingly. The values which have been estimated from control group includes 213.71 ± 0.40 µm, 10.28 ± 0.02 µm and 5.46 ± 0.09 µm for the mean hepatocyte count, hepatocyte size as well as their nucleus size correspondingly. **CONCLUSION:** Current findings dealt with the effects of Zinc chloride with Ciprofloxacin on post-natal albino rat litters which revealed that supplementation of Zinc chloride along with ciprofloxacin decreases the alteration rates of normal liver cells counts. Zinc chloride has minimized the hazardous effects of Ciprofloxacin on the mean hepatocyte count, their sizes and the morphological structure of their nuclei also found maintained after the addition of Zinc chloride supplements. **KEYWORDS:** Ciprofloxacin, ZnCl₂, Hepatocyte count per field, Hepatocyte size, Hepatocyte nucleus, immature Albino-rat litters.

1. Professor, Department of Anatomy Gambat Medical College Gambat / Pir Abdul Qadir Shah Jeelani Institute of Medical Sciences Gambat.
2. Assistant Professor Department of Physiology Peoples University of Medical & Health Sciences for Women Nawabshah.
3. Assistant Professor Department of Anatomy Makran Medical College Kech Turbat.
4. Assistant Professor, Department of Zoology University of Karachi.
5. Final year MBBS Indus Medical College Tando Muhammad Khan.

For Correspondence: Dr. Haji Muhammad Aslam Channa. Professor, Department of Anatomy Gambat Medical College Gambat H, No: 837/137-IIA.Golimar Nawabshah District Shaheed Benazir Abad. Email: drmaslamchanna62@gmail.com

How to cite this article: Channa HMA¹ Tanwani BM² Baloch WA³. Kawal R⁴ Channa MA⁵ "CIPROFLOXACIN-INDUCED HEPATOTOXICITY IS ATTENUATED BY PRETREATMENT WITH ANTIOXIDANT AGENT IN IMMATURE ALBINO- RAT LITERS". *JPUMHS*; 2021;11:03,100-105. <http://doi.org/10.46536/jpumhs/2021/11.03.325>

Received Julu 4th 2021, Accepted On 08 August 2021, Published On 30 September 2021

INTRODUCTION

Ciprofloxacin is a broad-spectrum fluoroquinolone antibiotic which has been used for the treatment of many bacterial infections. The formulation of fluoroquinolones have been considered as a milestone in the historical record of antibiotics. Being the broad-spectrum antibiotic, Ciprofloxacin acts opposed to

Gram-positive and Gram-negative microbial varieties. It is generally used for the treatment of skeletal system infections, abdominal infections, Urinary tract infections and respiratory tract infections. Ciprofloxacin involves in the inhibition of gyrase enzymes which is the active component of bacterial cells activity. Gyrase initiates the metabolic activities in the bacterial cells.²

Ciprofloxacin drug has been widely used for the treatment of different infections. Along with its curative ability it also has so many disastrous side effects on the tissues of receiver which includes the degeneration in the joint tissues and the also produce destructive changes in the cartilaginous tissues of young animals 3. There is so many evidence of allergic reactions, liver infections and secondary superinfections 4. The use of quinolones is restricted in toddlers and youngsters as they create central nervous system defects and results in cartilage deformation in children. Their use is not recommended during the pregnancy and for nursing mothers as they have the history of ulceration in liver and bile ducts of immature animals 5. There are several physicians who prescribe this drug to the patients without concerning their adverse effects and limitations. This careless behavior should be controlled by the government authorities 6.

Zinc is an important trace element which is necessary for the protein synthesis, DNA replication and RNA fabrication. Zinc is also responsible for enzyme production and embryonic development in fetus. It also acts as catalyst in many biochemical reactions. Zinc also involves in gene expression, stabilization of proteins and genomic structures of nucleic acids. Zinc also play an important role in maintaining the structural organization of cellular organelles including mitochondria 7.

Zinc also participates in cell synthesis mechanisms, produce enzymes which involves in cell division and multiplication 8. Zinc is also a vital element which is mandatory for regulation of various physiological functions, boosting up immunity, initiation of growth and reproduction 9. It is antioxidant in nature and involves in stabilizing many immunological functions. In spite of all the essential functions of zinc, the restricting position of zinc in cell proliferation is still uncertain 10.

The current study has been carried out to estimate the prophylactic function of zinc chloride after the treatment with ciprofloxacin on the parenchymal tissues in liver of post-natal Wistar albino rats.

MATERIALS & METHOD

The experiment was carried out on Wistar albino rats. Forty ovulating female rats and twenty male rats were selected having the age

of approximately 18 to 20 weeks. These experimental animals were collected from animal house. Mating of female rats was done with the males of similar variety following the technique explained (Luck 11). The mating of one male and two female rats in different cages were carried out. After twenty-four hours sign of mating were considered in female rats like vaginal secretions and mucous on vagina. These signs indicate the mating process and day zero of pregnancy was assumed 12. Total gestation period in albino rats is 21 to 23 days.

All the immature Albino rat's litters were divided in three groups A, B and C. Ten animals were included in one group. Animals in group A were treated with Ciprofloxacin injections with a quantity of 20 mg/kg weight (0.12 mg in 1.1 ml). Injections were applied in intra peritoneal two times in twenty-four hours (1st to 14th day from birth). While the animals included in group B were treated with Ciprofloxacin. Along with this they were also treated with the injections of zinc chloride 120 µg/100 G of body weight which was formulated with distill water (7.4 µg in 0.1 ml). These injections were also given intraperitoneally. The injection of zinc chloride was given two times a day and 30 minutes prior to the injection of ciprofloxacin. These injections were administrated for 14 regular days (1st to 14th day from birth).

The animals of Group C were considered as control group. They were administrated with normal saline (0.1ml). These injections were applied intra peritoneally for 14 regular days (1st to 14th day from birth). On the 15th day all Albino rat's litters were dissected after treating them with anesthesia. Liver was removed and fixed in 10% of buffered formalin. The paraplast was used for the process of embedding. Microtomical sectioning was done and a section of 3 µm were obtained by using rotary microtome device. Haematoxylin and Eosin (H&E) stains have been used for the process of staining. Histopathological studies were done, and the data was interpreted with statistical calculations." t" test was applied to calculate the significance of the results 14.

RESULTS

MEAN HEPATOCYTE COUNT PER FIELD IN TWO WEEKS POST-NATAL TREATED IMMATURE ALBINO-RAT LITERS

In Group A, the mean of post-natal hepatocyte count was calculated as 203.7 ± 0.41 . The mean value has shown a quick significant decline in the count of hepatocytes when the data was compared with the results of animals of Group B and C (Table 1). In Group B, the mean of post-natal hepatocyte count per field was estimated as 212.4 ± 0.47 . This result has shown the non-significant change in the mean count of hepatocytes. The value of significance i.e. ($P > 0.032$) was found after comparing the results with the results of animals of Group C (Table 1). In Group C, the mean of post-natal hepatocyte count per field was recorded as 213.71 ± 0.40 . A significant decrease in the values has been noted in the results of Group C after comparing the results with calculations of Group A while non-significant modification has been noted after comparing the results of group C with the calculations of Group B (Table 1).

TABLE – 1 COMPARISON OF HEPATOCYTE COUNT PER FIELD IN IMMATURE ALBINO-RAT LITERS BETWEEN POST-NATAL TREATED AND CONTROL GROUPS

	Group A Ciprofloxacin (n=10)	Group B Ciprofloxacin +Zinc Chloride (n=10)	Group A Control (n=10)
	Mean \pm SEM	Mean \pm SEM	Mean \pm SEM
No. Of Fields Observed (0.0324 Mm ² /Field	10	10	10
Postnatal (Day-14)	$203.7 \pm 0.41^{**}$	$212.4 \pm 0.47^{**}$	$213.71 \pm 0.40^{**}$

$p < 0.01$ highly significant as compared to Ciprofloxacin (A),

$p < 0.01$ highly significant as compared to Ciprofloxacin + Zinc Chloride (B)

$p < 0.01$ highly significant as compared to Control (C),

MEAN HEPATOCYTE SIZE PER FIELD IN TWO WEEKS POST-NATAL TREATED IN IMMATURE ALBINO-RAT LITERS.

In Group A, the mean hepatocyte size per field in post-natal was calculated as $08.12 \pm 0.06 \mu\text{m}$. As the result was compared with the significance of Group B and C, it was noted to attain a highly significant decrease in size (Table 2). In Group B, the mean of hepatocyte size per field in post-natal stage was recorded as $10.47 \pm 0.04 \mu\text{m}$. This result revealed that a non-significant change was

noted ($P > 0.05$) in group A after comparing the results with control group C (Table 2).

In Group C, the mean of hepatocyte size per field in post-natal was resulted as $10.28 \pm 0.02 \mu\text{m}$. Statistical analysis of results of Group C show the highly significant decrease after comparison with the results of Group A, while a non-significant change in size has been observed in size when compared with the results of Group B (Table 2).

MEAN NUCLEAR SIZE OF CHONDROCYTE PER FIELD IN TWO WEEKS POST-NATAL TREATED IMMATURE ALBINO-RAT LITERS.

In Group A, the mean hepatocyte size per field in post-natal was calculated as $4.37 \pm 0.12 \mu\text{m}$. As the result was compared with the significance of Group B and C, it was noted to attain a highly significant decrease in size (Table 2). In Group B, the mean of hepatocyte size per field in post-natal stage was recorded as $5.36 \pm 0.03 \mu\text{m}$. This result revealed that a non-significant change was noted ($P > 0.05$) in group A after comparing the results with control group C (Table 2). In Group C, the mean of hepatocyte size per field in post-natal was resulted as $5.46 \pm 0.09 \mu\text{m}$. Statistical analysis of results of Group C show the highly significant decrease after comparison with the results of Group A, while a non-significant change in size has been observed in size when compared with the results of Group B (Table 2).

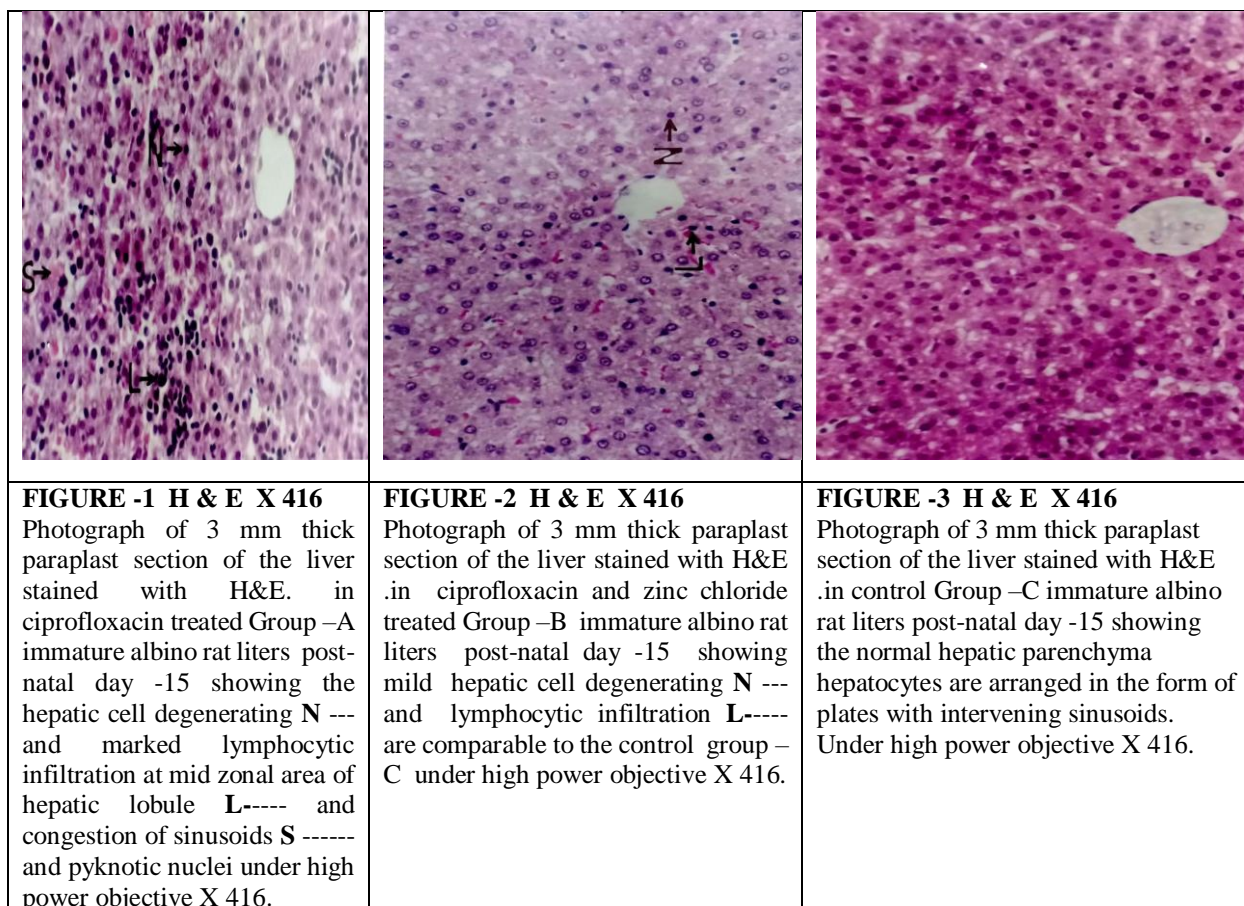
TABLE -2 COMPARISON OF HEPATOCYTE CELL SIZE (μm) AND THEIR NUCLEUS SIZE (μm) IN IMMATURE ALBINO-RAT LITERS BETWEEN POSTNATAL TREATED & CONTROL GROUPS

	Group A Ciprofloxacin (n=10)	Group B Ciprofloxacin +Zinc Chloride(n=10)	Group C Control(n=10)
	Mean \pm SEM	Mean \pm SEM	Mean \pm SEM
No. Of Cell Observed (0.0324 Mm ² /Field	125	125	125
No. Of Fields Observed (0.0324 Mm ² /Field	10	10	10
Hepatocyte Size - Cell (μm)	$08.12 \pm 0.06^{**}$	$10.47 \pm 0.04^{**}$	$10.28 \pm 0.02^{**}$
Hepatocyte Size - Nucleus (μm)	$4.37 \pm 0.12^{**}$	$5.36 \pm 0.03^{**}$	$5.46 \pm 0.09^{**}$

$p < 0.01$ highly significant as compared to Ciprofloxacin (A),

$p < 0.01$ highly significant as compared to Ciprofloxacin + Zinc Chloride (B)

$p < 0.01$ highly significant as compared to Control (C),



DISSUSSION

Current study has been carried out to investigate the consequences after the application of drug ciprofloxacin independently or administration of ciprofloxacin along with different doses of zinc chloride on the liver tissues of post-natal wistar albino rat's litters.

The drug has been used intraperitoneally in the body of immature albino rat litters. 20mg/kg body weight of the dose has been used. Histological studies of liver tissues of albino rats revealed that mean hepatocyte count has been found significantly decreased, while a high decline was also found in hepatocyte size and their nucleus size per field.

The histological evaluation confirmed the decline in the hepatocytes per unit area, significant decrease in the sizes of their nuclei as well as size of the liver cells were also modified and decreased. The results have been confirmed the findings of Hooper *et al.*, 15, Eric. S. *et al* 16, and Jain. S. *et al.*, 17. These researchers have discussed about the effects of Ciprofloxacin on liver cells and suggested the erosion and lesions in liver cells by the administration of ciprofloxacin drug. Cell necrosis and damage has also been observed by many of the researchers. The research on the effects of ciprofloxacin on the

cell's sizes and cell count per unit are and nuclei sizes has never been conducted before. That's why the new finding is not comparable with the previous research works.

The use of Zinc chloride was also a threshold in the studies of effects on ciprofloxacin as the current research aimed to find out the protective and curative position of zinc chloride in minimizing the adverse effects of ciprofloxacin on the liver tissues, sizes of hepatocytes and their nuclei. The administration of Zinc chloride to the animals of group B were found that the non-significant changes have occurred when these changes were related with the animals of group C (Control Group). These findings were line up with the findings of Kumar. S. D. *et al.*, 18 who have also studied on the curative role of zinc chloride and suggested that zinc is responsible for the formation of collagen fibers in the cells which initiates the cellular activities in the liver of albino rats.

Kloubert, V. suggested that Zinc is involved in the synthesis of DNA by increasing the enzymes activity and by attachment of F1 and F3 histone proteins to the molecules of DNA. This leads to the formation of more RNAs. Currents findings line up with the results of Channa, M. A., Nishada and MacDonald. They suggested that zinc is also involved in the stabilization of proteinic structures and provide steadiness in the formation of nucleic

acids molecules. These ultimately responsible for the assurance of stability of intracellular organelles 20, 21. Zinc also involved in cell multiplication, initiate enzyme production, which accelerate the process of cell division 22. Current studies also revealed the zinc chloride protect the hepatocytes from adverse effects of ciprofloxacin.

It is recommended that further research should be done on the effects of ciprofloxacin on the histology of post-natal albino rats' livers to confirm the least reactive dosages. Duration of the persistence of adverse effects of ciprofloxacin on the hepatocytes also needs to investigate.

CONCLUSION

The effects of ciprofloxacin along with zinc chloride has been studied in albino rat liver of post-natal stage. Post treatment effects were observed. Variation in sizes and numbers of liver cells have been noted. Variation in the sizes of nucleus were also noted.

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. Metallidis S, Kollaras P, Giannakakis T, Seitanidis B, Kordosis T, Nikolaidis J et al. A prospective, controlled, randomized, non-blind, comparative study of the efficacy and safety of a once daily high dose of ceftriaxone plus ciprofloxacin versus thrice daily ceftazidime plus amikacin in empirical therapy for febrile neutropenic patients, *Euro J Int Med*, 2008 19 (8): 619-624.
4. Santos AM, Wong A, Almeida AA, Fatibello-Filho O. Simultaneous determination of paracetamol and ciprofloxacin in biological fluid using a glassy carbon electrode modified with graphene oxide and nickel oxide nanoparticles, *Talanta*, 2017; 174: 610-618.
5. 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.

ETHICS APPROVAL: The ERC gave ethical review approval

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

FUNDING: The work was not financially supported by any organization. The entire expense was taken by the authors

ACKNOWLEDGEMENTS

I am thankful to and fortunate enough to get constant encouragement, support and guidance from all staff in laboratory of Anatomy Department Gambat Medical College / Pir Abdul Qadir Shah Jilani Institute of Medical Sciences Gambat for their timely support.

AUTHORS' CONTRIBUTIONS: All persons who meet authorship criteria are listed as authors, and all authors certify that they have participated in the work to take public responsibility of this manuscript. All authors read and approved the final manuscript.

CONFLICT OF INTEREST: No competing interest declared.

6. Channa HMA, Ashfaq M, Bangash R, Abbasi A, Qureshi MA. Preventive Role of Zinc Chloride against Toxicity of Ciprofloxacin on the Growing Cartilage of Wistar Albino Rat Litter. *J Ayub Med Coll Abbottabad*, 2008; 20(4): 77-81.
7. 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.
8. Kumar SD, Vijaya M, Samy RP, Dheen ST, Ren M, Watt F, et al. Zinc supplementation prevents cardiomyocyte apoptosis and congenital heart defects in embryos of diabetic mice. *Free Radic Biol Med*. 2012 ;53(8):1595-606.12
9. Küçüköglü M., Binokay US, Boğa PA. The effects of zinc chloride during early embryonic development in zebrafish (*Brachydaniorerio*). *Turk J Biol*. 2013; 37: 158-164.
10. Ryu JM, Lee MY, Yun SP, Han HJ. Zinc chloride stimulates DNA synthesis of mouse embryonic stem cells: involvement of PI3K/Akt, MAPKs, and mTOR. *J Cell Physiol*. 2015; 218: 558-567.
11. 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.
12. Chang HH, Schwartz Z and Kaufman MH. Limb and other postcranial skeletal defects induced by amniotic sac puncture in the mouse. *J Anat*, 2006; 189: 37-49.
13. Bancroft J D; Stevens A. *Theory and Practice of Histological Techniques*. 5th ed.

- Edinburgh: Churchill Livingstone, 2009; pp. 88,112, 232, 503.
14. Bland M. Introduction of medical statistics. 5thed. Oxford: Oxford University press, 2006; p.116–126.
 15. Hooper DC, Wolfson J S. Fluoroquinolone antimicrobial agents. *N Engl J Med* 2009; 324: 384-94.
 16. Eric S, Orman, Hari S, Conjee V, RajVuppalanchi, James W.Freston, et al . Clinical and Histopathologic Features of Fluoroquinolone-Induced Liver Injury. *Clinical Gastroenterol and Hepatol*, 2011; 9: (6) 517-523.
 17. Jain S, Kaplowitz N. Clinical Considerations of Drug-Induced Hepatotoxicity , *Comprehensive Toxicology*, 2010; 9: 369-381.
 18. Kumar SD, Vijaya M, Samy RP, Dheen ST, Ren M, Watt F,et al. Zinc supplementation prevents cardiomyocyte apoptosis and congenital heart defects in embryos of diabetic mice. *Free Radic Biol Med*. 2012 Oct 15; 53(8):1595-606.
 19. Kloubert V, Rink L.Zinc as a micronutrient and its preventive role of oxidative damage in cells. *Food Funct*. 2015 Oct; 6(10):3195-204.
 20. Channa MA, Kawal R, Tanwani BM, Gohar N, Channa MA, Rind S. protective role of zinc chloride against toxicity of ciprofloxacin on foetal hepatocytes of adult wistar albino rats. *JPUMHS* ; 2020 ; 10:04,37-43.
 21. Nishida K , Hasegawa a , Nakae S , Oboki K , Saito H, Yamasaki S , Hirano T . 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.
 22. Mac Donald RS. The role of zinc in growth & cell proliferation. *J Nutr*. 2007; 130(5):1500S-1508S.