Journal of Applied Veterinary Sciences, 8 (4): 37-42 (October, 2023)
ISSN: Online: 2090-3308, Print: 1687-4072
Journal homepage : https://javs.journals.ekb.eg

Biochemical and Histopathological Assessment of Zinc Acetate-Induced Nephrotoxicity in Male Albino Rats

Mahmood A. Hussein[1*], Maha T. AL-Saffar[2] and Karam H. AL-mallah[3]
[1]Ministry of Health, Nineveh Health Directorate, Mosul, Iraq
[2]Department of Dental Basic Sciences, College of Dentistry. University of Mosul, Mosul, Iraq
[3]Department of Pathology and Poultry diseases, College of Veterinary Medicine, University of Mosul, Iraq
***Corresponding Author:** Mahmood A. Hussein, **E-Mail:** mahmood.21dep69@student.uomosul.edu.iq

ABSTRACT

Despite the fact that zinc is recognized to be less hazardous than other heavy metals, e.g., cadmium, lead, mercury, and arsenic, it may cause acute and chronic toxicities in cases of exposure to high doses. To investigate the biochemical and histopathological toxic effects of zinc acetate on rats kidneys. Twenty-five healthy male albino rats were divided into five equal groups: Normal saline solution was given to the control group; groups 2, 3, 4, and 5 received zinc acetate (4, 8, 12, and 24 mg/kg), respectively. All the treatments were given intraperitoneally once every other day for 3 consecutive weeks for serum biochemical evaluation and renal histopathological assessment. There was a significant increment in serum urea concentration in group 5 in comparison with other groups, but there was no significant difference in creatinine concentration between all of the groups ($P \leq 0.01$). Histopathological examination of kidney sections of rats of different groups revealed different lesions that were more severe in the 4th and 5th groups. In conclusion, subacute zinc acetate toxicity produced dose-dependently significant effects on serum urea concentration in all the treated groups without any significant effects on creatinine concentration. This effect was reflected in renal histo-architecture, which was more severe in the 4th and 5th groups.

Original Article:
DOI:https://dx.doi.org/10.21608/javs.2023.218887.1248
Received : 03 July, 2023.
Accepted :27 August, 2023.
Published in October, 2023.

Keywords:Creatinine, Pathological changes, Urea, Zinc Nephrotoxicity. ***J. Appl. Vet. Sci., 8(4): 37-42.***

INTRODUCTION

Zinc is the second-most abundant transition metal in humans and many other living organisms' bodies after iron and the second-most abundant divalent cation after calcium, with an average of 2–3 gm. of body weight **(Gembillo *et al*., 2022).**

Essential biological processes involving zinc control a variety of cell processes, such as normal cellular growth, reproduction, foetal development, brain development, DNA synthesis, behavioural response, bone formation, and wound healing **(Chasapis *et al*., 2020).**

Zinc supplements are frequently used by the general public as self-medication at unknown dosages due to misconceptions about their availability, ignorance of zinc toxicity, and the ease with which many preparations of zinc salts can be found over the counter (OTC) in drug stores and health food stores, as zinc is an essential trace element and insufficient amounts of it cause poor appetite, skin changes, growth retardation, and testicular atrophy **(Calesnick and Dinan, 1988)**, These factors have encouraged the use of zinc supplements in the treatment and prevention of a wide range of diseases, including growth failure, skin diseases, infection, wounds, and cancer **(Chandra, 1984).**

Therefore, the present study has been done to clarify the biochemical and histopathological nephrotoxic effects of zinc acetate **(Chinni *et al*., 2021).**

MATERIALS AND METHODS

1. Experimental animals

Twenty five apparently healthy male albino rats, 250–360 g body weight, 2–3 months old, were obtained from the Animal House of the College of Veterinary Medicine, University of Mosul. All research procedures were carried out in accordance with the University of Mosul, policies regarding the handling and use of laboratory animals. The Research Ethics Committee of the College of Dentistry at the

University of Mosul (UoM.Dent/A.88/22) has approved this study. As bedding, homogenized wood shavings were used at room temperature (22°C±2°C) and ambient humidity (55%±5%). Food and water *ad libitum* were supplied in standard light conditions (12-hour light/12-hour dark cycle).

2. Experimental design

The rats were divided into five equal groups (5 rats/group): Normal saline solution was given to the control group intraperitoneally once every other day for 3 weeks; groups 2, 3, 4, and 5 received zinc acetate powder (BDH Laboratory Reagents®, England) at dose levels of 4, 8, 12, and 24 mg/kg B.Wt., respectively, intraperitoneally once every other day for 3 weeks **(Piao *et al.*, 2003).** A zinc acetate solution was prepared freshly by dissolving in distilled water.

All the experimental rats were euthanized by cervical dislocation on day 22 after ether an aesthesia for serum biochemical evaluation and histopathological assessment of the kidney.

3. Biochemical assays

Blood samples were collected from rats at the time of euthanasia from the retinal vein. The serum was separated using a centrifuge (800 electric, China) for 15 minutes at 3000 rpm. The separated serum was then taken out using a micropipette and put into Eppendorf tubes, where it was kept at (-20°C) until it was analysed using a spectrophotometer**.** The commercial kits (Biosystems, Spain) and (Biolabo, France) were used to measure levels of urea and creatinine, respectively.

4. Histopathological examination

Kidney specimens from rats of all groups were collected and fixed in 10% neutral buffered formalin. Paraffin sections of 5 µm thick were prepared, stained with Hematoxylin and Eosin, and examined microscopically **(Bancroft and Gamble, 2008).** All the observed changes were rated and graded according to their severity into four score values: (0=absent), (1=mild), (2=moderate), and (3=severe). Each specific lesion was assessed in five randomly selected fields at each section.

5. Statistical analysis

Utilizing the statistical software programme SPSS for Windows (version 22), data were analyzed statistically. Mean values between the groups were evaluated and compared using the one way analysis of variance (ANOVA) test and DUNCAN test **(Bailey, 2008).** All the values were expressed as the mean ± SDE.

RESULTS

1. Serum Level of urea and creatinine

As shown in Fig. (1), there was non-significant increase in serum urea level in groups 2 and 3. As compared to the control group, however, urea levels recorded a significant increase in groups 4 and 5 when compared to the control group ($P \leq 0.01$). On the other hand, Fig. (2) shows no significant change in serum creatinine concentration in different treated groups in comparison with the control group.

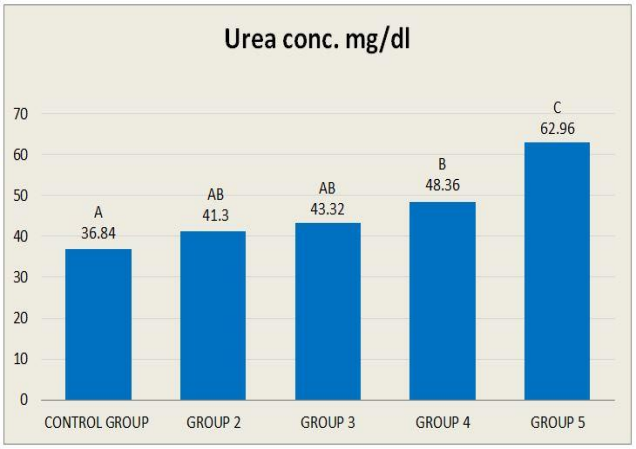

Fig.1: Serum urea level (mg/dl) in different treated groups

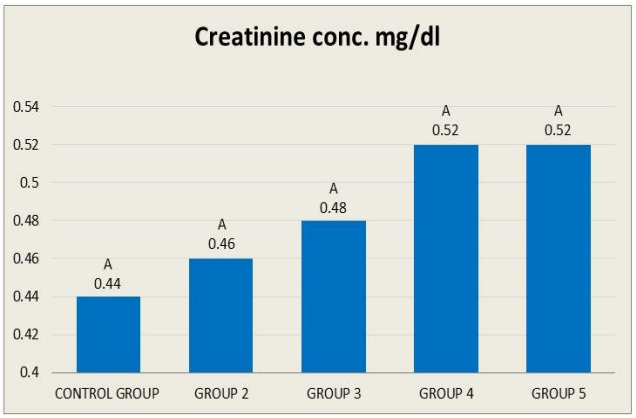

Fig.2: Serum creatinine level (mg/dl) in different treated groups

2. Histopathological findings

The control group showed a normal renal tissue architecture, including normal renal tubules, normal glomeruli, normal interstitial tissue, collecting tubules, and a normal pelvis, as well as a normal renal circulatory appearance upon histopathological examination (Fig.3).

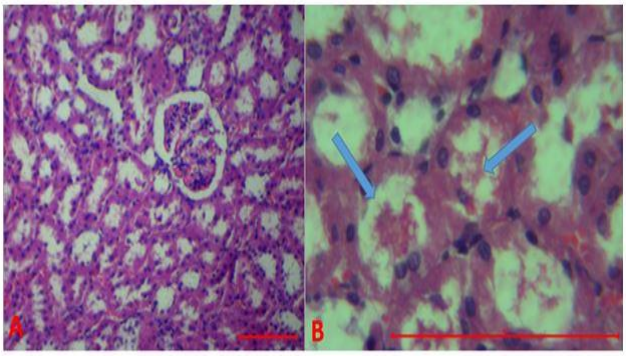

Fig.3: A: Photomicrograph of rats kidney sections from control group showing normal histological appearance. B: Magnified section showing normal renal tubules, normal circulation in blood vessels with presence of eosinophilic protein urea at some animals (Blue arrows). Staining H&E, Magnification 100 X and 400 X. Scale bar 100 µm.

Mild changes were noticed in group 2, which received 4 mg/zinc acetate. These changes were in the form of mild acute cell swelling (cloudy swelling), small interstitial foci of mononuclear inflammatory cells, and haemorrhage in some sections (Fig.4).

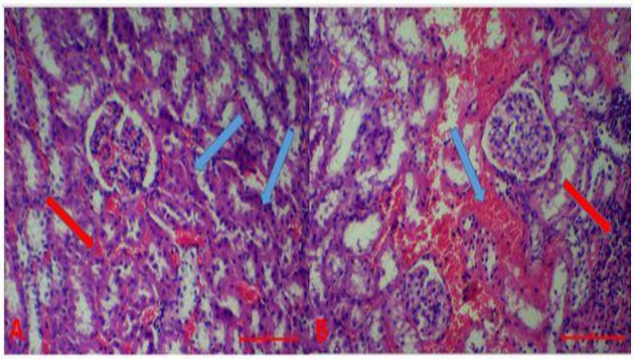

Fig.4: Photomicrograph of rats kidney sections from 4 mg/ zinc acetate treated group showing: A: Mild acute cell swelling (Blue arrows) and mild hyperemia of arterioles (Red arrow) .B: Small focus of hemorrhage (Blue arrow) and focal infiltration of mononuclear inflammatory cells one section (Red arrow). Staining H&E, Magnification 100 X. Scale bar 100 µm.

The lesions were more severe in the 3rd group; the cloudy swelling appeared clearly and diffusely few foci of coagulative necrosis were seen in some sections; and glomerulonephritis was noticed in a few sections with a variable degree of severity (Fig.5).

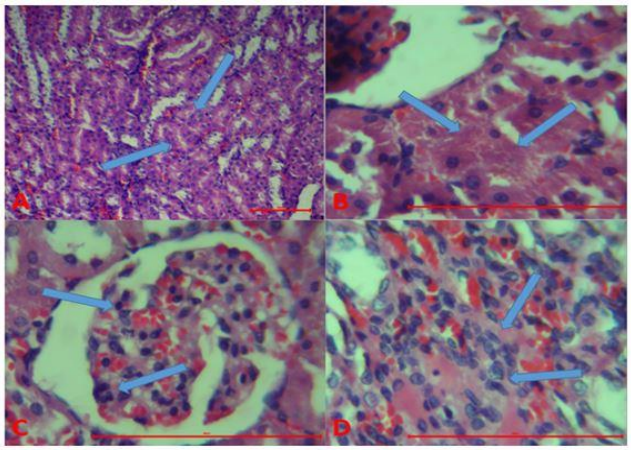

Fig.5: Photomicrograph of rats kidney sections from 8 mg/ zinc acetate treated group showing: A: Mild acute cell swelling (Blue arrows) .B: Focal coagulative necrosis of renal tubular cells (Blue arrows). C: Glomerulitis (blue arrows). D: Focal interstitial inflammatory cells infiltrations (Blue arrows) Staining H&E, Magnification 100 X and 400 X. Scale bar 100 µm.

In the fourth group, there was a severe form of congestion of blood vessels, cloudy swelling in the tubular epithelium, mild to moderate focal inflammatory cell infiltration in the interstitial spaces, foci of coagulative necrosis, hemorrhagic foci, and intratubular protein casts were noticed and glomerulonephritis was recognized in a few sections (Fig.6).

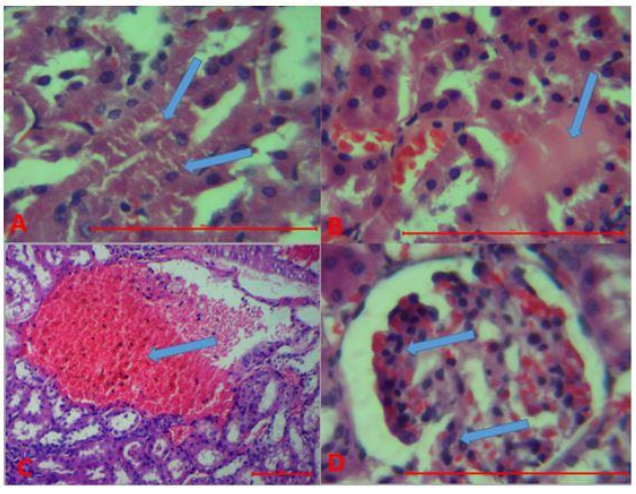

Fig.6: Photomicrograph of rats kidney sections from 12 mg/ zinc acetate treated group showing : A : Focal coagulative necrosis of renal tubular cells (Blue arrows) . B: Intratubular protein cast (Blue arrow) .C: Focal interstitial hemorrhage (Blue arrow). D: glomerulitis with shrinkage of glomerulus (blue arrows). Staining H&E, Magnification 100 X and 400 X. Scale bar 100 µm.

In the 5th group, glomerulonephritis was apparent at some sections, cloudy swelling, multifocal haemorrhages in the cortex and medulla, protein casts, focal infiltration of inflammatory cells, and deposition of giant cells (Fig.7 and table 1).

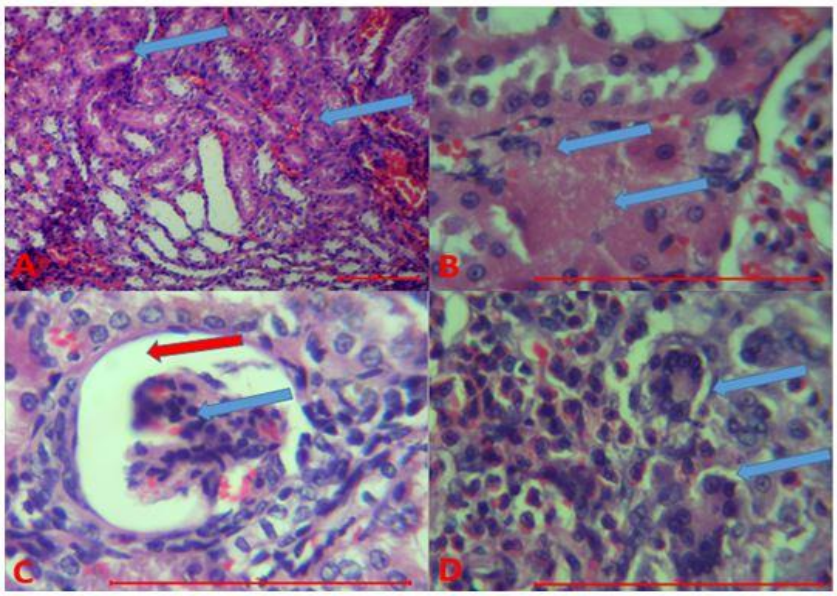

Fig.7: Photomicrograph of rats kidney sections from 24 mg/ zinc acetate treated group showing: A: Focal acute cell swelling of renal tubular cells (Blue arrows) . B: Coagulative necrosis of renal tubular cells (Blue arrow) .C: Glomerulitis (Blue arrow) with shrinkage of glomerulus (Red arrow). D: Giant cells with in focus of inflammation. Staining H&E , Magnification 100 X and 400 X. Scale bar 100 µm.

Table 1: The differences in the means of scores of renal lesions between the different groups of the experiment.

Group	Renal lesion score						
	Cloudy swelling	Coagulative necrosis	Focal hemorrhages	Vascular congestions	Glomerulone phritis	Focal interstitial nephritis	Protein cast
Control	1.33±0.90^{b}	0.00±0.00^{c}	0.33±0.90^{c}	0.53±0.16^{c}	0.00±0.00^{c}	0.06±0.66^{c}	0.66±0.06^{c}
Zinc acetate 4 mg	0.66±0.18^{b}	0.20±0.10^{c}	0.20±0.10^{c}	0.93±0.24^{cd}	0.20±0.10^{c}	0.13±0.09^{c}	0.66±0.06^{c}
Zinc acetate 8 mg	1.26±0.20^{c}	0.40±0.13^{c}	0.26±0.11^{c}	1.40±0.28^{cd}	0.53±0.19^{cd}	0.26±0.15^{c}	0.20±0.14^{cd}
Zinc acetate 12 mg	1.60±0.28^{c}	0.93±0.22^{d}	0.66±0.21^{cd}	1.33±0.28^{d}	0.53±0.23^{cd}	0.53±0.19^{cd}	0.73±0.26^{d}
Zinc acetate 24 mg	2.26±0.20^{d}	1.06±0.24^{d}	0.93±0.28^{d}	1.53±0.33^{d}	0.86±0.29^{d}	0.86±0.27^{d}	0.66±0.25^{d}

Values expressed as (Mean± Standard error). D for superiority followed by C and B. To be compared vertically.

DISCUSSION

There are many sources of zinc throughout the environment, so exposure and toxicity are not uncommon. Toxicity may result from occupational sources such as inhalation, excessive dietary supplement use, and improperly prepared complete parenteral nutrition **(Grissinger, 2011).**

Despite the fact that zinc is recognized to be less hazardous than other metals **(Claverie *et al*., 2000)**, it may cause acute and chronic toxicities after high dose exposure **(Barceloux, 1999)**. Metal fume fever, which affects people who inhale large amounts of zinc oxide vapours, is the main industrial risk **(Barceloux, 1999)**.

The levels of urea and creatinine are good indicators of efficient renal function. Urea and creatinine values will increase if renal function declines **(Pagana and Pagana., 1998).** The increase in serum urea concentration in groups receiving zing acetate at high doses runs in accordance with **Llobet *et al*., (1988),** who showed that the plasma levels of urea and creatinine significantly increased after high-dose exposure to zinc acetate dehydrate in drinking water.

Other than renal illness, there are recognized causes of urea increases, including protein breakdown **(Kellerman, 1995)**. In the same manner, **Yeh *et al*., (2011)** found a significant dose-dependent increase in blood BUN and creatinine upon administration of zinc in a diet for 8 weeks.

In the current study, the biochemical findings were supported by histopathological examination as microscopical examination of kidney sections of rats of different groups revealed the presence of pathological changes that appeared in the form of cloudy swelling of renal tubular cells, coagulative necrosis, focal haemorrhage, vascular congestions, glomerulonephritis, focal interstitial nephritis, and protein casts in renal tubules at all treated groups and were more severe in the 4th and 5th groups.

These changes could be attributed to the explanation of **Abdel-Aziz *et al*., (2018)** who recorded that the administration of rabbits with ZnO NPs by intraperitonial injections at doses of 100 ml/kg and 250 ml/kg once daily for 14 days produced histological changes in the kidney, including vacuolations of cytoplasm, loss of brush borders, infiltrations of inflammatory cells, an increase in Bowman's space, capillary congestion in between tubules, intratubular protein deposition, and destruction of distal convoluted tubule cells through oxidative stress.

Additionally, **Khorsandi *et al*., (2018)** showed that oral exposure to low dose ZnO NPs at a dose level of 5 mg/kg for 2 weeks would increase kidney weight, infiltration of leukocytes, congestion of blood vessels, and accumulation of fluids. The explanation for these histological findings may be attributed to oxidative stress contributing to progressive renal damage **(Chong *et al*., 2021).**

The cloudy swelling of renal tubular cells was apparent clearly in all zinc acetate treated groups, in agreement with **Hassan and Sahi (2019),** who recorded the appearance of renal tubular cells lacking nuclei and swelling of the tubular cells, and explained that this was a result of the accumulation of the irritant zinc-metallothionein complex in the kidney after its reabsorption by the renal tubules.

CONCLUSION

In conclusion, subacute toxic effects of zinc acetate (at doses of 4, 8, 12, and 24 mg/kg B.Wt.) may produce a significant increase in serum urea concentration at high doses without any significant increase in creatinine level. However, different renal lesions appeared in all of the groups in a dose dependent manner.

Conflict of interests

The authors declare no potential conflict of interest.

Acknowledgement

The authors would like to thank the College of Dentistry /University of Mosul for providing facilities to accomplish this work.

Funding

Self-funded

REFERENCES

ABDEL-AZIZ, H. O., HAMDAN, H. M., and RAGAB, E. E., 2018. The histological effects of zinc oxide nanoparticles on the kidney of adult male rabbits. Sohag Medical Journal, 22(2), 297-301. https://doi.org/10.21608/smj.2018.40959

BAILEY, R. A. 2008. Design of comparative experiments (Vol. 25). Cambridge University Press. https://doi.org/10.1017/CBO9780511611483

BANCROFT, J. D., and GAMBLE, M. (EDS.), 2008. Theory and practice of histological techniques. Elsevier health sciences. https://doi.org/10.1097/NEN.0b013e31817e2933

BARCELOUX, D.G. 1999. Zinc. Journal of Toxicology: Clinical Toxicology, 37(2), 279-292. https://doi.org/10.1081/CLT-100102426

CALESNICK, B., and DINAN, A. M., 1988. Zinc deficiency and zinc toxicity. American family physician, 37(4), 267-270. https://pubmed.ncbi.nlm.nih.gov/3358349/

CHANDRA, R. K. 1984. Excessive intake of zinc impairs immune responses. Jama, 252(11), 1443-1446. https://doi.org/10.1001/jama.1984.03350110043027

CHASAPIS, C. T., NTOUPA, P. S. A., SPILIOPOULOU, C. A., and STEFANIDOU, M. E., 2020. Recent aspects of the effects of zinc on human health. Archives of toxicology, 94(5), 1443-1460. https://doi.org/10.1007/s00204-020-02702-9

CHINNI, V., EL-KHOURY, J., PERERA, M., BELLOMO, R., JONES, D., BOLTON, D., and PATEL, O., 2021. Zinc supplementation as an adjunct therapy for COVID-19: Challenges and opportunities. British journal of clinical pharmacology, 87(10), 3737-3746. https://doi.org/10.1111/bcp.14826

CHONG, C. L., FANG, C. M., PUNG, S. Y., ONG, C. E., PUNG, Y. F., KONG, C., and PAN, Y., 2021. Current updates on the in vivo assessment of zinc oxide nanoparticles toxicity using animal models. BioNano Science, 11(2), 590-620. https://link.springer.com/article/10.1007/s12668-021-00845-2

CLAVERIE, C., CORBELLA, R., MARTIN, D., and DIAZ, C., 2000. Protective effects of zinc on cadmium toxicity in rodents. Biological Trace Element Research, 75, 1-9. https://doi.org/10.1385/bter:75:1-3:1

GEMBILLO, G., VISCONTI, L., GIUFFRIDA, A. E., LABBOZZETTA, V., PERITORE, L., LIPARI, A., and SANTORO, D., 2022. Role of Zinc in Diabetic Kidney Disease. Nutrients, 14(7), 1353. https://doi.org/10.3390%2Fnu14071353

GRISSINGER, M., 2011. A fatal zinc overdose in a neonate: confusion of micrograms with milligrams. Pharmacy and Therapeutics, 36(7), 393. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3171817/

HASSAN, I. J., and SAHI, I. S., 2019. Histopathological changes in the liver and kidney of albino mice on exposure to zinc toxicity. scopus ijphrd citation score, 10(7), 596. https://www.ijcmas.com/vol-4-7/Ateeq.%20M.%20J.%20Alarami.pdf

KELLERMAN, J. 1995. Blood Test. Signet Book, Chicago, USA, Reprint edition. https://www.amazon.com/Blood-Test-Signet-Jonathan-Kellerman/dp/0451154347

KHORSANDI, L., HEIDARI-MOGHADAM, A., and JOZI, Z., 2018. Nephrotoxic effects of low-dose zinc oxide nanoparticles in rats. Journal of Nephropathology, 7(3). http://dx.doi.org/10.15171/jnp.2018.35

LLOBET, J. M., DOMINGO, J. L., COLOMINA, M. T., MAYAYO, E., and CORBELLA, J., 1988. Subchronic oral toxicity of zinc in rats. Bull. Environ. Contam. Toxicol.;(United States), 41(1). https://doi.org/10.1007/bf01689056

PAGANA, K. D., and PAGANA, T. J., 1998. Manual of diagnostic and laboratory tests. St. Louis: Mosby Inc. https://cmc.marmot.org/Record/.b59437947

PIAO, F., YOKOYAMA, K., MA, N., and YAMAUCHI, T., 2003. Subacute toxic effects of zinc on various tissues and organs of rats. Toxicology letters, 145(1), 28-35. https://doi.org/10.1016/s0378-4274(03)00261-3

YEH, Y. H., LEE, Y. T., HSIEH, Y. L., and HWANG, D. F., 2011. Dietary Taurine Reduces Zinc-Induced Toxicity in Male Wistar Rats. Journal of food science, 76(4), T90-T98. https://doi.org/10.1111/j.1750-3841.2011.02110.x

How to cite this article:
Mahmood A. Hussein, Maha T. AL-Saffar and Karam H. AL-mallah, 2023. Biochemical and Histopathological Assessment of Zinc Acetate-Induced Nephrotoxicity in Male Albino Rats. Journal of Applied Veterinary Sciences, 8 (4): 28-36.
DOI: **https://dx.doi.org/10.21608/javs.2023.218887.1248**