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BIOCHEMICAL AND BIOLOGICAL STUDIES ON ZINC OXIDE NANOPARTICLES

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ABSTRACT: The current study endeavors to examine the synthesis of zinc oxide nanoparticles, which find utility in various domains such as biomedical, electrochemistry, and catalysis. The zinc oxide nanoparticles were fabricated through sol gel techniques utilizing zinc acetate and zinc sulfate as precursors. Subsequently, these nanoparticles were subjected to characterization using X-ray diffraction, FTIR analysis, and TEM analysis. Furthermore, the antioxidant activity of the zinc oxide nanoparticles was evaluated employing the DPPH assay, while their antimicrobial efficacy against Gram positive and Gram negative bacteria was also investigated. Additionally, the toxicity of the zinc oxide nanoparticles on adult male Westar rats was assessed by assessing liver function, kidney function, lipid profile, and blood sugar level. The outcomes of the study revealed a significant rise in all liver and kidney functions, glucose, cholesterol, low-density cholesterol, and triglycerides, in comparison to the negative control. However, the high-density cholesterol exhibited a decrease relative to the negative control.

Key words: Zinc oxide nanoparticles, toxicity, antimicrobial, antioxidant.

INTRODUCTION

Nanoparticle are defined as the particles having 1-100nm of size range. Nanotechnology is one of the most common and important developments in the twenty first century, and one of the fastest growing fields of science and technology is making remarkable progress Zhang *et al.*, (2013) Nanoparticles are found to possess specific characteristics like size, distribution, and morphology. The physicochemical properties of nanoparticles are substantially better and help to build many new structures, processes, nanoplatforms or devices with potential applications Mirzaei and Darroudi, (2017).

Many studies indicate reliance on natural products as antioxidants and antimicrobials, as well as to improve vital functions in experimental animals ((Abozid, and Farid, 2013; Abozid and Ahmed, 2013; Abozid *et al.*, 2014; Farid *et al.*, 2015; Ashoush *et al.*, 2017; El-Shennawy, and Abozid, 2017), but few of them are interested in studying different nanoparticle and evaluating their applied properties as antioxidants and antimicrobials.

In fields such as biomedical effects, electrochemistry, catalysis, sensors, biomedicine, pharmaceutics, healthcare, cosmetics, food technology, textile industry, mechanics, optics, electronics, space industry, energy science and optical devices, metal oxide nanoparticles display a very strong and widely applicable impact Hashmi *et al.*, (2017); Jiang *et al.*, (2018). Zinc is found to be an important element in humans, where various enzymes such as carbonic anhydrase, carboxy peptidase and alcohol dehydrogenase are inactive in the human system in the absence of zinc. Our body holds about 2-3 g of Zinc and has a daily need of about 10-15 mg. It is readily absorbed by the body from zinc oxide nanoparticles due to its smaller particle size, therefore it is used as a food additive. Indeed, Zinc has been found to regulates various physiological functions Siddiqi *et al.*, (2018).

Zinc oxide nanoparticles exhibit remarkable antibacterial efficacy against a wide range of bacterial organisms, each with distinct morphologies. These nanoparticles demonstrate antibacterial activity against both gram-positive and gram-negative bacteria, as well as foodborne

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pathogens like Escherichia coli O157:H7, Salmonella, Listeria monocytogenes, and Staphylococcus aureus (Sirelkhatim *et al.,* 2015; Xie *et al.,* 2011). The antibacterial mechanism of zinc oxide nanoparticles varies depending on their interaction with the bacterial surface and core. This mechanism can be determined by factors such as pH, temperature, area, and substrate concentration stability, which contribute to the absorption of associated substrates on the composite surface (Azizi-Lalabadi *et al.,* 2019; Beegam *et al.,* 2016). Zinc oxide nanoparticles have garnered significant interest due to their exceptional properties, including non-toxicity, biosafety, high electron transfer rates, excellent biological compatibility, ease of fabrication, enhanced analytical performance with heightened sensitivity, and cost-effectiveness (Hatamie *et al.,* 2015). This study aims to prepare zinc oxide nanoparticles and then conduct some tests on them to verify their formation in the first place and then the possibility of using them in some applications.

MATERIAL AND METHODS

The process of creating zinc oxide nanoparticles (A) (Zn-ONPsA)

ZnO nanoparticles were synthesized by a new sol gel approach according to the following manner. 8.1 gm $ZnSO_4.7H_2O$ (Merck) and 30 ml di ethylene glycol (Merck) were mixed, added 30 mL ethanol (99.7% Merck) and 900 mL distilled water. The mixed solution stirred with a magnetic stirrer at 85 C° for 2 hours to obtain the gel. The obtained gel was dried at 220 C° for 1 hour then ground into a fine particle. The temperature of the dried precursor powder was increased at the rate of 1 C° /min to attain the required temperature and then allowed the sample to stay at 500 C° for 3 hours to obtain the final product Jurablu *et al.,* (2015).

The process of creating zinc oxide nanoparticles (B) (Zn-ONPs B)

Was carried out using a sol-gel method, adhering to the subsequent procedure. In this case, a mixture of 6.585 $Zn(CH_3CO_2)_2 \cdot 2H_2O$ and 75 ml of isopropanol (C_3H_8O) was prepared, followed by stirring with a magnetic stirrer at a temperature of 60°C for a duration of 1 hour. To attain a clear and homogeneous gel, 50 - 75 ml of triethylamine (TEA) was added to the mixture. The resulting gel was then subjected to drying at a temperature of 60°C for 1 hour, after which it underwent grinding to obtain particles of a fine nature. Subsequently, the temperature of the dried precursor powder was increased gradually at a rate of 1°C/min until the desired temperature was achieved. The sample was then maintained at a temperature of 600°C for a total duration of 6 hours, leading to the formation of the final product.

Characterization of zinc oxide nanoparticles

X-ray diffraction

Were conducted in this research using the Philips PAN analytical machine. The range of scanning was set at 20˚-80˚ with a bond angle of 3˚, allowing for the determination of phase variety and particle size. The powder sample utilized for XRd analysis was placed on a glass slide for proper analysis.

For the study and characterization of the attached functional groups to the surface of the synthesized ZnO nanoparticles, Perkin Elmer Spectrophotometer

FTIR spectroscopy

One was used. The scanning range of 4000 – 400 cm-1 with a resolution of 4cm-1 was employed. To properly analyze the sample, it was mixed uniformly with solid KBr and compressed to form a thin transparent film. This film was then subjected to FTIR analysis by placing it in the instrument's chamber for scanning.

Transmission Electron Microscopy (TEM)

Is a microscopy technique that involves focusing a beam of electrons onto an ultra-thin specimen and transmitting it through. As the electrons interact with the specimen, an image is formed and magnified onto an imaging device such as a photographic film or fluorescent

screen. In this research, a Hitachi H-7100 electron microscopy was utilized for TEM studies. To prepare the sample for TEM, a small amount of powders were dispersed in ethanol and the suspension concentration was carefully controlled based on the material type. The solution was then poured onto the top surface of a copper grid, which was subsequently placed in an oven at 40 °C for one day.

Anti-oxidant activity of Zinc oxide nanoparticles

The measurement of free Radical Scavenging Activity of ZnONPs was carried out using the DPPH (1, 1-diphenyl-2-picrilhydrazyl) assay. The calculation of the free Radical Scavenging Activity was performed using the equation: % RSA = [(absorbance of control – absorbance of sample)/absorbance of control] ×100 (Velammal *et al.,* 2016). The absorbance of the control was determined by the DPPH solution, while the absorbance of the sample was determined by the solution containing ZnONPs added with the DPPH.

Antimicrobial activity of zinc oxide nanoparticles

Was assessed using the agar well diffusion method (Perez *et al.,* 1990). Nine bacterial strains, including Salmonella paratyphi, Salmonella typhi, Citrobacter sp., Proteus mirabilis, Escherichia coli, Shigella Sonii, Kelebsilla pneumonia, Pseudomonase aeruginosa, and Erwinia carotovora, were screened. Additionally, three bacterial strains, namely Enterococcus, Staph. Coagulase, and Staph. aureus, were tested. Different concentrations of nano-compounds (12.5, 25, 50, 100 µg ml-1) were utilized to evaluate the activity of the compounds. The materials were prepared in a 5% acetic acid solution and introduced into the wells using sterile micro pipettes. Simultaneously, the standard antibiotics were tested as a positive control against the pathogens. Imipenem was employed as the positive control. Subsequently, the plates were incubated at 37 °C for 48 h.

Toxicity of Zinc oxide nanoparticles

The objective of this investigation was to examine the impacts of zinc oxide nanoparticles on adult male Wistar rats. A total of twenty male Wistar rats were utilized for this study and they were divided into five groups, each consisting of four animals.

Experimental design

The initial group (1) was provided with regular feed devoid of any additives, which served as the negative control.

The second group (2) was given mixed fodder containing a concentration of 150 mg Zinc nano (A) per kilogram of diet.

The third group (3) was provided with mixed fodder containing a concentration of 300 mg Zinc nano (A) per kilogram of diet.

The fourth group (4) was given mixed fodder containing a concentration of 150 mg Zinc nano (B) per kilogram of diet.

The fifth group (5) received mixed fodder containing a concentration of 300 mg Zinc nano (B) per kilogram of diet.

Prior to receiving the experimental feed, the rats were granted a period of 7 days to adjust to their new surroundings. Subsequently, they were fed the experimental feeds for a duration of 1 week.

Blood Sampling and analysis

Alkaline phosphatase was examined using calorimetric methods following the approach of Bowers and McComb, who subsequently modified the procedure to a kinetic assay. The ALT (GPT) methods for analyzing ALT (GPT) were initially described by Henley in 1955 and later by Wroblewski and LaDue in 1956, utilizing U.V. procedures. Calorimetric analysis according to Jaffe (1886) was employed for the determination of creatinine and urea. Uric acid was also analyzed calorimetrically as outlined by Fossati *et al.* in 1980. The glucose level was determined using calorimetric analysis as described by Kaplan in 1984. For cholesterol and triglycerides, the calorimetric method according

to Kaplan *et al.* in 1984 was employed. HDL-cholesterol was determined following the method proposed by Lopez *et al.* in 1977. LDL cholesterol was calculated using the formula: LDL-cholesterol = total cholesterol - (HDL-cholesterol + Triglycerides / 5). The risk ratio was calculated according to the formula of Lopez *et al.* in 1977, where Risk ratio = total C / HDL-C. The atherogenic index was calculated using the formula proposed by Lopez *et al.* in 1977, with the following equation: Atherogenic index = LDL-C / HDL-C.

RESULLT AND DISCUSSION

The XRD pattern of the sample prepared in its original state indicates the absence of an induced crystalline phase of ZnO. Conversely, the powdered samples that underwent annealing demonstrate a well-defined long-range arrangement of sharp Bragg peaks. The miller indices assigned to each peak have been matched to the standard hexagonal wurtzite structure of ZnO. The Bragg peak patterns exhibited by the ZnO nanoparticles annealed at temperatures ranging from 300 to 600°C are comparable as in Fig. (1), (2) .

The FTIR spectrum

That was obtained revealed a series of absorption peaks within the range of 4000 – 400 cm-1. Two weak peaks were observed at 3369 cm-1 and 3160 cm-1, indicating the presence of the O-H stretching vibration of the intra molecular hydrogen bond. The peaks observed at 1520 cm-1 exhibit a doublet pattern and are associated with the symmetric stretching vibration of the zinc carboxylate C=O. Furthermore, the absorption peaks at around 1386 cm-1 are attributed to the bending vibration of the C-H stretching. Lastly, the sharp peak at approximately 685 cm-1 is ascribed to the Zn-O stretching mode as in Fig. (3), (4) .

TEM analysis

Revealed that the structural configuration of the ZnO nanoparticles has developed in a nearly hexagonal form. The visual representation demonstrates that the substance is composed of quasi-spherical nanoparticles. The preponderance of ZnO nanoparticles measured were situated within the diameter range of 30 to 50 nm, with an average diameter of 40 nm as in Fig. (5), (6) .

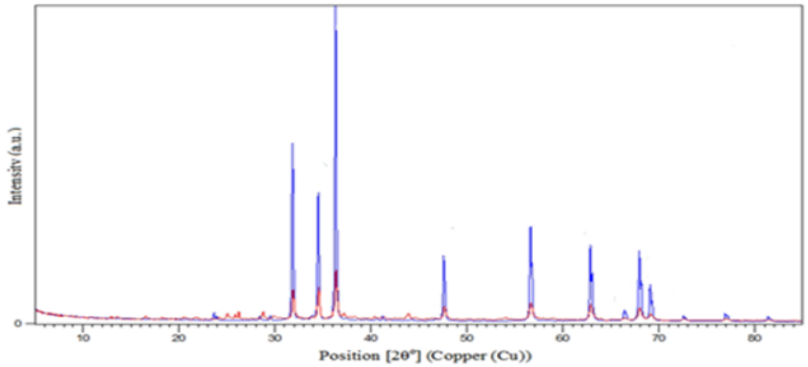

Fig (1): XRD patterns of ZnO nanoparticles. ZnO (A)

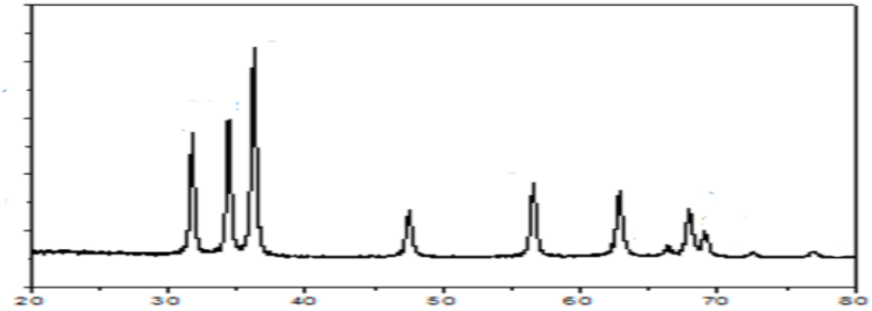

Fig (2): XRD patterns of ZnO nanoparticles. ZnO (B)

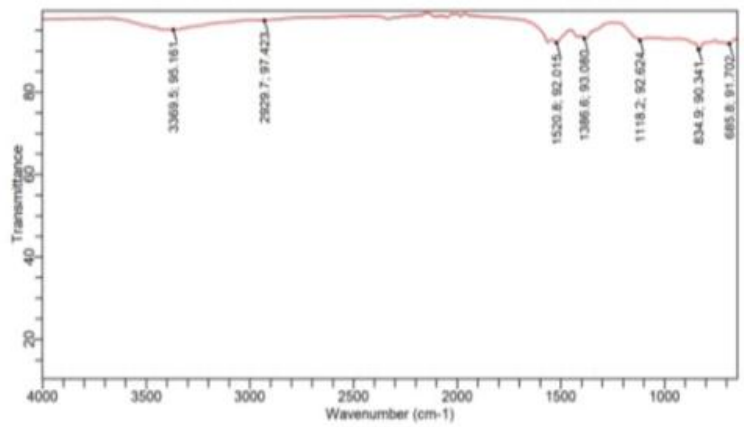

Fig (3): FTIR pattern of the zinc oxide nanoparticles (A)

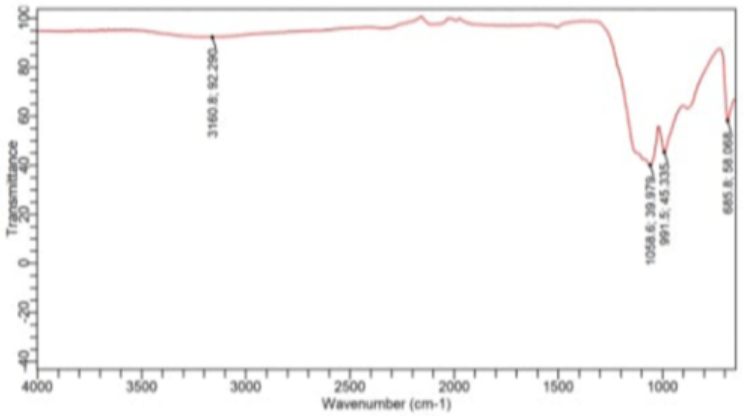

Fig (4): FTIR pattern of the zinc oxide nanoparticles (B)

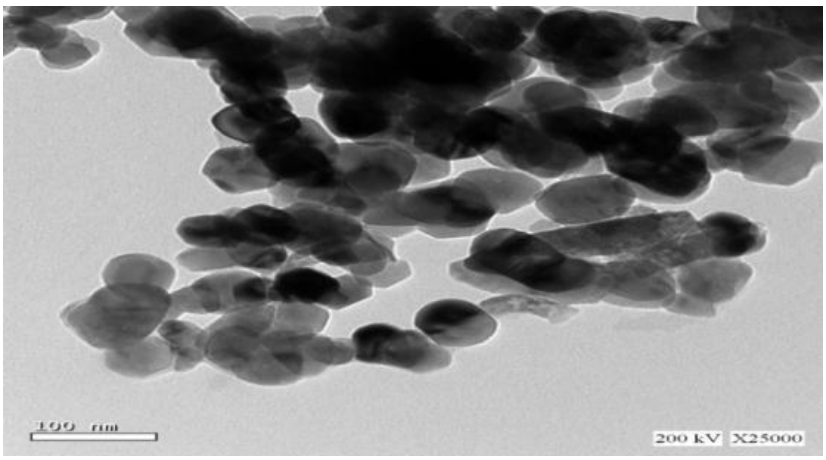

Fig (5) -TEM images of ZnO nanoparticle (A)

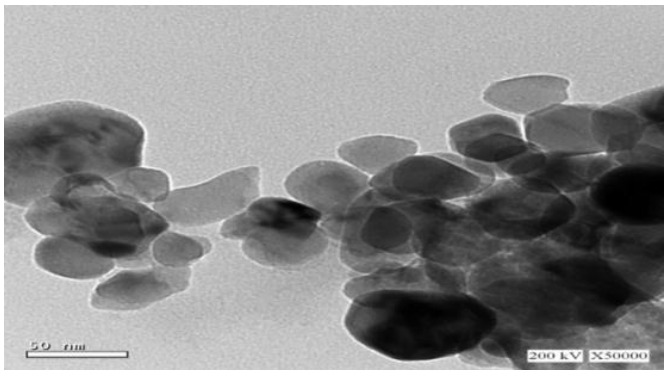

Fig (6): TEM images of ZnO nanoparticle (B)

Antioxidant activity of zinc oxide nanoparticles

An increase in antioxidant activity that was concentration dependent was noted. The information demonstrated that ZnO NPs were further emphasized by DPPH free radical quenching. In comparison to the absorbance values for ascorbic acid, which ranged from 95.2 to 95.9%, the absorbance values for zinc oxide nanoparticles (A) and ZnO NPs (B) varied from 49.25 to 53.79% and 58.24 to 59.54%, respectively. The following is the order of effectiveness: ZnO NPs (B) > ZnO NPs (A) > ascorbic acid as shown in Table (1).

Data on the antibacterial activity of zinc oxide nanoparticles

Revealed that ZnO NPs (A) and ZnO NPs (B) both possess enough antimicrobial activity. It suppressed the growth of all nine gm (-), three gm (+), three fungus, and three yeast organisms mentioned above. The suppression of growth is dose dependant. The zone of inhibition's diameter increases in tandem with the dose as shown in Table (2), (3).

Are zinc oxide nanoparticles toxic?

Using measurements of blood sugar, lipid profiles, renal function, and liver function, researchers examined the effects of micro ZnO on adult male Wistar rats. Whereas all liver and renal functions, glucose, cholesterol, low-density cholesterol, and triglycerides exhibited a considerable increase in comparison to the negative control, but high-density cholesterol shrank in comparison as shown in Table (4, 5, 6, 7,8).

Table (1): ZnO NPs (A) and ZnO NPs (B) in the DPPH test.

Concentration (µg/ml)	% inhibitaion		
	ZnO NPs (A)	ZnO NPs (B)	Vit. C
25	49.25	58.24	95.2
50	51.64	58.64	95.5
75	52.95	58.74	95.8
100	53.79	59.54	95.9

Tabe (2): Zones (mm) of growth inhibition by ZnO NPs (A) and ZnO NPs (B) on gm (-) microorganisms.

Organisms	IPM	ZnO NPs (A) µg ml $^{-1}$				ZnO NPs (B) µg ml $^{-1}$			
		12.5	25	50	100	12.5	25	50	100
Salmonella paratyphi	31	11	19	23	29	13	21	25	30
Salmonella typhi	33	13	20	25	31	18	26	30	36
Citrobacter sp.	29	11	17	23	29	12	17	22	27
proteus mirabilis	27	16	22	31	40	23	29	35	43
Escherichia coli	29	13	17	21	25	13	17	21	25
Shigilla Sonii	34	17	25	33	41	20	26	32	40
Kelebsilla pneumoniae	31	12	18	23	30	16	22	27	33
pseudom onase aeruginosa	29	11	15	20	27	7	11	13	19
Erwinia carotovora	25	11	16	22	27	13	19	24	29

Table (3): Zones (mm) of growth inhibition by ZnO NPs (A) and ZnO NPs (B) on gm (+) microorganisms.

Organisms	IPM	ZnO NPs (A) µg ml $^{-1}$				ZnO NPs (B) µg ml $^{-1}$			
		12.5	25	50	100	12.5	25	50	100
Enterococcus	22	9	14	20	26	12	16	23	28
Staph. Coagulase	37	8	13	19	25	17	23	28	34
Staph.aureus	31	13	19	24	29	21	27	33	39

Table (4): Effect of ZnO-NPs (A and B) on ALT , AST and ALP activity.

Group	ALT Level (IU/L)	AST Level (IU/L)	ALP Level (IU/L)
Negative Control	66.50^{a}± 3.1	239^{a}± 5.1	381.50^{a}± 6.55
ZnO-NPs A 0.15	96.75^{b}± 8.5	263.25^{b}± 8.1	402.75^{b}± 12.8
ZnO-NPs A 0.30	123.0^{c}± 3.9	295.50^{c}± 14.4	444.0^{c}± 5.29
ZnO-NPs B 0.15	95.75^{b}± 1.70	259.25^{b}± 1.70	385.0^{a}± 4.96
ZnO-NPs 0.30	102.25^{b}± 2.2	301.50^{c}± 7.3	407.25^{b}± 5.31

Table (5): Effect of ZnO-NPs (A and B) on total protein , albumin and total bilirubin level.

Group	Protein total (g/dL)	Albumin	Bilirubin total
Negative Control	5.95^{a}± 0.12	3.50^{a}± 0.08	0.56^{a}± 0.04
ZnO-NPs A 0.15	6.15^{ab}± 0.12	3.55^{a}± 0.12	0.59^{ab}± 0.03
ZnO-NPs A 0.30	6.17^{ab}± 0.12	3.52^{a}± 0.05	0.61^{ab}± 0.05
ZnO-NPs B 0.15	6.30^{c}± 0.21	3.52^{a}± 0.12	0.63^{b}± 0.01
ZnO-NPs 0.30	6.25^{c}± 0.12	3.50^{a}± 0.14	0.63^{b}± 0.02

Table (6): Effect of ZnO-NPs (A and B) on urea , creatinine and uric acid level.

Group	Urea (mg/dl)	Creatinine (mg/dl)	Uric Acid (mg/dl)
Negative Control	35.5^{a}± 1.29	0.45^{a}± 0.008	3.57^{a}± 0.009
ZnO-NPs A 0.15	53.75^{b}± 0.95	0.57^{b}± 0.008	4.06^{ab}± 0.32
ZnO-NPs A 0.30	62.0^{c}± 2.58	0.66^{c}± 0.005	4.42^{bc}± 0.23
ZnO-NPs B 0.15	52.5^{b}± 2.64	0.70^{d}± 0.009	4.8^{c}± 0.60
ZnO-NPs 0.30	75.75^{d}± 7.71	0.99^{e}± 0.008	5.47^{d}± 0.65

Table (7): Effect of ZnO-NPs (A and B) on blood glucose , total cholesterol , tri glycerides and HDL-cholesterol

Group	Glucose level (mg/dl)	Cholesterol (mg/dl)	Triglycerides(TG) (mg/dl)	HDL-Cholesterol (mg/dl)
Negative Control	$31.7^{a}\pm 3.3$	$99.75^{a}\pm 3.40$	$99.50^{a}\pm 2.88$	$35.25^{bc}\pm 0.50$
ZnO-NPs A 0.15	$38.75^{b}\pm 1.7$	$125.5^{b}\pm 6.65$	$130.0^{b}\pm 0.81$	$35.75^{c}\pm 0.95$
ZnO-NPs A 0.30	$43.0^{c}\pm 2.16$	$140.0^{c}\pm 3.47$	$170.25^{d}\pm 0.95$	$33.50^{b}\pm 1.29$
ZnO-NPs B 0.15	$37.0^{b}\pm 1.4$	$135.75^{c}\pm 6.55$	$148.75^{c}\pm 1.70$	$35.25^{bc}\pm 1.70$
ZnO-NPs 0.30	$41.75^{c}\pm 4.03$	$159.0^{d}\pm 2.16$	$183.5^{e}\pm 1.29$	$31.50^{a}\pm 1.29$

Table (8): Effect of ZnO-NPs (A and B) on LDL-cholesterol , atherogenic index and risk ratio.

Group	LDL Cholesterol (mg/dl)	Atherogenic index	Risk ratio
Negative Control	$59.25^{a}\pm 3.3$	1.68	2.83
ZnO-NPs A 0.15	$75.75^{b}\pm 7.41$	2.11	3.51
ZnO-NPs A 0.30	$87.50^{d}\pm 9.25$	2.61	4.18
ZnO-NPs B 0.15	$86.25^{bc}\pm 4.57$	2.4	3.85
ZnO-NPs 0.30	$96.25^{d}\pm 1.70$	3.05	5.05

Conclusion

The zinc oxide nanoparticles were fabricated through sol gel techniques utilizing zinc acetate and zinc sulfate as precursors. The zinc oxide nanoparticles appeared antioxidant activity by using DPPH assay, and showed antimicrobial efficacy against Gram positive and Gram negative bacteria. The outcomes of the study revealed a significant rise in all liver and kidney functions, glucose, cholesterol, low-density cholesterol, and triglycerides, in comparison to the negative control. However, the high-density cholesterol exhibited a decrease relative to the negative control.

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دراسات كيميائية حيوية وبيولوجية على جزيئات أكسيد الزنك النانوية

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الملخص العربى

تهدف الدراسة إلي القاء الضوء على أهمية الجسيمات النانوية لأكسيد الزنك التي تستخدم في مجالات مختلفة: مثل التأثيرات الطبية الحيوية ، والكيمياء الكهربائية وغيرها .

وفي هذا الصدد ، تم بتحضير جزيئات أكسيد الزنك النانوية من أسيتات الزنك وكبريتات الزنك بطريقتين مختلفتين عن طريق الجل المتماسك. ثم تم التعرف والتأكد من تحضير جزيئات اكسيد الزنك النانوية عن طريق :تحليل حيود الأشعة السينية (X-ray diffraction) وتحليل الاشعة تحت الحمراء ((FTIR وتحليل المجهر الالكتروني النافذ (TEM). ثم تم اجراء بعض التطبيقات لجسيمات أكسيد الزنك النانويةفقد تم اختبرها بواسطة مادة DDPH وأشارت النتائج إلي ان جسيمات اكسيد الزنك النانوية ذات خواص جيدة كمضادات أكسدة. كما تم ايضا تم اختبار النشاط المضاد لنم الميكوربات بطريقة ال Disk diffusion والتي أظهرت أن جسيمات أكسيد الزنك النانوية A , B لهما نشاط مضاد للميكروبات ضد تسعة من البكتريا السالبة لجرام، وثلاثة من البكتريا الموجبة لجرام، وثلاثة فطريات وسلالة خميرة .كما أظهرت النتائج التي تم الحصول عليها التأثير المبيد لجسيمات ZnO النانوية علي الحشرات وعند معاملة جسيمات النانوية لأكسيد الزنك على بعض القياسات الخضرية لنبات النعناع أظهرت البيانات أن هناك زيادة في طول النباتات بعد المعاملة بكبريتات الزنك (A) ZnO-NPs و (B) مقارنة مع المجموعة الضابطة. وأيضا كانت هناك زيادة في الوزن الرطب والجاف للنباتات وعند إجراء اختبارات لبيان سمية جزيئات أكسيد الزنك النانوية اظهرت النتائج أن هناك زيادة معنوية مقارنة بالمجموعة السالبة في جميع وظائف الكبد والكلى والجلوكوز والدهون الكاملة.