JOURNAL OF KUFA–PHYSICS
www.uokufa.edu.iq/journals/index.php/jkp | ISSN: 2077–5830

Toxicity of CaO Nanoparticles effect on Haematology and Histopathology of male Rabbits

***Rana Ismaeel Khaleel** **Asmaa Hadi Mohammed[1]**

Department of Physics, College of Science, Mustansiriyah University, Baghdad, Iraq .

*Corresponding Author E-mail: khleelrana79@gmail.com

ARTICLE INF

Article history:
Received: 10 AUG, 2021
Revised: 15 SEP, 2021
Accepted: 30 SEP, 2021
Available Online: 10 DEC, 2021

Keywords:

Calcium oxide nanoparticles,
Toxicity
Ld50.

ABSTRACT

Calcium oxide (CaO) nanoparticle (NP) was synthesized by simple chemical method .The CaONPs was characterized by X-ray diffraction (XRD), Scanning Electron Microscope (SEM) analysis and UV-absorption spectral studies. The average crystallite size was found to be ≈36nm and the band gap energy of CaO nanoparticle was 1.9 eV. Toxicity tests were performed on adult rabbits dosed with compound CaONPs for a period of (3 weeks).Areas of necrosis, haemorrhages and degenerations were seen histopathogically in kidney, liver after 3 weeks of administration of CaONPs, the data of hematologic obtained were statistically evaluated using Finney's Probit Analysis and Toxicity test performed revealed that Ld50 value was 0.5ml/kg. In this study found that an increase in the proportion of calcium oxide in the blood leads to damage to the organs, like the liver and kidneys that responsible for filtering the blood.

DOI: http://dx.doi.org/10.31257/2018/JKP/2021/130202

تأثير سمية أكاسيد الكالسيوم النانوية على أمراض الدم والتشريح النسيجي على ذكور الارانب

رنا أسماعيل خليل **أسماء هادي محمد**

قسم الفيزياء،كلية العلوم ،الجامعة المستنصرية.

الـــخُـــلاصـــة:

لقد تم تصنيع جزيئات أكاسيد الكالسيوم (*CaO*) النانوية (*NP*) بطريقة كيميائية بسيطة. تم فحص *CaONPs* بانحراف الأشعة السينية (*XRD*) وتحليل المجهر الإلكتروني (*SEM*) والدراسات الطيفية لامتصاص الأشعة فوق البنفسجية. ولقد وجد ان متوسط حجم البلورات ليكون (*36nm*) وكانت طاقة فجوة النطاق لجسيمات *CaO* النانوية(*1.9V*). تم إجراء اختبارات السمية على الأرانب البالغة المجرعة ب المركب النانوي *CaONPs* المركب لمدة ٣ أسابيع. شوهدت مناطق النخر والنزيف والانحلال في الأنسجة في الكلى والكبد بعد ٣ أسابيع من إعطاء *CaONPs* ، تم تقييم بيانات أمراض الدم التي تم الحصول عليها إحصائيًا باستخدام

الكلمات المفتاحية:

اكاسيد الكالسيوم النانوية
السمية
قياس الجرعة المميتة

تحليل بروبيت واختبار السمية الذي أجري لمعرفت أن قيمة *Ld50* كانت ٠.٥ مل / كجم.
ووجدت في هذه الدراسة أن زيادة نسبة أكسيد الكالسيوم في الدم تؤدي إلى تلف الأعضاء ، مثل الكبد والكلى المسؤولة عن تصفية الدم .

1. Introduction

Nanomaterials are widely used in many fields such as medicine, electronics, agriculture, textile production, industrial and biological applications due to their extraordinary chemical and physical properties, and their far-reaching benefits have reinforced concerns about potential health risks [1], and the biological and clinical toxicity of NPs is gaining increasing interest by researchers [2 ,3], The toxicity of nanomaterials depends greatly, on the special arrangement of many atoms, due to large differences in shape and chemistry even the smallest nanoparticles, with only tens of atoms, produce a large number of characteristic substances with the potential for extreme difference in physical properties. In recent years, great importance has emerged for healthcare, and recently several studies have facilitated the efficient coupling of biomolecules with organic and inorganic nanoparticles. But in this research, metal oxide nanoparticles (MONPs) with obvious and associated long-term effects study, which cause many diseases, including infections, impaired immunity, toxicity, tissue damage, and cancer when exposed for a long time [4]. Therefore, it has become necessary to create such kind of organic and inorganic nanoparticles which have the potential to reduce the undesirable effects due to long term exposure[5].Several experiments have also been conducted to study the antimicrobial effect of nanoparticles, but clinically it cannot be estimated due to their pest and toxic effects [6], especially for inorganic materials and their oxide, antimicrobial oxides are known for their high antimicrobial and heat resistance[7].As an example of these materials, the one we used in our research is calcium and its oxide, these nanoparticles is a good example of nanomaterials that at high levels in the blood cause serious damage to the internal organs, in the one research for example [8],they found that CaONPs Properties and the clinical applications of calcium oxide in the treatment of endodontics and the treatment of dental injuries including its antibacterial activity, antifungal activity, effect on bacterial biofilms. Some researchers have concluded that CaO can show severe toxicity in the event of an increase in its proportion from the normal level, and its effect appears in the liver and kidneys [9] It exhibits cubic lattice showing anisotropic catalytic behaviour and used as dopant to stabilize metal-oxide. Few research groups reported the synthesis of nano-CaO by using thermal decomposition and sol-gel techniques [10]. In the present work, the CaONPs synthesis was attempted used simple chemical method, and it was studied by SEM, XRD,UVand EDX . It was used in an integrated study to increase the percentage of MONPs toxicity in the body by studying a complete analysis of blood, kidney and liver histological effect .

2. Materials and method

The CaONPs was synthesized using a simple and low coast chemical method,$Ca(NO_3)_2$ at a concentration of (2.0 M) dissolved at (250 ml) distilled water and stirring continuously for a period of time (45) minutes and mixing (1M) of hydroxide Sodium NaOH with distilled water in a capacity flask (250 ml) and continuous stirring for (45) minutes, then adding one drop of sodium hydroxide to the salt of Ca in a reaction flask (capacity 500 ml) and mix well with magnetic vibrator (magnetic stirrer device) and after an hour at room temperature ,the result is a milky precipitate, filtered and washed several times with distilled water, and then dried in the oven and burn the residue for an hour, at a temperature of 100 ° C for a period of 3 hours in the oven at a temperature of 450 ° C , the resulting powder was dissolved in a normal saline solution.

Kind of animals Used

A healthy male of the white Malaysian Rabbits was used, totally 32 adult male rabbits were maintained with an average weight of 1.5 ± 2 kg in well-ventilated polypropylene cages.The animals were given food and fresh water.

Toxicity test

Acute toxicity of metal oxides was tested, the study population of 20 male rabbits were divided into groups (each containing 5 rabbits), received 3ml distilled water alone as a control, After an absolute diet for 12 hour, metal oxides were gavaged to the rabbits,0.3ml/kg,0.5ml/kg and 0.7ml/kg of CaONPs for Ld50 test, gavagation was performed daily for 2 weeks of administration .All rabbits were examined and the concentration which was killed half of animals was determined and considered as LD_{50}.

Experimental Design:

The adult male rabbits were divided into 3 groups, control 8 and for the test 24 rabbits, 8 animals for each group, since the daily oral therapeutic dose of CaONPs for animal's was 0.5ml/kg, t used protocol was approved by the institutional animal ethics committee (IAEC Ref No-79/2013).

Collection of liver and kidney

The rabbits were dissected as per guidelines given in [11]. A midline incision was given on the abdomen with the help of scalpel, the organs liver and kidney were identified and dissected out. The tissues were fixed in modified formaldehyde in and transferred to 50% of DDW for histological examination.

Histological studies

The organs (liver and kidney) were embedded in paraffin wax and blocks were prepared and labelled, 5µm thickness sections were cut using rotatory microtome. The sections were fixed on slides and stained using Haematoxylin and Eosin according to the Guidelines on staffing and workload for histopathology [12]. Assessment Stained sections were studied under (Olympus Research Microscope) (model cx21i) and photographed at 10X and 100X magnification in at least three random microscopic fields from each animal.

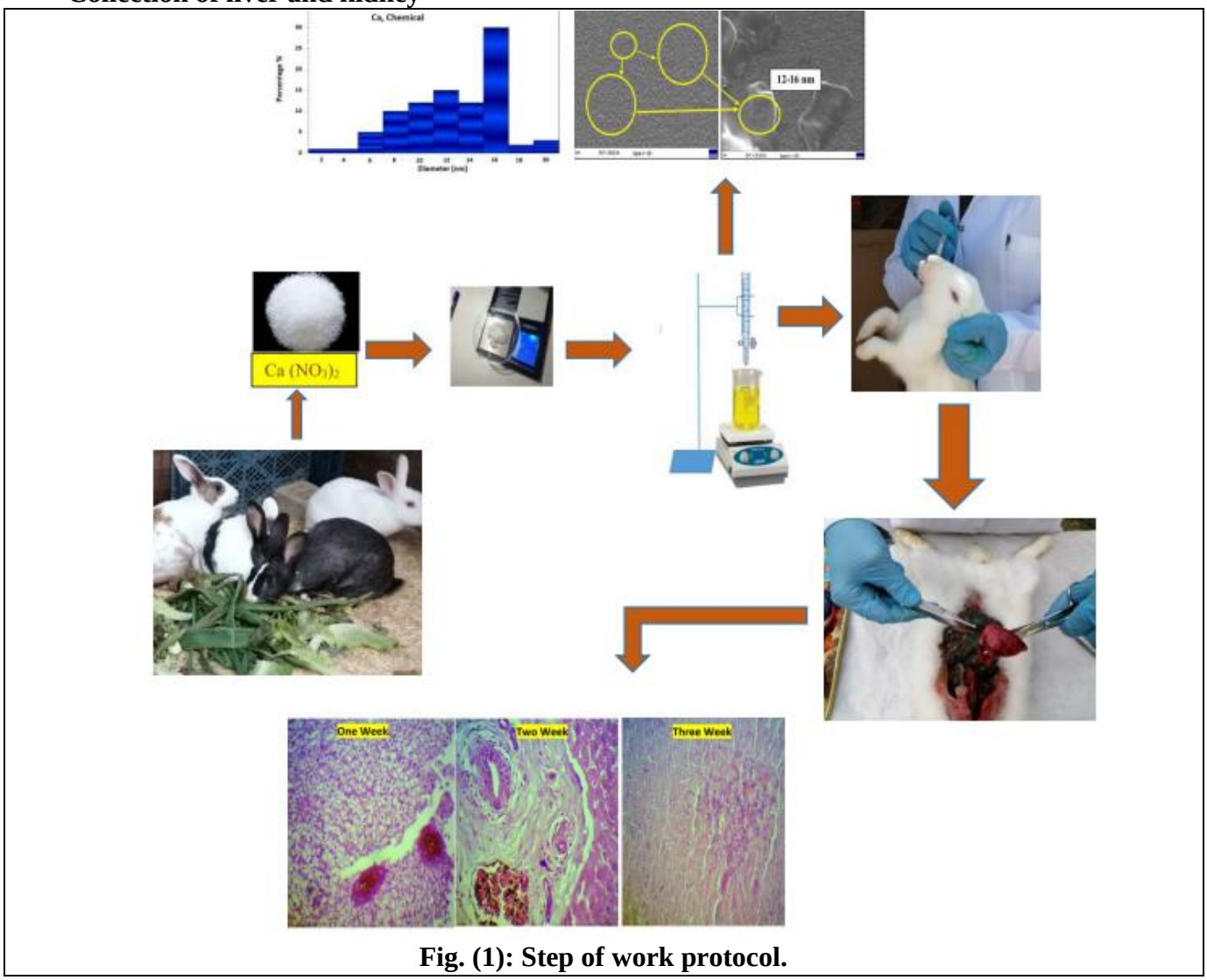


Fig. (1): Step of work protocol.

3. Results and discussion:

The SEM image in figure (2) showed that the nanoparticles are nearly spherical where they clump together as well as these agglomerates of small particles show a polycrystalline character of the NPs, other studies [13] have confirmed the spherical shape of the CaONPs and the average size of the CaONPs was estimated to be (20 nm), the diameter of the most CaONPs is (16) nm as shown in figure (2).

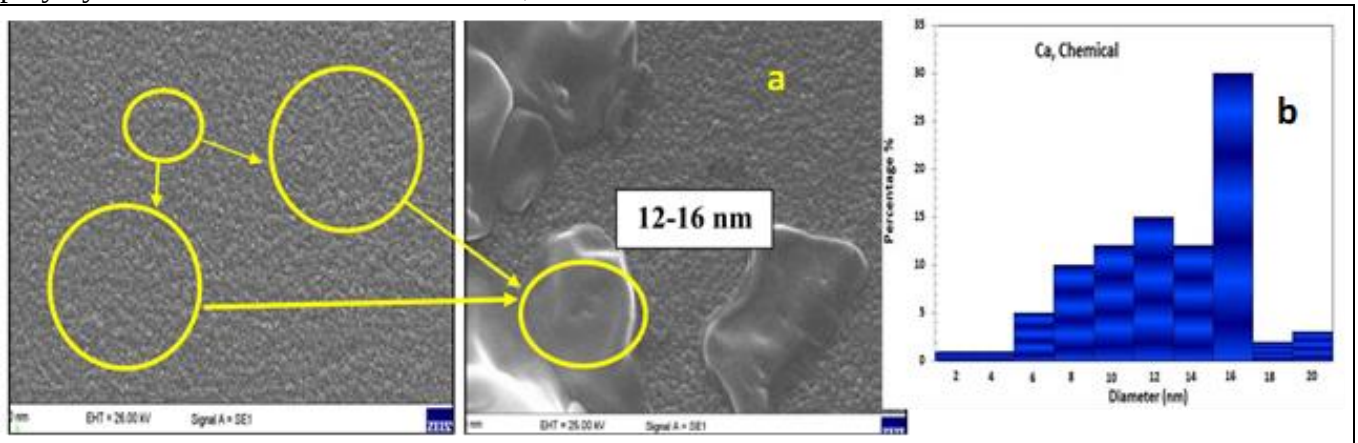


Fig. (2): a. SEM and b. histogram for CaONPs .

The crystalline structure of CaO NPs was studied by x-ray diffraction (XRD) (Fig.3). The results of the diffraction peaks corresponding to the diffraction planes with different (hkl) are well matches with standard results of ICDD (JCPDS card no. 37-1497). Full width at half maximum of the diffraction peak, and θ is the angular position of the peak. The average value calculated for the crystallite size for CaONPs was 35.94 nm.

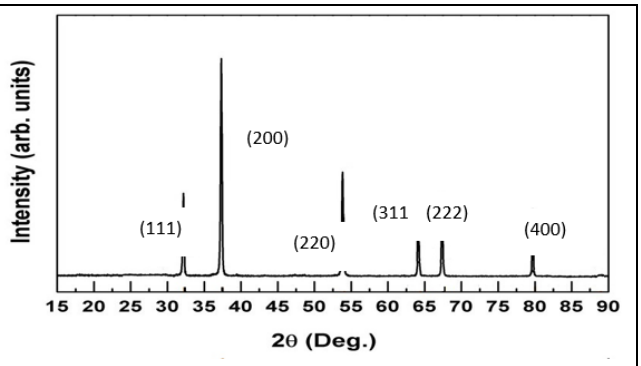


Fig. (2): XRD for CaONPs .

The Optical absorption spectrum depends on many factors like, crystal structure, energy of the incident photon, film thickness and film surface morphology. Figure (3) displays the variation of absorbance spectra with wavelength ranges from (200 to 900) nm of the CaONPs prepared using chemical method. It was appeared that nano biomaterials thin film has high absorbance in the UV region, indicating its applicability as an absorbing material. From the plots, it can be justified that the peak of the CaONPs in the range 500 to 900nm.

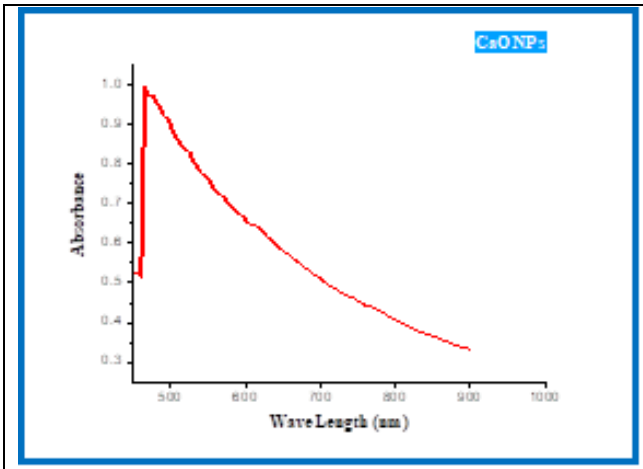


Fig. (3a): Optical absorbance spectrum

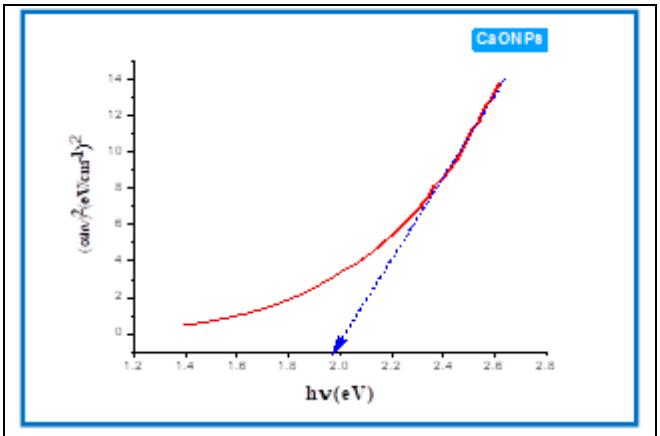


Fig. (3b): Energy band gap for CaONPs.

The results of EDX test for CaONPs showed that the elements contained calcium and oxide in different proportions were showed in figure (4), the Ca in almost up to 0.3 Kev and the O up little of 0.5 Kev, These two components were together form the CaONPs.

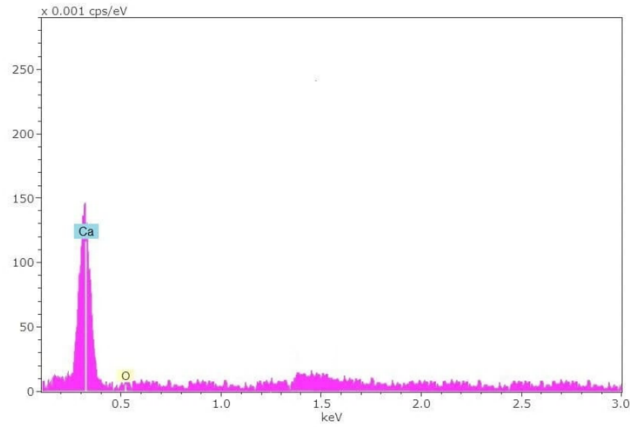


Fig. (4): EDX for CaONPs.

Examining of the sections obtained from livers of the control group showed normal histological structure. The liver was formed of classic hepatic lobules which were roughly hexagonal in shape with central veins forming their central axis. At their angles, there were portal areas containing connective tissue stroma and portal triads. The latter consisted of a terminal branch of the hepatic artery and a small branch of the portal vein as well as a bile ductile figure (5).

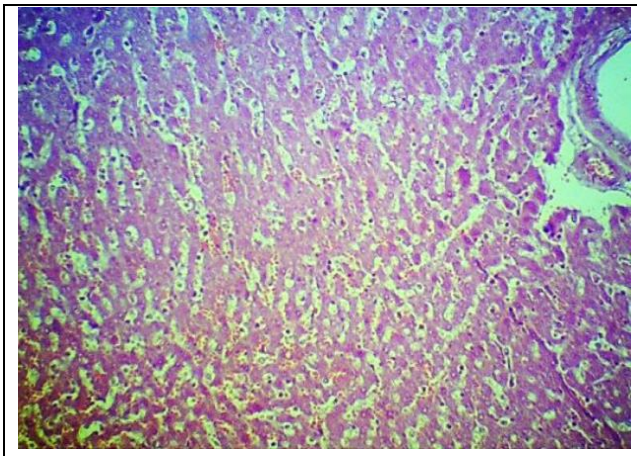


Fig. Figure (5): Liver section for control group.

Examining of livers sections obtained from this group orally dosing by CaONPs for two weeks, revealed that several histological changes in the form of disturbed hepatic architecture In addition to cellular infiltrations that can be seen around central veins and in between the components of portal tract and even between the hepatocytes as well as proliferation of bile ductules. Most of the hepatocytes showed variable degrees of cytoplasmic vacuolations, some contained multiple small vacuoles, some showed one large vacuole; the others appeared ballooned with peripheral nuclei. In addition, some nuclear changes were observed like darkly stained (pyknotic) nuclei, fragmented nuclei (karyorrhexis), and lysed nuclei (karyolysis) hepatocytes with nuclear vacuolations (glycogenated nuclei) were also observed. Multiple small cells with ovoid nuclei (most probably hepatic stem cells (HSCs) and arranged in rows were seen in between the hepatocytes.

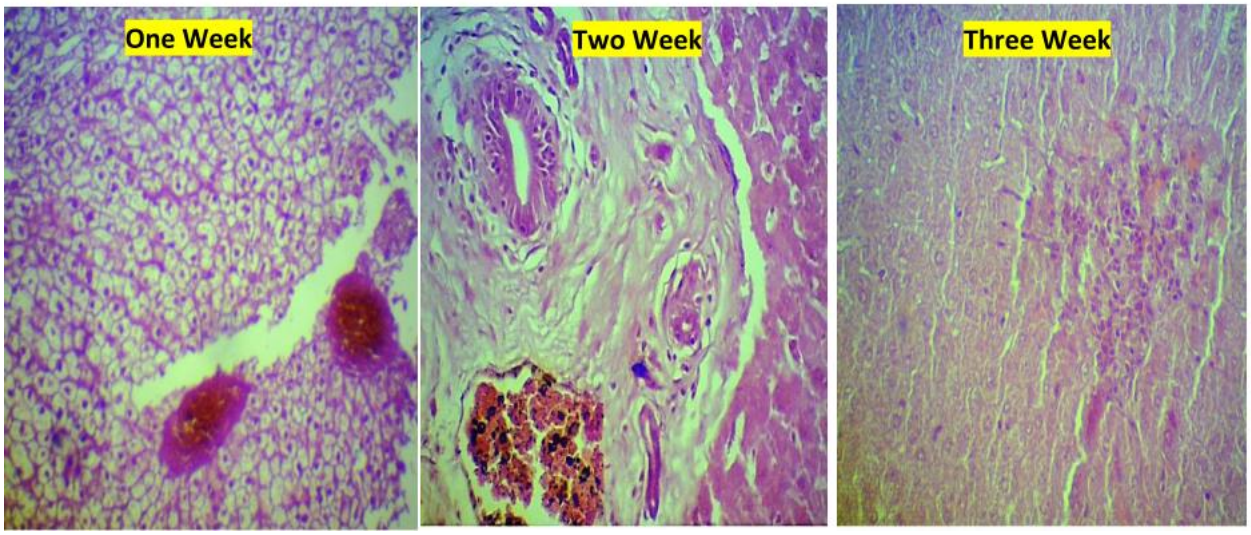


Figure (6): Liver section for damaging group (orally dosing CaONPs).

Representative micrograph showing kidney histomorphology of control rabbits fed with water only in figure (7). It has been seen regular shape and non-mild swelling of glomeruli and mild atrophy of renal tubules compared with the kidneys tissues of the dosed groups fed with nano-metal oxides.

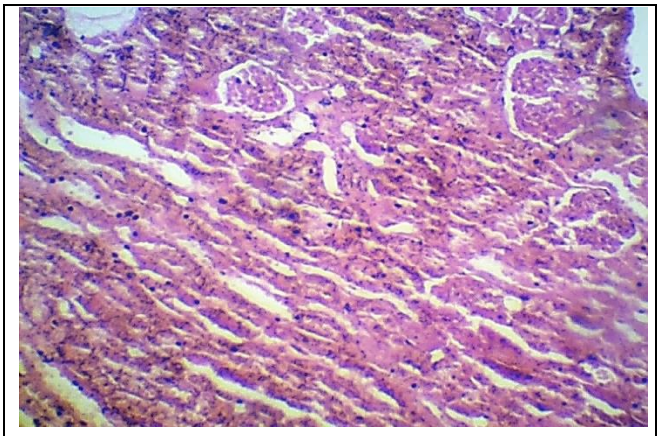
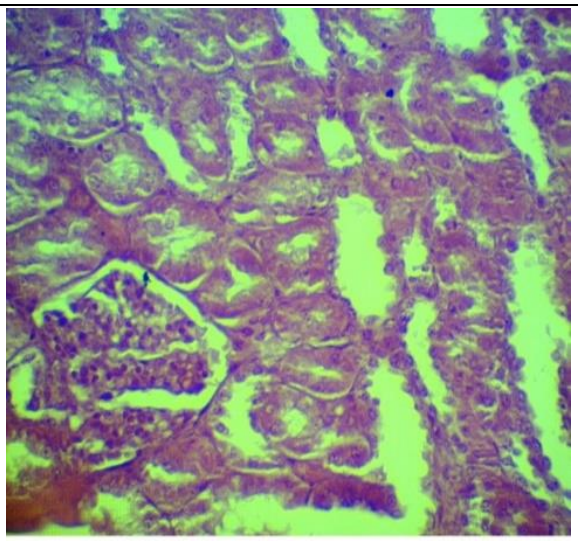
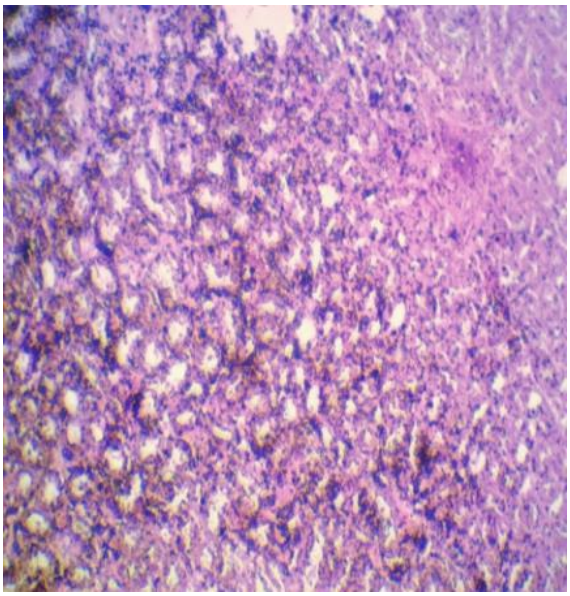


Figure (7): Kidney section for control group.

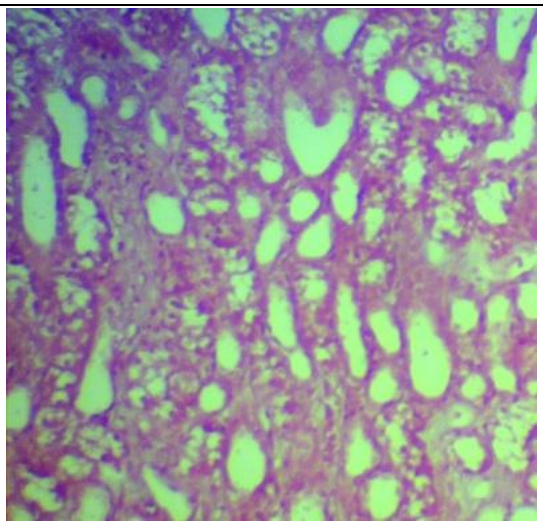
Rabbits orally dosed for 3 weeks with CaONps, expansion and hypercellularity of the interstitial area were noted in rabbits dosed CaONPs-orally, along with increased amounts of collagen figure (8). No glomerular or vascular injury was detected in any of the dosed groups.



(a) one week



(b) Two week



(c) three week

Figure (8): Kidney section for damaging group (orally dosing CaONPs).

4. Statistical analysis:

Table (1) shows the results of blood analysis after two weeks of taking CaONPs orally. A significant increase in the level of white blood cells was observed compared to the control group ($p \leq 0.01$, the results also showed a significant increase in the haemoglobin value for CaONPs compared to the control group ($p \leq 0.01$), in addition to a significant increase in the creatinine value compared to the control group ($p \leq 0.01$).Finally a significant increasein the second week in ALT enzyme was observed due to the presence of CaNPs effect and a significant increase in AST and ALP values in the second week of oral administration.

Table (1): blood analysis after two weeks of taking

Item	Grop 1 Control	Grop 2 CaONPs (1Week)	P-Value	Grop 3 CaONPs (2Week)	P-Value	Unit
WBC	7±0.23	7.5 ± 0.21	P.≤0.05	0.8± 4	0.01	×10*9/L
RBC	4 ± 0.12	3.22 ± 0.24	P.≤0.05	2.60± 2.5	0.01	×10*12/L
HGB	13 ± 0.11	6.6 ± 1.7	P.≤0.01	5.5 ± 3.8	0.01	g/dL
Blood Urea	21 ± 0.31	31 ± 0.05	P.≤0.05	41 ± 0.07	0.01	Mg/dL
S. Certain	0.8 ± 0.43	0.1 ± 1.3	P.≤0.01	9 ±0.01	0.05	Mg/dL
S. Ureic asid	4.1 ± 0.55	1.9 ± 3.1	P.≤0.01	5 ± 0.02	0.01	Mg/dL
A.L.P	98 ± 0.04	102 ± 0.08	P.≤0.05	64 ± 2.9	0.01	u/I
A.L.T	36 ± 0.52	13.1 ± 2.3	P.≤0.01	90± 0.05	0.01	u/I
A.S.T	47 ± 0.17	39.8 ± 0.09	P.≤0.05	53 ± 0.08	0.05	u/I

5. Conclusion:

In this work, CaONPs were prepared by simple chemical method. The crystallite size of synthesized nanoparticles prepared confirmed the cubic structure for CaONPs.The SEM analysis and the histogram diameter size revealed that the particle size of CaONPs was~ 12-16 nm. The absorption spectrum of nanoparticles shows the absorption in UV-region due to a large band gap of CaONPs. The acute toxicity of CaONPs in rabbits was investigated using H&E staining protocols. Sub-acute severe hepatic and tubular degenerations were observed in the liver and kidneys, respectively. Mild haemorrhage can be seen in the liver and cadaveric, which is attributed to the fact that blood crosses into the organs and infects them. It is suggested that CaONP cannot be used directly with the antibodies, it should be modified with an appropriate hydrophilic coating. However, these assessments require more careful studies.

6. References

[1] C. Buzea, Ivan. I. Pacheco Blandino, and Kevin Robbie," Nanomaterials and nanoparticles: Sources and

toxicity", Biointerphases vol. 2, issue 4, pages MR17 - MR172, (2007).

[2] J. Sobhanan, P. Jones, R. Kohara, S. Sugino and M. Vacha," Toxicity of Nanomaterials Due to Photochemical Degradation and the Release of Heavy Metal Ions", Nanoscale, (2020).

[3] A. Busra Sengu and E. Asmatulu," Toxicity of metal and metal oxide nanoparticles: a review", Environmental Chemistry Letters, 7 June (2020).

[4] Jossana A. Damasco , Saisree Ravi , Joy D. Perez , Daniel E. Hagaman and Marites P. Melancon ," Understanding Nanoparticle Toxicity to Direct a Safe-by-Design Approach in Cancer Nanomedicine", Nanomaterials , 10, 2186,(2020).

[5] Y. Yang, Z. Qina , W. Zenga , T. Yanga , Y. Cao, C. Mei and Y. Kuang," Toxicity assessment of nanoparticles in various systems and organs", Nanotechnol Rev ; 6(3): 279–289,(2017).

[6] R. AKÇAN1, H.Canberk, M. Şerif , B, Tastekinl , N, Saglam," Nanotoxicity: a challenge for future medicine", Turkish Journal of Medical Sciences, 50: 1180-1196, (2020).

[7] A. Sukhanova1, S. Bozrova, P. Sokolov, M. Berestovoy, A. Karaulov and I. Nabiev," Dependence of Nanoparticle Toxicity on Their Physical and Chemical Properties", Sukhanova et al. Nanoscale Research Letters, 13:44, (2018).

[8] Z. Mohammadi1 & P. M. H. Dummer," Properties and applications of calcium hydroxide in endodontics and dental traumatology", International Endodontic Journal, 44, 697–730, (2011).

[9] A. R. BUTTa , S. EJAZb , J. C. BARONb,d , M. IKRAMc , S. ALIa," CaO NANOPARTICLES AS A POTENTIAL DRUG DELIVERY AGENT FOR BIOMEDICAL APPLICATIONS", Digest Journal of Nanomaterials and Biostructures, Vol. 10, No. 3, July - September , p. 799 – 809,(2015).

[10] Zahra Mirghiasi a,b , Fereshteh Bakhtiari a,c, *, Esmaeel Darezereshki c,d , Esmat Esmaeilzadeh," Preparation and characterization of CaO nanoparticles from Ca(OH)2 by direct thermal decomposition method", Journal of Industrial and Engineering Chemistry 20 , 113-117,(2014).

[11] P. N. Nirmala, G. Suresh," International Journal of Recent Scientific Research", 809 4, 1320, (2013).

[12] Eila Kaliste," The Welfare of Laboratory Animals",2ed, Clive Phillips, Professor of Animal Welfare, Centre for Animal Welfare and Ethics, School of Veterinary Science, University of Queensland, Australia,(2007).

[13] Meysam Sadeghi , Mir Hassan Husseini." A Novel Method for the Synthesis of CaO Nanoparticle for the Decomposition of Sulfurous Pollutant", Journal of Applied Chemical Research, 7, 4, 39-49 (2013).

[14] N. Harcourt-Brown, M. Silkstone , T. J. Whitbread and F. M. Harcourt-Brown," RHDV2 epidemic in UK pet rabbits. Part 1: clinical features, gross post mortem and histopathological findings", Journal of Small Animal Practice ,Vol 61 , July ,(2020).