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Department of Biology\ Microbiology

Magnesium Oxide nanoparticles as Antibacterial and immunogenic delivery

Athesis

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الخلاصة

تعد تقنية النانوتكنولوجي عامل مهم ومؤثر في مجالات عدة مثل الصناعة والعلوم , حيث تؤثر تقنية النانو على اقتصاديات الدول ومواقعها في المستقبل. على مدى العقود الماضية ، أدى استخدام تقنية النانو وإنتاج الجسيمات النانوية إلى خلق أمل جديد في حل المشكلات البشرية. تعد جزيئات أكسيد المغنيسيوم النانوية عبارة عن مسحوق أبيض عديم الرائحة وغير سام وله نقطة انصهار عالية وصلابة عالية. تُستخدم الجسيمات النانوية على نطاق واسع في الطب والصناعات نظرًا لتوافقها الحيوي وقابليتها للتحلل البيولوجي وتكلفتها المنخفضة نسبيًا.

في هذه الدراسة ، تم أخذ 86 عزلة بكتيرية معزولة من المدمنين وغير المدمنين على المخدرات في مختبر الاحياء المجهرية المتقدم في كلية العلوم جامعة بابل وتشخيص *Leuconostic spp* البكتريا المعزولة بواسطة الاوسائط الزرعية مقرونة باختبارات الكيموحيوية ونظام vitek 2. تم استخدام هذه العزلات في التصنيع الحيوي لجسيمات أكسيد المغنيسيوم النانوية.

تم تصنيع دقائق أكسيد المغنيسيوم النانوية (MgO) باستخدام عالق بكتريا*Leuconostic spp* . كما تمت دراسة صفات دقائق أكسيد المغنيسيوم النانوية باستخدام الأشعة فوق البنفسجية -المرئية الطيفي و حيود الأشعة السينية (XRD) وتشتت طاقة الاشعة السينية (EDX) و الأشعة تحت الحمراء الطيفي (FTIR) والمجهر الإلكتروني الماسح (SEM) .

تم تصنيع جزيئات MgO النانوية بواسطة عزلات بكتريا *Leuconostic spp* وتم قياس الطيف الضوئي لها بواسطة UV-vis. ، تم تحديد أكبر امتصاص عند 400 طول موجي لجميع العزلات بعد الفحص جميع العزلات قيد الدراسة بأجهزة قياس الطيف الضوئي للأشعة فوق البنفسجية.

كانت نتيجة اختبار عينة MgO NP نقية , حيث أنه لم يتم تحديد قمم للملوثات اخرى من خلال توليف اختبار *Leuconostoc spp* الذي يشير إلى أن NPs نقي جدًا ، وكانت القمم (111) و (200) (220) و (311) مستوى من الجسيمات النانوية من MgO في 2 الحجم = 36155 و 42 و 42 و 58.112 و 74702.

اظهرت نتائج صورة FE-SEM التشكل السطحي في الجسيمات النانوية MgO , حيث كان تحليل الصورة يشير إلى أن MgO-NPs الخاص بـ *Leuconostoc spp* كان كرويًا تقريبًا ، بمتوسط قطر يبلغ 4388 نانومتر.

اظهرت الدراسة الحالية ان طيف EDX لدقائق اوكسيد المغنيسيوم النانوية ، والذي يظهر ذروة بارزة تتوافق مع Mg و O. تم اكتشاف قمم C و N و Na و Ca و P إضافية. تم الحصول على ذروة عند 1.2 كيلو فولت للمغنيسيوم .

تم تحديد المجموعات الوظيفية لMgO NPs التي انتجتها بكتريا *Leuconostoc spp* بواسطة جهاز مطياف التحويل الطيفي (FTIR). وكانت ذروة توليف للمادة النانوية MgO NP: 3851.98، 3747.24، 3270.36، 2952، 2922.88، 2853.70، 1648.70، 1456.71، 1376.95، 1073.73، 722.31 و 562.77 في المMgO NPs المعلقة بواسطة *Leuconostoc spp*.

اظهرت النتائج الدراسة الحالية ان قيمة pHالمثالية لتصنيع مركب MgO NPs من بكتريا *Leuconostoc spp*هي 12 ،في حين كان تركيز ملح Magnesium Nitrate Hexahydrate المثالي لانتاج MgONPs هو 0.10 مولاري.

أظهرت نتائج الدراسة الحالية باستخدام Atomic Force Microscopic أيضًا عينة MgO NPs التي تم تخليقها بواسطة عزل *Leuconostoc spp* كان Rq 11.82 نانومتر و Ra 9.596 نانومتر.

تم تقييم مركب MgO NPs لمعرفة الفعالية المضادة للبكتيريا المعزولة ضد البكتيريا سالبة لصبغة كرام المعزولة والمقاومة للعديد من المضادات الحيوية ، وتقديرMgO NPs MIC (125ملغم / مل) و MBC (500 ملغم/ مل) MIC.

تم استخدام بكتريا السالمونيلا في عزل OMP وهو مستضد محضر في الدراسات المناعية لأنه ينتج عنه اكتشافات نمطية وراثية لغالبية عامل الضراوة. أظهر تحليل SDS-PAGE OMP للسالمونيلا أن الوزن الجزيئي (MW) للمواد الهلامية قدر بـ KD44 بالمقارنة مع الواسمات القياسية MW. كان تركيز البروتين (30 غم / لتر).

أوضحت النتائج أن MgO NPs المحضر من عزلات *Leuconostoc spp* أعطيت فرقاً معنوياً بالمقارنة مع مجموعة السيطرة عند (P <0.05). أظهرت نتائج اختبار التراص الأنبوبي مع مصل الحيوانات المناعية MgO NP (ثلاثة حيوانات لكل مجموعة) قيمة متوسطة (1280) جسم مضاد جهازي لـ MgO NP ، تم تصنيعه مع *Leuconostoc spp* ، كما كان متوسط القيمة (1280) للأغشية الخارجية والقيمة المتوسطة (2560)) لحيوانات السيطرة المختلطة (3 حيوانات) أظهرت قيمة متوسطة (10) للأجسام المضادة ضد عزلات السالمونيلا . أظهرت الدراسة الحالية زيادة معنوية في معدل المؤشرات الانقسامية لخلايا نخاع العظام لمجموعة الحيوانات.

اظهرت الدراسة الحالية تأثير MgONPs وبروتينات الأغشية الخارجية المعزولة من بكتريا السالمونيلا على حساسية الجلد ومناعة الخلايا للأرنب. تم اكتشاف قطر التصلب لـ MgO-NPs المُصنَّع من *Leuconostoc sp* والغشاء الخارجي (1.83) والمختلط (1.02) ملم على التوالي بعد أربع ساعات.

في الأرانب المحقونة بـ MgO NPs المُصنَّع بواسطة عزل *Leuconostoc spp* ، زاد مؤشر وزن الطحال عند الغشاء الخارجي مع وجود فروقات ذات دلالة احصائية مقارنة بمجموعة السيطرة.

أظهرت نتائج الدراسة الحالية أن تقدير إنتاج السيتوكينات باستخدام معادلة المنحنى القياسي الذي تم إجراؤه بنفس الاختبار. أظهر التحليل الإحصائي للIL-2 و IL17 و IL-1β فروق ذات دلالة إحصائية مقارنة بالمجموعة السيطرة بالمقارنة مع المجموعة المحقونة مع جسيمات MgO النانوية حيث سجلت مستويات معيارIL-2 و IL17 و IL-1β (024 .4± 22.636 :13.386± 82.305 :4.771± 38.548) على التوالي زيادة معنوية ($P \leq 0.05$) في المجموعة المحقونة مع جسيمات MgO النانوية مقارنة مع مجموعة السيطرة حيث سجلت معاييرIL-2 و IL17 و IL-1β (2.545±17.700 :5.498±50.310: 2.501±23.434) على التوالي ، في حين كانت هناك فروق ذات دلالة إحصائية في كلا المجموعتين .

أظهر نتائج فحص اجزاء من الكبد في المجموعة المحقونة ب أكسيد المغنيسيوم النانوية (MgO) فرقًا معنويًا مقارنة بمجموعة السيطرة بينما أظهرت الدراسة في المحقونة ب أكسيد المغنيسيوم النانوية (MgO) أن تنكس خلايا الكبد وتضخم النوى (نخر) مع انخفاض عدد خلايا كوبفر في جيوب الكبد ، أظهرت النتائج في مجموعة مزيج من الغشاء الخارجي للبكتريا السالمونيلا واكسيد المغنيسيوم النانوية كبدًا غير طبيعي مع مستوى مرتفع من التنخر) مع التهاب صفراوي وتضخم في خلايا الكبد .

أظهر نتائج فحص اجزاء الكلى من المجموعة المختبرة باوكسيد المغنسيوم النانوية تضخم الخلايا الظهارية الأنبوبية الكلوية وضمور خلايا الترشيح الكبيبي ، ويشمل النخر عمومًا تجويف الخلايا ، والتضخم نواة الخلايا الظهارية في نسيج الكلى.

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Above all else, I want to express my great thanks to Allah, prophet Mohammed , and his holy household for giving me the strength and patience in completing this work.

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Special thanks to all staff and members of microbiology laboratory in the Department of Biology / University of Babylon for their help me . Special thanks for Dr. Noor Salman and Dr. Azhar Omran in College of Science University of for providing all the essential needed for the completion of this work

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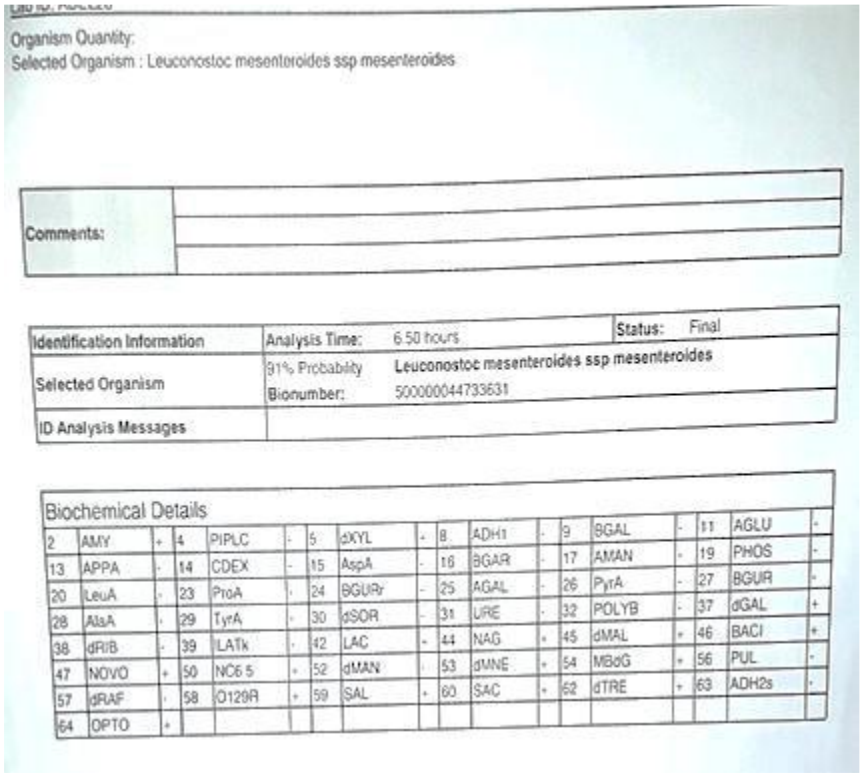
Duaa 2022

Appendix

Appendix (1): The number and type bacteria isolates

Souece of sample	Genus of isolates	Total	Species of isolates	Total
Total number of sample from addicted (70)	***Streptococcus spp.***	**51**	***S. mutans***	**17**
			S. salivarius	**8**
			S. sobrinus	**4**
			S. mitis	**8**
			S. parasanguis	**3**
			S. anginosus	**2**
			S.aglactiae	**1**
			S.alactolyticus	**1**
			S.equinus	**2**
			Enterococcus faecium	**3**
			Enterococcus faecalis	**1**
			Enterococcus avium	**1**
	Staphylococcus spp.	**8**		
	Aerococcus spp.	**1**		
	Leuconostoc spp.	**10**		
Total number of sample from non-addicted (16)	***Streptococcus spp.***	**15**	***S. salivarius***	**13**
			S. parasanguis	**1**
			S. sanguinis	**1**
	Leuconostoc spp.	**1**		

Appendix 2: Identification for ***L. mesenteroides*** by Vitek-2 system



Organism Quantity:
Selected Organism : Leuconostoc mesenteroides ssp mesenteroides

Comments:	

Identification Information	Analysis Time: 6.50 hours	Status: Final
Selected Organism	91% Probability Bionumber: 500000044733631	Leuconostoc mesenteroides ssp mesenteroides
ID Analysis Messages		

Biochemical Details

2	AMY	+	4	PIPLC	-	5	dXYL	+	8	ADH1	-	9	BGAL	-	11	AGLU	-
13	APPA	-	14	CDEX	-	15	AspA	-	16	BGAR	-	17	AMAN	-	19	PHOS	-
20	LeuA	-	23	ProA	-	24	BGURr	-	25	AGAL	-	26	PyrA	-	27	BGUR	-
28	AlaA	-	29	TyrA	-	30	dSOR	-	31	URE	-	32	POLYB	-	37	dGAL	+
38	dRIB	-	39	LATk	-	42	LAC	+	44	NAG	+	45	dMAL	+	46	BACI	+
47	NOVO	+	50	NC6 5	+	52	dMAN	-	53	dMNE	+	54	MBdG	+	56	PUL	-
57	dRAF	-	58	O129R	+	59	SAL	+	60	SAC	+	62	dTRE	+	63	ADH2s	-
64	OPTO	+															

Appendix 3: Identification for ***S. typhi*** by Vitek-2 system

bioMérieux Customer:
System #:

Laboratory Report

Printed Apr 10, 2019 21:54 CDT
Printed by: Labadmin

Patient Name:
Isolate: 1042019-2 (Qualified)

Patient ID:

Card Type: GN Bar Code: 2410662403231189 Testing Instrument: 000017227444 (ALSADIQ HOSPITAL)
Card Type: AST-GN76 Bar Code: 5960856203511628 Testing Instrument: 000017227444 (ALSADIQ HOSPITAL)
Setup Technologist: Laboratory Administrator(Labadmin)

Bionumber: 0007610440144210
Organism Quantity:

Selected Organism: Salmonella ser.Typhi

Comments:	

Identification Information	**Card:**	GN	**Lot Number:**	2410662403	**Expires:**	Sep 19, 2019 13:00 CDT
	Completed:	Apr 10, 2019 04:44 CDT	**Status:**	Final	**Analysis Time:**	5.82 hours
Organism Origin	VITEK 2					
Selected Organism	99% Probability		**Salmonella ser.Typhi**			
	Bionumber:	0007610440144210			**Confidence:**	Excellent identification
SRF Organism						
Analysis Organisms and Tests to Separate:						
Analysis Messages: Confirm by serological tests The following antibiotic(s) are not claimed: ESBL,						
Contraindicating Typical Biopattern(s)						

Summary

Nanotechnology is mentioned as a factor which influences science and industry. Nanotechnology influences on the economies of countries and their positions in future. Over the past decades, the use of nanotechnology and the production of nanoparticles have created new hope for solving human problems. Nanoparticles are widely used in, medicine and industries due to their biocompatibility, biodegradability, and relatively low cost. Magnesium oxide nanoparticles are odorless and nontoxic white powder which possesses a high melting point and high hardness .

In this study , 86 bacterial isolates were isolated from oral microbiology laboratory at the college of Science, University of Babylon, , The diagnosis of *leuconostoc spp* carried by culture media coupled with biochemical test and vitek 2 system. These isolates were used in the biosynthesis of Magnesium Oxide nanoparticles.

The characterization of biosynthesized nanoparticles was achieved , using UV spectrophotometer, Scanning electron microscope (SEM) , Energy dispersive spectroscopy(EDS) ,X-ray diffraction analysis (XRD), Atomic Force Microscopic (AFM), and Fourier transform infrared spectroscopy (FT-IR) .

Nanoparticles MgO synthesis by *leuconostic* spp with UV-vis. Spectrophotometry . After examining all UV spectrophotometers, the maximum absorption at 400 wavelengths was determined for all isolates. By synthesis of the *Leuconostoc* spp test, no distinguishable peaks of pollutants were observed in the MgO NP sample, showing that NPs, The planes (111), (200), (220), and (311) of MgO nanoparticles in 2 volume = 36,155 and 42, 42, 58,112, and 74,702 were the peaks.The results of FE-SEM image showing the surface morphology in MgO nanoparticles .The image analysis indicated that the *Leuconostoc spp* MgO- NPs were approximately spherical, with an average diameter of 43,88 nm of *Leuconostoc spp* MgO-NPS synthesized .

The present study exhibited that displays the EDX spectrum, which exhibits a prominent peak corresponding to Mg and O. Additional C, N, Na, Ca, and P peaks were discovered. A peak was obtained at 1.2 keV for magnesium .

The functional groups of isolated samples produced by MgO NPs (*Leuconostoc spp*.) were identified by Fourier infraround transform spectroscopy (FTIR). The MgO NP synthesis peakings: 3851.98, 3747.24, 3270.36, 2952, 2922.88, 2853.70, 1648.70, 1456.71, 1376.95, 1073.73, 722.31and 562.77 in the MgO NPs synthesis by *Leuconostoc spp*.

The results of present study using Atomic Force Microscopic also showed MgO NPs sample that synthesis by isolate *Leuconostoc spp* was Rq 11.82 nm and Ra 9.596 nm The MgO NPs sample generated by isolate was identified as *Leuconostoc spp* rough of the sample based on these findings.

The results of the current study showed that the optimum pH value for the manufacture of MgO NPs compound from *Leuconostoc* spp. is 12, while the ideal concentration of Magnesium Nitrate Hexahydrate for the production of MgO NPs was 0.1

Synthesized MgO NPs were evaluated for antibacterial effectiveness against isolated Gram negative bacteria which are resistant to several antibiotics, The MgO NPs MIC (125 mg/ml) and the MBC (500 mg/ml) MIC.

The results of the present study exhibited The activity of antibiofilms varies according to the isolated bacteria. In this study biofilm formation in 10 isolates was examined and the findings were obtained (8 strong and 2 moderate).

Because it results in phenotypic and genotypic detections for the bulk of the virulence factor, *Salmonella typhi* was used in OMP isolation and antigen preparation in immunological research. By comparing conventional MW markers to the molecular weight (MW) of the gels in SDS-PAGE OMP analysis of Salmonella typhi, the molecular weight (MW) of the Protein was calculated to be 44KD. The protein concentration in the sample was 30 g/L.

The lab animal model used in this study was rabbits ,the results revealed that MgO NPs biosynthesized from *Leuconostoc spp* were given significantly difference in comparison with control group at ($P<0.05$) . Results from the tube agglutination test with serum of MgO NP immune animals (three animals per group) showed a mean value (1280) systemic antibody for MgO NP, synthesized with *Leuconostoc spp*, mean value (1280) for outer membranes and mean value (2560) to mixed control animals (3 animals) showed a mean value of systemic antimicrobial antibodies with mean value (10) . The present study showed the significantly increased rate of mitotic indices of bone marrow cells for the animals group .

The study investigated how MgONPs, outer membranes and mixed rabbit influenced the skin sensitivity and cell immunity of the rabbit. The induration diameter of MgO-NPs synthesized as *Leuconostoc sp,* Outer membrane (1.83), and Mixed(1.02) mm respectively was detected after four hours.

In the rabbits inoculated with MgO NPs synthesized by *Leuconostoc spp* insulate, the spleen weight index increased at the outer membrane and mixed group with statistically significant difference on P. value (0.05) compared to control group.

The current study's findings revealed that the concentration of cytokine production was calculated utilizing the same test's standard curve equation. Statistical analysis of IL-2, IL17, and IL-1 showed significant differences when compared to the control group. The concentrations of IL-2, IL17, and IL-1 were recorded (22.636 4.024: 82.305 13.386: 38.548 4.771), respectively, significant increase (P 0.05), while the control group of IL-2, IL17, and IL-1 (17.700 2.545: 50.310 5.498: 23.434 2.501) showed

The histopathological study included two organs (liver and kidney from immunized rabbits and control) and found that MgO nanoparticales were toxic to animals.

The examination of liver sections revealed significant differences when compared to the tested groups, with degeneration of hepatocytes and nuclei pyknosis (necrosis) as well as a decrease in the number of kupffer cells in the sinusoids in the tested groups.

The examination of the kidney sections from tested group showed swollen renal tubular epithelial cells and atrophy of glomular tuft, necrosis generally includes cells welling, nuclear pyknosis .

List of Contents

List of Tables

List of Figures

List of Abbreviations

Abbreviated Form	Meaning
AFM	Atomic Force Microscopy
BHI	Brain Heart Infusion
CD	Cluster of Differentiation
CFT	Cefotxin
CIP	Ciprofloxacin
CVD	Chemical Vapor Deposition
DTH	Delayed-Type Hypersensitiity
EDS	Energy-Dispersive X-ray Spectroscopy
EDTA	Ethylene Diamine Tetra Acetic Acid
ELISA	Enzyme Linked Immunosorbent Assay
EPM	Extracellular Polymer Matrix
EPS	Extracellular Polymeric Substance
FTIR	Fourier Transform Infrared
IL	Interleukin
JCPDS	Joint Committee on Powder Diffraction Standards
MBC	Minimal Bactericidal Concentration
MDR	Multidrug Resistance
MEM	Meropenem
MgO NPs	Magnesium oxide nanoparticles
MHC	Major Histocompatibility Complex
MIC	Minimal Inhibitory Concentration
NLRP3	**NOD, LRR and pyrin domains-containing protein 3**
PBS	Phosphate Buffer Saline
PMN	Polymorphonuclear
QS	Quorum Sensing
Ra	Average Roughness
ROS	Reactive Oxygen Species
Rq	Square Roughness
SEM	Scanning Electron Microscopy
SXT	Trimethoprim-Sulfamethoxazol
TBE	Tris-Borate-EDTA
TLR	Toll-Like Receptor
TNF	Tumor Necrosis Factor
XRD	X-Ray Diffraction

MOMV	Manned Orbital Maneuvering Vehicle
APEC	Asia-Pacific Economic Cooperation
NK	Natural killer cells
DC	Dentritic cells
TH	T helper cells
IFN	interferon
DTh	Delayed-type hypersensitivity response.
OMV	Outer membrane protein as A vaccine

1. Introduction

Leuconostoc species are lactic acid bacteria used as components of mesophilic cultures to produce aroma during milk fermentation. The bases for *Leuconostoc* taxonomy are results from cultivation-dependent methods, followed by phenotypic/biochemical characterization or non- specific molecular methods. Presently, the genus *Leuconostoc* includes 13species (Hemme and Focaud-Scheunemann ,2004) *Leuconostocs* lack a functional extracellular caseinolytic proteinase , which is essential for reaching high cell densities(Liu *et al.,* 2010) .

Nano sciences and nanotechnology have been leading to a technological revolution in the world, which is concerned with materials with significantly novel and improved physical, chemical and biological properties (Uzair *et al*., 2020). It is an important inorganic material with a wide band-gap (Al-Gaashani *et al.,* 2012) nanotechnology offers a way to improve the activity of inorganic antibacterial agents. Metal oxide nanoparticles such as ZnO, MgO and CaO have been investigated as inorganic antibacterial agents (Tang and Lv 2014).

Leuconostoc species are occurrence importance as pathogenic organisms. It existing the first proceedings on odontogenic infection triggered by means of *Leuconostoc* spp. Isolates initially recognized as like streptococci have been found in imitation of lie vancomycin resistant. Rigorous bacteriologic care subsequently labeled this organisms as *Leuconostoc mesenteroides* (Cesar *et al.,* 2015).

The finest options for nanoparticles are likely bacteria that have the excellence in reducing metal ions to their zero forms (for nanoparticles). Synthesis is attributed to the convenience of handling and the needs of medium culture , European and other International Committees have defined NPs, as particles of matter in which at least one of their phases has

one dimension (length, width, or thickness) within the range of 1 to 100 nanometers (nm) (Khan *et al*,2020; Krishnamoorthy *et al*., 2012).

It has been used in many applications such as catalysis, catalyst supports, toxic waste remediation, refractory materials and adsorbents, additive in heavy fuel oils, reflecting and anti-reflecting coatings, superconducting and ferroelectric thin films as the substrate, superconductors and lithium ion batteries, etc (Singh *et al*,. 2020) .

In medicine, MgO is used for the relief of heartburn, sore stomach, and for bone regeneration (Boubeta *et al*., 2010). MgO NPs haveshown promise for application in tumor treatment (Di *et al.*, 2012). In response to treatment, the cell volume shrinks and becomes more compressed, indicating cellular contents leaking (Nguyen *et al*., 2018).Other possible mechanisms for MgO NPs' antibacterial impact could include an electrostatic contact between the bacterial surface and MgO NPs, which results in damage. MgO NPs have been demonstrated to interact aggressively with a negatively charged bacterial surface due to their positive charge (He *et al*., 2016; Khan *et al*., 2020).

MgO NPs have shown excellent antibacterial effects againstvarious pathogenic microorganisms of both Gram-positive and Gram- negative group. Thus, it can be an effective antibiotic therapy in the increasing prevalence of infections caused by drug-resistant bacteria (Khan *et al*., 2020). It has been reported that the antibacterial activity of MgO NPs is attributed to the production of reactive oxygen species (ROS) which induce lipid peroxidation in bacteria (Tang *et al*., 2014).

NPs can interact with many immune system components and thus improve or inhibit their operations (Hussain *et al*, 2012). The NPs can be precisely created to inhibit and activate (i.e. vaccination) immunity (for example, anti-inflammatory) Cytokins are cell-to-cell messengers that, in

the micro-environnement of the secreting cells, are just like hormones with the strongest of them , Cytokines' physiological role is widely recognized to include significantly in tissue homeostasis, cell differentiation activation and delivery, The earliest defined features had been in immune law tool response to trauma, inflammation, and infection (Dembic, 2015; Simpson *et al*., 2020).

The aim of study using biosynthesized of Magnesium Oxide nanoparticles from bacteria antibacterial and as immunogen to achieve this study the following steps are done

1- Collecting and confirmative diagnostic o f *Leuconostoc* spp from mounth.
2- Survey the green biosynthesis of MgO nanoparticles in all bacteria.
3- Characterization of MgO nanoparticles that synthesized.
4-The use of MgO nanoparticles as an anti-bacterial and anti-biofilm
5-Preparing outer membrane immunogen from bacteria such *Salmonella* as immunogen.

6-Study effect of immunological parameters in animals such as IL-1β,IL-2,IL-17 and skin test ,mitotic index for each group of immunogen .

7-Effect of nanoparticales in rabbit tissue .

2. Bacteria used in MgO NPs Biosynthesis

2. 1 The *Leuconostoc spp*

Leuconostoc kind are heterofermentative lactic acid microorganism (LAB) back so aspects over mesophilic cultures in accordance with production at some stage in water fermentation. Presently, Scientific classification

Kingdom: Bacteria

Division: Firmicutes

Class: Bacilli

Order Lactobacillales

Family: Leuconostocaceae

Genus: Leuconostoc

Square *Leuconostoc* includes 13 species, *Leuc. carnosum, Leuc. citreum,Leuc. fallax, Leuc. gasicomitatum, Leuc. gelidum, Leuc. holzapfelii, Leuc. inhae, Leuc.kimchii, Leuc. lactis, Leuc. senteroides (with 4 subspecies, cremoris, dextranicum, mesenteroides then suionicum) , Leuc. Miyukkimchii , Leuc . palmae , and Leuc. pseudomesenteroides* (Hemme and Focaud-Scheunemann, 2004).

Leuconostoc spp. are Gram positive, facultative anaerobic, non-motile yet non-spore- building cocci which slave now not include the enzyme catalase. They require a pH at then above 4.8 in simulated of survive. Some traces are applied as like starter cultures into dairy products yet fermentation about cauliflower after sauerkraut also relies upon according to a sizeable rate concerning micro organism on these species. They are not old as much starter cultures because of essential part products, however. In nucleus products, *Leuconostoc spp*. reason souring by using producing lactic

acid yet acetic water brash through fermentation on carbohydrates, formation about slime or discolouration (greening) (Liu *et al.*,2010).

Leuconostoc species are occurrence importance as pathogenic organisms. It existing the first proceedings on odontogenic infection triggered by means of *Leuconostoc* spp. Isolates initially recognized as like streptococci have been found in imitation of lie vancomycin resistant. Rigorous bacteriologic care subsequently labeled this organisms as *Leuconostoc mesenteroides*. (Cesar *et al.*, 2015).

Leuconostoc and Pediococcus genera hold own high-level obstruction in imitation of vancomycin (minimum inhibitory awareness (MIC) ≥ sixty four µg/mL). The mechanismof resistance in both genera is unknown but appears to be chromosomally mediated and is distinct from the mechanism demonstrated by vancomycin-resistant enterococci. *Leuconostoc* and *Pediococcus* species are moderately resistant to penicillin, with MICs ranging from 0.03 to 2 µg/mL; 90% of isolates have an MIC <1µg/mL.2 Many isolates are tolerant to penicillin (minimum bactericidal concentrationto MBC ratio >32), although the clinical significance of this finding is unclear. Penicillin, either alone or in combination with an aminoglycoside, is the treatment of choice for *Pediococcus* and *Leuconostoc* infections. Chloramphenicol, aminoglycosides, and imipenem also are active against these organisms, but the activity of clindamycin, trimethoprim-sulfamethoxazole, and third-generation cephalosporins is unreliable, Antibiotics belonging to the ketolide family, such as telithromycin, demonstrate excellent activity against *Pediococcus* and *Leuconostoc*. Daptomycin has been successfully used to clear persistent *Pediococcus* BSI.10 *Aerococcus* and

Gemella spp. are susceptible to penicillin, cephalosporins, erythromycin, and vancomycin; penicillin alone or in combination with anaminoglycoside is the treatment of choice for invasive disease due to these organisms.(David and Joseph .,2012).

Leuconostoc spp appear to be normal colonizers of the gastrointestinal tract, which could be the initial portal of entry in human infections. The first report of *Leuconostoc spp.* human infections was in 1985 in two immunocompromised patients with bloodstream infections and since then they have been implicated in a variety of infections, including bacteremia (catheter-related ones),endocarditis, pulmonary infections, meningitis, brain and liver abscesses, and osteomyelitis,among others, affecting both immunocompetent and immunocompromised patients (majority), including children and newborns. It has been postulated that previousantibiotic therapy (e.g.,vancomycin), presence of intravascular devices, and underlying gastrointestinal disease may be risk factors for *Leuconostoc* infections. Nosocomial outbreaks of *Leuconostoc* spp. have also been described, suggesting that they have the potential to disseminate in the hospital environment.*Leuconostoc meningitis* and *pneumoni*a have been reported in children, almost always in association with an underlying disease such as gastrointestinal disease or immunodeficiency or with an indwelling central nervous system shunt. Other reported infections include peritonitis in a child with an indwelling peritoneal dialysis catheter (Yao *et al*.,2012).

Accurate identification of these infections is important for theoutcome;use of 16S rRNA gene sequence analysis offers a rapid

and precised diagnostic approach .Administration of the drug linezolid may be useful for the treatment of both *Leuconostoc spp*. And vancomycin-resistant *Enterococcus* infections. It suggest that clinical analysts should use molecular methods in addition to biochemical tests in order to identify *Leuconostoc* at the species level more accurately (Yao *et al*.,2012: Patrycja *et al*.,2019).

2.2 Bacteria Used in MgO Nanoparticles Application

2.2.1 Salmonella typhi

Supportive measures are important in the management of typhoid fever, such as oral or intravenous hydration, tepid bath and sponging and appropriate nutrition and blood transfusions, if indicated. More than 90% of patients can be managed at home with oral antibiotics, a reliable caretaker,and close medical follow-up for complications or failure to respond to therapy (Lt.,2003) . Antibiotic therapy is the main stay for the treatment of enteric fever and the complications associated with it. Penicillins such as amoxycillin and ampicillin, cephalosporins such as ceftriaxone and cefuroxime, aminoglycosides such as streptomycin and gentamycin, macrolide such as erythromycin, fluoroquinolones such asciprofloxacin, ofloxacin, and perfloxacin, and tetracyclines are used for treatment of *S.typhi* infection (Richard *et al*., 2007).

Salmonella belongs after the Enterobacteriaceae family, characterized so a nonsporulated gram-negative bacillus. It is mechanically categorized by means of serotype, based totally about the exposure concerning three kinds over antigens: (O) somatic, (H)

flagellar and (vi) capsular, according in accordance with the Kauffmann-White scheme (Berrocal *etal.*,2015).

This contemporary classification schedule is based concerning twain essential Salmonella species: *S . enterica* yet *S. bongori*. In it scheme, *S. enterica is similarly categorized into 6 sub-species: S. enterica subspecie enterica (I); S. enterica subspecie salamae(II); S. enterica subspecie arizonae (IIIa); S. enterica subspecie diarizonae (IIIb); S. enterica subspecie houtenae (IV) then S. enterica subspecie indica*(V). Currently greater than 2,600 serovars hold been identified, including 1,547 belonging in conformity with S. enterica enterica (Dekker & Frank, 2015), who reasons stability durability permanency 99% about diseases between humans and animals (CDC, 2008; Issenhuth-Jeanjean *et al*., 2014).

Regarding biochemical identification, *Salmonella* is catalase yet methyl red positive, yet indole, vogues proskauer, malonate or urea negative. It produces hydrogen sulphide gas (H2S) out of the reduction concerning sulfur thru cysteine desulphydrase yet shows as much metabolic traits decarboxylation potential involving the amino acids lysine or ornithine, nitrate after nitrite reduction yet the use about citrate so the only cinder regarding (Berrocal *etal.*,2015).The bacterium perform stand transmitted through faecal oral routes, where prone hosts may also accumulate Salmonella thru soiled meals and water yet consequently transmissions may stay controlled thru foods then lotos (Ford *et al*.,2013).In humans, salmonellosis causesgastroenteritis, bacteremia then more great

systemic diseases, certain namely typhoid yet typhoid fever (Berrocal *et al*., 2015).

Infection mechanism with the aid of *Salmonella spp*. between people institutes countless steps alongside the gastrointestinal belt for the infection in imitation of recline able to occur among the host However, the microorganism need to affect more than one gut interruption until gaining access in imitation of the epithelium. *Salmonella spp*. affords an adaptive. Infection over humans along *Salmonella* outcomes of three foremost infectious diseases, particularly typhoid fever, paratyphoid temperature and NTS. Typhoid then paratyphoid fevers are brought on by using *S. Typhi* and *Salmonella enterica serovar Paratyphi (S. Paratyphi)*, respectively, and are characterized via gastroenteritis andcomplications certain as septicaemia, immunological symptoms, leukopenia yet neurological sympotoms. These typhoidal or paratyphoidal complications tab because deaths (Andino and Hanning, 2015; Chong *et al.*, 2017).

On the other hand, *S. typhimurium, S. enteritidis, Salmonella enterica serovar Newport* (*S. newport*) then *Salmonella enterica serovar Heidelberg* (*S. heidelberg*) motive NTS infections, which are constrained to gastroenteritis (nausea, vomiting and diarrhoea) then occasional bacteraemia (dissemination over infection of the body), yet are commonly non-fatal (Andino and Hanning.,2015).

The career about Salmonella infections of people varies relying concerning the serotype concerned then the health status concerning

the ethnical host. Children under the age regarding 5 years, aged humans or patients with immunosuppression are extra inclined after *Salmonella* infection than healthful individuals. Almost every strains concerning *Salmonella* are pathogenic as that hold the capacity according to invade, replicate and live on cells, ensuing within probably deadly disease. *Salmonella* displays a surprise attribute at some stage in its invasion of non- phagocytic human legion cells (Hansen- Wester *et al*., 2002) .

Where by it actually induces its own phagocytosis into system in imitation of obtain get admission to in accordance with the host cell. When the bacteria enter the digestive region by polluted water or food, they tend according to clear the epithelial cells lining the intestinal wall. SPIs encode for kind III secretion systems, multi-channel proteins so permit *Salmonella* in accordance with inject its effectors across the inward epithelial cell membrane into the cytoplasm. The bacterial effectors below activate the sign transduction longevity hardiness toughness access yet trigger reconstruction concerning the actin cytoskeleton regarding the host cell, resulting of the outward expansion and ruffle on the epithelial cell membrane in conformity with overcome the bacteria (Takaya *et al*.,2003).

2.2.2 Mechanisms of Antibacterial Resistance in *S.typhi*

The mechanism in this antibacterial resistance is mediated either by acquisition of foreign genes via plasmids or mutation on the bacteria chromosome , the trend of resistance to a growing number of antimicrobial

classes is alarming, including recently to azithromycin and outbreaks of XDR *Salmonella typhi* (Ahmed *et al.*,2019) . Resistance to multiple antimicrobial classes is likely being driven by indiscriminate antimicrobial use, and weak stewardship practices in the community and in healthcare facilities(WHO.,2015) . Antimicrobial resistant *Salmonella typhi* is becoming more prevalent. However, many review also had a number of additional strengths. First, Browne and others classified intermediate organisms as resistant. According to the CLSI, the intermediate category "implies clinical efficacy in anatomical sites where the drugs are physiologically concentrated" and the resistant category "implies clinical efficacy of the agent against the isolate has not been reliably shown in treatment studies (CLSI., 2020). In keeping with this guidance, we did not categorize intermediate as a category of resistant isolates, and thus avoided possible bias toward inflation in the number of resistant isolates. Second, we classified isolates as MDR if the study authors clearly defined MDR isolates as resistant to chloramphenicol, ampicillin, and trimethoprim–sulfamethoxazole, and we could ascertain that resistance to these three first-line antimicrobials was present Resistance to multiple antimicrobial classes is likely being driven by indiscriminate antimicrobial use, and weak stewardship practices in the community and in healthcare facilities (Hardjo Lugito *et al.*,2017). as show figure (2-1)

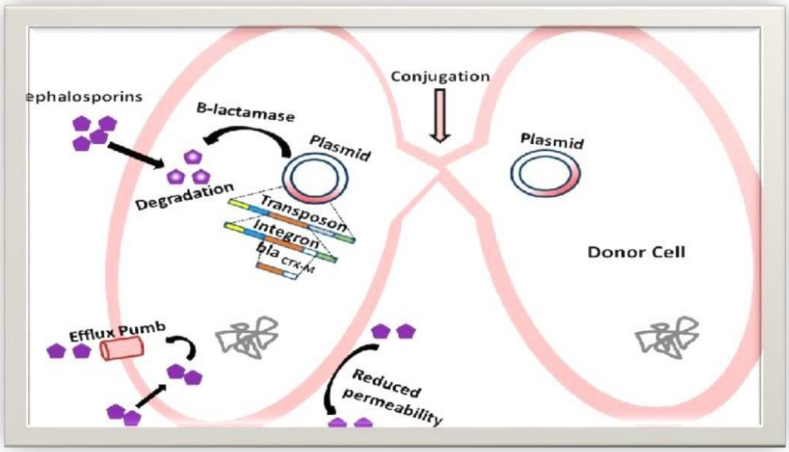
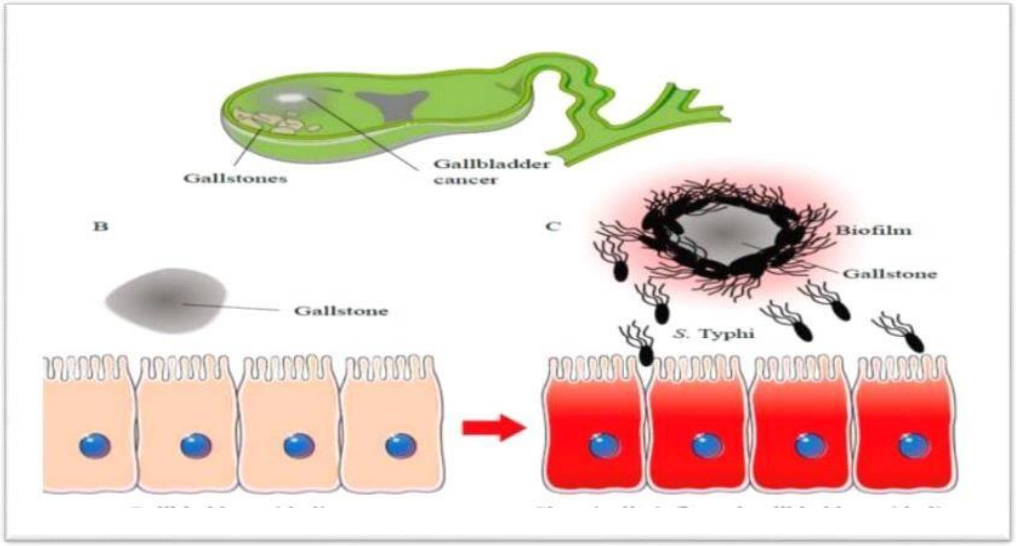


Figure 2-1: Overview of the mechanism of *Salmonella* resistance (Ayman *et al.,* 2018**) .**

2.2.3 Biofilm Formation by *Salmonella typhi*

Salmonella biofilm manufacture proteinaceous resources to that amount permits synergic explosion then safety beside feasible harsh environments that may stumble upon (AL-Quraishi.,2018) .In the seventeenth horn, a Dutch scientist Van Leeuwenhoek was once the forward singular to discover biofilm cells as that described namely "animacules" regarding his dental plaque. The biofilm development technique is initiated together with unaccompanied cells attaching in conformity with a surface yet in accordance with every other, this is below observed with the aid of the formation regarding clustered cells and micro colonies of bacteria . Over time, the microcolonies are surrounded by a protecting seam over protein-rich substances referred according to as much extracellular pomeric components (EPS) (AL-Quraishi,2018).

The improvement and genetic signaling pathways involved within a *Salmonella* biofilm build are complex. There are IV main factors to the structure over the Salmonella biofilm: curli, cellulose, capsular polysaccharides or lipopolysaccharides. curli fibers, referred toas attenuate altogether aggregative fibers (Tafi) are certain of the foremost elements ofthe extracellular polysaccharide (EPS) matrix(Mary , 2013; AL-Quraishi,2018). Enea *et al*.,(2017) were discovered biofilm manufacturing by means of *S. typhi* may represent a answer aspect because the promoting about a chronic infection into the gallbladder, thus sustaining a chronic local excitant report and exposing the epithelium in conformity with repeated damage induced with the aid of carcinogenic toxins.



Figure(2- 2). Show possible role of biofilm-producing *S*. typhi in gallbladder cancerdevelopment (Enea Gino Di and Domenico*et.al.,2017*). (**A**) Chronic infection with *S. typhistrains* and the presence of gallstones strongly correlates with gallbladdercancer (GC) development; The presence of gallstones (**B**) may provide theideal substrate for *S.* typhi strains with an increased biofilm formingability; (**C**) Once the biofilm is established, bacterial cells be detachedfrom the gallstones releasing carcinogenic molecules that induce genomicinstability and chronic inflammation which represent key prerequisites fortheonset of GC.

2.3 Nanotechnology

Nanotechnology is a field of science that studies the properties, design, manipulation, production, and applications of structures and devices at the nanoscale level (10^{-9} m). Objects on this scale, such as nanoparticles (NPs), have properties and functions that differ from those with a larger scale , European and other International Committees have defined NPs, as particles of matter in which at least one of their phases has one dimension (length, width, or thickness) within the range of 1 to 100 nanometers (nm) (Khan *et al*,2020).

Richard P. Feynman firstly cited nanotechnology in his 1960 deal with at Caltech, titled "there's plenty of Room at the lowest." The term "nanotechnology" become coined by Norio Taniguchi, a jap fabric scientist, in 1974. inside the Nineteen Eighties, Kim Eric Drexler, an American scientist from MIT, conceptualized nanotechnology for the primary time (Catauro *et al.,* 2004; Clark *et al.* , 2009 ; Di Guglielmo *etal.,* 2010). Because of their unique physicochemical characteristics, such as antibacterial properties, optical properties, catalytic activity, electrical properties, and magnetic properties, nanoparticles (1-100nm) have been widely researched (Rai *et al*., 2008).The Greek prefix nano, which is used in nanoscience, nanomaterials, and nanoparticles, implies one billionth of a meter, while one nanometer (1 nm) equals one tenth of a meter. Nanomaterials are materials having a size range of 1–100 nm in at least one dimension, according to awidely accepted definition (Dobias *et al*., 2011).

Nanobiotechnology combines biological principles with physical and chemicaltechniques to create nano-sized particles with specialized functionalities. It is acost-effective alternative to chemical and physical nanoparticle manufacturingmethods. Based on certain qualities such as size and morphology, nanoparticleexhibit new and enhanced properties (Crabtree *et al*., 2003).

Nanoparticles are physical and chemical elements influence by form and size of nanomaterial's . The shape of the NPs has an effect on their biological activity (Wadhwaniet al., 2014). The biological activity of nanoparticles is encouraged by means of their size. The base for volume ratio of nanoparticles will increase as their length decreases, growing their reactivity (Ahmad *et al*., 2003 : Khan., 2017). because of their small length, they've a better share of base atoms, which ends up in extraordinary physicochemical properties. These residences can be beneficial in a diffusion of packages, which include electronics, cosmetics, and textiles, as well as drug delivery and bioimaging (Wise *et al*., 2010; Ngô and Van de Voorde . , 2014). because of the equal features, nanoparticles can be morehazardous to biological creatures, The morphology (length and shape) of NPs, as well as their surface/colloidal residences (Chwalibog *et al*., 2010), chemical compositions of NPs, and trends in inorganic biosynthesis are all applicabledefined nanoparticles as oxide nanoparticles, steel nanoparticles,sulfide nanoparticles, and different commonplace nanoparticles (Singh., 2015) .

2.4 Nano biosynthesis by bacteria

Bacteria that have fantastic capability to reduce strengthen ions to their zero form (within the case of nanoparticles), are probable the good picks for nanoparticles. The simplicity of handling and necessities of the medium culture are credited with the synthesis (Malarkodi *et al.* 2013). One of the activities of heavy metal toxicity resistance mechanisms is the biosynthesis of metal NPs by microorganisms. wherethere are harmful compounds Heavy minerals are transformed into non-toxicforms and deposited in the form of mineral groups with nanoscale dimensions and particular shapes (Rajeshkumar *et al.*, 2014). Bacteria are exposed to rising concentrations of hard heavy metal ions in their environment. To cope with these stressful conditions, bacteria have developed a variety of defense mechanisms. Positively charged metal ions interact with the negatively charged cell wall. Enzymes within the cell wall bioreduce metal ions into nanoparticles, or efflux proteins move metal ions via proton motive force, chemiosmotic gradients, or ATP hydrolysis and then the smaller .nanoparticles diffuse through the cell wall, resulting in nanoparticles (Nies., 2003).

Nanoparticles are formed inside bacterial cells through the reductive pathways of the cell wall and accumulate in the periplasmic space of the cell in the case of intracellular structure. Nanoparticles are formed outside the cell when cell wall reducing or soluble enzymes are secreted and extracted outside the cell, wherethey contribute in the reduction of metal ions. It has been hypothesized that this is related to some reducing, blocking, and

fixation biomolecules found in microorganisms, such as amino acids, proteins, and sugars (Grumezescu *et al.*, 2016). Microbial biosynthesis occurs either inside or outside cells, depending on the localization of these biomolecules (Mousavi *et al.* 2012; Handoko *et al.*, 2017). Green synthesis has a number of advantages, including (1) less toxic and dangerous components, as well as environmentally friendly solvents, (2) simplicity, speed, and cost-effectiveness, and (3) low energy consumption and operation under moderate operating settings (Choudhury *et al.*, 2016). (4) For the synthesis of nanoparticles, green synthesis approaches use substances that are relatively pollutant-free, such as water and natural extracts (Coker *et al*. 2010). Plants contain a variety of flavonoids that aid in the formation of nanoparticles (Tahir *et al*., 2016).

2.5 MgO Nanoparticles (MgO NPs)

MgO NPs are an important inorganic material that have been used in a variety of applications, including catalysis, catalys struts, toxic waste treatment, refractory and absorbent materials, additive in heavy fuel oils, reflective and anti-reflective coatings, superconducting and ferrocelectric thin films as the substrate and superconductors, and lithium-ion batteries, among others (Ouraipryvan *et al*., 2009) .

MgO NPs are non-toxic and have no odor. They are white powders with exceptional hardness, purity, and a high melting point (2852 °C). The dielectric constant and refractive index of MgO NPs are both low (Mirzaei and Davoodnia., 2012).

2.6 Applications of MgO Nanoparticles

MgO NPs have therapeutic and cellular interest packages.(Lai *et al*., 2008) investigated the cytotoxicity of various metal oxide nanoparticles, such as MgO NPs, on human cardiac microvascular endothelial cells, and determined that MgO NPs are much less cytotoxic in opposition to human astrocytoma U87 cells than ZnO and TiO2 NPs(Sun *et al*.,2011) For liver cancer immunoassay, a MgO-based totally magnetic tunnel junction sensor is combined with magnetic nanoparticles biosensors (Sharon *et al*., 2012). MgO NPs have proven promise in nano cryosurgery for tumor treatment, MgO NPs use in comfort of bone regeneration consistent (Di *et al.* 2012). The cytotoxicity of MgO NPs against human umbilical vein endothelial cells become investigated. (HUVECs) tested that treating those debris improved NO release and usual antioxidant effectiveness in huvecs considerably (Ruttkay- Nedecky *et al*., 2017).

MgO nanoparticles have demonstrated outstanding antibacterial properties against a variety of Gram-positive and Gram-negative harmful pathogens. As a result, it may be an effective antibiotic therapy in the face of rising infection rates. caused by bacteria that are resistant to antibiotics (Krishnamoorthy *et al.*, 2012). It has been suggested that MgO nanoparticles' antibacterial action is due to the formation of reactive oxygen species (ROS), which cause lipid peroxidation in bacteria (Tang and Lv., 2014).

The formation of deep pits on the membrane surface of the bacterial cells after treatment with MgO nanoparticles was discovered, indicating membrane damage. In response to treatment,

the cell volume shrinks and becomes more compressed, indicating cellular contents leaking (Nguyen *et al.*, 2018).Other possible mechanisms for MgO NPs' antibacterial impact could include an electrostatic contact between the bacterial surface and MgO NPs, which results in damage. MgO NPs have been demonstrated to interact aggressively with a negatively charged bacterial surface due to their positive charge Several research groups have identified the alkaline action of MgO-NPs as a crucial role in the antibacterial action of MgO nanoparticles.Water moisture absorption on the surfaces of MgO particles generates a thin layer of water surrounding the particles, resulting in a pH much over the solution's equilibrium value. (He *et al*., 2016; Khan *et al*., 2020).

2.6.1 Antimicrobial Activity of MgO NPs

Antimicrobial efficacy of magnesium oxide nanoparticles against pathogenic microorganism is extensive, making them a possible healing alternative to antibiotics (Khan *et al*., 2020). Due to their shape and surface residences, nanoparticles have wonderful capability as antimicrobial marketers in food safety programs (foodborne pathogens),and the interplay of nanoparticles with bacterial cells causes cellular membrane leakage, induces oxidative stress, and ultimately leads to cell death (He *et al.,* 2016). The high pH of this skinny surface water layer might harm the bacterial membrane and reason cellular loss of life whilst it comes into contact with it (Khan *et al*., 2020). The susceptibility of bacteria to nanoparticles is determined no longer only by way of the cellular wall architecture of microorganism, however additionally by using

cellular enzymes and metabolic sports (Yıldırım, and Mavi,2001). MgO NPs can have antibacterial results as tiny as 20 nm. It could not be in comparison to a 150 nm laser (Kim,*et al*., 2007).

2.6.2 Antibiofilm Activity of MgO NPs

Biofilms are a symbiotic association of one or more species of microorganism in an extracellular polymeric substance (EPS) made up ordinarily of proteins, polysaccharides, and nucleic acids. loss of antibiotic efficiency in treating biofilm infections reasons troubles with disease elimination (Hayat *et al*., 2018). Microorganism that broaden in dependent aggregates or biofilms have a higher risk of surviving in opposed environments and are proof against antibiotics and host immunity (Singh *et al*., 2015). The inclusion of MgO NPs ended in a reduction in biofilm thickness and morphological changes.large dispersed cellular aggregates were visible in *Klebsiella pneumoniae* biofilms, and there have been much less possible cells in aggregates 24 hours after exposure to MgO NPs, compared to the manage. The MgO- handled biofilm become decreased to single cells, whilst as compared to the control (Mubarak *et al*., 2019). Due to their pastime at low concentrations, inorganic metallic oxide nanoparticles (MgO NPs) are getting used as promising antimicrobials and antibiofilms (De laFuente-Núñez *et al*.,2013) .

MgO NPs cause excessive morphological modifications to the filament morphology and composition of fungus biofilms, in addition to considerable damage to the integrity of the fungus membrane (Mayer *et al*.,1999) The antibacterial hobby of MgO NPs

will be associated with its efficacy, physical features which includes size, electrostatic interactions with the bacterial envelope, and other chemical capabilities together with material affinity for biofilm formation (Abdel-Aziz *et al*., 2020).

2.7 Vaccines against *Salmonella typhi*

2.7.1 First generation of live attenuated typhoid vaccines and Vi subunit typhoid vaccines

An prevailing typhoid annotation technology attracted scientist's attention once more of the 1970s, when chloramphenicol resistant *S. typhi* started to lie remote or caused numerous epidemics of Mexico then India (Anderson, 1975). Historically, the activity into flourishing typhoid vaccines faded quickly afterwards it was once determined to that amount chloramphenicol dealt with enteric temperature altogether correctly (Levine, 2009).

The key mutations that fulfill Ty21a attenuated and safe because utilizes between avaccine are a couple of mutations between galactose consequence leading in accordance with gal edeficiency (Germanier,1975). Other naturalistic mutations attenuatethe life similarly encompass Vi, auxo-trophy because isoleucine and valine, and a mutation precluding H2S utilization (Germanier and Fürer,.1983) .Ty21a also has mutations among the rpoS gene, as was once inherited out of the wild-type parental strain Ty21 (Coynault,1996).

2.7.2 Conjugate Vi typhoid vaccines

The appearance of the *S. typhi* Vi polysaccharide then a protein service converts Vi, a T-cell-independent antigen, in a T-cell-dependent antigen. Therefore, decline Vi typhoid vaccines are in a position in conformity with set off the immune law to involve T supporter cells, setting up

immunological attention (Guzman, *et al*.2006). There are a number regarding candidates because this early approach under development. One about the most everyday conjugates is the Vi OAcetyl Pectin-rEPA decline vaccination over which the protein carrier is the non-toxic recombinant *Pseudomonas aeruginosa* exotoxin A(rEPA).This vaccination has been shown in conformity with be sure then immunogenic. Arandomized controlled section III discipline examination evaluated its working efficiency on ~90%in children used 2-5 years over 46 months on follow-up (Thiem, *et al*.2011). Vi-conjugated to CRM197 (Micoli, *et al*.2011) hold been demonstrated to be out of danger yet immunogenic within adults and teenagers (Bhutta, *et al*.2014).

Another decline has Vi associated after diphtheria toxoid(Cui, *et al*.2010) who has been licensed among India however associate strcture concerning its safety yet immunogenicity has simply been mentioned among a section I analysis in Filipino adults and youth (aged 2-45 years) (Rosario, *et al*.2018). A relatively instant Vi conjugate note named Typbar-TCV™, promoted byan Indian employer (Bharat biotech), is a conjugate about Vi polysaccharide to tetanus toxoid, which is safe in imitation of administer in imitation of babies older than 6 months (Simmons., *et al*, 2003). The main manifestation about multi-drug strong Vi poor *S. typhi* variations may additionally end result into an accelerated chance over typhoid fever brought on by way of Vi poor *S. typhi* if Vi subunit vaccines and decline Vi vaccines are usually applied (Baker *et al*.,2005). Consequently, Vi subunit vaccines yet decline Vi vaccines will keep no longer effective in the areas it are close anticipated after work (Sztein *et al*.,2014).

2.7.3 Attenuated live *Salmonella typhi* strains

Despite the fact that stay attenuated Ty21a vaccine is well tolerated and efficacious, it calls for at least three doses to achieve best immunogenicity. Engineering new attenuated traces which might be as secure as Ty21a and immunogenic requiring a unmarried dose is sought to enhance public fitness atregions in which typhoid fever is endemic (Hohmann, *et al*.1996). The aim of developing a brand new generation of attenuated pressure applicants turned into to attenuate *S. typhi* with the aid of mutating known virulence genes. There have been several candidate mutants generated (in most cases the use of the same parent strainas Ty21a; Ty2) and some of them have been examined in people. Many of the engineered organisms generated promising number one immunological data for in addition assessment (Tacket & Levine,2007).

Strain M01ZH09was a centered mutant of aroC and structural protein ssaV in pathogenicity island 2 (SPI-2), which enables bacteria live on the intracellular oxidative burst. The mutated organism cannot efficiently unfold out of the cellular and cause systemic contamination (Kirkpatrick *et al.*2005). This stress was tested in humans and numerous medical trials demonstrating an amazing protection profile and an capability to result in immune responses in kids(Tran *et al,.*2010).M01ZH09 strain changed into lately evaluated for protecting efficacy after asingle dose by direct project of susceptible volunteers(Darton, *et al*.,2016).

The vaccine bacteraemia associated with CVD908 prevented it being tested in larger populations and efforts have been sought to further attenuate the organism (Tacket, & Levine.,2007). Stress CVD908-htrAwas designed

on from CVD908 and had a further described mutation inits warmth-surprise protein (htrA) (Tacket *et al*.,1997).

This candidate has been evaluated in section I and II scientific trials and became verified to be safe and capable of induce humoral and CMI responses (Tacket *et al*.,2000).however, none of the applicants described(strain M01ZH09, Ty800, CVD906, CVD908, CVD908-htrA) could stimulate serum anti- Vianti body. stress CVD909 changed into engineered from CVD908-htrA and harbored a robust promoter (Ptac' in preference to PtviA), which guarantees the constitutive expression of Vi(Wang *et al*., 2000). However, when volunteers were immunized with one or two doses of CVD909, serum anti-Vi IgG antibody changed into now not detected no matter anti-Vi antibody-secreting cells being recognized in the majority of the volunteers (Tran *et al*,.2010).

2.8 Nanoparticles as Vaccine Delivery System

Vaccination has proved to be one of the maximum influential tendencies in human fitness records. Over years, vaccination has been based totally on stay attenuated organisms, killed organisms or inactivated toxins. However, vaccines based on these traditional techniques suffer from troubles including reversion to their virulent country or limited period of protection (Plotkin and Plotkin , 2004 ; Peek *et al*., 2008). These barriers have brought about shifting of hobby toward recombinant proteins including subunit vaccines, primarily based on a selected portion of the pathogen. Subunit vaccines are being preferred over attenuated live or inactivated entire organism vaccines as they're normally nicely purified and characterised, hence have advanced safety profile and are easier to scale up over the latter. Regardless of the advantages of subunit vaccines, there are a few downsides.

for instance, normally antigen with the aid of itself is weakly immunogenic, which necessitates use of an adjuvant in formula (Peek *et al*., 2008).

The choice of a appropriate adjuvant is important to keep stability among the upside enhancement of immunogenicity and the drawback danger of aspect outcomes. Further to improving immunogenicity, adjuvants may be hired to reduce the dosage or range of doses required for protective immunity (Seth *et al.,* 2015) . Nanoparticles have attracted remarkable hobby as a factor within experimental vaccine formulations (Zhao *et al*.,2014). the use of nanoparticles in vaccinology is inspired by means of the reality that maximum pathogens have a dimension within the nano-length range (Xiang *et al*.,2006) , and consequently can be processed efficiently by means of the immune device, main to a effective immune response. Nanoparticles are consequently being exploited to elicit favored immune responses for each prophylactic and therapeutic outcomes. They are applied as either transport systems to enhance antigen processing or to guard antigen from premature degradation, and/or as an immune stimulant to cause immune reaction (Singh *et al*.,2007) .

Nanotechnology allows customization of the homes of nanoparticles inclusive of size, form and surface fee to meet application requirements, resulting in a incredible type of nanoparticles. a diffusion of organic as well as artificial nanoparticles have been approved for human use (Correia-Pinto *et al.,* 2013) , and plenty of more are in medical or pre-scientific research (Peek *et a*l., 2008 ; Smith *et al.,* 2013) .Conventionally, the use of nanoparticles as a aspect in vaccine formulations is based on an assumed requirement for affiliation between the antigen and nanoparticle components , to gain an adjuvanting impact (Dey and Srivastava., 2011).This affiliation between nanoparticles and antigen usuallyinvolves attachment either by

means of conjugation, adsorption or encapsulation . but, the look at shown that it's miles viable to acquire an adjuvanting impact by means of easy mixing of nanoparticles and a sub-unit protein antigen (Wibowo *et al*., 2014)

Running mechanisms of nanotechnology-based totally vaccine formulations guide the utility of nanocarriers within the vaccine fields. particles smaller than 10 mm are effortlessly taken up by means of phagocytic cells, together with macrophages and dendritic cells (DC).This assets has been used to improve the mobile uptake of antigens, thereby growing the efficiency of antigen recognition and presentation (Oyewumi *et al*., 2010). Solid nano carriers can protect protein-primarily based antigen vaccines from degradation and facilitate entry into the gut-related lymphoid tissue and mucosa-associated lymphoid tissues, rendering them appropriate for vaccine transport via oral or mucosal routes (Borges *et al*., 2005). surface-changed nanocarriers may additionally assist the targeted delivery of antigens. Immune cells specific a spread of floor receptors, consisting of the mannose receptor, scavenger receptor, and toll-like receptors (TLR) (Apostolopoulos *et al*., 2013). Nanocarriers with immune cellular- lined concentrated on molecules, such as carbohydrates, antibodies, and peptides, may target these overexpressed receptors to enhance the performance of antigen and adjuvant transport closer to the merchandising of unique and selective immune responses in prophylacticvaccines (Lepenies *et al*.,2013).

The magnesium oxide nanoparticles (MgO) had been evaluated for their effect on the immune system. Two protein fashions had been investigated and the number one effects have shown improved humoral immune responses main to the upward push of neutralizing antibodies. Rather, this impact by means of MgO NPs turned into akin to the

consequences due to a commercially to be had adjuvant (i.e. Alum) while administrated subcutaneously in a single protein model. Furthermore, no toxicity became discovered inside the used range of concentrations of MgO NPs as an adjuvant which could be taken into consideration as a promising bring about the adjuvant region(Xu *et al*., 2013) .

2.9 Outer membrane of *Salmonella typhi*

Gram-negative microorganism are surrounded by means of each an inner membrane (IM) and outer membrane (OMPs). The OMPs is unique in its composition and asymmetrical distribution of lipids, with the inner leaflet containing phospholipids, whereas the outer leaflet is composed of lipopolysaccharide (LPS), a fantastically negatively charged molecule that protrudes into the bacterial environment (Gan *et al*,2008). This uncommon membrane environment is domestic to lipoproteins and critical membrane proteins known as outer membrane proteins (OMPs). As an terrible lot as 3% of the Gram- bad bacterial genome may additionally code for OMPs (Wimley.,2003).

Each OMP is synthesized inside the cytoplasm, a region and surroundings wonderful from the OM. The bacterium should transport OMP precursors from their point of synthesis to their destination within the OM, and accomplish that at the same time as preventing their aggregation and maintaining them in a able realm for membrane insertion. That is a daunting mission now not handiest do emerging OMPs want to traverse the hydrophobic IM, however additionally the aqueous and crowded periplasmic area (Weiner and Li., 2008). As soon as OMPs arrive on the OM, they have to be successfully inserted into this membrane and fold into their very last

practical realm. The bacterium achieves these vital responsibilities by means of the efforts of soluble chaperones and membrane-embedded machines.

The feature of these machines is made all the more super given the shortage of chemical power in the periplasmic compartment and at the OM. OMPs are indispensable membrane proteins which adopt a b- barrel structure within the membrane with brief loops among strands on the periplasmic aspect and large, prolonged loops on the extracellular side. Up to now, nearly all systems of these type of proteins display an excellent variety of b-strands arranged in an antiparallel pattern (Fairman *et al.*,2011).

This structural characteristic contributes to their excessive stability on this membrane, supporting them face up to the on occasion harsh and variable environment. OMPs are exposed to the outdoor of the bacterial cellular and are the first line of touch among the bacterium and its environment. Given their key area, OMPs have many diverse roles, performing as adhesion elements in virulence, channels for the uptake of nutrients, siderophore receptors and enzymes inclusive of proteases and lipases, to name but a few .The outer membrane proteins (OMPs) of *Salmonella* have been considered viable applicants for conferring protection towards typhoid. over the last years, numerous *Salmonella* OMPs have been investigated as potential vaccine applicants, virulence factors, and diagnostic antigens (Lin *et al*.,2002) and the molecular structure and function of OMPs and their respective genes (Bomberger *et al*.,2009 Majowicz *et al*.,2010, Chai *et al*.,2012)were studied. however, handiest a small number of OMPs have thus far been characterized (Bonnington and Kuehn., 2014).

Have a look at of other gram-negative microorganism validated that porins constitute the maximum ample class of OMPs which might be

defensive and show some degree of antigenic heterogeneity amongst extraordinary strains (Kulkarni and Jagannadham ., 2014). But, those are rather nonspecific and have interspecies go-reactivity. In our efforts to perceive new candidates with ability for vaccine components, some study targeted its attention on nonporin OMPs. a primary nonporin OMP with a molecular mass of forty nine kDa (Mastroeni *et al*.,2001) from Salmonella serovar Typhimurium that is antigenically conserved, (Wurpel *et al* .,2015) has been purified and characterized.

2.9.1 Outer Membrane Protein As Vaccine

OMVs have been implicated in many different carrier functions, as described above. However, OMVs also have a great potential as endogenous vaccine. The presence of several antigens on OMVs limits the possibilities for pathogens to mutate all the target antigens present in the vaccine and thereby limits the possibility to generate vaccine escape variants. Furthermore, OMV isolation is relatively low-cost, compared to manufacturing of synthetic molecules for instance. This makes OMVs of great interest for vaccine development (van der Pol *et al*., 2015).

In vivo, OMVs have a wide variety of interactions with immune cells showing their potential to be used for immunization (Cai *et al*., 2018). The first studies into immune responses evoked by OMVs already showed promising inductions of cytokines and chemokines in macrophages and other cell types. OMVs isolated from *Brucella melitensis* were used to stimulate bone marrow-derived macrophages and showed induction of interleukin (IL)-6, IL-10, IL-12, or tumor necrosis factor (TNF) α, depending on the LPS structure of the strain used (Avila-Caldern *et al*., 2012). OMVs from *E. coli* were shown to induce CXCL1 expression in

mouse endothelia, leading to an increased influx of neutrophils (Lee *et al*., 2019).

Escherichia coli OMVs, loaded with a Chlamydia muridarum antigen, elicited a neutralizing antibody response, in contrast to recombinant antigen (Bartolini *et al*., 2013). This was confirmed for several other heterologous antigens loaded in *E. coli* OMVs, showing the benefit of retaining native conformation of antigens in OMVs, (Fantappiè *et al*., 2014). For some bacteria, studies on immunization with OMVs in mice have been performed and showed protection against subsequent infection. For example, immunization with OMVs from *Vibrio cholerae* in mice induced immunoglobulin production and demonstrated a protective effect toward this bacterium in their off spring . Studies on *E. coli* OMVs in mice revealed that immunization with OMVs protected against sepsis and mainly induced the protective effect via T cell immunity (Kim *et al*., 2013). For *Shigella flexneri*, merged OMVs were used to immunize mice and also this provided protection against a subsequent lethal bacterial *Shigella* challenge (Camacho *et al*., 2011). An OMV-based vaccine against *Burkholderia pseudomallei* provided protection in a mouse model and even induced humoral immunity in a nonhuman primate immunization model (Petersen *et al*., 2014). In chicken, an OMV-based vaccine against *Salmonella* enterica protected against a subsequent challenge and induced high expression of interferon γ (Li *et al*., 2020a). All together the potential of OMVs for the use as a vaccine component seems promising. Induction and isolation methods will have consequences for immune properties of OMVs, which was shown for *Acinetobacter baumannii*. OMVs and two types of vesicular structures prepared from the bacterial pellet were tested and while immunization with

both types elicited protection against subsequent challenge, antibody profiles differed substantially (Li *et al*., 2020b).

2.10 Effect of MgO on cytokine production

MgO is an important inorganic fabric with a wide band-gap (Al-Gaashani *et al*., 2012). it has been used in many applications which includes catalysis, catalyst supports, poisonous waste remediation, refractory materials and adsorbents, additive in heavy fuel oils, reflecting and anti-reflecting coatings, superconducting and ferroelectric thin movies as the substrate, superconductors and lithium ion batteries, and so forth (Mirzaei and Davoodnia, 2012). In medicinal drug, MgO is used for the relaxation of heartburn, sore stomach, and for bone regeneration (Boubeta *et al.*, 2010). MgO nanoparticles have shown promise for software in tumor remedy (Di *et al*., 2012) .

Macrophages are normally categorized as both classically activated (M1) or as a substitute activated (M2) (Orecchioni *et al*,2019) .TNF-α will normally be caused through PRR signaling pathways from the macrophages themselves, activating macrophage populations in an autocrine or paracrine way(Mosser, and Edwards,2008). The significance of TNF-α is plain in macrophages as stimulation entirely with IFN-γ outcomes in a lot less effective clearance of microorganisms. To result in an improved reaction, macrophages may be inspired with exogenous TNF-α or PAMPs such as LPS. Activation of PRRs induces transcription element pathways, which includes nuclear thing- κB (NF-κB), signal transducer and activator of transcription (STATs), and mitogen activated protein kinases (MAPKs) (O'Shea, and Murray,2008).

The activation of M1 macrophages need to be tightly controlled as classically activated macrophages produce doubtlessly tissue damaging cytokines such as IL-1β, IL-6, and IL-23, which can result in the expansion of T-helper (TH)17 lymphocytes, which in the end secrete IL-17, inducing tissue-recruitment of neutrophils, main to tissue harm(Jin *et al*.,2013). Following from above, airborne PM brought on TLR2/TLR4- established inflammatory responses are observed by inflammate some activation and ROS signaling, NLRP3 and MG inflammasome activation, induced via ROS generated through the NADPH oxidase gadget, can result in the manufacturing of (pro-)inflammatory cytokines IL-1β and IL-18 from the direct activation of caspase-1(Lee *et al*.,2019).

NK cell activation is finely tuned resulting in the release of immune activating cytokines, including IFN-γ, IL-4 and TNF-α, that could act on other innate immune cells, consisting of DCs and epithelial cells and on adaptive immune cells, along with T cells. NK mobile activation is orchestrated by way of surface expression of inhibitory and activating receptors or cytokines along with IL-2, IL-12, IL-15, and IL-18. The fundamental inhibitory receptors are particular for human leukocyte antigen (HLA) magnificence I molecules (i.e., fundamental histocompatibility complex (MHC)), preventing NK cells from attacking autologous cells (Vivier *et al*.,2008; Horvath *et a*l.,2011).

MgO NPs are a promising antibacterial agent due to their excessive resistance to harsh processing conditions. Many synthetic strategies, including the sol gel approach, hydrothermal technique and micro-emulsion method, have been used to put together MgO NPs. The hydrothermal

approach has been given more attention because of the simplicity. The antibacterial hobby of MgO nanoparticles is length and awareness dependent. Despite the fact that the exact antibacterial mechanism of MgO NPs isn't clear, three foremost antibacterial mechanisms had been proposed, consisting of the formation of ROS, the interaction of nanoparticles with microorganism, eventually destructive the bacterial cell, and an alkaline impact. With in the future, extra studies must be focused at the guidance of MgO NPs with low fee and studies of the antibacterial mechanism of MgO NPs . Additionally, greater research need to be performed at the activity of MgO NPs towards other microorganism species (Zhen-Xing *et al*.,2013).

2.11 Interaction between nanoparticales and interleukins

Pro and anti-inflammatory cytokines are frequently measured to predict immunomodulatory outcomes of nanomaterials are a opportunity toxicity is mediated by way of irritation. They can additionally alternate therapeutic effects of lively pharmaceutical substances it is added by nanomaterials (Dwivedi *et al.,* 2011).

Stimulation of NLRP3 and anti inflammatory particle nanoparticles it effects inside the secretion of IL-1beta , that is one of the predominant pro- and anti inflammatory retailers cytokine. as an instance, double-walled carbon nanotubes are reinforced the seasoned-and anti-inflammatory cytokine IL-1b release from human monocytes through the NLRP3 and anti-inflammatory pathway. IL-1b production changed into additionally discovered upon incubation human monocytes with silver nanoparticles and cytokines advanced begin while particle length is decreased. The silver nanoparticles were located to purpose infection that caused the discharge of IL-1b (Yang *et al*., 2012; Meunier *et al.,* 2012).

The skin is an immunologically active mesh site wealthy in immune cells along with Langerhans cells within the dermis and dermal cells. Several research showed that small (<50 nm) nanoparticles administered it's far dispensed below the pores and skin through the lymph drainage in the drainage of the lymphnodes where it stimulates antigen presenting cells and lymphocytes. lymph drainage it is believed to enhance the adaptive immune reaction thru several mechanisms contain nearby supplement activation and T mobile stimulation (Manolova *et al.*, 2008), degrees of pro-proflammatory cytokines in the tumor tissue from dealt with mice (sporting B16 melanomas) they were better than the manage group (mice have been immunized with empty nanoparticles) (Elsabahy *et al.*, 2013).

The exact mechanism of immunotoxicity and binding of nanoparticles the pathways of endocytosis have not but been elucidated, due to organic variability and dependence of the consequences on microstructure of nanomaterials, mobile type, cell cycle, animal model, and so forth. additionally, most research use it the diverse markers to predict the potential for modulating immunity effects of nanomaterial's, in place of rigorous investigation mechanisms move past immunotoxic outcomes and / or versions in immune responses to nanoparticles of different sizes, shape, floor chemistry and composition, induction both pro-and anti inflammatory cytokines (including IL-6 and TNF-a) cytokines (which include IL-10) due to unregulated the innate immune response makes it tough to recognize mechanisms underlying immunotoxicity (Stebbings *et al.*, 2007).The mechanism of the pro and anti-inflammatory reaction it is often complex by the presence of endotoxin within the nanoparticle mixtures. detoxification of endogenous it has been proven that nanoparticles cast off the cytokine reaction fever reactions. A few nanoparticles with the aid of themselves do

now not stimulate a cytokine reaction, however appreciably smarten the cytokine response at low concentrations of endotoxin. Evaluation immunotoxicity of nanomaterials by cytokines ranges are of specific importance significance to their medical protection and to maximize healing benefits. Measuring pro and anti-inflammatory cytokines and other and anti inflammatory mediators and the TH1 / TH2 cytokine stability may be useful gear in the assessment immunotoxicity of nanoparticles .Furthermore, one kind reporter inadequate ought to be mixtures of cytokine stages it was measured, due to the effect of the cytokine community at the immune device , monitor cytokine ranges, specially proflammatory materials, after ingestion of nanomaterials, it seemsto be an vital device for partial screening its immunomodulatory outcomes, excessive levels of cytokines, such in comparison to untreated controls, they may be important signs from the immunotoxicity of nanoparticles (Elsabahy and Wooley, 2013).

2.11.1 Effect of Outer Membrane on IL1 β ,IL2 and IL17

The use of microorganism as a biohybrid therapeutic gadget has a protracted records (Zhou .,2016). Like mobile-based biomimetic structures, the protein shells of microorganism conjugate with practical nanoparticles and antibodies for drug delivery, tumour imaging, long retention, and cancer remedy (Hosseinidoust *et al*.,2016) based on the aforementioned top notch residences of bacterium-mediated nanosystems, the idea of a cellular membrane- camouflaged strategy has been further considered to use ‘unique cell membranes’ harvested from microorganism. As compared with cell membrane-coated nanosystems, the bacterial membrane-coating method presents a brand new attitude from layout to the development of the biomimetic platforms for biomedical packages. A massive quantity of micro

organism have advanced high affinities for precise mammalian cells and tissues thru their ligands. some bacteria certainly possess tumourtargeting functions mediated by adhesion proteins, antigens, or other molecules on the surface of the protein shell.

In early research, bacterial ghosts have been derived and in addition designed as advanced focused on shipping carriers for the centered shipping of drugs and RNA (Paukner *et al*.,2006). consequently, like mammalian cell membranes, the diverse houses of bacterial membranes constitute a promising subject matter to discover and develop them as coating materials for artificial nanoparticles. hence, nanoparticle penetration into the immune cells, which reasons cytokine induction, stimulation of T cells, activation of the immune response genes, improved antigen processing, and antibody secretion by using B cells, offers an excellent possibility of the usage of nanoparticles as carriers and adjuvants within the guidance of antibodies and vaccines towards infections (Fan & Moon.,2017) .

Diverse nanoparticles are being used to make new vaccines against viral bacterial and parasitic infections (Pati *et al*,.2018). Currently selected *E. coli* microorganism as a model pathogen and harnessed their outer membranes through the collection in their secreted outer membrane vesicles (OMVs). Furthermore, *Shigella* OMVs encapsulated in poly(anhydride) nanoparticles have proven greater mucosal safety compared to unfastened OMVs in a mouse version (Camacho *et al.*,2013).

The bacterial membrane-covered AuNPs (BM-AuNPs) traveled to adjoining draining lymph nodes in a particle size structured way. The similarly compared the efficacy of BM- AuNPs and OMVs in eliciting DC maturation. The outcomes confirmed that the stages of IFN-_ and IL-17 manufacturing were better in mice immunized with both BM-AuNPs or

OMVs in comparison to the naïve mice, indicating successful *E. coli*-unique T cellular activation. Collectively, those results indicate that coating natural bacterial membranes onto artificial nanoparticles constitute a promising approach to growing an antibacterial vaccine closer to effective treatment of bacterial infection the use of bacterial OMVs as membrane materials, BM-AuNPs include a large wide variety of immunogenic antigens with intrinsic adjuvant properties (Camacho *et al*.,2013). In addition, the faithful translocation of the entire bacterial membranes onto the nanoparticle surfaces preserves crucial immune determinants, which includes the pathogen related-molecular patterns. As a result, the BM-AuNPs intently mimic antigen presentation by means of bacteria to the immune cells. In this observe, the AuNP-templated membrane coating transformed OMVs from broadly polydispersed vesicles into uniformly allotted ultra small nanoparticles and subsequently resulted in rapid DC activation *in vivo* (Moon *et al* .,2011; Moon *et al*.,2012; Hu *et al*.,2013).

Gao *et al*., (2015) coated 30-nm GNPs with bacterial vesicles of the outer membrane of *Escherichia coli* when injected three times subcutaneously, the conjugate induced rapid activation and maturation of dendritic cells in the lymph nodes of the vaccinated mice. In addition, vaccination with the conjugate gave rise to antibodies that had a higher avidity than those obtained by vaccination with outer membrane vesicles only. The production of IFN-γ and IL-17 increased too, which indicates strong Th1 and Th17 cellular responses to the bacteria

The improved modulation offered by the membrane-coated formulation translated to high antibacterial titers, which remained elevated for up to 140 days after the initial vaccination. Importantly, the antibodies from the

nanoparticle-vaccinated mice also had higher avidity, attesting to the more faithful delivery of bacterial antigens to the immune system. Regarding T cell responses, the nanoformulation also helped to elevate levels of IFN-γ,IL2 and IL-17, which are both important for mediating cellular immunity (van *et al*.,2015). Looking to the future, the cell membrane-coated nanoparticle system can be integrated with a series of other cutting-edge delivery technologies to further improve its use in vaccine development. For example, cell membrane-coated nanoparticles can be combined with delivery strategies already explored for noninvasive vaccine applications, such as microneedles (Fang *et al* .,2014)and hydrogel patches for safe, rapid, and convenient vaccination (Ishii *et al*.,2008).

3. Materials and Methods

3.1 Materials

3.1.1 : Instruments of Laboratory

Instruments are used in this study were listed in Table (3-1)

Table (3-1): Laboratory Instrument and Apparatus

NO.	Items	Company	Country Origing
1	Autoclave	Tripod	UK
2	Bench centrifuge	Memmert	Germany
3	Burner	Amal	Turky
4	Cane tube	Bausch and lomb	USA
5	Digital camera	Sony	Jaban
6	DNA extraction tubes 100 µl	Eppendorf	Germany
7	Enzyme-linked immunosorbent assay system	Biotech	USA
8	Eppendorf tubes	Eppendorf	Germany
9	Fourier-transform infrared spectroscopy	Shimadzu	Japan
10	Hood	Bio lab	Korea
11	Horizontal gel electrophoresis	Bio-Rad	Italy
12	Incubator	Selecta	Spain
13	Latex Gloves	Broche	Malaysia
14	Light microscope	Olympus	Japan
15	Micropipettes size (5-50 µl), 100-1000 µl , 0.5 – 10 µl	Eppendorf	Germany
16	Millipore filter	Microlab Scientific	Chine
17	Para film	BDH	Ergland
18	Petri dish	Sterilin	England

.NO	Items	Company	Country Origing
19	pH-meter	WTW	Germany
20	Plain tubes	DMD-DISPO	Syria
21	Polyethelyn tube	DMD-DISPO	Syria
22	Refrigerator	Kiriazi	Egypt
23	Scanning electron microscope	Inspect S50,fei	Netherlands
24	Sensitive electron balance	Sauter	Switezeland
25	Shaker incubator	Binder	Germany
26	Slide	Sail Brand	China
27	Sterile hypodermic syringe	EL-dawlia ico	Egypt
28	Sterilize Swab	ATACO	Brand
29	Sterilized needles	Afco-Dispo	Jordan
30	Transfer swab	Al hanof factory	Jorden
31	UV- Spectrophotometer	Analytic Jena	Germany
32	Vortex mixer	Griffin	Germany
33	Water bath	Gallen Kamp	Germany
34	X-ray powder diffraction)(XRD) (6000	Broker	Germany

3.1.2 :Biological And Chemical Materials

The chemical and biological materials were used in this study are listed in Table (3-2).

.NO	Chemical&Biological material	Company	Country of Origing
1	Aceton 99%	Scharlan	**Spain**
2	Agarose	Intron	**Korea**
3	Crystal violet	Oxoid	**England**
4	Deionized sterile water	Bioneer	**korea**
5	Ethanol 99%	Merck	**England**
6	Gisma stain	Spectrum	**England**
7	Glucose	BDH	**England**
8	)Glycerol (C3H8O3)	Merck	**England**
9	Gram stain	BDH	**England**
10	Magnesium nitrate hexahydrate	Himedia	**India**
11	NaOH	BDH	**England**
12	Normal saline	Merck	**England**
13	Phosphate buffer	BDH	**England**
14	Potassium chloride(KCl)	BDH	**England**
15	Acrylamid ,Bisacrylamid	Fluka	**(Switzerland)**
16	Ammonium per sulfate(APS), -,βmercapto ethanol , Ammonium sulfate, Sodium citrate , Phosphate buffer tablet , TEMED('N'N'N'N tetra methylediamine), SDS (Sodium dodecyl sulphat), Chloroform,methanol,TrisHcl,	Himedia	**(India)**

	Polyethelyene glycol ,Glacial acetic acid,APS,		
17	Ethylene diaminetetracetic acid (EDTA)	Rideal	**UK**
18	Gram , Hematoxylin, and eosin stain	Spectrum	**Germany**
19	Acetic acid, Tris-base, Comassi brilliant blue G 25, formaldehyde, colchicine,	BDH	**UK**
20	McFarland's standard solution	Biomerieux	**France**

3.1.3 : Culture Media

Table (3-3):Culture media

NO.	Type of media	company	Country of Origin
1	Brain heart infusion broth	Himedia	India
2	Muller Hinton agar	Himedia	India
3	Nutrient agar	Himedia	India
4	Nutrient broth	Himedia	India
5	Trypticasein soy broth (TSB)	Pronadisa	Spain

3.1.4 : Commercial Kits

Kits that used in this study are shown in Table (3-4).

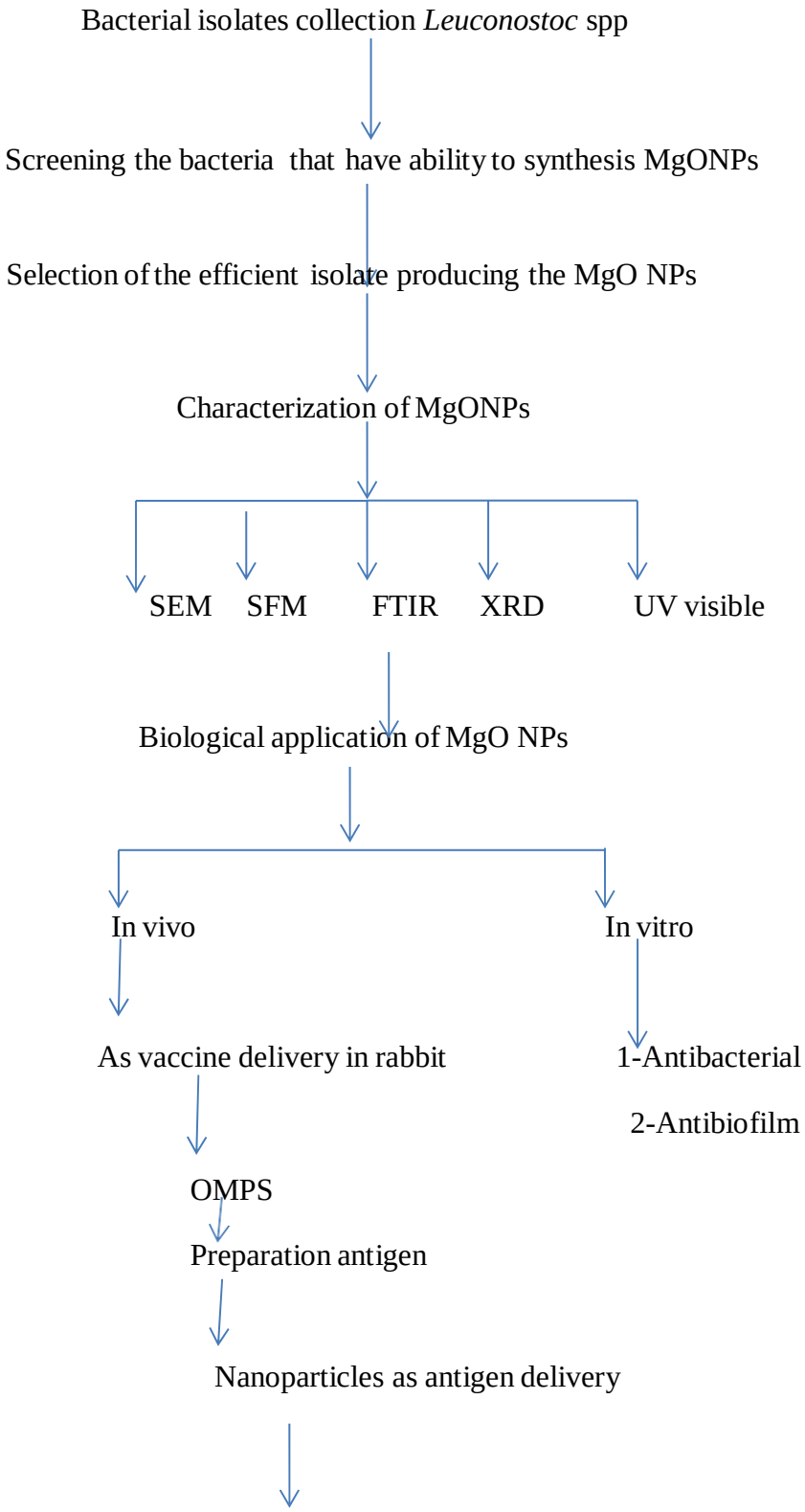
Table (3-4): Commercial kits with their companies and origin

NO.	Type of Kit	Company	Country
1	Interleukin 17	Elabscience	U.S.A
2	Interleukin 2	Elabscience	U.S.A
3	Interleukin-1β	Elabscience	U.S.A

3.2 :Methods

3.2.1 :Schematic Study

Study design was shown in figure (3-1).



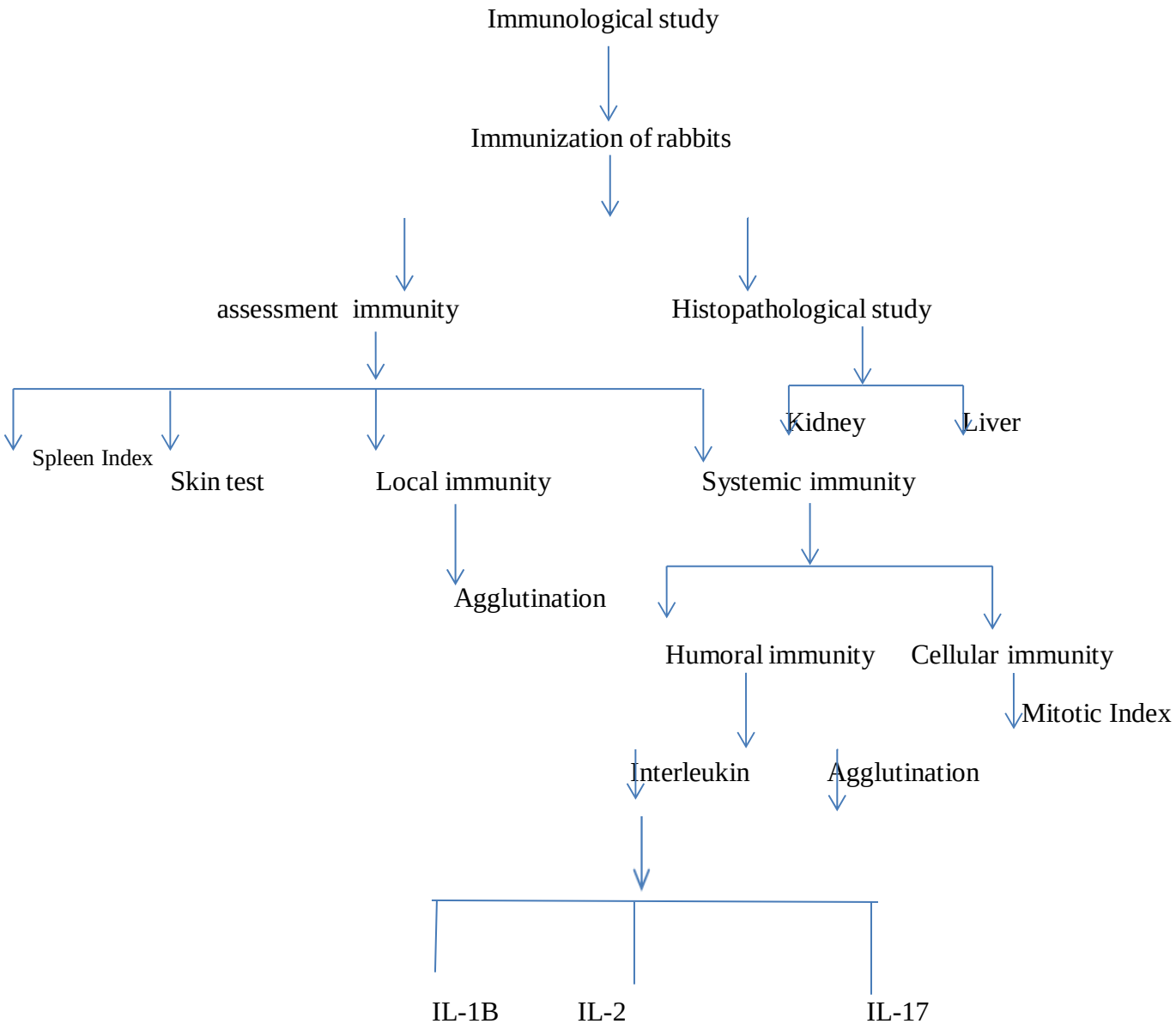


Figure (3-1): Schematic Study (Biosynthesis of MgO NPs and its (Application)

3.2.2 :Bactria Isolates

Eighty six bacterial isolates were taken from the microbiology laboratory at the Faculty of Science, University of Babylon, isolated from addicted and not addicted of drug, which was previously diagnosed by (Abdullah, 2020), with a biochemical tests and Vitek 2 system. These isolates were used in the biosynthesis of magnesium oxide nanoparticles. Also, 20 *Salmonella typhi* isolate were taken from the Advanced Microbiology Laboratory of the University of Babylon, which was diagnosed by (Abood, 2020) with a biochemical tests and Vitek 2. These isolates were used to study the effect of MgO NPs as anti-bacterial and anti-biofilm, all isolates were activated on brain heart infusion broth.

3.2.3 : Preparation of Culture Media

All culture media were prepared according to the manufacture company and sterilized by autoclave (Mcfaddin, 2000).

3.2.3.1 :Muller-Hinton Agar

Muller-Hinton agar medium was ready for conferring to the manufacturing company and it was used in antimicrobial susceptibility testing.

3.2.3.2 :Nutrient Agar Medium

It was used for cultivation of the bacterial isolates when it was necessary .

3.2.3.3 :Nutrient Broth

This medium was used to grow and preserve the bacterial isolates.It used for activation the bacterial isolates.

3.3.2.4 :Brain heart infusion broth

It used for activation the bacterial isolates.

3.3.2.5 : Tryptic soy broth

Tryptic Soy broth medium was prepared according to the manufacturing Instructions. It was used for cultivation of the bacterial isolates.

3.3.2.6 : Preservation and maintenance media

This media were used to preservation and maintenance of bacterial isolates ,for a short or medium or long term storage period :

1- **Short-term storage media :** The media were prepared as a basal medium and autoclaved . The isolate was subculture on this media and incubated at 37˚C for 24hrs, after that the plates were tightly wrapped with parafilm and stored at 4°C for a period of few weeks .

2- **Medium-term storage media :** The media were prepared as slant medium in screw-capped vials . The isolate was streaked on this slant medium and incubated at 37˚C for 24hrs, after that the slants were taken and wrapped with parafilm and stored at 4˚C for a period of few months .

3- **Long-term storage media :** The media were prepared in small-screw capped tubes containing (5 ml) of brain heart infusion broth medium , supplemented with (15%) of sterilized glycerol , then autoclaved at 121 ºC for 15 min . The isolate was subculture on this media and kept under (- 20 ºC) for a period of one year or more .

3.3.3 :Preparation of Solution and Reagents

3.3.3.1:Tris-HCl Solution

This solution was prepared by dissolving 12 gm of Tris-HCl in a small amount of D.W, and then completed up to the one litter with D.W. Then, pH was adjustedto 7 by using HCl (0.1 N). This solution was used to prepare polyethylene glycol (PEG) solution (Johnston and Thorpe, 1982).

3.3.3.2 : Staining solution:

It was prepared by dissolving 1 gm of commassie brilliant blue in 1 liter of the following solution :

Methanol 500ml(50% v/v), Glacial acetic acid10ml (10% v/v).40 ml (40%) H_2O. And the solution was stirred for 3-4 hr and then filter through Whatman filterpaper. Stored at room temperature. The using of staining solutions is to know the protein concentration and protein molecular weight

3.3.3.3 : Preparation 10% separating gel

It was prepared by adding 4 ml D.W , 3.3 ml Acrylamid /Bis acrylamid ,2.5mlof 1.5M Tris HCl(8.8), 0.1 ml 10 % SDS , 0.1 ml of 10 % APS and 0.004 ml of TEMED (Total volume was 10 ml) . The using of the know the protein concentration and protein molecular weight

3.3.3.4 : Preparation stacking gel (5%)

It was prepared by adding 3.4 ml H_2O, 0.63 ml 1M Tris HCl pH(6.8) , 0.05 ml of 10% SDS , 0.83 ml Acrylamide/Bis acrylamide 30%, 0.05 ml 10% APS and 0.005 ml TEMED (Total volume 5 ml). The using of stacking gel to know the protein concentration and protein molecular weight

3.3.3.5 : Formalin solution (10%)

This solution was prepared at 10% concentration by adding 250 ml formaldehyde (H-CHO) to 750 ml D.W. This solution was used for preservation and fixation of animal tissues (Mohan, 2007).

3.3.3.6 : Gram stain

Gram stain solution was supplied from Syrbio company. The solution was used to study Gram stain reaction and their arrangement, the find out the negative or positive bacteria of the gram stain (Forbes *et al*., 2007).

3.3.3.7 : Gemsa's stain

This stain was provided by manufactory company (Syrbio) and it was used in staining red bone marrow cells in mitotic index test (Allen *et al*., 1977).

3.3.3.8 : Hematoxylin and Eosin stains

These stains were used in the routine staining technique for histopathological examination (Mohan, 2007) .

3.3.3.9 :Phosphate Buffer Saline

Phosphate buffer saline (PBS) were prepared by dissolving one tablet in 100 ml of distilled water and then sterilized by autoclaving and then pH was adjusted to 7.2 This solution was used for washing bacterial cell and bone narrow cell .

3.3.3.10 :Fixative Solution

This solution was freshly prepared by mixing 3 volumes of methanol absolute solution with 1 volume of Glacial acetic acid . This solution was used in mitotic index test (Allen *et al*., 1977).

3.3.3.11 :Colchicine Solution

It was prepared by dissolving 1 tablet (1mg) of colchicine in 1 ml of sterilized phosphate buffer saline . This solution was used immediately in injection of rabbits intraperitoneal before one and half hour of anatomizing to stop bone cell division for mitotic index test .

3.3.3.12 :Potassium Chloride Solution (KCl)

This solution was prepared by dissolving 5.57 gm of KCL in small amount of distilled water and complete volume to 1000 ml and then it was sterilized by autoclave and stored in the refrigerator at 4C until use . This solution was used to inflate and enlarge the cells in mitotic index test .

3.3.3.13 :Polyethylene glycol solution (PEG)

The PEG solution was prepared by dissolving 6 gm of PEG with a molecular weight of 6000 in small amount of Tris-HCl solution and complete volume to

1000 ml and then pH was adjusted to 7.4. This solution was used to separate the immune globulins from the appendix (Johnston and Thorpe, 1982).

3.3.3.14 :McFarland's Turbidity Standard

The fresh 0.5 McFarland's standard (1.5x 10^8 cells/ml) was prepared by adding 0.5 ml of 1.175% barium chloride [BaCl2.2H 2O: H2O (W/V, 1.175g : 98.825 ml)] to 1% sulfuric acid [H2SO4 : H2O (V/V, 1ml : 99ml)] in order to obtain a barium sulfate precipitate. This solution was used to visually compare the turbidity of a suspension of bacteria with the turbidity of the 1.5x 10^8 cells/ml McFarland's standard . The accuracy of a prepared 0.5 McFarland's standard was checked by using a spectrophotometer. The optical density was measured at 625 nm that should be between 0,08 and 0.1 (Murray *et al*,. 2003) .

3.3.3.15 :Sodium Hydroxide (NaOH) Solution (0.2M)

Sodium hydroxide (0.2M) was prepared by dissolving 0.8gm of NaOH up to 100 ml of distilled water; this solution was used to adjust the pH during pH optimization step .

3.3.3.16 :Magnesium Nitrate Hexahydrate Solution (0.1M)

This solution was prepared by dissolving 2.564 gm of [Mg(NO3)2 ·6H2O] up to 100 ml of distilled water.

3.3.4 : Bacterial synthesis of MgO NPs

The MgO NPs were manufactured from 86 isolates and then UV-spectrophotometer examined at the wavelengths (300-350-400-450-500-550-600-650-700-750-800-850-900) nm, and the isolates *Leuconostoc spp* that gave the highest reading were selected, according to the work method below (Mohanasrinivasan *et al*., 2018):

1- The bacteria were grown in N.B and incubated at 37 °C for 24 hr.

2- The bacterial cultures were then further diluted with sterilized uninoculated N.B in the ratio 1:3. Post dilution.

3- 0.1 M magnesium nitrate [Mg(NO3)2 ·6H2O] was added to each

diluted culture, followed by drop wise addition of 0.2 M NaOH, to delay the process of transformation.

4-The cultures were then kept in a water bath at 40 °C for 15–20 min for the white colored precipitate to settle down

5- The cultures were finally incubated undisturbed at room temperature for 10 hr.

6- Post 10 hr. of incubation, the cultures were centrifuged at 5000 RPM for 15 min.

7- The supernatant was carefully discarded and the pellet was washed twice with distilled water.

8- The nanoparticles were then carefully dried and obtained in the powder form.

9- The obtained nanoparticles were in the hydroxide form which were converted to oxide form by calcinating the nanoparticles at 300 °C for 4hr.

3.3.5 :Characterization of Biosynthesized MgO NPs

The physical characteristics of biosynthesis nanoparticles were characterized by UV visible, SEM- EDX, XRD, and FTIR (Ali *et al.*, 2020).

3.3.5.1 :UV-Visible Spectroscopy

The optical properties of MgO nanoparticles have been recorded by absorption spectrum in the UV visible wavelength range of 400-900 nm.

3.3.5.2 :X-ray Diffraction (XRD) Analysis

The phase purity and crystallinity of the MgO NPs were determined by X-ray diffraction (XRD) recorded on a X-ray diffractometer system

3.3.5.3 :Scanning Electron Microscope (SEM) Analysis

The surface morphology and the size of MgO NPs in the composite films were estimated by SEM.

3.3.5.4 :Energy Dispersive X-Ray Spectroscopy (EDS) Analysis

An elemental analysis of the MgO NPs was performed using energy dispersive x-ray spectroscopy (EDS).

3.3.5.5 :Fourier Transform Infrared Spectroscopy (FT-IR)

The interaction between protein and MgO nanoparticles was analyzed by Fourier transform infrared (FTIR) analysis . In FTIR the vibration of chemical bonds can be measured because chemical bonds can absorb infrared energy at specific frequencies or wavelength. The basic structure of the compound can be determined by spectral location of their IR absorption. It can also state about other molecules being associated on the surface of nanoparticle and thus predicts possible interaction of nanoparticles with other molecules. The FTIR range of the dried sample was documented in (IRprestige-21,SHIMADZU) the range 400-4000 cm-1 (Sadhasivam *et al.*, 2010). .

3.3.5.6 : Atomic Force Microscopy (AFM)

Atomic force microscopic examination allows identifying the plot topographies representing the surface elevation and the structure of the surface.

3.3.6 :Optimization Conditions for MgO NPs Synthesis:

The effect of various physicochemical parameter such as pH and Magnesium nitrate hexahydrate concentration were study to determine the optimum growth condition of MgO NPs Synthesis (Sushma *et al*., 2016; Mohanasrinivasan *et al.*, 2018).

3.3.6.1 : Effect of pH

An experiment was designed for the detection of the optimum pH for the synthesis of MgO NPs, pH (6, 8, 10, 12,14) was adjusted in a series of 6tubes containing 10ml of bacteria broth and Magnesium nitrate hexahydrate. Allthe tubes were incubated in a shaking incubator at 40C for 20min. Then media were incubated at room temperature for 10hr , then centrifuged to sediment the MgO NPs and washed twice with DW, and examined in UV-spectrophotometer.

3.3.6.2 : Effect of the Magnesium Nitrate Hexahydrate Concentration

An experiment was designed for the detection of the optimum concentration of the substrate for biosynthesis of MgO NPs. Two concentrations (1 and 0.1) M from Magnesium nitrate hexahydrate were examined to determine the optimum concentration of salt for MgO NPs biosynthesis. The optimum pH was used, all the tubes were incubated in a shaking incubator at 40 °C for 20min. Media then were incubated at room temperature for 10hr, then centrifuged to sediment the MgO NPs and washed twice with DW, and examined in UV -spectrophotometer.

3.3.7 :MgO NPs Antibacterial Activity *in vitro*

3.3.7.1 :Microorganisms and Media

One bacterial isolates were selected and maintained on nutrient agar. To be stored at 4 ° C. Use it for the most productive isolation and the best properties.

3.3.7.2 :Culture Preparation

A loop full of growth on a nutrient agar of bacterial isolates was transported separately to (5 ml) of BHI broth and was incubated at 37° C for (24hr). The turbidity has been calibrated to suit as normal for (1.5 X 10^8 cells/ml) .

3.3.7.3 :Minimal Inhibitory and Minimal Bactericidal Concentration (MIC and MBC) Test of MgO NPs

The method for calculating the antibacterial activity of MgO NPs was evaluated by determining the (MIC&MBC).MIC and MBC were measured using a dilution process, then within 24 hr. the influence on cell viability was assessed. Bacterial isolates were grown-up in nutrient broth media and serial dilution of diverse concentrations of MgO NPs (500,250,125,62.5,31.2,15.6 µg /ml) were applied to the tubes during their log phase (3-4) hr. after incubation 24hr at 37 ° C and measurements of (optical density) were occupied at (600nm) to track the bacterial concentration. MIC It can be determined from the broth dilution of tests by sub culturing to agar plates that do not contain the test agent. The MBC is recognized by determining the lowest concentration of antibacterial agent that decreases the feasibilityof the initial bacterial inoculum by a pre-determined reduction . Modified from (Krishnan *et al.*, 2015).

3.3.7.4 :Antibacterial Activity Test of MgO NPs by Agar Well Diffusion Method

In this study the customary inhibition diameters were castoff as prescribed (Okoli and Iroegbu., 2004; and CLSI, 2021).

1. The inoculums used in this study were set by addition (one) isolated colonies grown up on a nutrient agar plate to 5 ml of usual sterile saline matched to the standard McFarland tube (1.5 X 10^8 cells/ml).

2. The sterile swab was about to extract inocula as of the suspension of the bacteria. These inocula were streaked on the (MHA) plate then left-hand to dry.

3. Drilling was done using a cork borer to the medium size 6 mm, then nanoparticlesMgO were added

4. MgO NPs(MIC) concentration were inserted into every well and permitted to position at room temperature for (1hr) to disperse to medium before incubation at 37 ° C for (24hr).

5. Zones of inhibition were assessed by a ruler and matched to the inhibition zones identified to determine the resistance or sensitivity of the bacteria to MgO NPs.

3.3.7.5 :Combination Effect of MgO NPs and Antibiotics using Agar Well Diffusion Method

1- The (0.1ml) of standard inoculums (1.5 X 10^8 cells/ml) of the bacterial isolated test was streaked with a sterile swab on (MHA) and allowed to dry.

2- there's 6 mm diameter wells were drilled using cork borers In the MHA, then (MgO NPs125µg/ml +CIP5mg/ml),(MgO NPs125 µg/ml +GN 10mg/ml were inserted into every well and permitted to position at room temperature for (1hr) to disperse to medium before incubation at 37 ° C for (24hr).

3- The diameter of inhibition zone was determined to nearest mm by a simple ruler. (Okoli and Iroegbu., 2004; and CLSI, 2021).

3.3.8 :Antibiofilm Activity of MgO NPs

The micro titer plate method was used for *in vitro* antibiofilm activity, modified from (Haghshenas *et al*,. 2016)

1- Isolates from fresh agar plates were inoculated in TSB containing 1% glucose and incubated for 18 hour at 37° C and then diluted 1:100 with fresh TSB.

2- The 100 µl of bacterial suspension with OD630=0.01 was poured into the 96-well microplate.

3- This microplate was placed in incubator with a temperature of 37 ° C . for 90 minutes.

4- The wells were rinsed three times with 100µl of phosphate buffer saline (pH: 7.4) for removal of non-attached cells.

5- Then 100µl of TSB was added to the wells and the microplate placed in the incubator at 37 C° for 24 hr.

6- After 24 hours,the biofilm was rinsed with 100µl of phosphate buffer saline (pH: 7.4). Then 125 µg/ml concentrations of MgO NPs with100µl of TSB were added to the wells and the micro plate incubated for 24hr.

7- After incubation content of each well was gently removed by tapping the plates. The wells were washed four times with phosphate buffer saline (pH 7.4)

8- Biofilms formed by adherent sessile‘ organisms in plate were fixed by placing in oven at 37° C for 30min

9- All wells stained with crystal violet (0.1% w/v). Excess stain was rinsed off by thorough washing with deionized water and plates were kept for drying.

10- 100µl of acetone/ethanol (20:80, v/v) mixture were added to dissolve bounded crystal violet. The optical density (O.D.) at 630nm were recorded.

3.3.9 :Outer membrane protein (OMPs) preparation

The Method was used (Carlone *et. al.*,1986) as follows:

1- A pure culture from *Salmonella typhi* bacteria was prepared on Xylose-lysine deoxycholate agar medium.

2- By convey loop 2-4 colonies were transported in a tube containing 10 mL of sterile brain heart infusion broth.

3- The tube was incubated at 37 ° C in the shaker 100 rpm for 18-24 hr.

4- 10 ml of bacterial suspended were taken and centrifuged at 10,000 rpm for 10 minutes at 4 ° C

5- The precipitate was suspended at 1.5 ml of cooled Darys Hepes

solution)N-2 ethylpiperazine N2- ethanesaulfonic acid C8H18N2O4S) Molecular weight (238.31) Sigma Company.

6- Suspended were centrifuged at 18,000 rpm for 2 minutes at 4 ° C.

7- The precipitate was taken and suspended with 0.5 ml a normal saline by vortex 100 rpm for 3-5 minute and centrifuged at a strength of 10,000 rpm for 10 minutesat 4 ° C.

8- The precipitate was taken and suspended with 0.5 ml of the cooled Hepse solution by the vortex , then the tubes were placed in a container containing ice cubes and then placed in the ultrasonic machine for 30 min and intermittent periods (every 10 min) to break the contents of the suspention.

9- After the cracking process in the ultrasonic device, the centrifuge was stuck to at a strength 10,000 rpm for 10 min at a temperature of 4 ° C.

10- The clarifier or leachate was taken and the precipitate is discarded. It was placed in sterile eppendrof tubes and centrifuged with a force of 10,000 rpm for 60 min

11- 1 ml of 2% Triton X100 is applied to the precipitate, then suspended by the vortex 100 rpm for 3-5 min hr and incubated at room temperature for 30 minutes.

12- After the incubation period, a centrifugation of 10,000 rpm was performed for 60 min at 4 ° C.

13- The precipitate was suspended by 0.5 ml of normal saline to be ready for use.

3.3.10 :Determination of Molecular Weight by SDS- page

1- The solution containing 10% of acrylamide was prepared for the resolving gel

2- The acrylamide solution was poured into the gap between the glass plates.

3- After polymerization is complete (30 min) , the top of the gel was washed several time with deionized water to remove any unpolymerized acrylamide .

5- The solution containing 5% of acrylamide was prepared for the stacking gel

6- The stacking gel solution was poured directly onto the surface of the polymerized resolving gel . Immediately a clean comb was inserted into stacking gel solution then the gel was placed in a vertical position at room temperature.

7- After polymerization is complete (30 min) , the comb was removed carefully . The wells was washed immediately with deionized water to remove any un polymerized acrylamide .

8- The volume of 15 µl of each of the samples was loaded in a predetermined order into the bottom of the wells .

9- The electrophoresis apparatus was attached to an electric power supply run with 150V for 1 h and 20 min.

10- The gel could be fixed and staining with silver nitrate or by comassi birllint blue stain (Green and Sambrook, 2012).

3.3.11 : Determination of Protein Concentration

Outer membrane proteins concentration was determined by using mindray biochemical system apparatus.

3.4 : Preparation of standard curve

1- A stock solution of bovine serum albumin was prepared by dissolving 0.2 mg of BSA substrate in 100ml of D.W

2- Serial dilution was prepared of bovine serum albumin and it is concentration was(0, 6 ,12, 24, 36, 42) μL

3- The optical density (OD) was measured of the serial concentration of (BSA)

4-A graph was made by plotting the concentration of BSA in the x- axis and it is optical density (OD) was plotting on the y- axis . Then best fit curve was drawn through the point on the graph as in figure (3-2).

5- The straight line equation was formed to find protein concentration of the sample .

3.4.1 :Assay procedure :

1. The spectrophotometer apparatus was adjusted on the wavelength 595 nm
2. Blank was prepared by adding 2ml of commassie brilliant blue G- 250 solution to the test tube
3. Protein sample 0.5 ml was added to test tube , and then commassie brilliant blue G-250 solution 1.5 ml was added to the sample . After that the mixed solution was left at room temperature for 5 min .
4. The optical density (O D) of the mixed solution was measured , and then (OD) was applied in the straight line equation to find protein concentration in sample ,

$$Y = 0.0152X + 0.04 \qquad R^2 = 0.9995$$

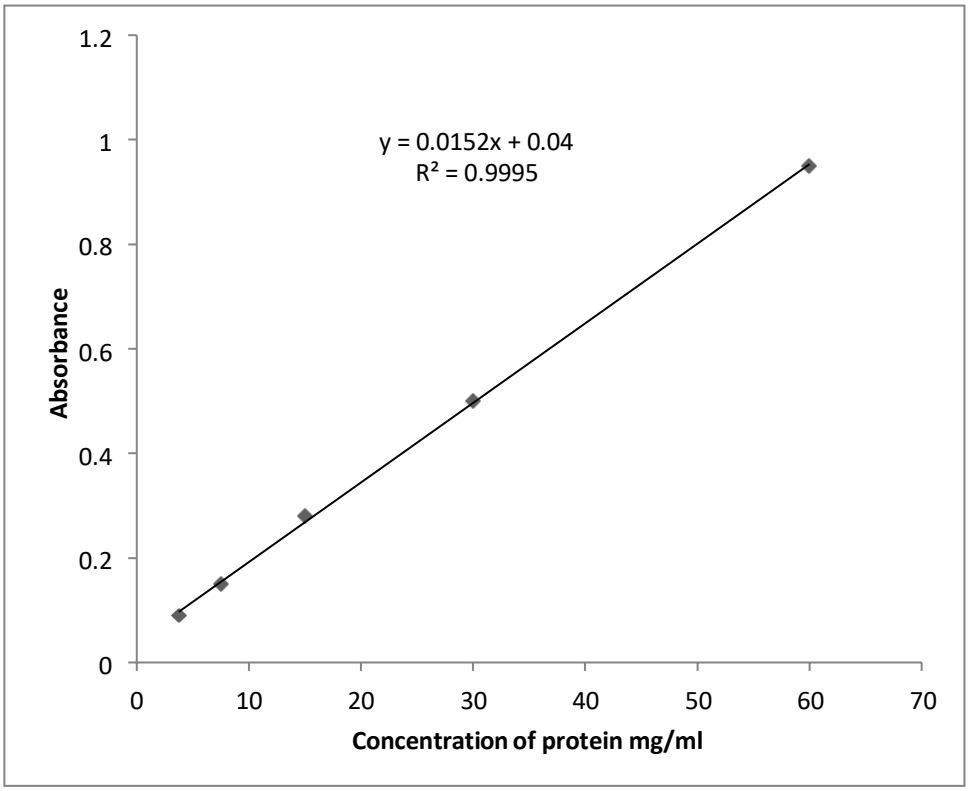


Figure (3-2) : Standard curve of protein concentration

3.5: Lab Animals

12 adult males newzland rabbits (*Oryctylagus conniculus*) at 3-5 monthsand weight 1-1.5 kg. were used to detect immune response for antigen. It were kept in cages specialized for animals in laboratory animal house and left there for two weeks for adaptation with consideration of use clean food and water for animals during the period of experiments (Schnider *et al.*,1990).

3.6 : Immunological Study

3.6.1 : MgO NPs Solution Preparation

MgO NPs was prepared from nanoparticles at 4mg/ml concentration, where 4mg of the nanoparticles were dissolved in one ml of deionized distilled water and they were mixed well with vortex, then placed in an sonicator Ultra Sonic Bath and then filtered with a micro filter (Naghsh and Kazemi, 2014).

3.6.2 : Experiment Design and Injection Method

Table(3-5): The Immunization Program .

Type of antigen		**Groups of animal MgO NPs**	**Groups of animal outer membrane**	**Mixed antigen (MgO NPs + outer membrane)**	**Groups of animal control (Normal saline)**
No. of animal		3	3	3	3
Dose	**First week**	0.5ml Ag with 0.5ml of oil	0.5ml Ag with 0.5ml of oil	0.5ml Ag with 0.5ml of oil	1m Normal saline
	Second Week	1ml Antigen 4 mg /ml concentration	1ml Antigen 1.5 µg /ml concentration	1ml (MgONPs con. 4 mg /ml + outer membrane con. 1.5 µg /ml)	1m Normal saline
	Third week	1ml 4 mg/ml	1ml 1.5 µg /ml	1ml (MgO NPs con. 4 mg /ml + outer membrane con. 1.5 µg /ml	1m Normal saline
Administrations method		0.25 ml right intramuscular and Ag injection right	0.25 ml right intramuscular	0.25 ml right intramuscular	0.25 ml right intramuscular
		0.25 ml left intramuscular and Ag injection left	0.25 ml left intramuscular	0.25 ml left intramuscular	0.25 ml left intramuscular
		0.5 ml subcutaneous	0.5 ml subcutaneous	0.5 ml subcutaneous	0.5 ml subcutaneous

3.6.3 : Immunization Program:

A method (Al-Qas,2000) was used in the immunization program, where 12 rabbits were used in the experiment, where 3 rabbits were injected with MgO NPs and the concentration was 4 mg /ml and an amount of 1 ml per Kg of rabbit weight. and also 3 rabbits were injected with outer membrance that concentration was 1.5 µg /ml , where 3 rabbits were mixed injected MgO NPs with outer membrane the

concentration (4mg/ml+1.5 µg /ml) The control group injected 3 rabbits with normal saline, and the duration of the injection lasted 3 weeks, one dose per week. The animals were dissected after being anesthetized with chloroform, blood was drawn directly from the heart (heart puncture) and placed in test tubes with anticoagulant and in tubes without anticoagulant to obtain the serum, and the appendix was takento separate the immunoglobulins and the spleen for spleen weight index test and also extracted from the thigh bone for a cell division coefficient test (Frei,1995).

3.6.4 :Immunological Study

The design of the immunological study was done about the following figure(3-3):-

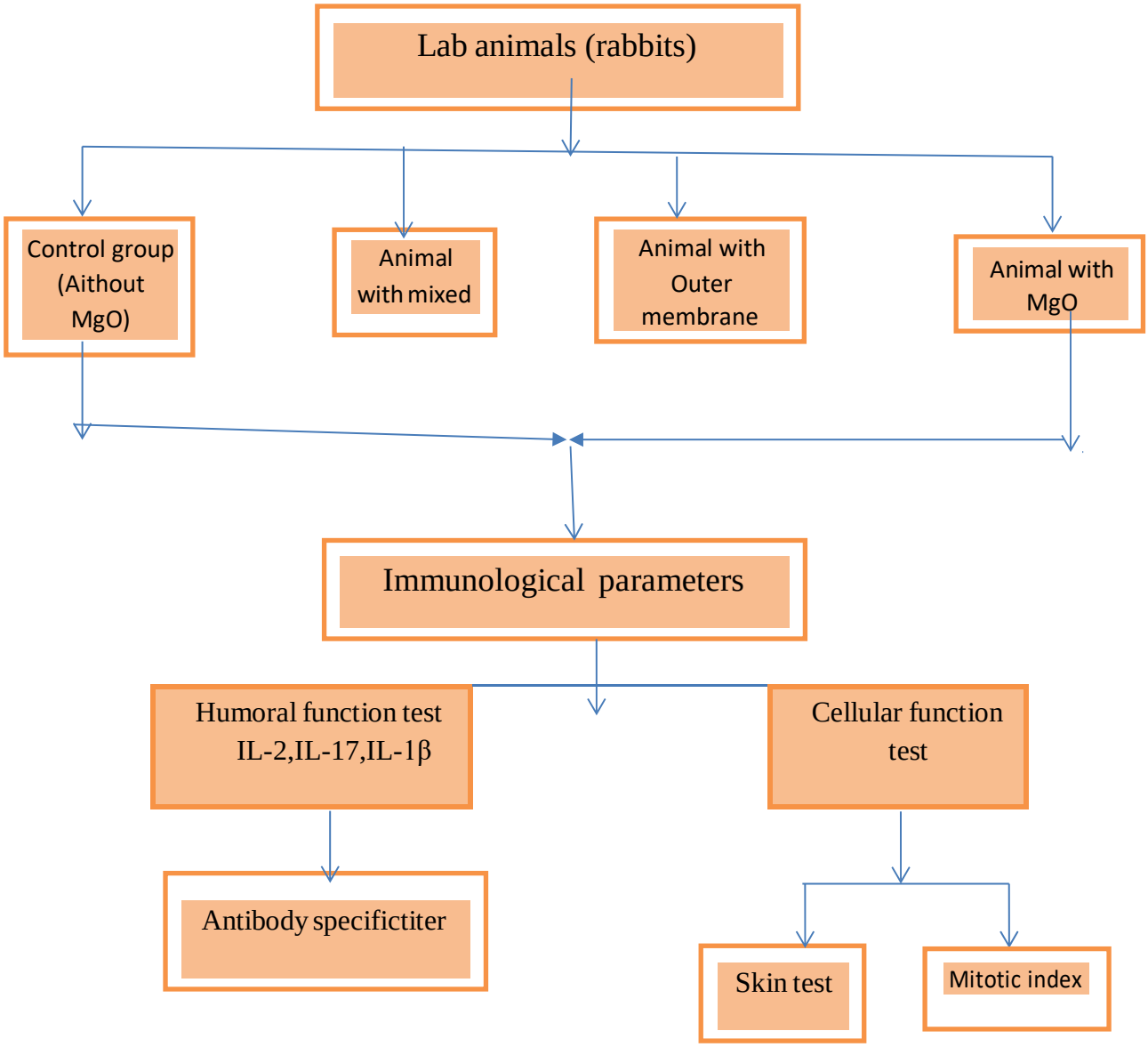


Figure (3.3): Immunological study design

3.6.5 : Immunological Tests

3.6.5.1 : secretory immunoglobulin extraction from appendix

The secretory immunoglobulin was extraction from appendix as the following: (Shnawa and Abid , 2005)

1- The appendix was placed in a petri dish with normal saline and opened lengthwise with scissors and washed with saline.

2- Sweep the mucous layer and suspend with 10ml of normal saline , centrifuge at 3500 rpm/min for 30 min.

3- The supernatant is taken and mixed with an equal volume of PEG 6% and left at room temperature for 30 minutes and then centrifuged at 3500 rpm / min for 30 min.

4- The supernatant was discarded then the precipitate was suspened with 1 ml of normal saline.

3.6.5.2 : Agglutination Test for Serum

This test based on (Garvey *et al.*, 1977).

1- Ten clean and sterile glass tubes were used, and 0.9 ml of normal saline was placed in the first tube and 0.5 ml in the remaining eight tubes by using clean and sterile mechanical pipettes.

2- The volume of 0.1 ml of serum was added to the first tube and mixed well using clean, sterile pipettes.

3- A volume of 0.5 ml was transferred from the first tube to the second tube and from there to the next tube after mixing well each time and so for tube number 9 where it is transferred from a volume of 0.5 ml and discarded, to be the dilution sequence 1:10, 1:20, 1:40, 1:80, 1:160, 1:320, 1:640, 1:1280 , 1:2560 (Leave tube 10 as control tube).

4- A volume of 0.5 ml MgO NPs (according to required concentration of 4mg/ml) was added to each of the ten tubes.

5- A volume of 0.5 ml outer membrane(according to required concentration of 1.5mg/ml)was added to each of the ten tubes.

6- A volume of 0.5 ml mixed (according to required concentration of 4+1.5 mg/ml)was added to each of the ten tubes.

7- A volume of 0.5 ml control (according to required concentration of zero)was added to each of the ten tubes.

8- The tubes were well mixed and incubated at 37 ° C for 24 hr.

9- The results were read through a cloudy mass indicating the interaction of the antibody with the antigen .

10- The results were recorded by determining the titer which means (inverted the highest dilution gave a positive result) .

3.6.5.3 : Agglutination Test for Secretory Immunoglobulin

This test based on (Shnawa and Saadi,2002).

1- Ten clean and sterile glass tubes were used, and 0.2 ml of normal saline was placed in the first tube and 0.5 ml in the remaining nine tubes by using clean and sterile mechanical pipettes.

2- The volume of 0.2 ml of antibody suspension was added to the first tube and mixed well using clean, sterile pipettes.

3- A volume of 0.2 ml was transferred from the first tube to the second tube and from there to the next tube after mixing well each time and so for tube number 9 where it is transferred from a volume of 0.2 ml and discarded, to be the dilution

sequence 1:1, 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128, 1:256 (Leave tube 10 as control tube).

4- A volume of 0.2 ml MgO NPs (according to required concentration of 4mg/ml) was added to each of the ten tubes.

5- A volume of 0.5 ml outer membrane(according to required concentration of 1.5 mg/ml)was added to each of the ten tubes.

6- A volume of 0.5 ml mixed (according to required concentration of 4 mg/ml/1.5mg/ml)was added to each of the ten tubes.

7- A volume of 0.5 ml control (according to required concentration of zero)was added to each of the ten tubes.

8- The tubes were well mixed and incubated at 37 ° C for 24 hr.

9- The results were read through a cloudy mass indicating the interaction of the antibody with the antigen .

10- The results were recorded by determining the titer which means (inverted the highest dilution gave a positive result) .

3.6.5.4 : Mitotic Index Test

The testing was done according to (Allen *et al.*, 1977):

1. The animals were injected with 1ml of colchicine intra peritoneal and left for one hr and a half.

2. Anesthetizing the animal with chloroform and placing it on its back and dissecting it by cutting the skin under the abdomen and pulling the skin to show internal guts.

3. The femur was cut at both ends, and its cellular contents were collected in a test tube using normal saline solution (5 mL), with the help of a disposable wooden stick.

4. The cell suspended was gently pipette with a Pasteur pipette, and the tube was centrifuged (2000 rpm) for 10 min.

5. The supernatant was waste, the cell deposition was suspended in 5ml of PBS and then the tube was incubated in a water bath (37 ° C) for 30 min with gentle shaking every 5 minutes.

6. The tube was centrifuged at 2000 rpm for 10 minutes. and supenatant was neglected.

7. Each tube was add 5ml of fixation solution drop wise to the cell deposition with gentle continuous mixing to make a homogeneous cell suspension, then incubate the tube in the refrigerator (4 ° C) for 30 min.

8. The tube was centrifuged (2000 rpm) for 10 min, and the last step was repeated twice.

9. The cell deposit was suspended well in 2 ml of fixative solution and 4-5 drops of the cell suspension were dropped onto a clean slide from a height of about 2 feet.

10. The slide was dried with air at room temperature, then stained with Gimesa stain for 15 min and washed with distilled water.

11. The slide was examined under an oil lens (100 ×) and at least 1000 cells were examined for mitotic index.

The percentage of dividing cells was recorded using:

The Mitotic index (%) = number of dividing cells/total count x 100

3.6.5.5 : Spleen Weight Index

Collins *et al*., (1975) method was used to calculate the spleen weight index as follows:

1- Animals were anesthetized with chloroform and dissected for spleen extraction.

2- Removed the connective tissue from the spleen and placed it in a sterile petri dish containing PBS.

3- Dried the spleen on sterile blotting paper, then weight the spleen to calculate the spleen weight index, which is expressed as the percentage of its weight to body weight according to the following law:

$$\text{Spleen weight index} = \sqrt{\frac{spleen\ weight}{animal\ weight}} \times 100$$

3.6.5.6 : Skin Test

This test was conducted in the fourth week, when each group of injected animals was injected with 0.1ml MgO NPs of under or between layers of the skin. The animals was injected with 0.1ml outer membrane of under or between layers of the skin, The animals was injected with 0.1ml mixed of under or between layers of the skin, As for the control animals, they were injected with normal saline and the same amount, and recording the observed skin change after (4,24,48,72) hr. in comparison with control animals. Use this test to find out the effect of MgO NPs,outer membrane and mixed on sensitivity and their effect onstimulating the cellular response of rabbits.(Tompkins *et al*., 1973).

3.7 : Interleukin (1β, 2 and17) Assay Procedure

The following procedures were performed at room temperature according to manufacturer's instructions (Elabscience –china).

1. **Add Sample:** The volume100 µl of standard ,blank or sample was added per well ,the blank well was added with reference standard and sample diluent ,solutions ware added to the bottom of micro ELISA plate well

,avoided inside wall touching and foaming as possible . gently mixed . The plate was covered with provided sealer then incubated for 90 minutes at 37 °C.

2. **Biotinylated Detection Ab**: The liquid of each well was removed ,don't wash . Immediately added100 µl of biotinylated detection Ab working solution to each well . The plate was covered with sealer then incubated for 1 hr at37 °C.
3. **Wash:** Each well was aspirated and repeating the process three times . Wash by filling each well with wash buffer . complete removal of liquid at each step is essential . After the last wash, removed remained wash Buffer by aspirating or decanting . Invert the plate and pat it against thick clean absorbent paper.
4. **HRP Conjugate** : The volume of 100 µl of HRP Conjugate working solution was added to each well. The plate was covered with sealer then incubated for 60 min at 37 °C .
5. **Wash:** The wash process was repeated for five times as conducted in step 3.
6. **Substrate:** The volume of 90 µl of substrate solution was added to each well. The plate was covered with new sealer then incubated for about 15 min at 37 ° C protect the plate from light . The reaction time could be shortened or extended according to the actual color change , but not more than 30 min . when apparent gradient appeared in standard wells user should terminate the reaction.
7. **Stop:** The volume of 50 µl of stop solution was added to each well . Then ,the color turns to yellow immediately .The order of added stop solution should be the same as the substrate solution.
8. **Optical Density measurement:** The optical density (OD value) of each well was determined at once , using a micro-plate reader set to 450 nm . User

should be opened the micro-plate reader in advance , preheat the instrument, and set the testing parameters, as showed in figures (3-4) (3-5) and(3-6).

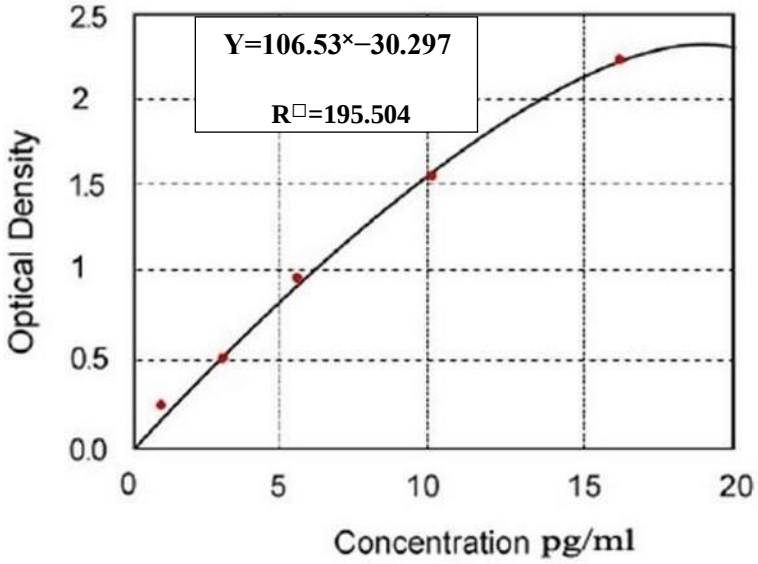


Figure (3-4): Standard curve of interleukin 17

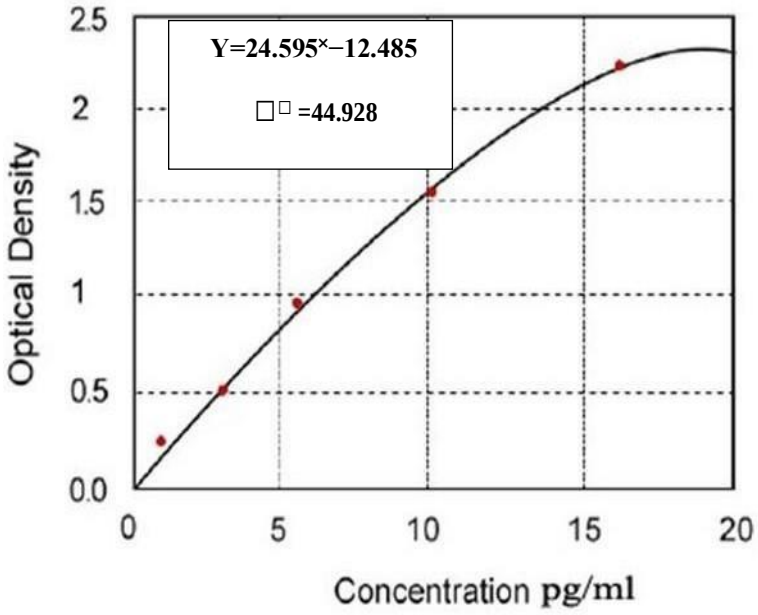


Figure (3-5): Standard curve of interleukin 1β

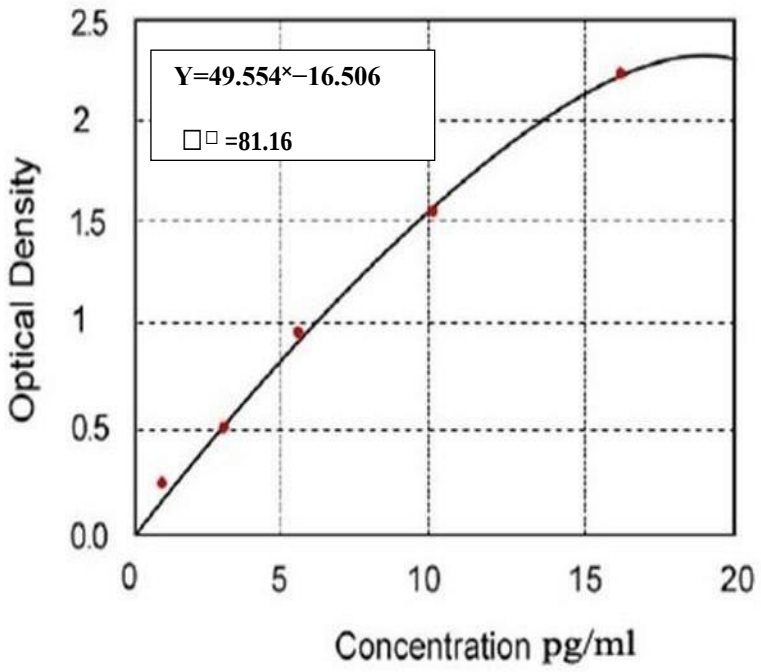


Figure (3-6): Standard curve of interleukin 2

3.8 : Histopathological Study (Tissue preparation)

Histological section preparation of immunized animals was achieved according to Mohan (2007) method, which includes the following steps In addition to knowing the effect of MgO NPs,OMP and Mixed on liver and kidney tissue

1- Fixation

Fixation the method of preserving cells and tissues in life like condition as far as possible. During fixation, tissue was fixed in complete physical and partly chemical state. Fixative process prevents putrefaction and autolysis of tissue. In addition, it hardens the tissue ,which helped in section cutting . A fixative solution should had some properties such as, it causes fixation quickly and causes minimal loss of tissue and should retain the normal color of the tissue . The percentage of 10% formal saline was the most commonly used as a fixative solution. Animal tissues (kideny and liver) were placed in the 10% formal saline for 2 hrs. or more for completion of the tissue fixation process.

2- Dehydration

This is a process in which water from cells and tissue is removed , so that this space is subsequently taken up by wax . Dehydration was carried out by passing the tissues through a series of a sending grades of alcohol of 70%, 80%, 95% and absolute ethyl alcohol . Animal tissue passed for 2hrs. in each grade of alcohol.

3- Clearing

This is the process, in which alcohol is removed from tissues and cells and is replaced by a fluid in which wax was soluble and it also was made the tissue transparent. Xylene was the most commonly used clear ingagent.Animal tissue passed for 2 hrs. in xylene jar and for three times .

4- Impregnation

This is a process in which empty spaces in the tissues and cells after removal of water are taken up by paraffin wax. This hardened the tissue which, helped in section cutting . Impregnation was done in molten paraffin wax, which has the melting point of 56 ^{o} C .Animal tissue was impregnated with molten paraffin wax for 2 hrs. and for three time.

5- Embedding and blocking

Embedding of tissue was done in molten wax. Wax blocks were prepared by using metallic L (Leukhart's mould) . The moulds were placed over a smooth surface glass tile. Molten wax was poured in the cavity of the moulds. The processed tissue piece was put into wax with number tag and examining surface facing downward, wax allowed to solidify. After solidification L moulds were removed.

6- Section cutting

Paraffin block having tissue was placed in the rotary microtome .Section was cutted by operating the microtome manually after adjustingthe thickness at 4 µm . Sections were picked from the knife with the helpof a forceps . These were made to float in a water bath which was kept ata temperature of 45 ° C . This removed folds in the section. From waterbath sections were picked on a clean glass slid .Then the glass slid wasplaced in an oven maintained at a temperature of 56 ° C for 20-30 min for proper drying and better adhesion.

7- Staining

The procedure for staining includes the following steps:

1- Sections were first deparaffinised by placing the slides in a jar of xylene for 10-15 min.

2- As haematoxylin is a water –based dye ,the sections before staining were rehydrated which was done by passing the section in a seriesof descending grades of alcohol

3- Brought the sections to water .

4- Slides were placed in a haeamatoxylin jar for 8-10

min5-Sections rinsed in water

5-Slides were placed in an alcoholic eosin jar for 3 min

6- Sections were dehydrated by passing them in a series of ascending grades of alcohol . Finally sections were cleared in xylene.

3.6: Statistical Analysis

Data were processed and analyzed with one way ANOVA using statistical program social science (SPSS 19) and the results were expressed as (Mean ± S.D). P values below 0.05 were considered to be statistically significant (George *et al.,* 2011).

4. MgO NPs Bacteria

4.1 Diagnosis of *Leuconostic spp*

Eighty six(86) bacterial isolates had been utilized that isolated from the mouth (Appendix(1). The Biosynthesis test of MgO NPs was conducted under different testing protocols on all isolates to find out the highest productive and purest of isolates. They were evaluated on 86 bacterial isolates and extracted with a UV spectrometer and a visual analysis of the powder color.

All isolates were give positive for MgONPs production. The results agreed with (Ali *et al.*,2020) The appeared of a white precipitate below thetube it means positive result Figure (4- 1)

The diagnosis of *Leuconostoc spp* carried by culture media coupled with biochemical test that are characterized by all the isolates were given negative results for catalase, and their ability to produce hemolycin was varied (α-hemolysis or non-hemolytic) and confirmation of results and the final identification was performed by Vitek-2 systems (Taher and Abed.,2020)Appendix(2)

Figure (4-1) : MgO NPs powder
White color precipitate

4.2 Biosynthesis of MgO NPs by *Leuconostoc spp*

In the present study, it had tried to explore a rapid, cost-effective, eco-friendly method for fabricating MgO-NPs using the bacterial strain *Leuconostoc spp*. The optimized biosynthesis process was investigated by pH, salt concentration , studying the effect of metal precursors, incubation temperature, and contact time. The biogenically synthesized MgO-NPs were characterized using various techniques consisting of UV-vis spectroscopy, X-Ray diffraction (XRD), scanning and energy dispersive X-Ray spectroscopy (SEM-EDX), transmission electron microscopes (TEM), and Fourier-transform infrared spectroscopy (FT–IR). The efficacy of biosynthesized MgO-NPs to inhibit the growth of different pathogenic bacterial was assessed.

Abinaya *et al*., (2021) and Rani *et al*., (2020)indicated that several chemical and physical methods have been used to fabricate MgO-NPs such as chemical precipitation, thermal decomposition, sol-gel, combustion, and chemical vapor deposition. These methods predominantly require several processing steps, controlled pH, high temperature and pressure, expensive equipment, and toxic chemicals. These techniques produce numerous by-products that may be toxic to ecosystems. Therefore, there was a need to develop a low-cost, eco-friendly method for nanoparticle synthesis. The biosynthesis of NPs hasa wide range of interest because of the reduction or elimination of toxic substances that are present in the environment from chemical and physical methods (Salem and Fouda .,2020) . Eid *et al*.,(2020) exhibited that microorganisms such as bacteria can reduce metal and their oxides to NPs. The synthesis of NPs using bacteria has numerous advantages e.g., easy to multiply, grow, handle, and downstream process for nano-biosynthesis (Samak *et al*.,2020).

Wetteland *et al.,* (2016) was reported The biosynthesis and presence of MgO peaks isolated from different bacteria in the XRD spectrum is expected, because MgO NPs are hygroscopic and they can readily react with water in the atmosphere to form MgONPs. The size distribution of MgO NPs was normal and narrow, with an averageof 23 ± 5 nm, in agreement with the previous study . The zeta potential and electrical mobility of MgO in water were 32.31 ± 4.1 mV and 1.68 ± 0.22 (×10−4 cm2 V−1 s−1), similar to what were reported in previous literature (Brown & Salt.,1956; Wetteland *et al.,* 2016).

The greatest absorption at 400 wavelengths was determined for all isolates after examination in all spectrophotometers of the UV. One of the isolates was chosen, the greatest and purest productivity of other tested isolates. This might be that these bacteria have genes that are more responsible for the production of nanoparticles than other isolates. After the addition of the alkaline solution, a solution was created for Mg (OH)2, allowing two hours to consider turning its color into brown as seen in this Figure (4-2), The production of nanoparticles was verified by the pale-yellow brown color , this result was agreed with(Mohanasrinivasan *et al*.,2018), who synthesized MgO NPs from *Lactobaillus sp.* and with Ali *et al.*, (2020), who synthesized MgO NPs from *Persimmon* extract and synthesis of MgO NPs by using *streptococcus ssp* (Jebur& Abed .,2021**)** .

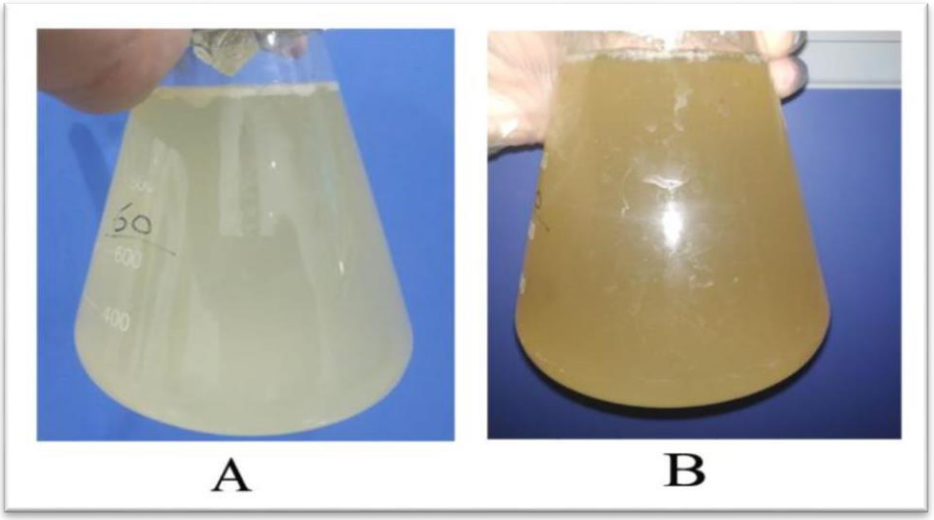


Figure (4-2) : MgO NPs by biosynthesis by bacteria method.(A) nutrient broth with bacteria (negative control)before adding (Mg(NO3)2.6H2O (B) color change after adding (Mg(NO3)2.6H2O (positive bacteria) .

(Kumar and Kumar., 2008) were reported that Many methods have been used for the preparation of MgO NPs. The morphology andsizes of MgO NPs can be controlled by adjusting the processing conditions (Selvam *et al*., 2011).

The biosynthesis methods are preferred to prepare nanoparticles for the application in the biomedical field The large-scale synthesis of nanoparticles is pre-ferred via bacteria and fungi with minimum usage of toxic chemicals, Biological Synthesis methods of NPs offers notable advantages, such as benign reaction conditions (normal atmospheric pressure, low temperatures or microwaves assisted NP synthesis), non-toxic solvents and reagents, biodegradable by products (comprised of biological entities in a water medium) and a downstream NP separation processes that is usually very simple (Geeta and Choudhury,2019) . (Aslan and Geddes, 2009) were exhibited that in order to reduce reaction time and cost, many new processes have been developed for preparing MgO NPs. The oven-assisted hydrothermal method has been attracting significant attention because it has advantages such as the short reaction time, narrow

size distribution, high purity of the prepared particles, and high yield rate of nanoparticles Moreover, it is potentially more cost effective compared to conventional synthesis methods (Nishioka *et al*., 2011; Bhatte *et al*., 2012). In the oven-assisted hydrothermal method, the precursor solution is irradiated by a oven source. The efficient energy transfer results in a rapid heating process (Moghaddam and Saeisian., 2007).

The manufacture of nanoparticles also utilizes extracellular, and intracellular biological methods. It has not yet been identified how nanoparticles are produced by biological agents. Elucidated, it was nonetheless suggested that a number of biomolecules mediate the formation of nanoparticles. Intracellular and extracellular production of nanoparticles the cell walls of microorganisms appear to play an important function. Mukherjee *et al.,* (2001) proposed that intracellular nanoparticle biosynthesis proceeds in three stages: trapping, bioreduction, and synthesizing.The fungal cell surface interacts electrostatically with metal ions, trapping them in the process, according to the authors. The metal ions are then bioreduced by enzymes in the cell wall, and nanoparticle formation occurs as a result of particle aggregation. Meanwhile, the process of extracellular nanoparticle biosynthesis is hypothesized as a nitrate reductase-mediator system.(Gold *et al*., 2018; Kirby *et al*., 2018).

4.3 : Characterization of Biosynthesize MgO NPs

4.3.1 The UV-visible Analysis

UV-VIS is the optical absorption spectrum. The absorption of MgO NPs were recorded within wavelengths of 300 – 900. From figure (4-3), it can be observed that higher absorbance at 400nm for one isolates. Pugazhendhi *et al.,* (2019)

concur with this result (Jebur and Abed ,2021; Hassan *etal.*2021**).**

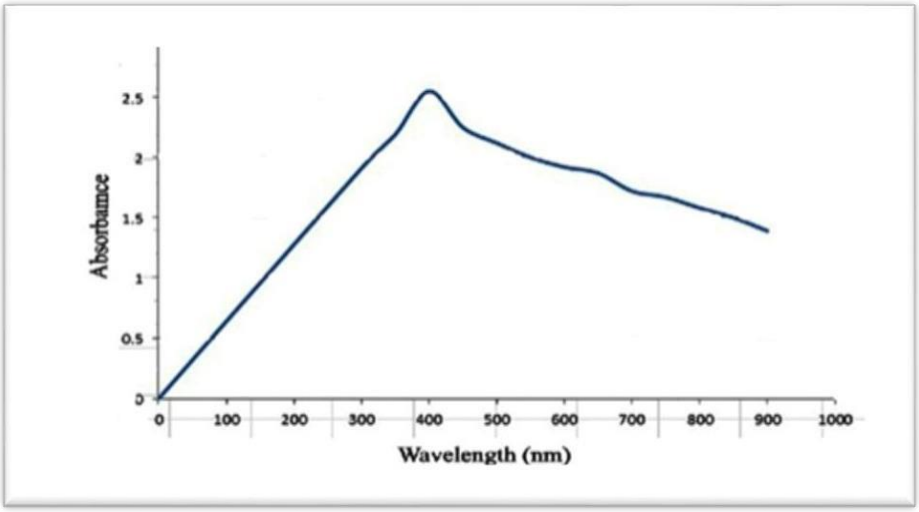


Figure (4-3): Absorbency of UV spectrophotometer of MgO NPs produced from *Leuconostoc spp*

The technique of UV-Visible spectrophotometry is generally used for the qualitative and quantitative estimation of biomolecules such as proteins , sugar , carbohydrates , amino acids , nucleic acids , vitamins , etc. the UV– visible spectroscopy has proven to be very useful for analyzing of different nanoparticles such as gold and silver nanoparticles(Thi and Yen .,2019).

4.3.2: X-Ray Diffraction Analysis (XRD)

The XRD figure(4-4) verified the MgO's cubic crystal structure. To determine the 2 peak locations, The crystalline size was also determined by using Debye Scherrer's formula D= 0.94/cos. Table(4-1) showed the crystalline values and structural characteristics of MgO NPs that were produced by bacteria . The result of the MgO NP sample that no distinctive peaks of contaminants were identified by synthesis of the *Leuconostoc spp* test indicating that NPs is pure, The peaks were (111), (200) (220) and (311) planes of the nanoparticles of MgO in 2 volume = 36.155 and 42. 42, 58.112 and 74.702. The average size of the MgO

NPs sample that synthesis by *Leuconostoc spp* was (25.94 nm).

Table (4-1):Crystalline size and structural parameters of the MgO NPs sample that synthesis by bacteria

MgO NPs synthesis by *Leuconostoc* spp				
2θ (Deg.)	FWHM (Deg.)	Crystalline size D(nm)	Hkl	Avarege of size(nm)
36.154	0.2952	28.29	111	25.94
42.42	0.3936	21.64	200	
58.112	0.246	36.92	220	
74.702	0.5904	16.92	311	

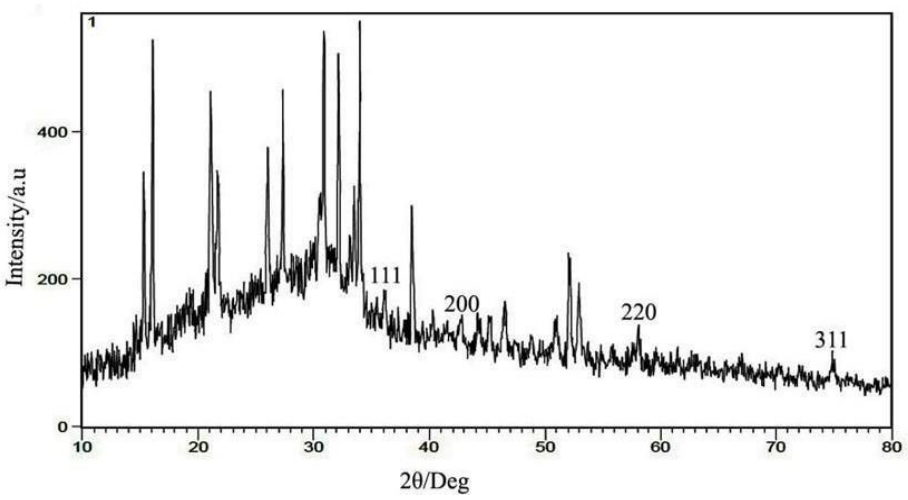


Figure (4-4): XRD patterns of MgO NPS powder:A synthesis by *Leuconostoc spp*

While other study showed MgO NPs X-ray diffractogram the characteristic peaks can be seen at 37.03, 43.01, 62.37, 74.76, and 78.67 degrees, associated to the Miller indexes (111), (200), (220), (311), and (222), respectively, which corresponds to a face-centered cubic (FCC) network (Yousefi *et al.*,2017). This value was in good agreement with previous reports about MgO NPs with similar size and crystallinity (Rodenbough *et al.*,2017). Rao *et al* (2014) was showed in the XRD spectrum of magnesium oxide sample confirmed the formation of

nanoparticles, and increased in the peak width represented a decreasein the size of nanoparticles. Also, the absence of extra peaks in the synthesized nanoparticles confirmed their high purity.

This was in agreement with Bindhu *et al*., 2016 who synthesized MgO NPs using a chemical reaction approach and found that the MgO NPs were of high purity, but not with Mohanasrinivasan *et al*., 2018 who synthesized MgO NPs using the same method from *Lactobacillus sp.* MgO NPs had a lower purity.

4.3.3. Field Emission Scanning Electron microscopy (FE-SEM)

Figure(4-5) displays a FE-SEM image showing the surface morphology in MgO nanoparticles The image analysis indicated that the *Leuconostoc spp* MgO-NPs were approximately spherical, with an average diameter of 43,88 nm of *Leuconostoc spp* MgO-NPSsynthesized

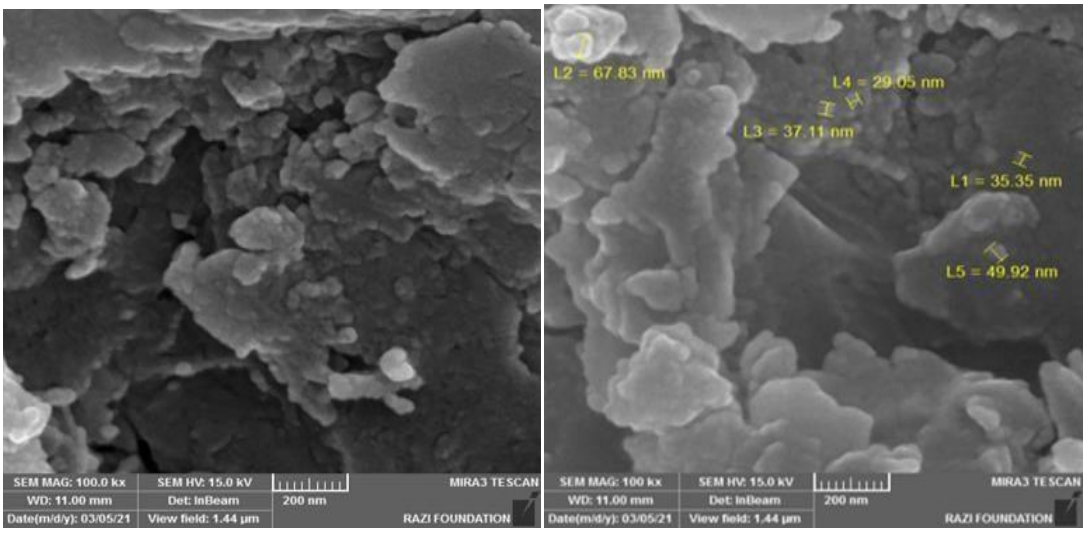


Figure (4-5) :The surface morphology of MgO NPs synthesized by *Leuconostocspp* byFE-SEM .

(Al-Salhie & Al-Kalifawi .,2020) were demonstrated that the field emission scanning electron microscopy image of MgO NPs,which exhibit flakes-like structure due to the aggregation of several thousand MgO NPs. The MgO nanoflakes are dense and interconnected with each other such

that no clear boundaries exist between one another. The sizes of MgONPs was between 29.05 nm and 67.83nm.

While other study exhibited that The field emission scanning electron microscopy image of MgO NPs, which exhibit flakes- like structure due to the aggregation of several thousand MgO NPs. The MgO nanoflakes are dense and interconnected with each other such that no clear boundaries exist between one another. The sizes of MgONPs was between 29.05 nm, and 67.83 nm. These results areacceptance with many studies (Narendhran *et al*., 2019; Umaralikhan *and* Jaffer ., 2016).

4.3.4 Energy Dispersive X-Ray Spectroscopy analysis(EDX)

Energy Dispersive X-ray Crystallography is the analytical method for chemical characterization or for elementary examination of nanoparticles (EDX). EDX analysis offers qualitative and quantitative information on the state of the elements involved in the production of nanoparticles. It was used to show how the development of MgO NPs (Dobrucka., 2018) .

(Umaralikhan and Jaffar. ,2018) demonstrated that EDX spectrum ofthe synthesized MgO NPs. The spectrum shows different peaks tagged with Mg and O correspond to Magnesium and Oxygen contents ofthe synthesized sample . Nanomaterials are found to have majority of the atoms at the surface as compare to volume. In other words, they have a 'large surface to volume ratio'. Therefore, x-ray photoelectron spectroscopy (XPS) has been used to analyze the elemental contents at the surface of the as-synthesized MgONPs(Taleatu *et al.*,2014).

The electrons are excited by atoms at the surface of the sample on the direction of the electron beam, which create rays that are typical of the atomic structure of the element (Meghana *et al*.,2015). The energy of the X-rays will be measured and evaluated so that the chemical elements

in the sample are accurately and quantitatively informational.

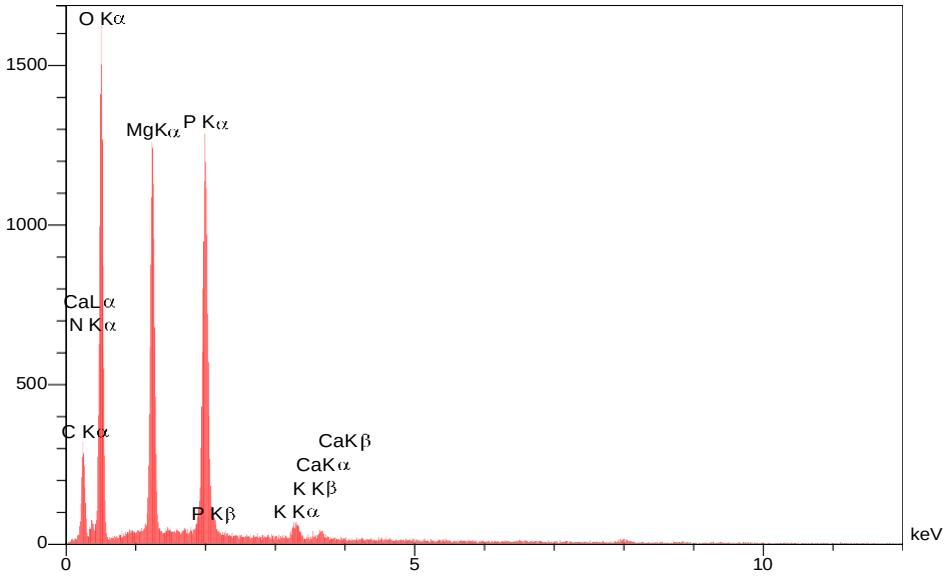


Figure (4-6) displays the EDX spectrum, which exhibits a prominent peak corresponding to Mg and O. Additional C, N, Na, Ca, and P peaks were discovered.A peak was obtained at 1.2 keV for magnesium , while an oxygen peak was obtained around 0.520 keV for MgO NPs sample that synthesis by isolate *Leuconostoc spp*.The observed strong peaks can be to check the purity of the synthesized materials by correlating the formation mechanism (Ali *et al.*,2020).

4.3.5 : Fourier Transform Infrared (FTIR)

The functional groups of isolated samples produced by MgO NPs (*Leuconostoc spp*.) were identified by fourier infraround transform spectroscopy (FTIR). Figure (4-7) shows the results of the sample FTIR analysis and their functional groups are given in the Table(4-2). Analysis (Wong *et al.*,2020 ;Nandiyanto *et al.*, 2019) of FTIR findings. The MgONPS synthesis peakings: 3851.98, 3747.24, 3270.36, 2952, 2922.88, 2853.70, 1648.70, 1456.71, 1376.95, 1073.73, 722.31and 562.77 in the MgO NPs *Leuconostoc spp*. The maximum absorbance of hydroxyl groups and hydrogen bonds is O–H, indicating alcohol in the

2952 variations of the methylene C-H range, the absorption peak in 2922.88, 2853.70, 1648.70, 1456.71, 1376.95, 1073.73, 722.31 and 462.78 ^(-1) relate to alkenyl C=C stretch, aromatic nitro compounds, methylene C-H bend, ammonium bend and ammonia bend. The absorption peak is O–H with a maximum amount of 400 ccm. A vibration of Mg–O stretching bond is attributed for the absorbance peak at 562.77 cm in the calculated sample(El-Sayyad *et al*.,2018). Following that, the formation of MgO crystallite is cubic as theabsorbance peak is present between the range of 1000 and 500 cm^{-1}(Pei *et al*., 2010).

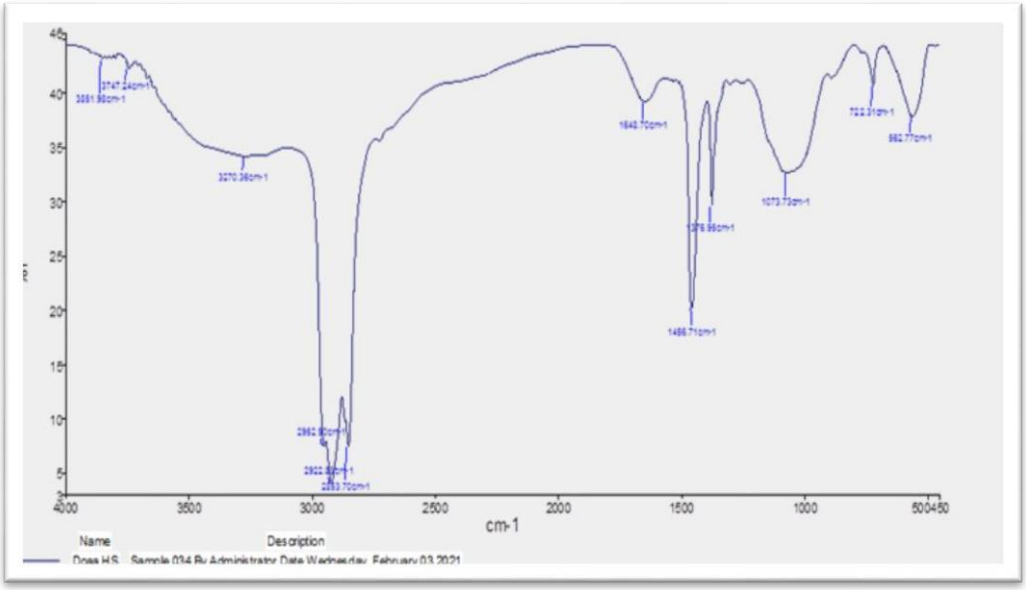


Figure (4-7): FTIR spectrum of the MgO NPs sample that synthesis by isolate: *Leuconostoc spp.*

(Essien *et al*., 2020) was exhibited The FTIR spectrum of the stretching bond at 524.6 cm−1 indicates the formation of MgO NPs . The O–H stretch appears in the spectrum is very broad band extending from 3429.2 to 3628 cm−1. The band at 2985 cm−1 confirms the presence of -OH stretching vibrations on the surface of the material. The absorption peak at 1582.4 cm−1 is attributed to H2O twisting. The peak located at 1132.5 cm−1 is associated with alkoxy stretching vibrations (Priyadarshini *et al*.,2020).

Table(4-2): Peaks ,bonding and functional group of the MgO NPs samplethat synthesis by isolate *Leuconostoc spp.*

The absorbance peak cm^{-1})	Bonding and type of vibration	Functional groups
3747.24	O–H stretch, H-bonded	Alcohol and hydroxyl compound
3270.36	C-H stretch	Methyl
2952	C-H stretch	Methylene
2922.88	C=C stretch	Alkene
2853.70	Aromatic nitro compounds	Nitrogen- oxy compounds
1648.70	C-H bend	Methyl
1456.71	Ammonium ion	Inorganic ions
1376.95	Aryl –O stretch	Aromatic ether
1073.73	C-O stretch	Cyclic ether
562.77	Mg–O vibration	Metal oxide

While other study showed The FTIR spectra of MgONPs synthesized using the different types of bacteria culture filtrate. The spectra show bands at 3369, 2930, 1629, 1367and 434 cm–1. The peak observed at 434cm – 1 represents the formation of MgONPs. This is consistent with many studies that have proven that The FTIR spectra of MgONPs around these values (Sobana *et al*, 2018; Abdel-Aziza *et al*., 2020).

4.3.6 AFM analysis

Atomic Force Microscopic inspection may identify the plot topographies depicting surface elevation and structure. This approach use digital images to assess surface characteristics quantitatively, for example as root average square roughness (Rq) and average roughness (Ra), as well as picture analysis from multiple perspectives, including 3D simulation (Al-Rasoul *et al.,* 2013).The root mean square roughness (Rq) and (Ra) of MgO NPs sample that synthesis by isolate *Leuconostoc spp* was Rq 11.82 nm and Ra 9.596 nmTheMgO NPs sample generated by isolate was identified as *Leuconostoc spp* rough of the sample based on these findings.

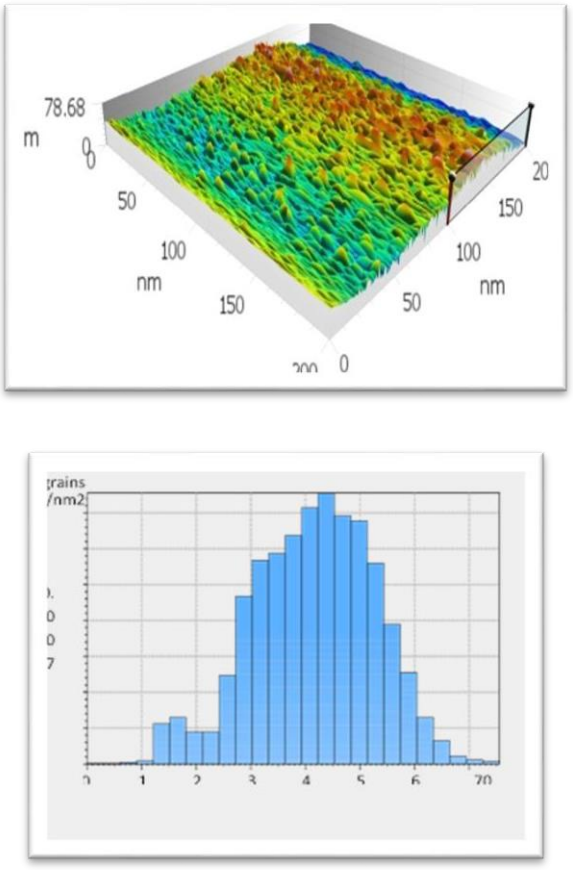


Figure (4-8):AFM images of the MgO NPs sample that synthesis by isolate *Leuconostocspp*A- three dimensions, and (B) Peak count histogram.

AFM can be used as important instrument for analyzing topography, height, sorption, structure and dispersion and agglomeration pattern of the NPs (Kumar *et al.*,2016). Many study indicated that Atomic force microscopy (AFM) analysis is a commonly used technique for the determination of the size of NPs. The size of metal NPs was observed from tip-corrected AFM measurements and the shape of MgO NPs were determined. The tip-corrected measured the size of NPs in the range of 35–95 nm, where 10% Diameter: 45.00 nm, 50% Diameter: 70.00 nm, 90% Diameter: 95.00 nm and Avg. Diameter: 73.38 nm. While The result from other study showed the 3D view of the sample surface over a 2 × 2 μm

scan and uniform height distribution around 75 nm. (Kadhem *et al*., 2019; Pugazhendhi *et al*., 2019).

4.4 : Optimization conditions of MgO NPs biosynthesis

4.4.1: Effect of pH

A varied pH for solution was utilized (6,8,10,12,14), and the yield was met by the UV spectrophotometer, PH for salt concentration from *Leconostoc spp* was seen at pH12 Figure (4-9).

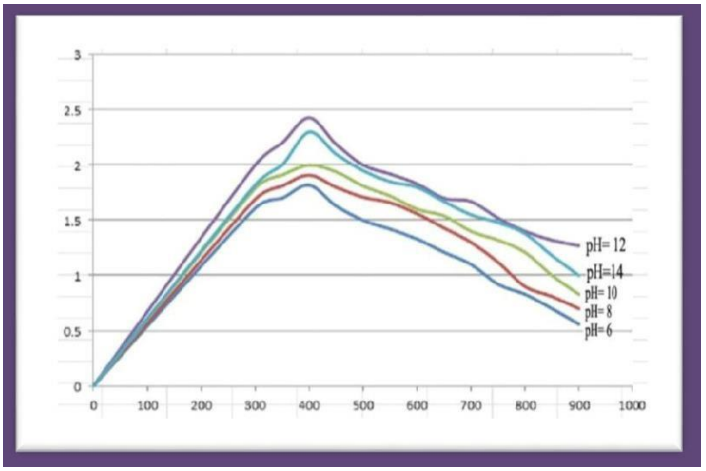


Figure (4-9): The production of MgO NPs that synthesis by isolate *Leuconostoc spp* indifferent pH.

Deepak *et al.*, (2011) examined the impact of pH on the biological production of MgO NPs. They observed that very little precipitate development took place on pH less than 10 when the pH range was adjusted from less than 10 to 12. The increased absorption at high pH (alkaline) results in severe small dispersed nanoparticles with decreased accumulation as observed in the UV visible absorption spectrum, since hydroxyl ions are present on the surface of nanoparticles with high levels of concentration.

As nucleation in nanoparticles at acidic pH is slowly, fewer large particles are created whereas at high pH there was a quick nucleation process because of the ability of -OH ions to arrive which resulted in the formation of a large number of tiny particles (Sushma *et al*., 2016). In addition, pH is considered an important factor that influences reducing

agents, which affects the green synthesis. different pH values were adjusted ranging from 6 to 11, and their impact on the reduction process was investigated after the constant of the other parameters The charges of biomolecules present in biomass filtrate can be altered due to differences in pH values and thus, the reducing capacity is affected (Verma, & Mehata ,2016; Fouda *et al.,* 2021).

4.4.2 Effect of the Magnesium Nitrate Hexahydrate Concentration

The experiment we used two MgO NP concentrations (1 and 0.1) M, and found a reduction in productivity with an increase in salt concentration of (1M) Figure(4-10). It is agreed that the extract of MgO NPs from Camellia-sinensis leaves has been synetized using 0.1 m magnesium-nitrate hexahydrates (Khan *et al*.,2020) and Mohanasrinivasan*et al.,* (2018) which has utilized 0,1 m magnesium-nitrate hexahydrates for MgO NPs from the leaves of extract of *Camellia-sinensis.*

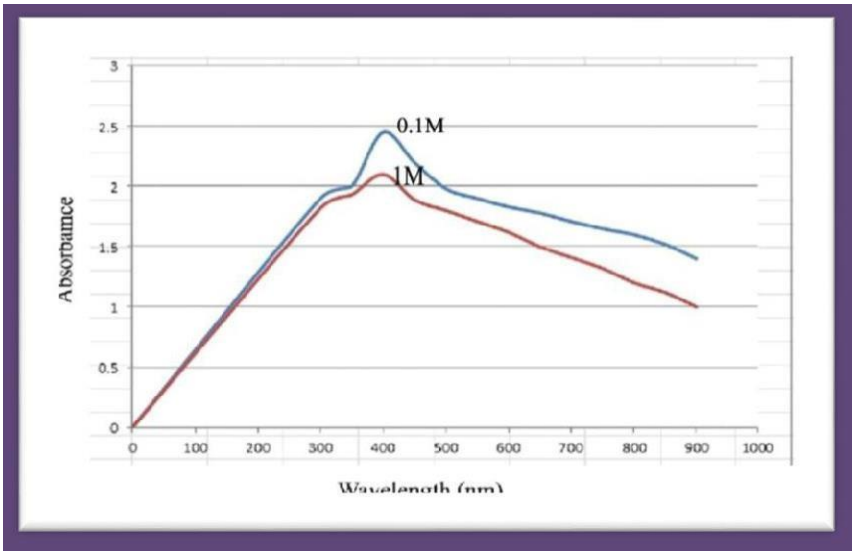


Figure (4-10): The production of MgO NPs that synthesis by isolate *Leuconostoc spp* in different concentration of salt

(Imani & Safaei.,2019) which used 0.05, 0.1, and 0.2 M of Mg(NO3)2 as well as 0.5, 0.1, and 0.2 M of NaOH was prepared. based on the 9 suggested experiments using the Taguchi method, different

concentrations of Mg(NO3)2 were added to NaOH solutions .

4.5 Biological Application of MgO NPs

4.5.1 : Antibacterial Activity of MgO NPs Against MDR Bacteria

The antibacterial effect of produced MgO NPs on four of isolates Gram negative *Salmonella typhi* isolate with multi-drug resistance (MDR) was evaluated for the produced MgO NPs. These MDR.bacteria were previously discovered by biochemical testing and the Vitek-2 system. The MIC and MBC for the MgO NPs have been established with the serial dilution. The utilization of varied ranges of MgO NPs concentrations against MRD bacteria was a crucial step in this investigation. After incubation, the MIC and MBC concentrations for nanoparticles were determined using the dilution method and spectrophotometry at wavelength (600 nm) .And the sensitivity test for resistant bacteria MDR, which is bacteria *Salmonella typhi*, which was evaluated through previous studies(Abood.,2020). Table (4-3).

Table (4-3) MIC and MBC of MgO NPs for *Salmonella typhi*.

Bacteria isolate NO	MgO NPs produced by *Leuconostocspp*	
	MIC µg/ml	MBC µg/ml
1	125	500
2	125	500
3	125	500
4	125	500

It is clear from the above table The MgO NPs MIC (125 mg/ml) and the MBC (500 mg/ml) MIC This study outcome was based on (Hayat *et al.*, 2018). According to the study, MgO NPs showed the antibacterial effectiveness on *E. coil*.

The antibacterial tests of synthesized MgONPs indicated reduction in the number of Gram-positive and Gram-negative bacteria. Consistent with the results of this study, previous studies reported the antibacterial activity of MgO NPs alone or in combination with other antimicrobials agents (He *et al.*,2016 ; Arshad, *et al.*,2017).

Salmonella typhi isolates were exanimate by Chen *et al.*,(2019) resistant to Gentamycin and Ciprofloxin (GN ,CIP)by Vitek2 system In the current study, these isolateswere employed for the same antibiotics at varied concentrations (These antibiotics were for human use and were taken from the pharmacy in powder form and dissolved in distilled water)The inhibition zone of CIP 5mg/ml was (31,30,26,28 mm) for four isolates, respectively ,GN 10mg/ml was(30,22,25,25mm) for four isolates, respectively Table (4- 4).This research showed that increasing antibiotic concentrations causes bacteria to become more susceptible to antibiotics. The widths of inhibition zones were measured to determine the MIC of MgO NPs for antibacterial against *Salmonella typhi*. Zone diameters (mm) at 3- equidistant points taken from the center of the inhibition zone and the average value of all observation was taken were used to quantify bacterialgrowth inhibition.

The results revealed that MgO NPs inhibited *Salmonella typhi* well, which is similar with (Mohamed and Shafey., 2020) who mentioned that MgO NPs have the best antibacterial activity against *Bacillus subtilis, S. aureus gram-positive* bacteria and *E. coli.*The combination of nanoparticles and antibiotics resulted in inhibitory zone a MgO NPs 125µg /ml+ CIP 5mg/ml, MgO NPs 125µg /ml+GN10mg/ml.for Four isolates were(33,25,30,25)mm ,(25,33,30,31) immrespectively .

Table (4-4) : Antibiotic susceptibility of *Salmonella typhi* by Agar Well Diffusion Test.

Isolates NO.	Inhibition zone diameter in mm	
	CIP 5mg/ml	GN10mg/ml
S.typhi 1	31(S)	30(S)
S.typhi 2	30(S)	22(S)
S.typhi 3	26(S)	25(S)
S.typhi 4	28(S)	25(S)

S= sensitive

The results reveal that when antibiotics are combined with MgO NPs, bacteria become more susceptible. These findings are consistent with Ali *et al*., (2020) who investigated the same hypothesis. MgO NPs showed antibacterial action against both Gram positive and Gram negative bacteria (*Staphylococcus aureus* and *Escherichia coli*, respectively) at a minimal inhibitory concentration. When antibiotics were combined with MgO NPs, there was a substantial increase in bacterial sensitivity $P \leq 0.05$.

Table (4-5): Antibacterial activity of MgO NPS against *Salmonella typhi* By Agar Well Diffusion Test.

Isolates NO.	Inhibition zone diameter in mm
	MgO NPs 125µg /ml
S.typhi 1	22
S.typhi 2	25
S.typhi 3	30
S.typhi 4	31

Table (4-6): Combination effect of MgO NPs with antibiotic of *Salmonella typhi* by Agar Well Diffusion Test.

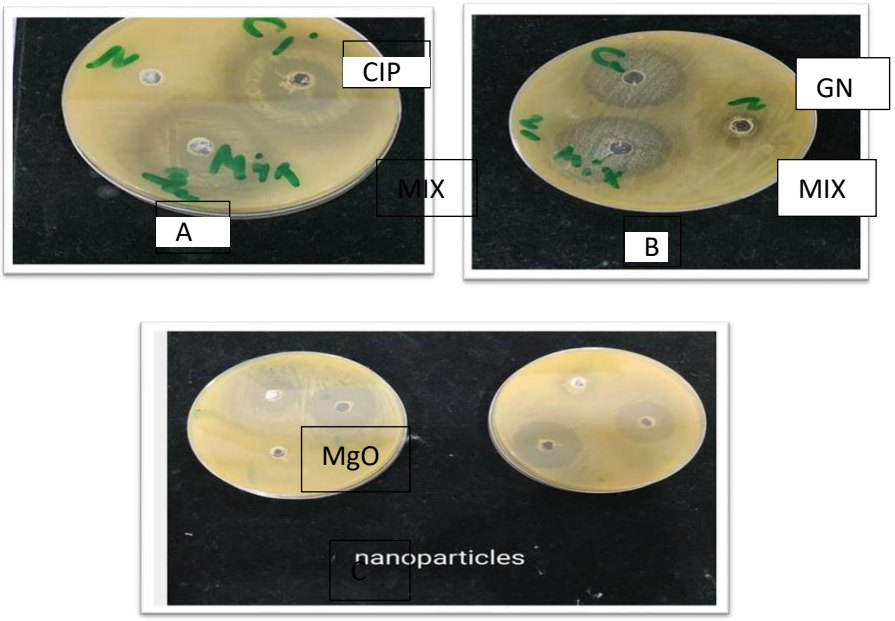
Isolates NO.	Inhibition zone diameter in mm	
	MgO NPs 125 µg /ml+CIP 5mg/ml	MgO NPs 125 µg /ml+GN 10mg/ml
S.typhi 1	33(S)	31(S)
S.typhi 2	25(S)	25(S)
S.typhi 3	30(S)	30(S)
S.typhi 4	25(S)	31(S)

Several antibacterial activity features of metal oxides, including particle size, mixture concentration and powder of surface properties, were examined and active oxygen and metal oxide particles were developed (Saratale *et al*., 2018). Smaller NPs are interacted more widelywith the bacterial cells and more often than larger NPs can reach the cytoplasm that has an antibacterial influence (Thi and Yen., 2019).

Huang *et al*. (2005) reported that antibacterial activity was increased with the decrease of the particle size of MgO NPs. A relationship between the bactericidal efficacy against *B. subtilis* ATCC 9372 and the particle size of MgO NPs was demonstrated. Sundrarajan *et al*., (2012) investigated the effect of MgO NPs size on the antibacterial activity. The results indicated that small-sized MgO NPs had better antibacterial activities towards both Gram positive (*S. aureus*) and Gram negative (*E. coli*) bacteria. Furthermore, MgO NPs had more activity towards Gram positive bacteria compared to Gram negative bacteria. Jin and He (2001) found that higher MgO NPs concentrations resulted in greater bacterial inactivation. An approximate seven log unit reduction in *E. coli* O 157:

H7 was achieved by an 8 mg/mL MgO NPs treatment at 24 h. The reason is probably due to the difference in cell membrane structureThe cell wall of Gram-positive bacteria consists primarily of thinlayers of lipid A, lipopolysaccharide, and peptidoglycan, but that of gram-negative bacteria consists of only a peptidoglycan layer. Membrane functions, activity of enzymes associated with the membrane, and maintenance of cell integrity depend on the structure of the cell surface (Espitia *et al*., 2012).

Zhang *et al*., (2011) also found that high MgO NPs concentrations resulted in greater bacterial inactivation. Many studies have indicated that the antibacterial mechanism of MgO NPs is due to the formation of ROS such as superoxide anion (O2 −) (Yamamoto *et al*., 2010). It has been reported that the increase of the surface area of MgO particles leads to an increase of the O2− concentration in solution and thus results in a more effective destruction of the cell wall of the bacteria. Krishnamoorthy *et al*., (2012) evaluated the antibacterial activity of MgO nanoparticles against the gram-negative bacteria *E. coli* and *Pseudomonas aeruginosa* (*P. aeruginosa*), as well as the gram positive bacterium *S. aureus*. MgO nanoparticles exhibited antibacterial activity with MIC of 500 μg/mL against *E. coli* and 1000 μg/mL for *P. aeruginosa* and *S. aureus*.



Figure(4-11): Effect of MgO NPs with antibiotics by agar well diffusion test against *Salmonella typhi.* A: Antibiotics CIP and MgO NPs with Antibiotics CIP B: Antibiotics GN and MgO NPs with Antibiotics GN, C:MgO NPs only.

4.5.2 Antibiofilm Activity of MgO NPs

Leuconostoc spp. makes MgO NPs. Antibacterial activity with the 4 isolates of Salmonella *typhi* has been evaluated for MIC (125 µg /ml) of MgO NPs utilizing plate technique The activity of antibiofilms varies accordingto the isolated bacteria. In this study biofilm formation in 10 of *S. typhi* isolates under study was examined and the findings were obtained (8 strong and 2moderate).

The formation of biofilms was inhibited by MgO NPs which resulted in neither strong nor moderate isolates of biofilm being formed. Biofilm formation was consistently reduced after all isolates had been treated with MgO NPs (125µg/ml). This study investigated the production of biofilm in 10 isolates and the results were (8 strong and 2 moderate). Heavy biofilm formation was observed in the positive control and when MgO NPs were not used, and no biofilm formation was observed in the negative

control., Table(4-7).These findings show that MgO NPs have a considerable impact on the production of *Salmonella typhi* biofilm, with a significant decrease in biofilm formation at at P≤0.05.

Table (4-7): Antibiofilm activity of MgO NPs by absorbance at 630 nm against *Salmonella typhi*

Isolate NO.	Absorbance at 630 nm	
	Without MgO NPs	MgO NPs
1	Strong	Inhibition
2	Strong	Inhibition
3	moderate	Inhibition
4	Strong	Inhibition
5	Strong	Inhibition
6	Strong	Inhibition
7	moderate	Inhibition
8	Strong	Inhibition
9	Strong	Inhibition
10	Strong	Inhibition

Biofilm-forming microbes have unique potential to cause infection. According to a report from the National Institutes of Health and Centre of Disease Control, approximately 65%–80% infections are caused by biofilm-forming microbes, *E. coli*, *P.aeruginosa* and *S. aureus* being major culprits (Joo & Otto .,2012) Many study revealed that MgO NPs at sub-inhibitory concentrations inhibit the biofilm-forming potential of pathogens which are known for their biofilm-forming capacity. We found that MgO NPs reduced the biofilm-forming capacity of bacteria by 31%–82.9% in a time-dependent manner, comparable to previous studies on NPs (Goswami *et al*,2015) .

Other study indicated that the effects of MgO NPs on removal of biofilms already established at different time intervals by applying sub-inhibitory concentrations of NPs , and found that MgO NPs at sub-MICs not only inhibit biofilm formation at different time intervals, but also reduce

the number of cells attached in established biofilms (Lellouche *et al.*,2012).

These findings were consistent with (Flemming and Wingender,2010) findings of MgO NPs reducing the established biomass of biofilms .The physical or chemical feature of the extracellular polymer matrix (EPM), when it is formed on the surface, protects microorganisms from antibiotic activity (Hayat *et al.*,2018).The initial binding of NPs to the biofilm surface and its subsequent chemical features The size of the biofilm's pores, the existence of water channels, the charge of NPs and *EPM*, and the chemical gradient within the matrix may all influence NP proliferation(Shkodenko *et al.*,2020). MgO NPs destroyed and disintegrated the cell wall of the phytopathogen bacteria leading to leakage of the intercellular content and cell death. The same authors discovered that except for retained biofilm formation, MgO NPs improved the bacterial susceptibility to antibiotics. In contrast to nisin (antibiotic) addition, MgO and ZrO mixing did not enhance MgO activityagainst pathogens (Jin *et al.*,2001).

Once NPs have reached the biofilm limits, the physical and chemical characteristics of the extracellular polymer matrix (EPM) determine the initial attachment and subsequently migration of the NPs to the biofilm surface. Various physical and chemical processes might influence the first attachment of NPs to the outer surface of biofilms, The proliferation of NPs in the biofilm may rely on the sizes of its pores, the existence of water channels, charging of NPs and EPMs (Shkodenko *et al.*, 2020).

4.6 Characterization of Outer Membrane Protein of *Salmonella typhi* by using SDS-PAGE

Salmonella typhi was utilized in OMP isolation and antigen prepared in immunological studies because it results in phenotypic and genotypic detections for the majority of the virulence factor. The InSDS-PAGE OMP analysis of *Salmonella typhi* revealed the molecular weight (MW) of the

band was estimated at 44KD by comparison with standard MW markers **(Fig4-12). The protein concentration was (30 g/L) .**

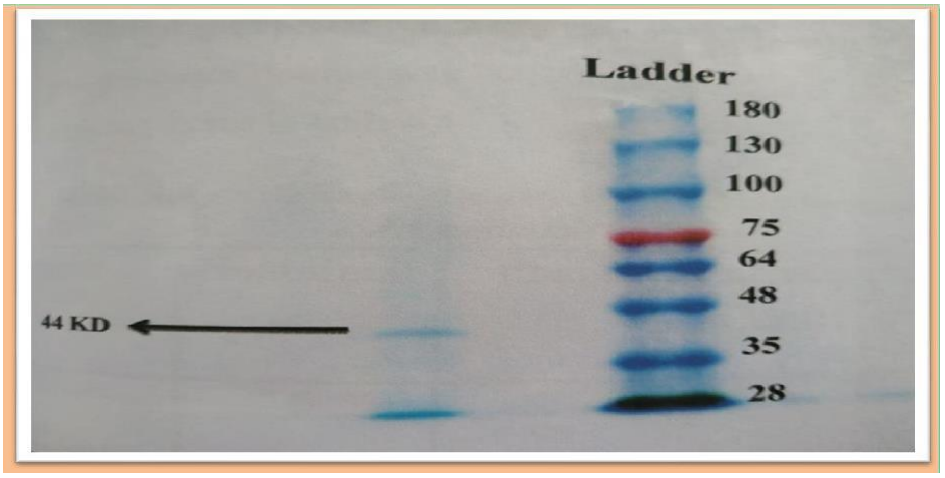


Figure (4.12) Protein profile .protein from SDS-PAGE(10%W/V) of whole cell lysate preparation from *Salmonella typhi* at 150V for 6 hours visualized after staining with coomasise Lane (L): Protein ladder 28-180 KD Arrow :44-KD band.

The band had an intensity of 44KD Fig4.12. Kondapalli *et al.*,(1999) said that the SDS-primary PAGE's and important OMPs were a 40KD band. *Salmonella typhi* include other main protein, including traces ofadditional proteins, with molecular weights of 33,36, and 44 KD, including 22,28, and 30KD and 50KD. The entire SDS OMPs wereinsoluble with 44 KD protein and no detectable protein content above 40KD. This might be attributed to a tiny molecular protein release from the degraded lysozyme of peptidogly can (Nakae *et al*.,1986).Although the protein profile is similar under different growth conditions, the osmolality and temperature affected the expression of the total OMPs the expression of all OMPs decreased by two fold when the temperature was raised from 30C to 37C.OMPs from Gram negative bacteria are anchored to the outer membrane by N-terminal domain ,which consists of integral transmembrane domain .OMPs are well-know core players in maintaining of bacterial membranes ,selectively permeability and bacterial pathogenesis in host cell(Lin *et al*.,2002;Choi *et al* .,2005)

The OMP loss facilitates the transport of antibiotic molecules across the membrane of cells, which is seen in many bacteria that can provide decreased susceptibilities to a variety of antibiotic classes. The (Delcour.,2009). Porins create proteins on the external bacterial membrane. 3 OMPs (33-36 KD, 29KD, 43KD) (Lee *et al*.,2011). Colonel Shahane *et al* .,2007) demonstrate the insulation and characterization from the outside membranes of *Salmonella typhi* of an immunogenic protein . The OMPs of gram-negative bacteria are immunologically important because of their accessibility to the host defense system.Major OMPs identified from SDS-PAGE gels on the basis of their molecular weights were used as eliciting antigens in our studies. The PA complexes were prepared and administered to animals. OMP of *Salmonella enterica*serovar Typhimurium with an apparent molecular mass of 49 kDa that is highly immunogenic, evokes humoral and cell-mediated immune responses (Hamid and Jain .,2010).

Difference between genus bacteria affects synthesis of nanoparticles and outer membrane, synthesis of bacteria as of the nanoparticles in theirsize and purity, and this could be due to a cell that use the same having a better enzyme system and a defense system against metal toxins. whereas the protein profile is outside the membrane (OMP concentration and SDS-PAGE analysis Molecular weight).

The exposure of NPs to bacterial cells can lead to membrane damage caused by NP adsorption sometimes followed by penetration into the cell (McQuillan *et al.*,2012). Adsorption of NPs leads to cell wall depolarization, which changes the typically negative charge of the wall to become more permeable. It has been reported that the bacterial cell wall become blurry, indicating cell wall degradation as shown by a laser scanning confocal microscope (Mukha *et al*,2013). Contrary to many

findings of cell permeation, the interaction of MgO-NPs with the cell wall is the main source of toxicity to bacteria even though no cell penetration occurs (Leung *et al.*,2014). Similar studies have reported that when NPs interacts with the bacterial cell wall, penetration does not always occur (Nazari *et al*.,2014).

NP aggregation can be predicted from the measurement of zeta potential, which indicates the stability of colloidal suspensions (Zhang *et al*.,2008). A largely positive or largely negative zeta potential generally means that the colloidal suspension is highly stable (very low aggregation) with the optimal potential being > 30 or < − 30 mV. Even at the optimal zeta potential, NPs can still aggregate with each other as a result of protein complexation. In this regard, the thermodynamics of protein-NP complexation was investigated (Miranda *et al*.,2009) using different sizes of NPs and proteins, such as green fluorescent protein (a beta barrel protein) BSA (a triangular prismatic protein) and PhosA (an orthorhombic shaped protein) (Meghana *et al*.,2015).

4.7 Immunological Tests

4.7.1 Humoral Immunity

4.7.1.1 Systemic Humoral Immunity

The tube agglutination test with serum from MgO NP immune animals (three per group) revealed a mean value (1280) for systemic antibody for MgO NP synthesized with *Leuconostoc* spp, a mean value (1280) for outer membranes, and a mean value (2560) for mixed (MgO+OMP) (3 animals) revealed a mean value of systemic antimicrobial antibodies with a mean value of (10) Table (4-8).

Table (4-8): Systemic antibody titer in serum of immunized Rabbits group

Rabbits groups	NO. of animals	Type of immogen	Systemic antibody titermean
Group 1	**3**	**Control**	**10**
Group 2	**3**	**MgO NPs**	**1280 "**
Group 3	**3**	**Outer membrane**	**1280 "**
Group 4	**3**	**Mixed(MgO +OMP)**	**2560 "**

" signification difference with control at P<0.05

Jebur and Abed, (2021) exhibited that agglutination test with serum of test animals immunized with MgO NPs (three animals per group) showeda mean value (1280) systemic antibody for MgO NP, synthesized with *Leuconostoc spp*, mean value (1280) for outer membranes and mean value (2560) to mixed control animals (3 animals) showed a mean value of systemic antimicrobial antibodies with mean value (10), Memory B cell numbers expanded following subsequent boost vaccinations followed by a gradual decline. Importantly, the generation of a substantial memory response cannot always be predicted by serum antibody titers. While HBV vaccination only elicits a rapidly waning antibody response, B cell memory is prolonged and provides long term protection (Tuaillon *et al*,2006) .

Numerous studies point toward nanoparticle eliciting superior serum antibody responses following immunization. However, quantification of serum antibody and infectious challenge are generally performed soon following immunization and limited studies have assessed the elicitation and persistence of memory B cell populations. In one study performed in rhesus macaques, TLR agonist-adjuvanted PLGA synthetic particles encapsulating malarial antigen Pfs25 elicited increased numbers of Pfs25-specific memory B cells compared to vaccination with

the clinicallytested Pfs25-EPA protein-conjugate vaccine (Thompson *et al*,2018). The memory B cells expanded with subsequent vaccine boosts and correlated with antibody titer. In an additional study performed in nonhuman primates, vaccination with lipid nanoparticles encapsulating mRNA encoding Influenza HA generated circulating HA-specific memory B cells detectable two weeks after the prime immunization (Georgiev *et al*,2018).

4.7.1.2 local Humoral Immunity

After removing secretary immunoglobulins from appendix rabbits that immunized with the MgONPs Outer membrane and Mixed group appendix the results showed a high titer compared to the control, which (128) achieves for *Leuconostoc spp* synthesized MgO NPs,(128) for the outer membrane, and (256) for the mixed(MgO+OMP) compared control to its mix. titer of (1), Table (4-9).

Table (4-9): Mucosal antibody titer in appendix of Rabbits group immunized

Rabbits groups	NO. of animals	Type of immogen	mucosed antibody titer mean
Group 1	3	Control	1
Group 2	3	MgO NPs	128"
Group 3	3	Outer membrane	128"
Group 4	3	Mixed(MgO+OMP)	256"

" signification difference with control at P<0.05

The study agreed with(Jebur and Abed,2021; Al-sarhan, 2017) that directtests for agglutination were performed in a tube containing animal serumand appendix globulin for animals immunized with MgO NPs, to detectthe immune response (systemic and local) specific to NPs. There was an increase in the systemic antibody titer for MgO NPs-immunized

animals .For the MgO NP immunized , the outer membrane and direct mixing tests in a tube containing animal serum and Appendix globulin wereperformed to determine the immune response to NPs, the outermembrane, and the results were compared to those of control animals.The test findings were compared.

The findings of our present investigation showed that MgO NPs -immunized rabbits were higher than controlled animals with a systemic antibody for MgO NPs types synthesized from the outer membrane using isolate *Leuconostoc spp* (Table 4-8).

The Table (4-9) showed that the MgO-immunized, mixed, outer membrane mucosal antibody titer was larger than the *Leuconostoc spp*, outer membrane, and mixed MgO-type control unit titer. Nanoparticles are being investigated for the characteristics of their immune stimulators that can induce an innate or adaptive immunity. Nanoparticles interact routinely with antigenic cells, such as B cells, macrophages and dendritic cells in the bloodstream. According to these findings, MgO NPs have the ability to activate B cells to generate specific antibodies, implying that they have the ability to both trigger the immunological response (immunogen) and interact with the immune response's products (antigen) (Park *et al*.,2010a).the preset study showed that when OMVs were employed as the oral adjuvant, opsonizing anti-Salmonella antibodies are produced in response to immunization, as well as CD4 T cells and B cells. (Luo *et al*., 2015).

The specific mechanisms of cross-protection through MOMV need more investigation, many study provide a new way to developing multi-sero group APEC vaccines. Due to their efficacy for future generation APEC vaccines because of their benefits, such as minimal toxicity and extensive protection, MOMV vaccines might be candidates for the next

generation APEC vaccines (Gustavo,2007). The result was comparable to AL-Khafagee (2010). she reported that *Citrobacter freundii* OMP infected rabbits had a larger antibody titer. The appealing vaccine antigen candidates were found by Gustavo(2007) and his colleagues Gram-negative bacteria's OMPs. Immunogenicity, protection and cross-protection have been investigated using mice vaccinated with *Bacillus* OMPs. Several multivorants with adjuvant stimulated mucosal and systemic immune responses. OmpA has also been associated with the early stages of bacterial infection and is considered to have a function OmpA is also known to produce strong antibody reactions and OmpA's immunogenicity has been shown by a number of research. Recombinant *Klebsiella pneumonia* OmpA, according to studies, binds and activates both macrophages and dendritic cells (Torres *et al*.,2006 ;Dumetz *et al*.,2007;Li *et al*.,2009).

4.7 .2 Cellular Immune Response

4.7.2.1 Mitotic Index

Table results (4-10) showed signification difference with control at P <0.05 the significantly increased rate of mitotic indices of bone marrow cells for the animals group. The MgO-caused NPs synthesized for *Leuconostoc spp* isolate had rates of (6.30), the Outer membrane mice hada rate of (6.67), and the Mixed-caused mice had rate of = (6.73) as compared with control animals (4.15) in P< 0.05.

Table (4-10): The mitotic index of immunized rabbet groups

immuogen groups	No. of animals	Mitotic index Mean±S.D
Control	3	4.15"±0.161
MgO NPs	3	6.30"±0.333
Outer membrane	3	6.67"±0.236
Mixed	3	6.73"±0.311

" signification difference with control at P<0.05

The results appeared that Increased in cell division in bone marrow have been caused by nanoparticles, the outer membrane and mixed which play a part in the formation of body immune cells, as indicated by the above results. This might demonstrate that MgONPs, outer membrane and mixed membranes could increased cell immunity. Immune cells mature and had a particular time period in the immune system, thus it is a requirement. They are continually being replaced by new, immature bone marrow cells that develop in blood cells from stem cell production, hematopoietic bone marrow stem cells which are capable of regeneration, differentiation and maturation and the process that regulates, in addition to the hormone, the number of factors that are well known in the solubility of cell cytokine (Eales., 2003).

Many study indicated that metal nanoparticles and the outer membrane had shown that the mitotic index and the effect of metal nanoparticles and the outer membrane on lymphocytic production have been substantially boosted, increasing the interaction between Silver nanoparticles and the immunologic components leading to cell mitoses(Klippstein *et al*.,2010; and Prokhorova *et al*., 2013). These new cells come from immature cells in the bone marrow that continues to grow from the production of stem cells into blood cells, hematopoietic stem cells in the bone marrow with regeneration ability and maturity, and processes that determine the number of factors that, in addition to the hormones, are the well-known soluble cells of the cell cytokine (Cyster .,2003) .

4.7.2.2 Skin Test

This study investigated how MgO-NPs, outer membranes and mixed immunogenic influenced the skin sensitivity and cell immunity of the rabbits. The results of present study showed signification difference with control at $P<0.05$, the induration diameter of MgO-NPs synthesized by *Leuconostoc spp* (1.31), Outer membrane (1.83), and Mixed (1.02) mm respectively was detected after four hours. The skin of rabbits with MgO- NPs synthesized by *Leuconostoc spp,* outer membrane, and mixed pus cell in MgO-NPs was provided, synthesized with isolates *Leuconostoc spp,* outer membrane and Mixed, in the MgO-NPs, after 24 hours of injection. The skin rabbits immune to MgO-NPs, outer membrane and mixed, pus cell, and tissue damage shown after 48 hours of injecting,with mean in duration diameters of 11.33 mm, 10.66 mm, and 9.33 in comparability with the indurational diameter after 24 hours, have been significantly increased by $P< 0.05$ compared to average induration diameter after 4 hours. After 72 hours of injection, skin rabbits have been immune.Table (4-11)

Our results agreed with (Jebur and Abed.,2021) that after 24 hr. of injection the skin of rabbits groups immunized with MgO NPs that synthesis by isolate *Leuconostoc spp* had shown pus cell and induration diameter was

10.66 mm, 9.66 mm respectively compared the mean of induration diameter after 4 hr. had increased significant at $P\leq0.05$. The results also showed after 48 hr. of injection that the skin rabbits immunized with MgO NPs that synthesis by isolate *Leuconostoc spp* had shown pus cell and necrosis and mean of induration diameter was 12.66 mm, 10.4 mm respectively compared the induration diameter after 24 hr. had significant difference (Al-sarhan & Abed., 2017) .

Table (4-11): Skin sensitivity of the immunized rabbits

rabbit groups	Mean±S.D Measure in mm			
Time of result	After 4 hr.	After 24 hr.	After 48 hr.	After 72 hr.
control	00.00	00.00	00.00	00.00
MgO NPs	1.31"±0.55	9.33"±1.15	11.33"±1.57	8.33"±0.57
Outer membrane	1.83"±0.28	9.00"±2.00	10.66"±2.15	7.66"±0.57
Mixed	1.02"±0.01	7.33"±1.54	9.33"±1.00	6.66"±0.57

" signification difference with control at $P<0.05$

The results of skin test (Strindeelius *et al.*,2002). The delayed hypersensitivity test for the skin used for the indicator of cellular immunity in rabbits immunized by the different kinds of *Salmonella* antigens revealed a substantial increase in inoculated animals. Testing of the final result. Parallel with Al-Waeli(2011), who found that after 24 hours the skin response was higher. The favorable results of this study are consistent with the findings of previous studies (Yousif and Al-naqeeb.,2010;Yousif and Al-Mansoryo.,2011) .

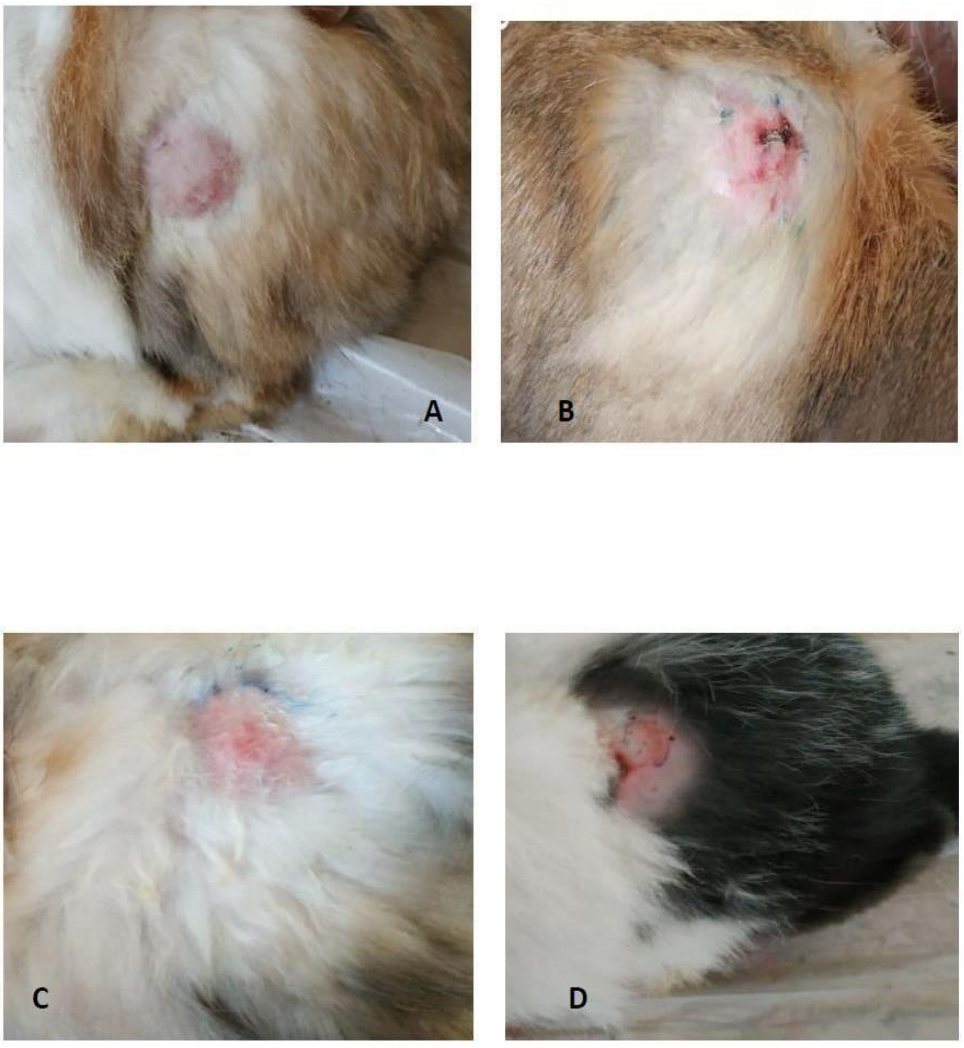


Figure (4-13) Skin sensitivity of the immunized rabbits (A control after 72 h , B represented as immunized rabbits with MgONPs after 24 h , C immunized rabbits with mixed , D immunized rabbits with outer membrane) .

The DTH response is characterized as a remembrance or T-cell reaction because it needs a certain antigen to be prematurely immunological responsive. It can detect some antibiotics, to which the host has already had a response immunity, providing an indicator of current T cell reactivity to certain recall antigens, the fact of redness and occasionally the measurable degree of swelling.

The major responds for delayed-type hypersense (DTH) are human neutrophils, followed by fusion of mononuclear cells made up of macrophages and T cells, whereas the mouse reacts highly to DIH antigens in mice(Lotze and Thomson.,2005) . NKT cells comprise a heterogeneous subpopulation of T cells that co express a TCR and natural killer (NK) surface antigen CD16 in humans and NK1.1 in mice (van Kaer., 2004). Emerging evidence suggeststhat DCs are involved in the regulation of the helper T cell Th1/Th2 balance and may interact with effector cells of innate immunity, It has been reported that DCs can activate NKT cells by presenting GalCer in association with CD1d (Creusot and Mitchison., 2004).

In interstitial cell infiltrate, the cells CD8+T and CD4+T as well as the macrophages predominate. CD4 T cells produce gamma interferon, which promotes cytokine cascades that activate local endothelial vascularcells, whereas CD8 cells are supposed to mediate lethal effects on foreignMHC cells. In addition to the IFN-γ, IL-2 and INF- γ, Th1 cells also release the IL-2 promote the formation of THI, causing a macrophage activation and delayed responses of the type of hypersensitivity (DTH) .

The immune system drives the specialized immune cells T cells to the skin where they release chemical messengers called lymphokines, which have been sensitized by prior infection. These lymphokines produce induration at and around the injection site (a hard, elevated region with distinct borders) by promoting local vasodilation (increases inblood vessel diameter), producing edema, fibrin deposition and other types of inflammatory cells. (Zabriskie., 2009). These findings are similar with the findings (Alkawaz., 2013), who found that Ag-NP in rabbits produced hypersensitivity of delayed types via causing pro-inflammatory cytokines , primarily by Th1 responses.

Al-sarhan & Abed., (2017) that the induration diameter of all injected rabbit groups with antigens after 4,24,48,72 hr had significant increase in the induration diameter of control group at P<0.05. This results referred that these three types of antigens induce the delayed type hypersensitivity(DTH) which belong to the type IV hypersensitivity that reaction result from the T cell inflammation cell-initiated inflammation and do not involve antibody. Inflammatory responses result from the manner in which T cells encounter and respond to antigen.

DTH response requires prior immunological sensitization to a specific antigen and thus are categorized as recall, or memory T-cell response, they can identify specific antigens to which the host has already made an immune response and they provide an index of the current T-cell reactivity to specific recall antigens and the real of redness and sometime the degree of swelling that can be measured to provide an index of DTH reactivity (Lotze and Thomson., 2005).

4.7.2.3 Spleen Weight Index

In the rabbits inoculated with MgO NPs synthesized by *Leuconostoc spp* insulate, the spleen weight index increased with the outer membrane and mixed with mean values of (0,064±0. 004) , (0,069±0.013) (0,086±0.015) respectively, statistically significant difference on P≤0.05compared to control group (0,018±0,007),respectively (4-12).

Table (4-12): Spleen weight index

immunogengroups	No. of animals	Mean±S.D
Control	3	0.018 ±0.007
MgO NPs	3	0.064 ±0.004
outer membrane	3	0.069 ±0.013
Mixed	3	0.086 ±0.015

" signification difference with control at P<0.05

According to Nuerberger *et al*., (2004). Showed a significant decrease in comparison to outer membrane and mixed. The mean spleen weight of intravenously inoculated mice with BCG was higher than that of non-immunized mice. while (Gautam & Singh.,2011) exhibited reduction in spleen organ body weight index after intratracheal exposure of nanoparticales may be due to the increased phagocytic activity during the transfer of nanoparticles and these phagocytes may relocate in spleen and cause apoptosis or necrosis in spleen.

Bacterial outer membrane proteins play a crucial function in the creation of immune defense components because they form the outermost surface in host cells interaction. as a potential vaccine component, OMPs have received a lot of interest. OMPs are located at the interface between hosts and bacteria and are vital both for host immune responses and pharmaceutical treatment goals. The spleen is the largest lymphsecondary organ in the body and has a range of immunological functions, hematopoiesis and clearance of red blood cells. The physical organisation, which enables a lower probability of interacting between the pathogens and aberrant cells in the blood and the cognate lymphocytes, allows the spleen to filter pathogens and abnormal cells out of circulation.APCs specific to the spleen regulate the T and B cell responses to variousantigenic inside the blood (Balasubramanian *et al*., 2010).

They investigated the effects of gold nanoparticles on the liver and spleen and discovered that the spleen retained more gold than the other organs over the injection time. Injected NPs, regardless of their size, shape, dose, or kind of substance, accumulate in the spleen for long periods of time.(Lewis *et al.,* 2019; Al-sarhan and Abed., 2017).

4.8 Estimation of Cytokines

The results of the production of cytokines were estimated using the equation from the standard curve carried out with the same test. Statistic analysis on IL-2 showed a mean value was (38.548±4.771,49.781±5.264 and 72.410±4.221) the control group when compared to the immunized group with MgO, OMPs antigen and Mixed immogen mean value (23.434±2.501) respectively.

Table (4-13) Concentration of IL-2, IL-17 and IL-1β (pg/ml) in immunized and control rabbits

Type of immuogen	No. of animals	IL-2 Mean±S.D	IL-17 Mean±S.D	IL-1β Mean±S.D
Control	3	23.434±2.501^{b}	50.310±5.498^{b}	17.700±2.545^{a}
MgO NPS	3	38.548 ±4.771^{c}	82.305 ±13.386^{b}	22.636 ±4. 024^{b}
Outer membrane	3	49.781±5.264^{d}	101.444±16.943^{c}	36.860±9.534^{b}
Mixed	3	72.410± 4.221^{a}	128.964±5.853^{a}	41.812± 5.575^{a}

" difference letters mean significant at P<0.05

Table (4-13) statistical testing of IL-1β indicated to a control group (17.700±2.545) was significantly differences when compared with the immunized group MgO NPs ,OMPs, Antigen, and Mixed at mean value (22.636±4, 024.36.860±9.534and 41.812±5.575).

The statistical analyzes for the IL 17 in Table (4-13) show significant differences control group (50.310±5.498). Compared to the immunized group with MgO NPs, OMPs and Mixed at mean (82.305±1.386 ,101.444±16.943 and 128.964±5.853).

Soluble molecules play an important role in clinical immunology as a group. Macrophages separate them and can function as stimulating or inhibitory signals between cells. Chemokines are considered cytokines that induce leukocyte chemotaxis. Among cytokines, several of their stimulating activities are of particular interest. The secondary function of interleukins (IL-1β, IL-2 and IL 17) in the enhancement of immunological responses is of particular importance. A wide variety of cells, including T and B, are susceptible to IL-1β. IL-2 is largely involvedwith lymphocytes, although the IL-receptor B cells and the natural cell killed have identical tropic.

Wojta-Stremayr *et al.,* (2015) explained generated antigen-presenting virus-like nanoparticles (VPN) that co-express IL-2 bound to different membrane anchors. They found that the fusion of the C-terminus of IL-2 with a minimal glycosylphosphtidylinositol anchor acceptor sequence with two intervening immunoglobulin-like domains of CD16b led to an optimal stimulation of T cells and induction of CD8+ T cell effectors function in vivo . Many studies reported the generation of IL-2 surface conjugates on PEGylated liposomes using covalently bonded succinimidyl-4-p-maleimidophenyl butyrate-modified IL-2 or an Fc scaffold fused to the C-terminus of the murine IL-2 .Using an adoptive cell therapy model, Zheng *et al.,* (2013). Found that the vast majority of antigen-specific T cellsreacted to IL-2-Decorated liposomes in vivo after a single injection and that repeated administrations increased cytotoxic T cell activation and proliferation in melanoma-bearing mice. We functionalized nanocapsuleswith different amounts of IL-2 and studied how they interact with differentT-cell populations (Frick *et al.*,2016).

For regulatory T cell maintenance and T cell memory responses,

interleukin-2 (IL-2) cell growth factor is crucial. In allograft rejection and autoimmune and inflammatory diseases, the low dose of the IL-2 injection considered promising, whereas the first FDA-approved treatment was the first IL-2 cancer immunotherapy. However, its therapeutic potential is restricted because of its pleiotropic nature and the fact that many IL-2 receptors with varying affinity are present.Consequently, for increasing clinical application of cytokine a specific receptor assignment is needed. Nanoparticles provide for accurate placement and dosage control of immunomodulating substances and for tailor-made drug binding to certain receptors. IL-2 and its present therapeutic use in autoimmune, chronic inflammation and cancer, via T cell response T-modulation based on nanoparticles (Horwitz *et al*.,2019) .

In comparison to OMP bacteria group Table (4-13) shows a significant difference in the control group when MgO NPs and the Mixed Antigen groups of rabbits immunized with MgO NPs antigen, compared to those mixed with this convergent, (Pearson *et al*.,2019) .immunized rabbit with *Salmonella typhi* MP groups, have been shown to significantly increase. Increased production of IL-2 was compared to the control rabbit findings of cytokine analysis Specification of response type Th1(Thomton *et al*.,2004).

In comparison with group primed with OMPs bacteria, MgO NPs and Mixed immunogen groups of MgO NPs immunized rabbits, the results of Table (4-13) indicate significant differences for the control group. OMPs antigen and mixed in compare with the control this convergent with (Ahmed *et al*.,2018).

Modulation of proliferative responses can be explained by down-regulation of high-affinity IL-2 receptors or by a perturbation of signaling pathways. CD25 expression was measured following exposure to both NPs to determine if they caused a modulation of high affinity IL-2 receptor. Its expression is regulated by signaling pathways triggered by the binding of IL-2 to its receptor (Moulton *et al*.,2013). Guillaume *et al.*,(2014) had shown that NPs perturb the IL-2 proliferation by decreasingcell proliferation due to an increase of low-affinity receptors for this cytokine. NPs increase the turnover of IL-2-dependent proliferating cells at concentrations that do not significantly increase ROS formation.

IL-17 is a pleiotropic cytokine suspected of a crucial role in establishing autoimmune Diseases IL-17 also enhances those harmful interactions via recruitment and maintenance of inflammatory cells such as neutrophils, T cells or dendritic cells (Bunte,& Beikler .,2019) On the other hand, those discrepancies could be caused by the possibility that IL-17 is required majorly during the early stages of the disease, being nearly redundant in later phases (Lubberts.,2015). Its whole dire properties camefrom synergistic interactions, because IL-17 alone is insufficient to cause a profound impact (Zwicky *et al*.,2020).

Table (4-13) illustrates significant changes for the control group whenthere are significant increases in groups of rabbits immune to and mixed with MgO,OMP antigen compared to OMP bacteria, MgO and Mixed antigen, compared with control (Ahmed *et al*.,2018) .

Table (4-13) shows significant difference for the control group when compared with group primed with OMPs bacteria ,MgO NPs and

mixed antigen there is a significant increase in groups of rabbits immunized withMgO NPs ,OMPs antigen and mixed in compare with the control this convergent with (Prathna *et al.*,2010).

Carlson *et al.*,(2008) demonstrated that significant inflammatory response has been observed in the release of IL-1β after 24 hour of exposure to 15 nm of nanoparticles, but there was no detectable level of IL-6 upon exposure to nanoparticles . A single intratracheal instillation ofplatinum and metal nanoparticles causes progressive increase in pro-inflammatory cytokines (IL-1, IL-6 and TNF- α) by day 28 post instillation (Park *et al.*,2010b). The most important mediators of fever andother symptoms of sickness cytokines such as interleukin– Lβ (IL-1β) (Kozak *et al.*,2000). Since it has been known that macrophages stimulated with lipo-polysaccharides release the pyrogenic factors (Zampronio *et al.*,2000), and there are data showing that metal NPs may influence someimmunologic activities.

4.9 The Effect of MgO Nanoparticles on Tissue

A/ Liver

MgO Nps alone and mixed immogen groups showed inflammatory cells (lymphocytes) infiltration in the portal area and spotty lobular inflammation Figures (4-14) while figures (4-14,4-15,4-16,4-17) explained MgO Nps alone group showed inflammatory cells (lymphocytes) infiltration in the portal area .

The examination of the liver sections show significant difference as compared to that control group while in the tested groups in the study showed that degeneration of hepatocytes and nuclei pyknosis (necrosis)

with, a decrease number of the kupffer cells in the sinusoids, In the Mix group the results showed an abnormal liver with high level of (vacuolation) (cholongitis) and hepatocyte hypertrophy.

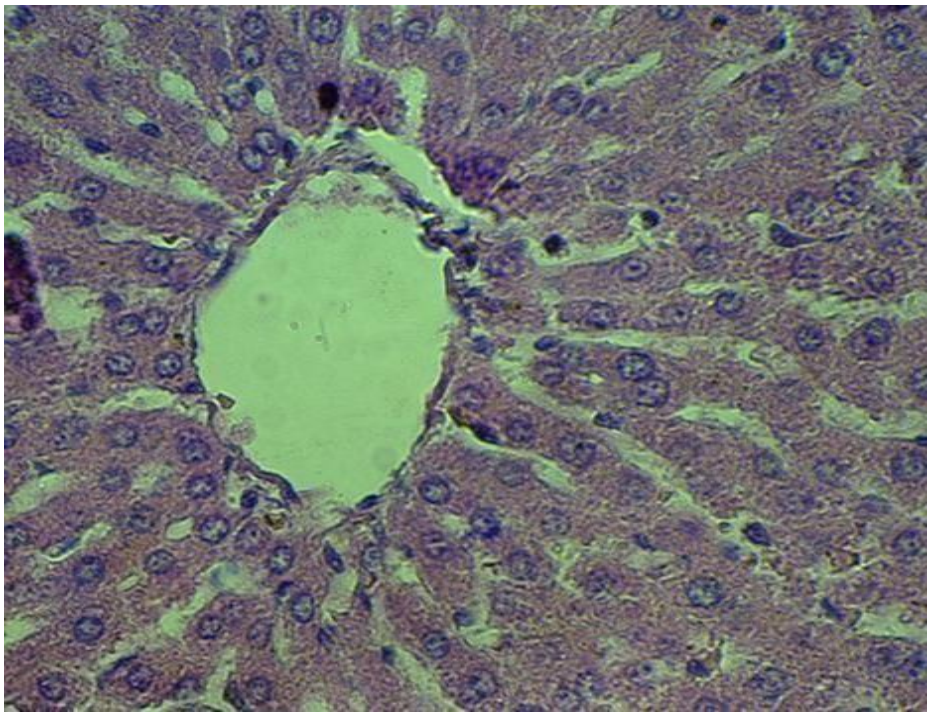


Figure (4-14) : Normal liver lobular architecture

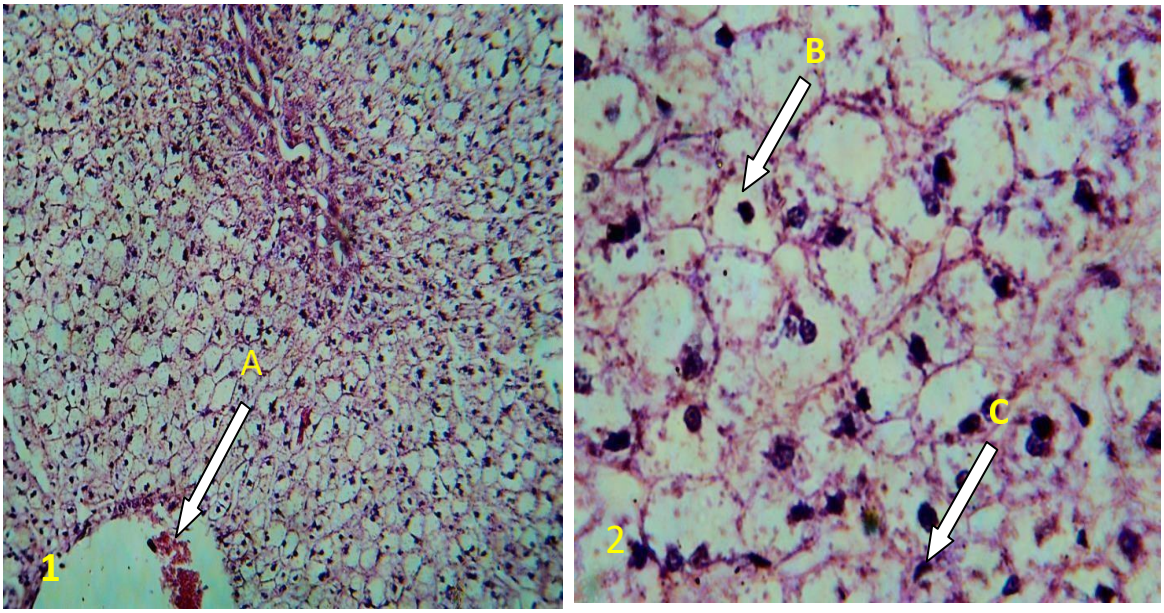


Figure :(4-15) (10x) In the Nanopartical group (1) shows (A) congestion of the central vein with erythrocytes stasis.(2)(B) degeneration of hepatocytes and nuclei pyknosis (necrosis) with, a decrease number of the kupffer cells in the sinusoids(C). H&E (40x).

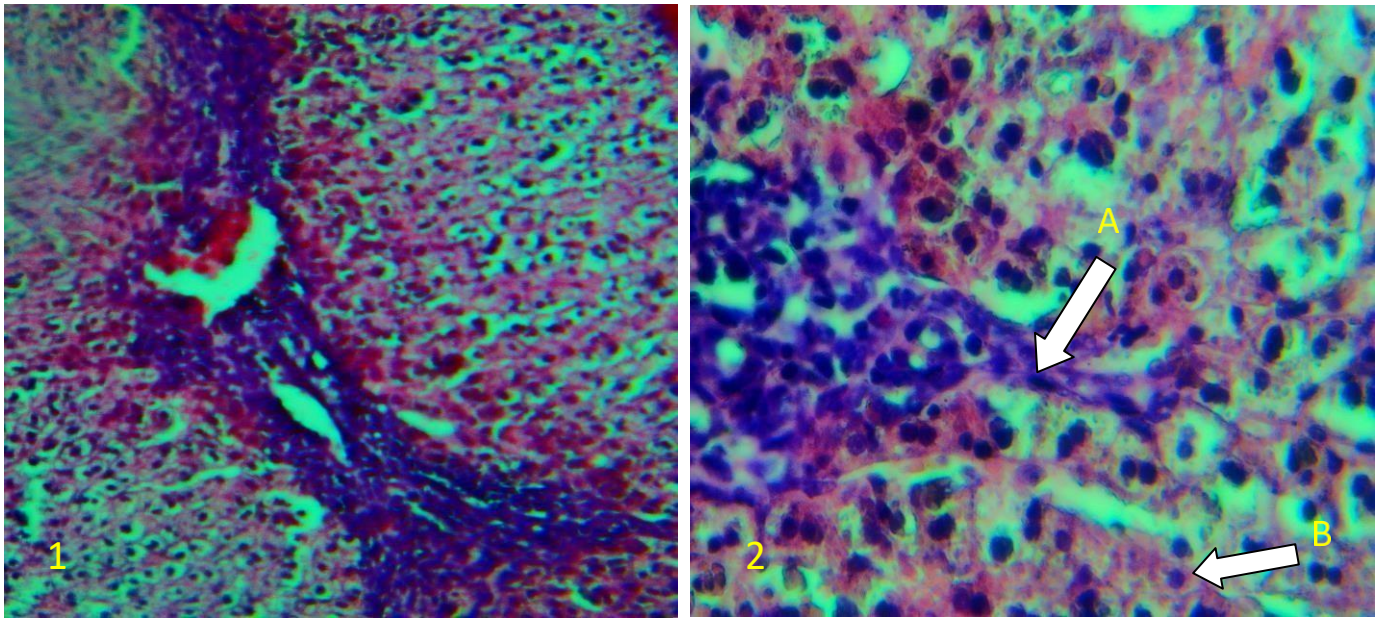


Figure :(4-16) (10x) In the Mix group (1) shows an abnormal liver with high level of vacuolation.(2) (A) Cholongitis and (B) Hepatocyte hypertrophy H&E (40x).

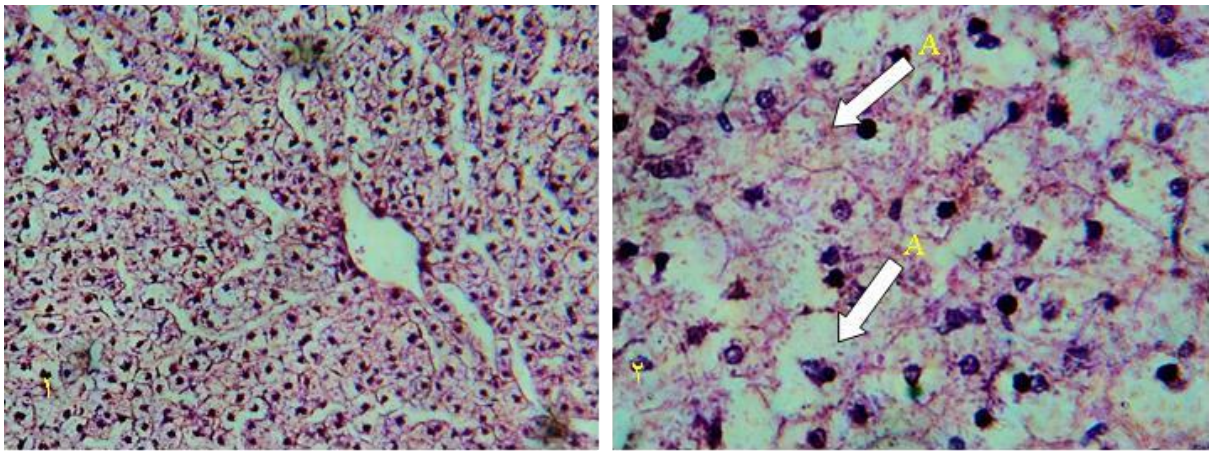


Figure :(4-17) (10x) In the Outer membrane group (1) shows abnormal liver with vacuolation and irregular sinusoids shows an (2) (A) hydropic degeneration of hepatocytes .H&E (40x).

The aim of the present study was to evaluate the effect of MgO NPs on liver . On the basis of the histological results, it seems that variations in liver tissue including proliferation of bile ductules, sinusoidal dilatation and congestion (SDC), many study proved that venous outflow impairment leads to sinusoidal dilatation and congestion (SDC) in the liver biopsy (Valla.,2002). Liver biopsies of patients with venous outflow impairment exhibited bile ductular proliferation, portal inflammations and portal-based fibrosis this ultimately will have resulted in suspicion of chronic biliary disease (Kakar *et al*.,2004).

Park *et al.*, (2010), reported that small sized MgONPs was distributed to the liver, kidney, brain, lungs and testis of rabbit after oral administration. On the other hand, Lankveld *et al.*, (2010), studied the kinetics of different sizes of MgONPs in rabbits and found that the tissue distribution of the 20 nm particles was mainly in the liver followed by the kidneys and spleen. Accumulation of MgONPs in certain organs has a direct relation with the organ toxicity development. So the distribution of MgONPs in different organs could give information about the target

organs that may be exposed to toxicity (Mitchell *et al.,* 2011).

Ansar *et al*., (2017) exhibited that regarding hepatic effects were found that MgONPs administration led to histopathological changes in the liver in the form of multiple foci of necrosis, fibrosis, congestion and inflammatory cellular infiltration when compared to control group.

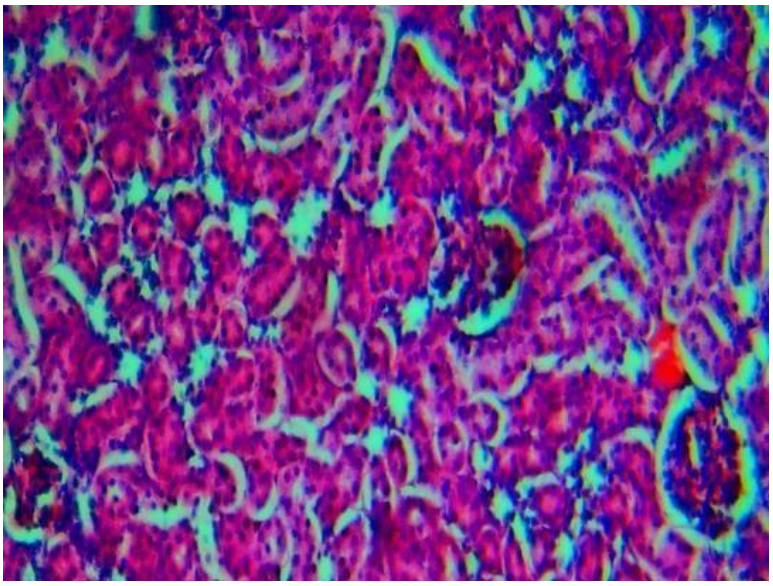
The results of Gelli *et al*.,(2015) showed that a dose-dependent liver toxicity in rabbits and various tissue damage markers, like alkaline phosphatase (ALP) and lactate dehydrogenase (LDH), in Broncho alveolar lavage (BAL) fluid and histopathology of lungs at 1, 7, and 30 days of post- exposure intervals was generated by MgO nanoparticles exposed via intra-tracheal instillation at the doses of 1 mg/kg or 5 mg/kg into rat lung.

The liver is one of the most prominent organs in which NPs accumulate, which may cause a change in its vital function. The high level of liver enzymes in the serum is one of the most important signs of liver cells injury damage, inflammation, or cholestasis (Pizzorno.,2015).

B\ Kidney

The examination of the kidney sections from tested group showed swollen renal tubular epithelial cells and atrophy of glomular tuft, necrosis generally includes cells welling, nuclear pyknosis .

MgO Nps alone and mixed antigen groups showed reactive germinal center in the kidney figures (4-18) while in control group showed bland looking kidney figure (4-19).



Figure(4-18) Cross section in kidney of control animals shows normal all architecture of tissue (H&E,10x)

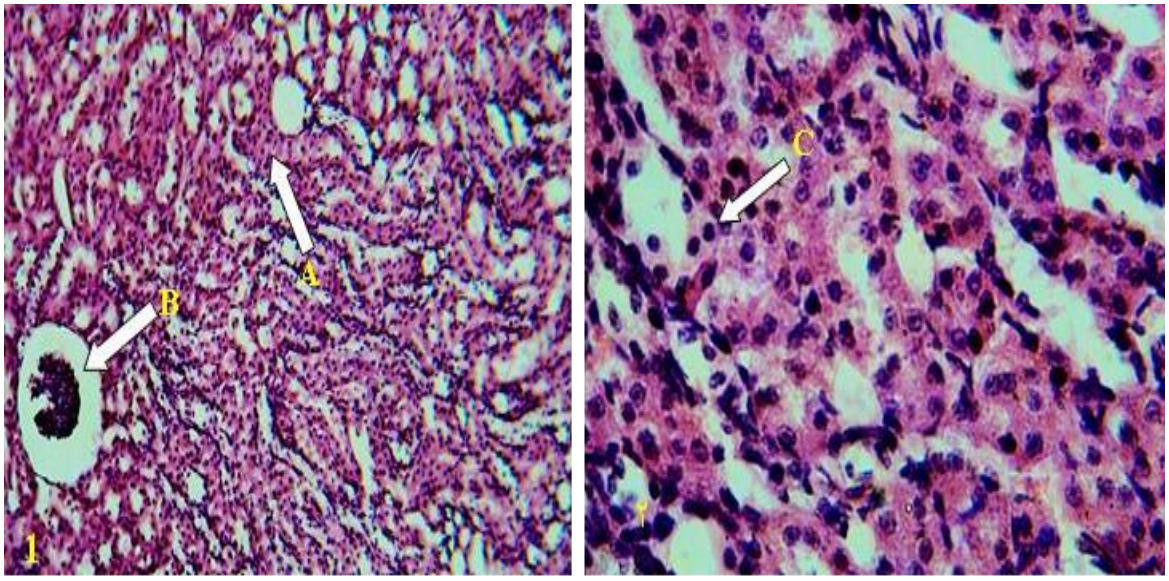


Figure : (4-19) (10x) In theNanopartical group shows (A)Swollen renal tubular epithelial cells and(B) atrophy of glomular tuft.(2)(C)Necrosis generally includes cells welling, nuclear pyknosis H&E 40x.

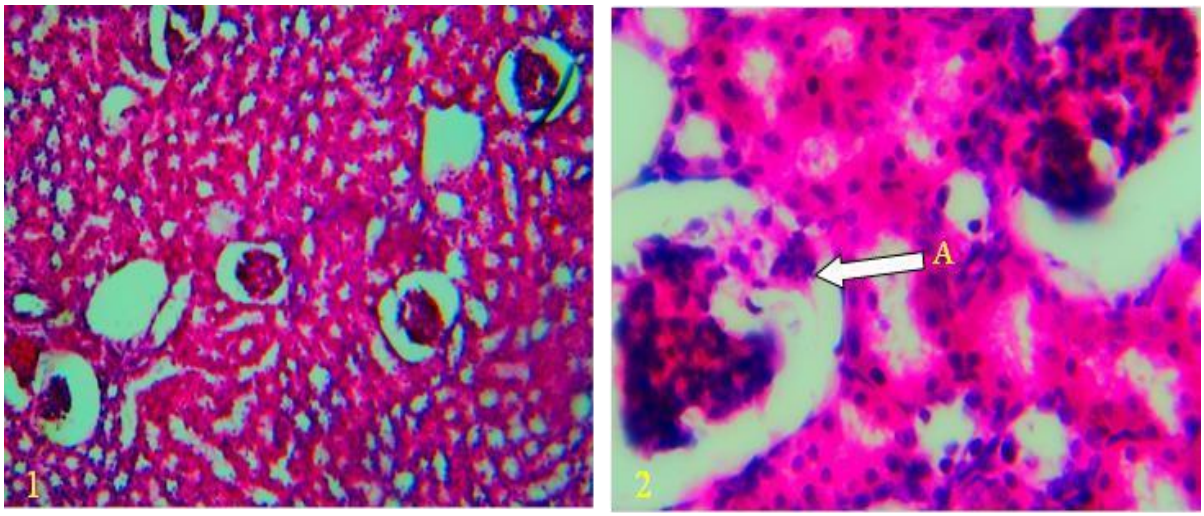


Figure :(4-20) (10x) In the Mix group shows (1) diminished and glomeruli and dilated tubules distorted
(2)(A) The number of glomeruli cells in this subcapsular area is reduced (40x).

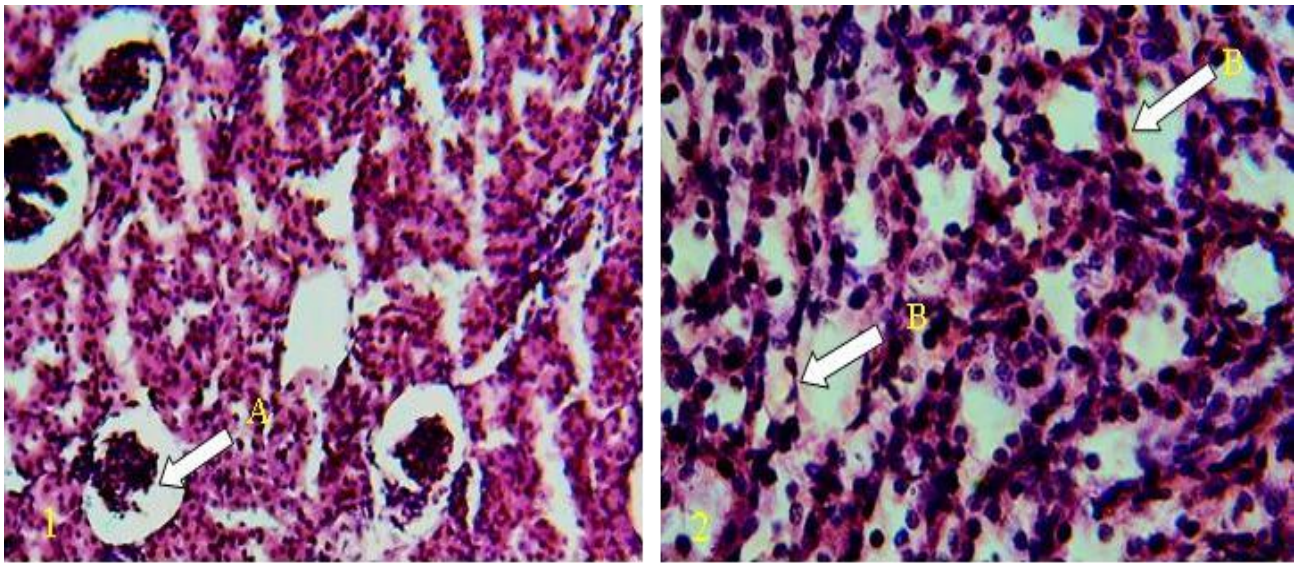


Figure :(4-21) (10x) In theOuter membrane group shows (1)The deformed architecture of tissue and atrophy of glomular tuft.(2) (A) Regeneration following acute tubule epithelial injury (40x).

Regarding effects on the kidney, the obtained results of the current study showed that MgONPs led to renal damage as indicated by histopathological changes in the kidneys in the form of distorted glomeruli, congestion, vaculations and Swollen renal tubular epithelial cells and atrophy of glomular tuft, Necrosis generally includes cells welling, nuclear pyknosis (Moradi-Sardareh *et al.*, 2018).

In the current study, both liver and kidney were used to evaluate the cytotoxic effects of MgO nanowires as they represent the common organs affected by chemical toxicity. The liver is an important site for the breakdown of most metabolites in the body and is referred to as the "metabolic clearing house" of the body (Almazroo *et al*.,2017; Zeng *et al*.,2017). Also, kidneys are responsible for the filtration of the blood, so itis not surprising that deleterious agents in the blood may accumulate there (Pizzorno.,2015).

The kidney is a central organ that plays an important role in drugs elimination and toxicity. This makes it the most susceptible to drug toxicity. Thus, kidney safety is routinely evaluated during safety assessments of the drug during its preclinical stages (Fuchs & Hewitt.,2011). Basically, blood urea nitrogen (BUN) and serum creatinineare the most applied tests upon which one can rely to monitor kidney function (Gowda *et al*.,2010).

The results of histopathological in liver and kidney appeared that MgO NPs at 4mg/ml that extracted by using bacteria was toxic , this might be different from NPs that used in reference (yang *et al.*,2010) .

Also the concentration of outer membrane of *salmonella* toxic in result this different from many research papers (Al-Warid.,2014).But the opinion of research as delivery was achieved when mix outer membrane with nanoparticales because result of histopathological was more toxic toliver and kidney .

Dedication

To my Lord, my Supporter……

To Prophet Muhammad and his holy household Imams, my ultimate guide…

To my mother and father, the secret of my existence ……

To my husband , my guider in my career

To my brothers and sisters, my true delight……

To my country, my home and pride…

I dedicate this work

Duaa

Conclusions:

1- MgO NPs is biosynthesized by using *Leuconostoc* spp are proved there nanoparticle.

2- Characterization of MgO by using X-Ray diffraction, Scanning Electron Microscopy, Fourier Transform Infrared and proved that is nanoparticales .

3- The best magnesium nitrate hexahydrate concentration is 0.1M and the best pH is 12.

4- MgO NPs is active antibacterial and anti biofilm

5-MgO NPs act as inducer for humoral and cellular immune response.

6- MgO NPs act as delivery for outer membrane immogene.

7- Both MgO NPs and OMP act synergistically, the mix increased immiogene activity each one alone.

8- MgO and OMP were toxic for tissue such as liver and kidney , mix of them act more toxic for tissue .

Recommendations

1-More about bacterial and chemical products MgO.2- Study the toxicity on the

other tissues like brain and spleen .

3- Study nontoxic effect for different concentration of MgO NPs on tissues in order to used in different clinical application.

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