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Effect of Acute Toxicity of Zinc Oxide Nanoparticles in Male Albino Rats

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Abstract

Last decades, nanotechnology technique attracted attention of researchers, it's considered an important tool in many applications such as healthcare. In this study, Zinc Oxide Nanoparticles (ZnONPs) were used as a parameter for some histological and biochemical changes in male rats. Primary results showed that treated animals with 100 mg/ml on nanoparticles daily showed a response to some histological changes in hepatic and renal tissue. this variation includes characterized degeneration, necrosis, and apoptosis with vascular changes accompanied by inflammatory reactions of hepatic tissue. Regarding histological changes in renal tissue, results showed generation of necrotic changes in epithelium of renal tubules as well as vasculitis , degeneration, and necrosis of the renal corpuscles. Results also displayed an increase in values of Serum acid, Serum creatinine, Serum glutamic oxaloacetic transaminase (GOT), and Alkaline phosphatase in comperes with a control group. however, there was a reduction in values of blood urea and Serum glutamic pyruvic transaminase (GPT) observed in treated animals. In contrast, biochemical results of blood showed an increase in GOT and GPT (serum acid, blood urea, serum creatinine, serum (GOT), serum (GPT), and alkaline phesion. phosotase). Aim of this study was to estimate toxic effect of Concentra.

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تأثير السمية الحادة لجسيمات أوكسيد الزنك النانوي في ذكور الجرذان البيض

ريا غالب السلطان هناء خليل إسماعيل ازهار البكري
اسرار الخفاف

الخلاصة

خلال السنوات القليلة الماضية اهتم الباحثون كثيرا بتقنية النانو، والتي أصبحت أداة مهمة في العديد من المجالات مثل الرعاية الصحية والعلوم المختلفة. في هذه الدراسة تم استخدام جزيئات أوكسيد الزنك النانوية (ZnONPs) zinc oxide nanoparticles كمؤشرات لبعض التغيرات النسجية البيو كيميائية في الجرذان البالغة وكذلك تمت دراسة التغيرات النسجية على بعض أعضاء الجسم مثل الكبد والكلية. حيث أظهرت نتائج هذه الدراسة أن الحيوانات المعاملة بجسيمات النانو النانوية مع جزيئات أوكسيد الزنك النانوية بمعدل 100 ملغ / مل يومياً ظهرت عليها تغيرات نسجية في كل من الأنسجة الكبدية وفي الانسجة الكلوية التي أظهرت تغيرات أساسية تمثلت بانحلال، تنكس وتنخر خلايا النسيج، كما ظهرت تغيرات في نسيج الاوعية الدموية في الكبد تمثلت بأضرار تنكسية، تنخرية، وعائية وموت الخلايا المبرمج مع تغيرات التهابية لأنسجة الكبد. أما بالنسبة للتغيرات النسيجية لأنسجة الكلى فقد أظهرت تغيرات تنكسية وتنخرية في ظهارة النبيبات الكلوية وانحلال، وتنكس وتنخر في الكبيبة الكلوية. كما وأظهرت النتائج زيادة في قيم حمض المصل، مثل مصل الكرياتينين، مصل الناقل الاميني الجلوتاميك أوكسالوكسيتيك ترانس أميناز (GOT) Glutamic oxaloacetic transaminase، وقيم إنزيم الفوسفاتيز القلوي. مقارنة مع المجموعة الضابطة، ومع ذلك، فقد لوحظ انخفاض قيم اليوريا في الدم وفي مصل الناقل الاميني الجلوتاميك بيروفيك ترانساميناز (GPT) glutamic pyruvic transaminase في الحيوانات المعاملة. بينما أظهرت النتائج البيوكيميائية زيادة الدم في GOT و GPTحمض المصل، يوريا الدم، مصل الكرياتينين، مصل(GOT) ، مصل (GPT)، phesionالقلوي (phosotase) .ان الهدف من هذه الدراسة هو التحقق او دراسة التاثيرات السمية لأوكسيد الزنك النانوية.

الكلمات المفتاحية: جزيئات أكسيد الزنك النانوية، الفئران، الكبد، الكلى، التغيرات النسيجية المرضية.

Introduction:

Nanotechnology has become a new revolution in biology fields that may bring considerable enhancement to human health in terms of the application of nano diagnostics, nanomaterials as well as nanorobotics. Fortunately, nanotechnology has developed rapidly along with a strategic alternative for producing residue-free nanocomposites. Recently, many different nanoparticles such as Ag, CuO, MgO, and ZnO have been developed with drugs and chemical materials against different orders. (Hagens *et al*, 2007; Kumar *et al*, 2013 and Al-Saeedi,2024). ZnONPs have become one of the most common metal oxide nanoparticles in

biological applications due to their excellent biomaterials and non-toxicity (lim, *et al* 2011; Sharma *et al* 2012; Jiang *et al* 2018; Hana *et al* ,2020).

Several studies showed that ZnoNPs treatment process results in unexpected relationships, it reaches different organs in the body such as the heart, spleen, liver, and brain, as well as the respiratory system and digestive system (Hagens *et al*, 2007). Oral administration of ZnNPs causes histopathological changes to occur in organs such as (the liver, lung, and kidney), when oral administration of ZnNPs and the toxicity effect depends on the size of synthesized ZnNPs ing upon the size of ZnNPs (Vandebriel *et al*, 2012). Studies have revealed that most internal organs including in addition to the brain can get and are get affected by nanomaterials in different ways. routes of entries Nanoparticle of zinc oxide are the most amply used nanomaterials as the worldwide production of ZnO NPs is valuation to be 0.1 to 1.2 million tons per year (Kumar *et al*, 2013). The study (Al-Baker *et al* 2020) clarified the effect of nanoparticles on some blood parameters of rats. Thus, the current was carried out to examine So this study to estimate the effect of oral administration of ZnO NPs on histopathological structure changes of the liver, and kidney and on the serum function of both organs. testes of kidney and liver

Materials and Methods:

Experimental design

The experimental animal (adult male albino rats) with weight of (250±10 gm) were brought from approved animal house at Education College for Girls / University of Mosul. These aimals were kept under intial conditions in plastic cages with wood chip bedding, at temperature 25◦±2◦ and light 14 hours' light 10 hours' dark and given normal food and tap water. The animals were randomly separated into two groups after one-week adaptation. The first group named: Groups I (control group which given normal saline for ten days, while the second one named GroupII 100 mg/ml orally given 100 mg/ml from ZnO NPs orally by using oral gavage daily. At the end of experiment all rats were sacrificed. Tissue samples of liver and kidney were removed immediately after necropsy excised and fixed in 10% neutral buffered formalin (Suvarna *et al*, 2019; Raghad *et al*, 2022) for histopathological estimation. The organs were processed (dehydration) by grading ethanol alcohol, clearing in xylol embedding in paraffin was, then prepare 5-6-micron thick paraffin sections, then staining the tissue section by hematoxylin and eosin stain. Light microscope was used for histopathological examination (Al-Hajj 2015).

The blood samples were collected for estimation of Serum acid, Blood urea, Serum creatinine, Serum glutamic oxaloacetic transaminase (GOT), Serum glutamic pyruvic transaminase (GPT), Alkaline phosphatase. In this study, zinc oxide nanoparticles used at 5 µm, preparation by SIGMA-ALDRICH company – Baghdad (Reagent Plus®, powder, <5nm particle size, 99. 9%).Statistical analysis: -

Data were subjected to statistical analysis using SPSS, the values were expressed as the mean ± Standard deviation analyzed with one-way ANOVA. Post hoc test was performed using the Duncun test. Mean values were statistically significant at $p \leq 0.05$

Results:

Static analysis results revealed that the treated animals is proportional to the values of Serum acid, Serum creatinine, Serum glutamic oxaloacetic transaminase (GOT), while,Alkaline phosphatase incomparism compared to the with control group. , However there was reduction in the decrease the values of blood urea and Serum glutamic pyruvic transaminase (GPT) Was observed in treated animals compared to the control group. ..

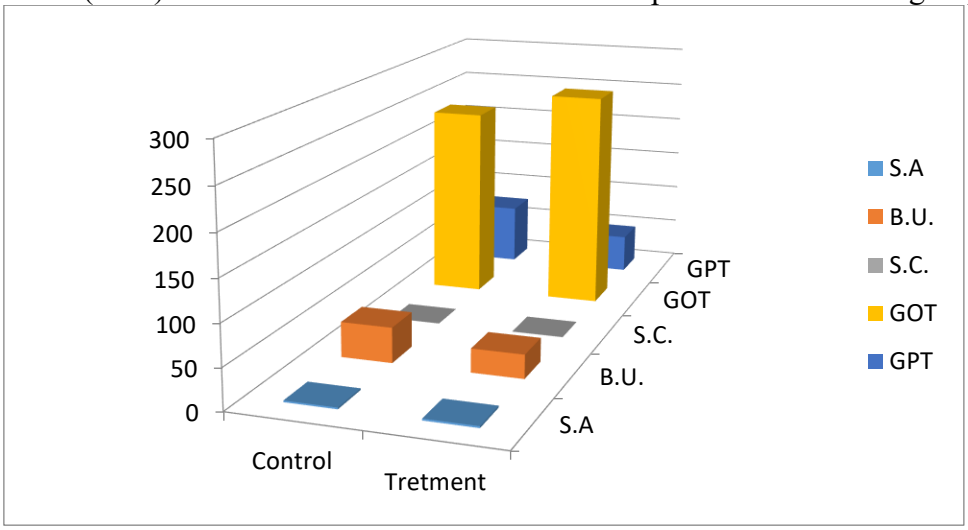

Figure (1) Effect of zinc oxide NPs on some parameters in rats.

Table -1- Effect of ZnO NPs **on parameter (100 mg/kg) of blood rats**

parameter(mg/l)	**control**	**zinc oxide NPs**
Serum acid	1.933 a	2.2 b b
Blood urea	43.5 a	29.33 b
Serum creatinine	0.353 a	0.493 b
Serum glutamic oxaloacetic transaminase (GOT)	239 a	270.3 b
Serum glutamic pyruvic transaminase (GPT)	75.33 a	47.56 b
Alkaline phosphatase	1050 a	1586.93 b

*Significant difference from the control group (*P*, 0.05); **significant difference from the control group (P, 0.01)

The results also showed that zinc oxide nanoparticles cause different changes in the hepatic architecture of the treated animal, such as hyperplasia of Kupffer cells (Figure 3), congestion of vessels in the portaltraid, and centeral veins (figure 4) (figure 5). Inaddition, there was also infiltration of inflammatory mononuclear cells in the portal traids and around the centeral vein. Vacuolar degeneration of hepatocytes around the centeral vein, apoptosis of hepatocytes, and coagulative necrosis of hepatocytes also observed. (figure6) (Figure 7). Other sections showed recent thrombus in both central and portal veins (Figure 8).

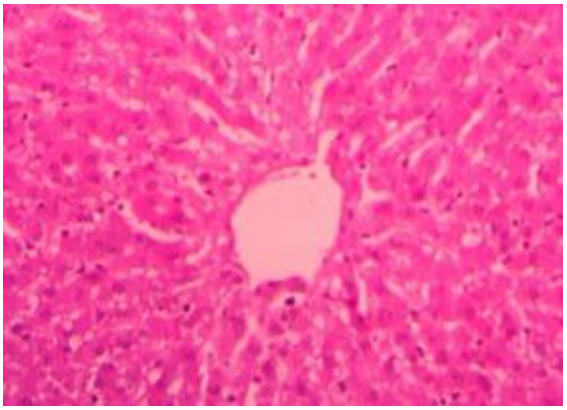

Figure 2: Photomicrograph of section of control group showed normal architectural of liver 10X (H & E Stain).

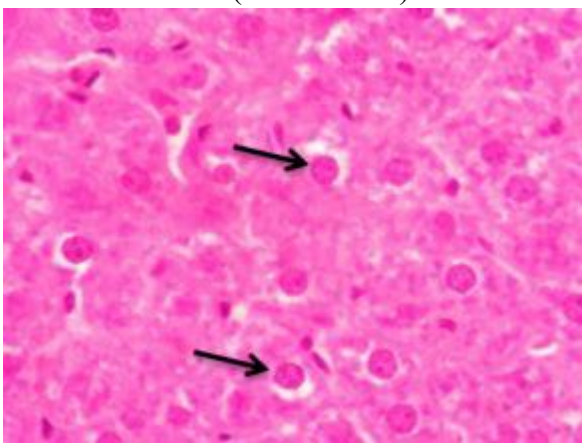

Figure 3: Photomicrograph of section of liver showed hyperplasia of Kupffer cells 40X, (H & E Stain).

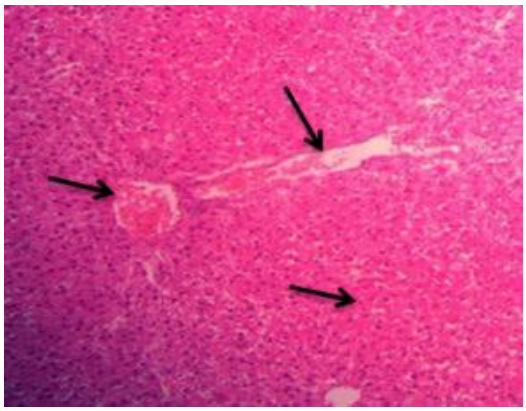

Figure 4: Section of liver showed congestion of blood vessels in portal area 10X, (H &E stain).

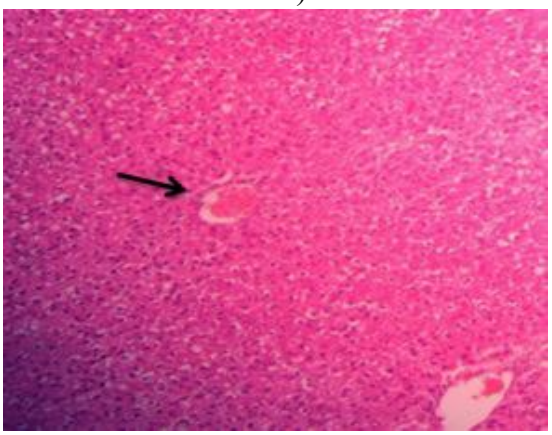

Figure 5: Section of liver showed congestion of c.v. surround by necrotic hepatocytes 10X (H and E stain).

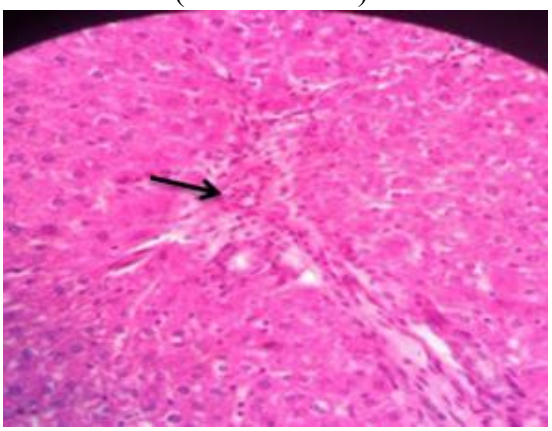

Figure 6: Section of liver showed infiltration cells and degeneration and necrosis of hepatocytes (H and E stain).

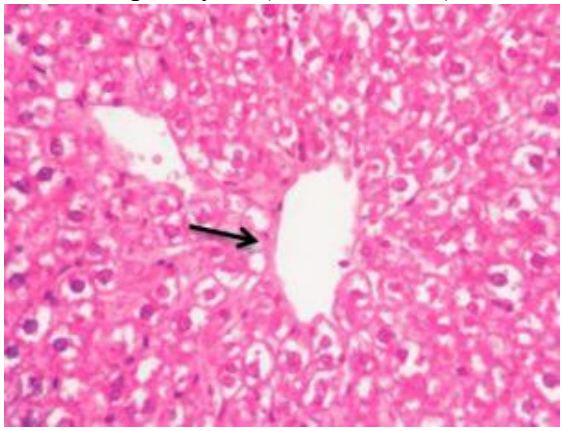

Figure 7: Section of liver showed vacuolar degeneration coagulative necrosis of hepatocytes 10X (H and E stain).

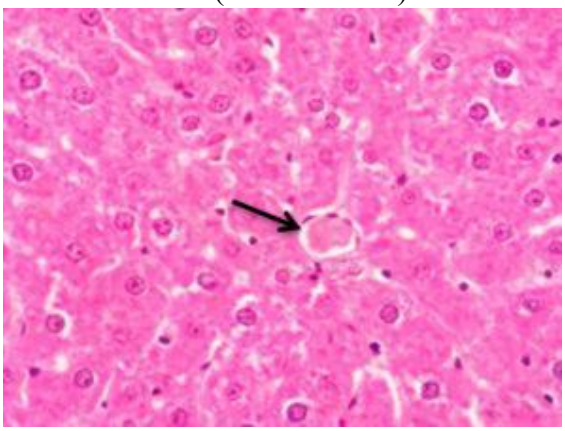

Figure 8: Photomicrograph of section of liver showed apoptosis of hepatocyte, with coagulative necrosis of other hypatocytes 40X, (H and E stain).

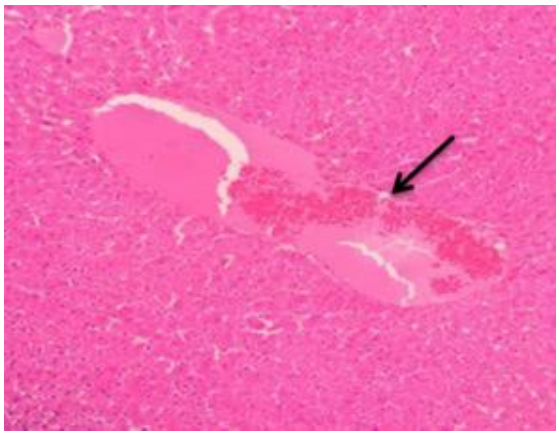

Figure 9: Section of liver showed recent thrombus of blood center vein 10X (H and E stain).

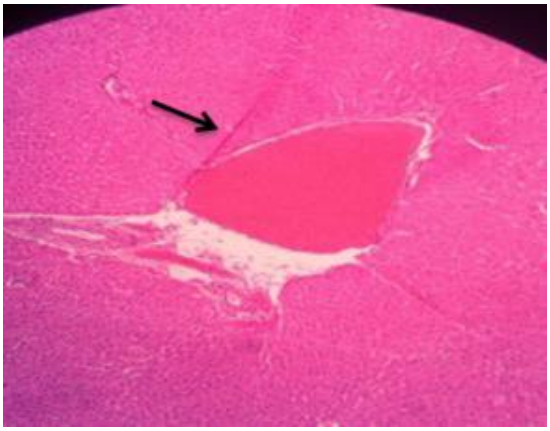

Figure 10: Section of liver showed thrombus in blood vessel in portal traid 10X (H and E stain).

In terms of the kidney, the current study showed histopathological including multifocal areas (figure11), sever haemorrhage in the interstitial tissue(figure 12) with generalized blood vessels congestion, congestion of glomeruli, swelling of cuboidal cells lining convoluted tubules leading to stenosis and obstruction of renal tubules lumen, thickening of blood vessel wall (figure 13) (figure 14), infiltration of inflammatory cells around the blood vessels (vasculitis) degeneration and necrosis of renal corpuscle atrophy and shrinking of some renal corpuscle was also observed(figure 15) (figure 16), these result were compared with histology of control group.

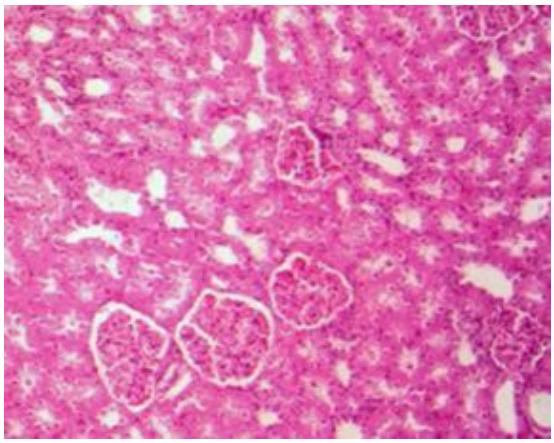

Figure 11: Photomicrograph of section of kidney control group 10X l (H and E stain)

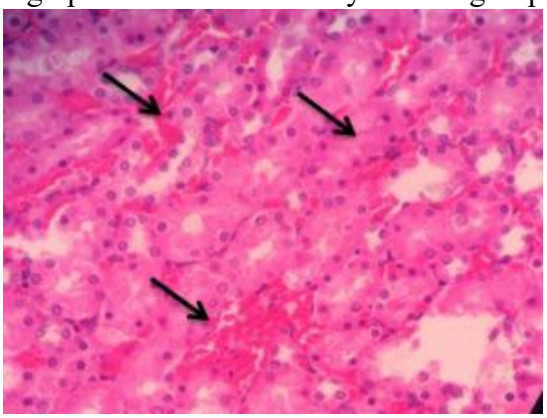

Figure 12: Showed normal architectural of renal tissue section of kidney heamorrhage of treated animal 40X (H and E stain)

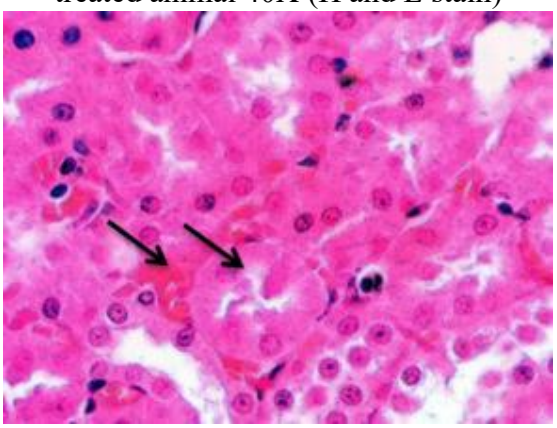

Figure 13: Sction of swelling of epithelial cells with stenosis and obstruction of lumen 40X, (H and E stain)

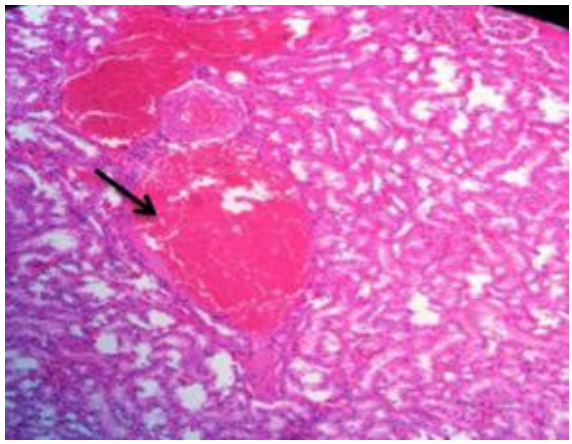

Figure 14: Section of vasculitis congestion of blood vessels 10X, (H and E stain)

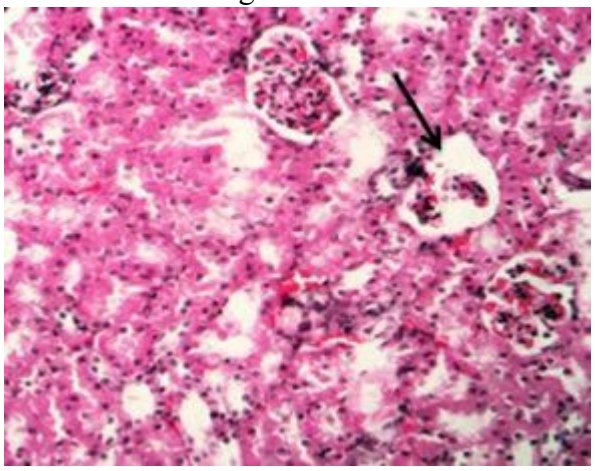

Figure 15: Section of necrosis and shrinking of renal corpuscles 10X, (H and E stain)

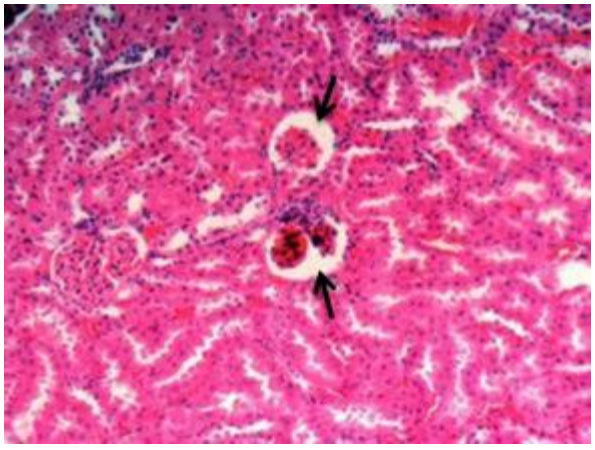

Figure 16: Section of degeneration and necrosis of renal corpuscles 10X, (H and E stain)

Discussion

The current study showed that the rats treated with 50, 100 and 200 ppm of ZnONPs causes increasing ed in the of liver enzymes compared with control group, this means that ZnO nanomaterial had toxic on liver enzymes by causing e damaging effects on of the body metabolism (Fazilati 2013) The effect of treatment male with ZnONPs explained more significant changes in liver enzymes, oxidative stress, liver and renal tissue at 50 gm/Kg (Abbasalipourkabir *et al* 2015). The ZnONPs-induced oxidative stress and apoptosis (Attia *et al* 2018, Alsultan, 2024). When male rats given 5, 7.5 and 10 mg/kg of zinc oxide NPs three times weekly at eight weeks cause reduction of enzymes: glutathione, catalase, superoxide dismutase and reduced of kidney function parameters: Creatinine, urea, uric acid after treatment with ZnO-NPs (Bashandy *et al*, 2018).

When treatment, adult male rats of Zn nanomaterial at 50 mg/Kg gave toxicity effect and histolgical changes in liver and kidney (Abbasalipourkabir *et al* 2015). There are some factors increase of kidney to the nephrotoxic reason via the drugs and toxins, that well cause injury and disease of kidney (Perazella, 2009), And the ZnONPs consider one of medicinal material (Jiang *et al*, 2018). The over dose because toxicity change in liver and kidney at 300mg/ml during 14 days when treated mice (Salman, 2018).

Conclusions: The current study proved that the severity of histological and biochemical changes dopend on the dose used in the study. The male rat's treatment of zinc nanoparticles orally causes sever histopathological changes in liver and kidney at 100gm/k at 100µ size.

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