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EVALUATION OF THE ANTIBACTERIAL ACTIVITY AND THE TOXICITY OF MAGNESIUM OXIDE NANOPARTICLES ON MICE USING THE LD50 TEST

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Abstract-*In this research, synthesis of MgO nanoparticles by Co-precipitate method. Results showed that MgO nanoparticles with an average grain size (17) nm. Characterization of MgO nanoparticles was done by X-ray diffraction (XRD), Fourier transforms infrared spectroscopy (FTIR), UV–visible ,Transmission Electron Microscopy (TEM) and Scanning and Electron Microscopy (SEM) were made. The antibacterial activity of MgO nanoparticles (NPs) showed higher inhibition of gram negative bacteria (E.coli) compared to gram positive bacteria (S. aureus) . Thirty-five male albino mice, divided into seven groups based on concentrations prepared from MgO NPs, each group having five mice and administered for 30 days. The calculated of LD50 was 1200 mg/kg bwt. It has been found that there were non-significant in the organ index of all organs compared to control group with the exception of the liver at a concentration of 1200 mg/kg bwt was found significant elevated. No change in the histology of brain, heart and kidneys organs while there was a significant change in the liver histology at the concentration of 1200 mg/kg bwt of MgO NPs. Mild inflammation of the portal vein and necrotic liver was observed.*

Keywords: *Magnesium oxide NPs, XRD, FTIR, UV–vis, TEM, SEM, Antibacterial activity, LD50 test, Organ Index and Histology study*

I INTRODUCTION

Nanotechnology deals with the applications and manipulationse of material at the nano scale of (1-100 nm). The creation of structures, devices and systems that have novel properties and functions may imply to any innovation depending on markable features of these materials[1].

Nanomaterials are any materials that have unique or novel properties due to nano-scale structuring that show interesting electrical, optical, and magnetic properties and catalytic activity[2]. They can be fabricated with the top down or bottom up approaches. With the top down principle; very small structures are produced

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starting with a large part of material through processes of mass removal. On the contrary, with bottom up technique nanoscale materials is produced by building up atom by atom and molecule by molecule[3].

Nanomaterials are classified depending on the dimension into four main types include zero dimension, one dimension, two dimensions and three dimensions [4].

Nanoparticles of metal oxides have better properties than the original materials. The size of the nanoparticles, shape, surface, purity, stability and the method of synthesis are responsible for the interaction of nanoparticles with biomolecule[5].

MgO is an important inorganic oxide, odorless, non-toxic, high hardness , high purity high melting point and it appears in white powder form[6].

Because magnesium is widely used as a medical treatment, magnesium oxide is also used in this field due to magnesium oxide is the main source of magnesium. Magnesium oxide has used as a treatment of heartburn, sour stomach and laxative [7]. *In 2016, Thirunavukkarasu S et al* [8], observed that the obtained results suggest the MgO Nps have an important role in the drug delivery. *In 2017, Islam MI et al*[9] detected that the MgO Nps as an inhibitor against breast carcinoma. Although nanomaterials are widely used in treatment and medicine, there are adverse health effects for humans if the concentrations of these nanomaterials are very high, among the nanomaterials are MgO, ZnO and TiO_2 nanoparticles[10].*R. S. Kumaran et al*[10], reported that the toxicity of MgO was lower than ZnO, and TiO_2.

II MATERIALS AND METHODS

Synthesis of MgO nanoparticles

MgO nanoparticles were prepared by co-precipitation method. At which $MgNO_3$ of (1M) was dissolved by 50 ml of deionized water and also NaOH (1M) solution was prepared using 50 ml of deionized water. The solution of NaOH was added to a mixture solution of $MgNO_3$ and PVP under stirring at 24°C. After the reaction process was completed, a precipitate was observed as a white powder. Followed up the step by filtration and washing the precipitate. It has been washed several times using deionized water, then dried the precipitate for 1 hour at 80°C, to obtained $Mg(OH)_2$. For the purpose of obtaining pure MgO nanoparticles, the calcination process is performed using a muffle furnace for 3 hours at a temperature of 350°C. The reactions occur according to the equation referred to below [7].

$$\mathbf{Mg(NO_3)_2 + 2NaOH \longrightarrow Mg(OH)_2 + 2NaNO_3} \qquad \mathbf{(1)}$$

$$\mathbf{Mg(OH)_2 \xrightarrow{350\ °C,\ 3hr} MgO + H_2O} \qquad \mathbf{(2)}$$

Preparation for bacterial activity experiment

Antibacterial activity of MgO nanoparticles was carried out by t disc diffusion method. The MgO NPs was prepared in different concentrations (1,2,3,5 and 7) mg/ml to measure the diameter the inhibition effect of bacteria which included; Gram-positive (Staphylococcus aureus) and Gram-negative bacteria (E. coli). To grow bacteria on the petri plates , nutrient agar media was used then 5 mm diameter of wells was made using the

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sterilized cork borer. The MgO nanoparticles were filled into the wells. The plates were incubated for 24 hrs. at 37 ºC.

Animal samples

Thirty-five male albino mice were obtained from Mustansiriya University/ Iraqi Center for Cancer Research and Medical Genetics, weighing 25-35 grams housed in plastic cages and were maintained at temperature between 22 to 24ºC. The mice were divided into seven groups. Each group having 5 mice and oral dosage for 30 days with different concentrations of MgO NPs ranging from low to high concentration (0,200,300,500,700,1000 and 1200) mg/kg body weight. MgO NP solution was prepared with deionized water and with the use of sonication and vortexing to obtain uniform solution of all the particles of MgO NPs, in addition to stable for few minutes.

Preparation of histopathological samples

Histological study was achieved on brain, heart, liver and kidneys. These organs were fixed in 10% of formalin, then the tissues were embedded in paraffin blocks by instrument (Leica) and sliced into 3 mm thick sections. After that the hematoxylin and eosin (H & E) stain being used on the slides .Finally, the slides were analyzed by microscope (Olympus, Japan).

Statistical test

A statistical test was done by Microsoft office (SPSS no.24) which included Mean ± standard deviation, Student t- test and $P \leq 0.05$ was considered significant. The results were analyzed by one- Way ANOVA.

III RESULTS

Characterization of MgO nanoparticles

X-Ray Diffraction (XRD)

The structure of the mgo nanoparticle is investigated by X-ray diffraction. Figure 1 shows the mgo nanoparticles are in the cubic phase and there is no impurity phase in this sample. The average crystallite size of MgO NPs was 17 which were calculated by Debye Scherrer formula.

$$D = \frac{k\lambda}{\beta COS\,\theta} \qquad (3)$$

Where k =0. 9 scherrer co 54 nm is the wavelength of the source radiations, β = width at half maximum while θ is the angle of the Bragg diffraction peak. Five major diffraction peaks are seen at 2Θ = 36.92◦, 42.85◦, 62.18◦, 74.56◦, and 78.45◦ correspond to orientations of peaks (111), (200), (220), (311) and (222) matching the cubic MgO (JCPDS PDF 45-946). The results in agreement with *A. Kadari et al* [11]

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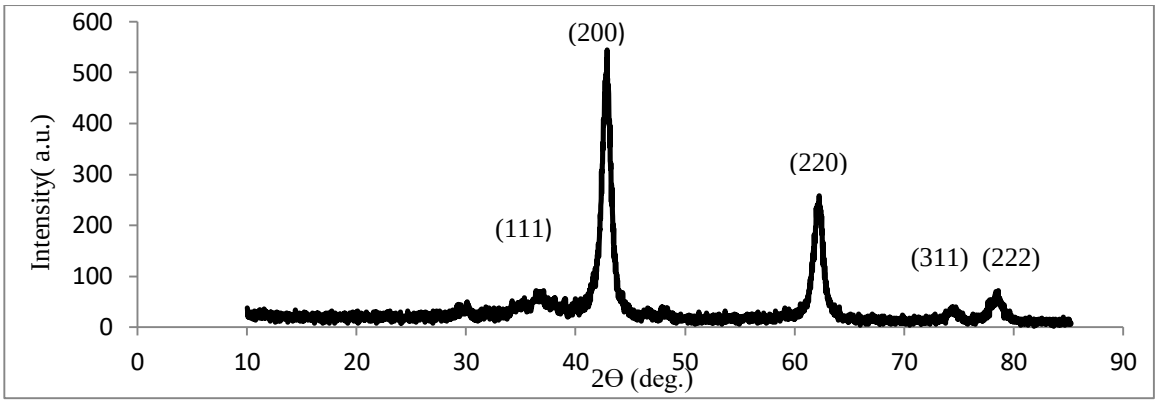
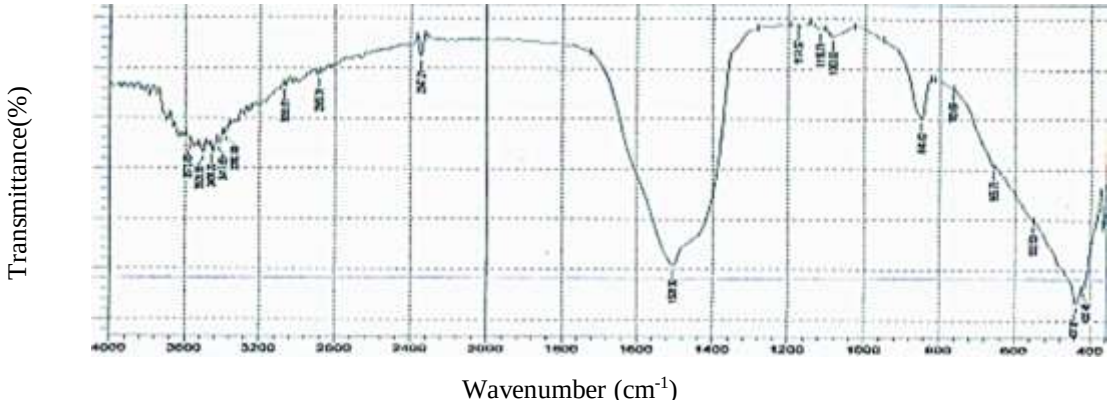


Fig.1: XRD of MgO nanoparticles

Fourier-transform infrared spectroscopy (FTIR) Analysis

Figure 2 represents the FTIR spectrum of MgO nanoparticles in the range 400-4000 cm^{-1}. The peak at 437 cm^{-1} indicates the presence linkage of magnesium metal to oxygen vibrations. The absorption peak at 1506 cm^{-1} is the bending the vibration of the OH bond. The peak at 3417 cm^{-1} attributed to the O–H stretching due to moisture with the powder of MgO nanoparticles [12].



nm. Fig. 3 shows the maximum peak was at 218 nm with band gap 5.69 ev, the formation of MgO Nps was approved by agreement with *M.R. Bindhu et al*[13] and *N. Badar et al* [14], they observed that the band gap energy is increasing with decreasing in the particle size of MgO nanoparticles. Other studies confirmed these results where they reported the average size of 20 nm when the band gap is 5.6 eV for the MgO nanoparticles[8].

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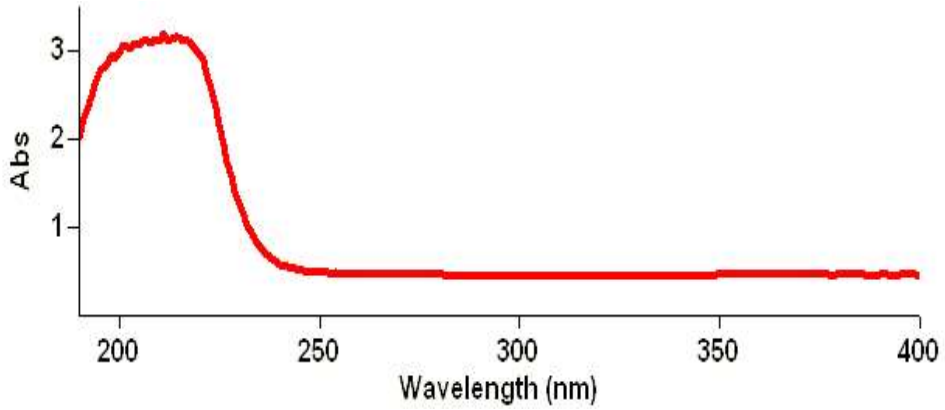

Figure3: UV-Vis spectra of MgO nanoparticles

Morphology analysis of MgO NPs

The morphology of MgO NPs was investigated using Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM). Figures 4 and 5 show the SEM and TEM images respectively of samples prepared by Co-precipitate method. The obtained average particle size about 28 nm measured by SEM and 20 nm measured with TEM. Most of the particles in these images had a spherical shape.

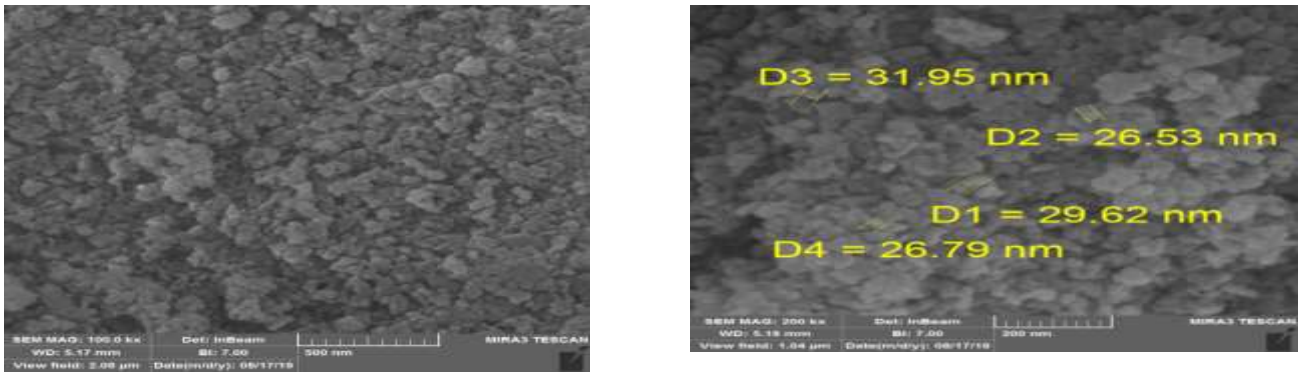

Figure 4: SEM images of MgO nanoparticles

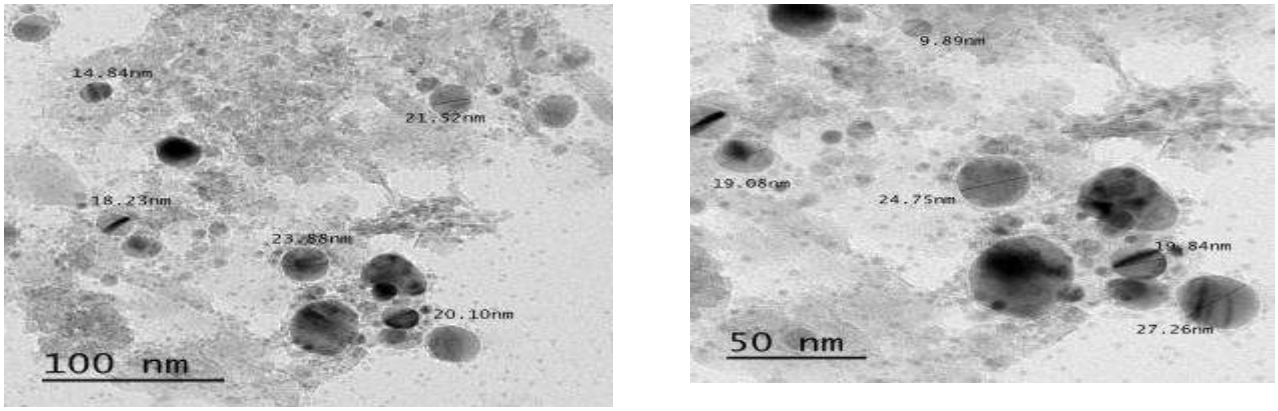

Figure 5: TEM images of MgO nanoparticles

Study the effect of MgO NPs on bacterial activity

Two pathogenic bacteria (*Escherichia coli, Staphylococcus aureus)* were chosen in the present study. The results exhibited as shown in table 1 and fig.6 the antibacterial activity of MgO NPs against gram positive bacterial more effective than gram negative bacterial. It was observed that the concentrations of MgO NPs at (1, 2 and 3) mg/ml did not affect the inhibition activity of both bacteria, whereas the concentration of MgO NPs at 7 mg/ml showed a higher inhibition result than concentration 5 mg/ml gram-negative bacteria (*E.coli*) compared to gram- positive bacteria *(S. aureus*) .

Table 1: Inhibition Zone of bacteria using different concentrations of MgONPs

Bacteria types	Zone of inhibition (mm)				
	1 mg/ml of MgONPs	2 mg/ml of MgONPs	3 mg/ml of MgONPs	5 mg/ml of MgONPs	7 mg/ml of MgONPs
E.coli	-	-	-	18	22
S.aureus	-	-	-	15	19

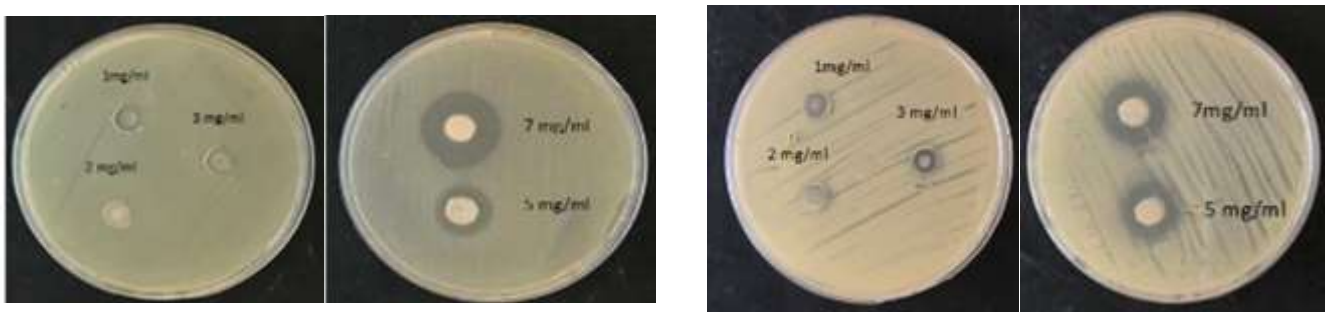

Fig.6: Antibacterial activity of MgC
E.coli S.aureus

LD50 measurement of chronic oral toxicity

The LD50 for 30 days was measured after observing the number of dead mice and was at 1200 mg/kg of body weight. Changes in the weight of the mice and its organs including Brain, Heart, liver and kidneys were evaluated due to the administration of various doses of the MgO NPs according to the organ index formula[15]:

$$\text{Organ Index} = \frac{\text{Wt. of experimental organ /Wt. of the experimental mice}}{\text{Wt. of control organ /Wt. of the control mice}}$$

Table 2 clarifies the variations of organ index of Brain, Heart, liver and kidneys of mice when compared with normal organs obtained from the control mice. There were a non-significant in the organ index of all organs

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mice compared to control group ($p>0.05$) with the exception of liver at a concentration of 1200 mg/kg of MgO NPs significant increase was observed compared to control group ($p<0.05$).

Table 2: Organ index of Brain, Heart, liver and kidneys of mice exposed to MgO for 30 days compared with normal organs obtained from the control mice

Organ index								
groups	*Brain*	*P value*	*Heart*	*P value*	*Liver*	*P value*	*kidney*	*P value*
Control Mean±SD *MgO NPs 200 mg/kg Mean± SD*	0.981±0.054 1.048±0.08	p>0.05	1.016±0.069 1.055±0.076	p>0.05	1.157±0.052 1.16±0.114	p>0.05	1.03±0.056 0.995±0.09	p>0.05
Control Mean± SD *MgO NPs 300 mg/kg Mean± SD*	0.981±0.054 0.921±0.094	p>0.05	1.016±0.069 1.07±0.086	p>0.05	1.157±0.052 1.175±0.086	p>0.05	1.03±0.056 1.123±0.096	p>0.05
Control Mean± SD *MgO NPs 500 mg/kg Mean±*	0.981±0.054 1.065±0.036	p>0.05	1.016±0.069 1.121±0.098	p>0.05	1.157±0.052 1.188±0.103	p>0.05	1.03±0.056 0.976±0.06	p>0.05

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SD								
Control Mean± SD *MgO NPs 700 mg/kg Mean± SD*	0.981±0.054 1.086±0.04	p>0.05	1.016±0.069 0.987±0.093	p>0.05	1.157±0.052 1.045±0.105	p>0.05	1.03±0.056 0.966±0.082	p>0.05
Control Mean± SD *MgO NPs 1000 mg/kg Mean± SD*	0.981±0.054 1.168±0.026	p>0.05	1.016±0.069 1.155±0.061	p>0.05	1.157±0.052 1.208±0.112	p>0.05	1.03±0.056 1.121±0.072	p>0.05
Control Mean± SD *MgO NPs 1200 mg/kg Mean± SD*	0.981±0.054 1.19±0.07	p>0.05	1.016±0.069 1.18±0.085	p>0.05	1.157±0.052 1.525±0.043	p<0.05	1.03±0.056 0.93±0.07	p>0.05

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Histopathology studies

The organs of the mice such as brain, heart, liver and kidneys were analyzed for the purpose of identifying the changes that occurred after 1200 mg / kg bwt oral administration of the MgO NP. There are no changes in the morphological of these organs excepted liver organ compare with organs of control mice, as shown in fig.7 and 8. Mild inflammation of the portal vein and necrotic liver was observed.

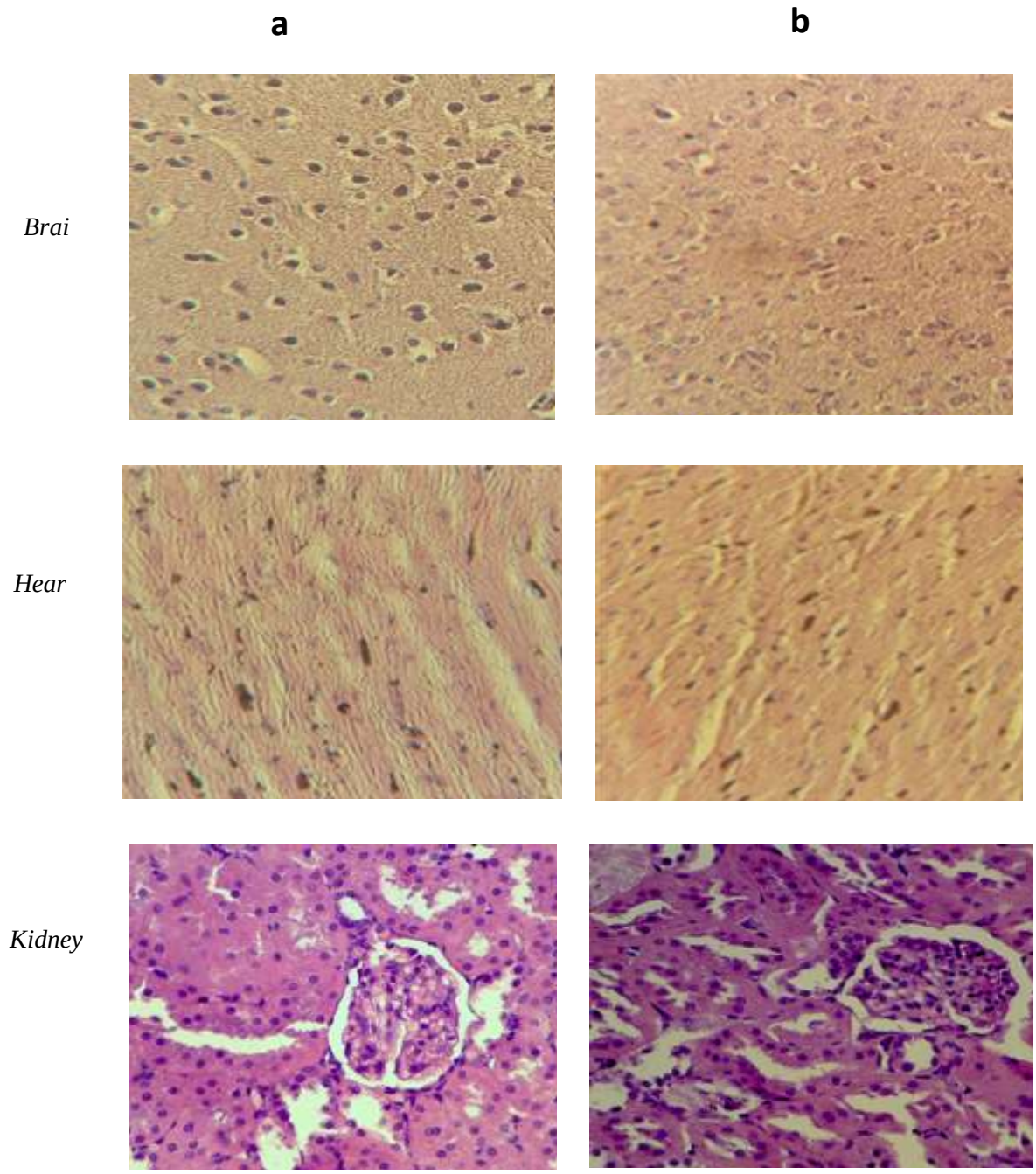

Figure 7: Normal histopathology organs of the mice for brain, heart and kidneys. They were seen at 40x magnification.

a) Before 1200 mg / kg bwt oral administration of the MgO NP

b) After 30 days of 1200 mg / kg bwt oral administration of the MgO NP

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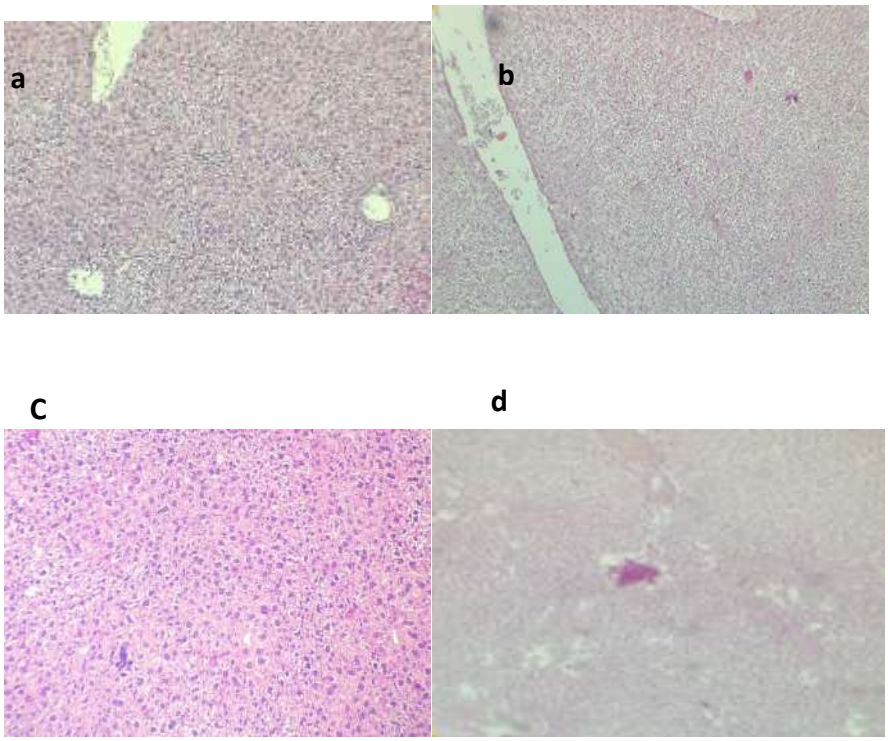

Figure 8: Histopathology images of liver mice at 10x magnification. (a) and (c) are normal mice liver. After 30 days of 1200 mg / kg bwt oral administration of the MgO NP (b) showing mild inflammation of portal vein (d) liver necrosis .

IV DISCUSSION

This research showed useful applications of MgO NPs in terms of its usage as a bacterial inhibitor and low toxicity in the presence of high concentrations of MgO NPs. This results of anti-bacterial agree with N.Y.T. Nguyen *et al*[16], they noticed nanoparticles of MgO can penetrate the cell wall of gram-negative bacteria (E.coli) due to having had a thin wall of peptidoglycan causing the cell wall deformity and cell death. While, MgO NPs will adhesive in the thick peptidoglycan layer of the gram-positive bacteria and therefore cannot penetrate it. *Moreover, Y.He et al*[17], reported that MgO NPs possesses strong antibacterial inhibition efficacy due to a number of mechanisms such as induction of oxidative stress and disruption of membrane integrity in bacterial cells.

The organ index of brain, heart, liver and kidneys for mice was studied to monitor the changes in these organs that occur due to the exposing mice to MgO NPs. In our results, there were a non-significant in organ index of mice after oral administration of MgO NPs compared with mice before oral administration of MgO NPs ($p>0.05$) whereas, the liver at a concentration of 1200 mg/kg of MgO nanoparticles significant increase was observed compared to control group. The agreement of this work comes also with *Peter et al*[18] who they reported that the organ index is useful for drug toxicity determination and important for identification of potentially harmful effects of drugs on organs.

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In the present study there was no change in the histology of brain, heart, and kidneys organs except liver and in the concentration of 1200 mg/kg of MgO NPs. *This matches the Mazaheri. N et al*[19] who did not show histological changes in the kidney, but showed changes in some regions of the liver with a high dose of MgO NPs. They clarified that MgO NPs stimulated inflammation in liver tissues and apoptotic cells.

Other studies have shown that the level of damage caused by doses of the MgO NPs on the liver is higher than the kidneys, then the heart and brain respectively. In addition, significant amounts of magnesium were excreted in the body through urine and gradually over time[20].

V CONCLUSION

Pure MgO NPs was obtained by Co-precipitate method. Magnesium oxide nanoparticle is more effective inhibiting against (*E.coli*) bacteria than *(S. aureus*) bacteria. LD50 test was studied on mice. Changes in the weight of the mice and its organs including Brain, Heart, liver and kidneys were measured using an equation to calculate the organ index to identify the changes on these organs due to different doses of the MgO NPs. There was a non-significant in organ index of mice before oral administration of MgO NPs compared with mice after oral administration of MgO NPs whereas, the liver at a concentration of 1200 mg/kg of MgO NPs significant increase was observed compared to the control group.

There are no changes in the morphological of these organs excepted liver organ compare with organs of control mice. We observed a mild inflammation of the portal vein and necrotic liver.

VI ACKNOWLEDGEMENTS

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