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INFLUENCE OF *BRUCELLA MELITENSIS* ON MDA OXIDATIVE STRESS AND COMET ASSAY DNA DAMAGE AND PATHOLOGICAL CHANGE IN THE INTERNAL ORGAN OF FEMALE RATS IMMUNIZED WITH REV-1 AND GOLD NANOPARTICLES

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Abstract

In order to investigate the role of the *Brucella melitensis* infection in induce oxidative stress and the role of gold nanoparticle with and without Rev-1 vaccine and with freund complete adjuvant in protective of female rat against *B. melitensis* infection and effects on DNA damage. And the current study showed the role of the *Brucella melitensis* infection induce comet assay DNA damage with all rat immunized with Rev-1 and with or without gold nanoparticles and freund complete adjuvant. 60 female rats divided into six group treated as following. 1st group administered with Rev-1vaccine live attenuated of *Brucella melitensis* S/C. 2nd group administered with gold nanoparticle and Rev-1vaccine S/C. 3rd group treated with gold nanoparticle. 4th group administered with gold nanoparticle with Rev-1 and freund complete adjuvant S/C. 5th group served as control positive and six group served as control negative. all groups infected with *Brucella meletinsis* of challenge dose after 30 day post immunization. At the end of experimental blood sample collected from animals under euthanized and ethical condition and taken to evaluated the MDA oxidative stress and comet assay DNA damage. The result showed low oxidative stress of MDA in 1st group and more low in 2nd group but show most lowest in the 4th group. And showed highest level of MDA in control positive group infected only with *Brucella melitensis* of challenge dose. And the result showed low comet assay DNA damage in 1st group and more low in 2nd group but show most lowest in the 4th group. And showed highest level comet assay DNA damage in control positive group infected only with *Brucella melitensis* of challenge dose.

Keyword : *Brucella melitensis*, Rev-1, Gold nanoparticles and Freund complete adjuvent.

Introduction

Brucellosis is one of bacterial zoonotic diseases that affect different mammals include animals and humans around the world. The disease of Brucellosis caused by different species of the genus Brucella, Brucella characterized by gram negative bacteria, non spore forming, uncapsulated and intracellular coccobacilli (Al-Khafaji, 2003; Bechtol *et al*., 2010; Jaff, 2016). The humans infected with Brucellosis through ingestion of contaminated food or through the close contact with animals infected with Brucella, by handling the placentas or aborted fetuses of infected animal (Dean *et al*., 2012), also the Brucellosis transfer to other people through blood transfusion and bone marrow transplantation (CDC, 2016). Brucellosis characterized by pyrogenic disease without appear of any clinical signs in patient and the Brucella bacterial infection localized in organ of anybody system specially in joint, in farm animal the Brucellosis evident as abortions or breeding disorders (Bosilkovski *et al*., 2009). Clinical symptoms include severe disease but may include frequent fever, night sweats, sleeplessness, art hralgia, headaches, weakness, irritation, neuralgic symptoms and joint, muscle, and/or back pain (Pappas *et al*., 2006; Mohammed, 2015).

Zoonotic diseases is wide in Iraq such as *Anaplasma phagocytophilum* in sheep and brucellosis, so, animal vaccination is the best method for eradication of this bacterial zoonotic disease particularly in infection herd (Mohammed, 2015; Hamzah and Hasso, 2019; Hasso and Al-Janabi, 2019). The best method for prevent infection in human not eating undercooked meat and prevention of ingestion unpasteurized dairy products and using disinfectant instrument when handling tissues are the best safest ways to avoid transform infection for humans (Dado and Abdullah, 2000).

In small ruminant there several vaccine available to prevention of infection with Brucellosis but Re-1 of *Brucella melitensis* is the best vaccine which available used in control of Brucella infection (Blasco, 1997, 2006; Munoz *et al*., 2008).

Rev-1 live attenuated vaccine of *Brucella melitensis* it is until well known to eradication of Brucellosis (Elberg, 1981; Elberg, 1996), it is administration through subcutaneous route at standard doses, induces good protection against B in sheep and goats. Abortion linked to melitensis (OIE,2016)(Munoz *et al*., 2008).

There is no specific vaccine against *Brucella ovis* caused caprine Brucellosis but used of Rev-1 *B. melitensis* live attenuated vaccine more commonly in ovine , it is also act against *B.ovis* to prevention of Brucellosis (Blasco and Molina-Flores, 2011) . However, Rev-1 is virulence against human and cause abortion when vaccination of pregnant animal (Blasco and Molina-Flores, 2011) human infected with Brucellosis treatment with streptomycin it is the best choice of antibiotic (Ariza *et al*., 2005) And the researcher recommended to use the Rev-1 vaccine against Brucella infection in small ruminant because it is better control of this wildlife reservoirs (ANSES, 2015; Thébault *et al*., 2015). Nanotechnology is the science deals with and applied in many areas, including chemistry, biology, physics science, materials of engineering and field of healthcare, also from the other properties of metallic NPs support and biomedical applications (AbouEl-Nouretal, 2010).

There are several studies deals with of AuNPs are most extensively of NPs served as nobel metal because have various surface function and special plasmon reasons used in several ways Radetal., 2011; Ghosh *et al*., 2008).

The researcher showed the AuNPs can be paired with multiple and different nanobiological supplier such as drugs, antibodies and proteins. And the binding of these biomolecules with AuNPs can affects on the resonance (SPR) of surface plasmon, and increase conductivity and activity, thereby enhancing of their flexibility (Karuppiah *et al*., 2015).

The application of AuNPs in the biomedical field including used as therapeutic agent, drug delivery agent and act as antimicrobials effect (Shahzad *et al*., 2017) .

Materials and Methods

1. Rev1 Vaccine of ***Brucella melitensis*** (Abrovac Turkey).
2. Gold Nanomaterial Metals plates gold ounces are purchased from Al-Rafedian bank, with high purity listed of (99.999) for Au foil. The plates were polished, washed in ethanol and DDDW and cut off to pieces with dimensions to suite the experimental arrangement. The surface of the noble metals plate (ounce) was polished with 600-grade emery paper and applying to ultrasonically rinse in organic solvents before being prior to each experiment.

X-ray Diffraction

XRD pattern of gold nanoparticle prepared by laser ablation has been shown in figure (2). Generally of gold nanoparticle the peak of the XRD were observed between (20 and 100 degree).The presence of diffraction peaks indicates that the film is polycrystalline and no amorphous phase is detected. It is revealed that the nanoparticle has peak corresponding to (111), (200), (220), (311), (222) and (400) directions of the gold nanoparticle crystal structure.

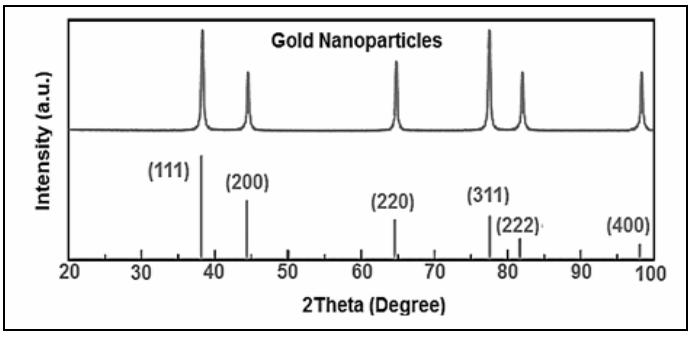

Fig. 2 : Generally of gold nanoparticle the peak of the XRD were observed between (20 and 100 degree)

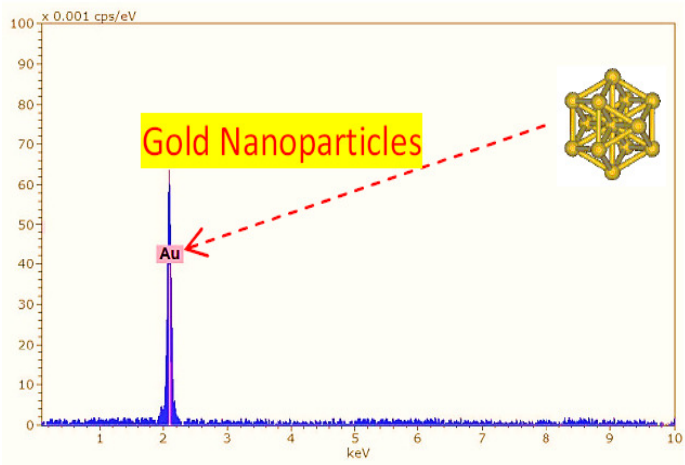

Fig. 3 : shows Energy Dispersive Spectrometry (EDS), it can be seen only demonstrating the element of Au, further confirms the high purity of gold nanomaterials and agreement with the XRD results.

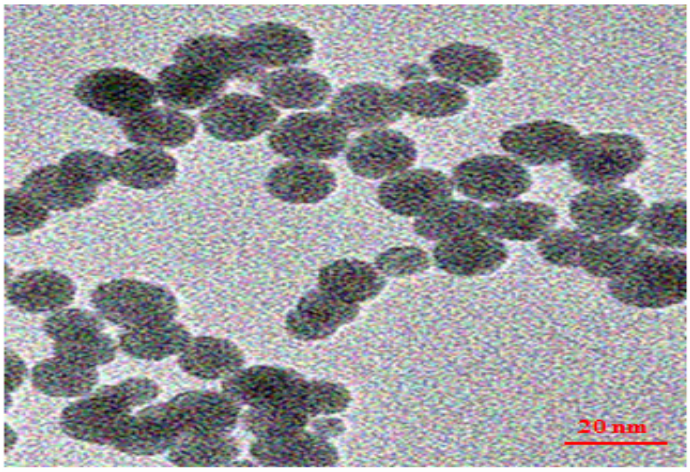

Fig. 4 : Show TEM picture, it can be observed that the morphology of the product is particle-like and nearly uniform in diameter (10 ± 5 nm).

3. ***Brucella melitensis*** **strains:**

Brucella melitensis Was obtained from the College Vet. Medicine in Baghdad condition, were cultured on routine culture media and confirmed diagnosis again by:

❖ **Biochemical test for routine bacterial identification**

❖ **Polymerase Chain Reaction (PCR):**

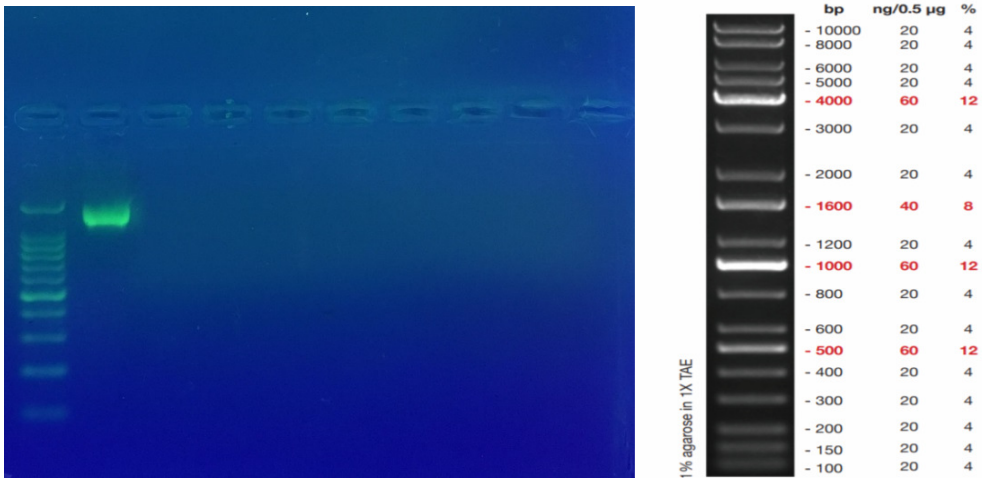

Fig. 5 : PCR product the band size 1250 bp. The product was electrophoresis on 2% agarose at 5 volt/cm^2. 1x TBE buffer for 1:30 hours. N: DNA ladder (100).

4- Freund's Complete adjuvant is a solution of antigen emulsified in mineral oil and used as an immunopotentiator .(FCA)(Manufactured by santa cruz USA) (Ramasamy *et al*., 2012)

5- Challenge dose of *Brucella melitensis* $2x10^4$ according to (Zeki yumuk *et al*., 2000) .

6- Determination of serum malondialdehyde (MDA) concentration: Done according to (Gilbert *et al*., 1984).

7- Dose of Rev-1 vaccine determination according to (Utba and Qays, 2015).

8- Determination the dose gold nanoparticle dose according to (Laima *et al*., 2012).

9- Comet assay determine DNA damage. Oxiselect comet assay kit was done according to (Olive *et al.,* 1990; De Boeck *et al.,* 2000).

10- 60 Female Rats divided into six groups.

Experimental design:

1. 1st group immunized with Rev-1 live attenuated vaccine *Brucella melitensis* (0.3ml) .
2. 2nd group will immunized with Rev1 Vaccine of *Brucella melitensis* two dose two week interval and treated with gold nanomaterial adjuvant(0.3ml) . .
3. 3rd group immunized with 0.3 ml of mix groups texture consist from 0.3 ml of rev-1 vaccine and 0.3 of gold nanoparticle adjuvant.
4. 4th group immunized as 2nd group and treated with Freund's Complete adjuvant (FCA).(0.3 ml S/C).
5. 5th group control positive infected with *Brucella melitensis*.
6. 6th group control negative.

At 30 days post immunization blood samples collected to determine the immune response

Result

Table 1 : Level of Malondialdehyde concentration(mM/dl) in female Rat with different treatments.

Oxidative stress (MDA)(Groups)	Mean ±Std. Error
G 1 immunized with Rev-1	23.34±0.52c
G 2 immunized with Rev-1 + gold nanoparticles	13.57±0.89d
G 3 treated with gold nanoparticles	33.34±0.58b
G4 immunized with Rev-1 + gold nanoparticles + Freunds complete adjuvent	5.48±0.51e
G 5 control +	45.80±1.65a
G 6 control -	0.38±0.10f
LSD	2.513

Table 2 : Mean and stander error of DNA damage in female Rat with multiple treatment:

Comet assay DNA damage (Groups)	Comet extent	Tail length	Tail DNA	Head DNA
	Mean ±Std. Error			
G1	100.23±0.58c	17.90±0.37c	19.70±0.65c	80.29±0.65d
G2	74.12±0.94d	17.05±0.83c	16.43±0.65d	83.59±0.66c
G3	110.08±0.54b	23.58±0.50a	33.39±0.99b	66.61±0.99e
G4	54.33±1.06e	13.89±0.65d	12.04±0.51e	87.96±0.51b
G5(C+)	125.06±1.47a	30.05±0.36a	45.93±0.41a	54.06±0.41f
G6(C-)	48.62±0.67f	8.76±0.63e	4.78±1.07f	95.21±1.07a
LSD	2.7381	1.7121	2.2121	2.2164

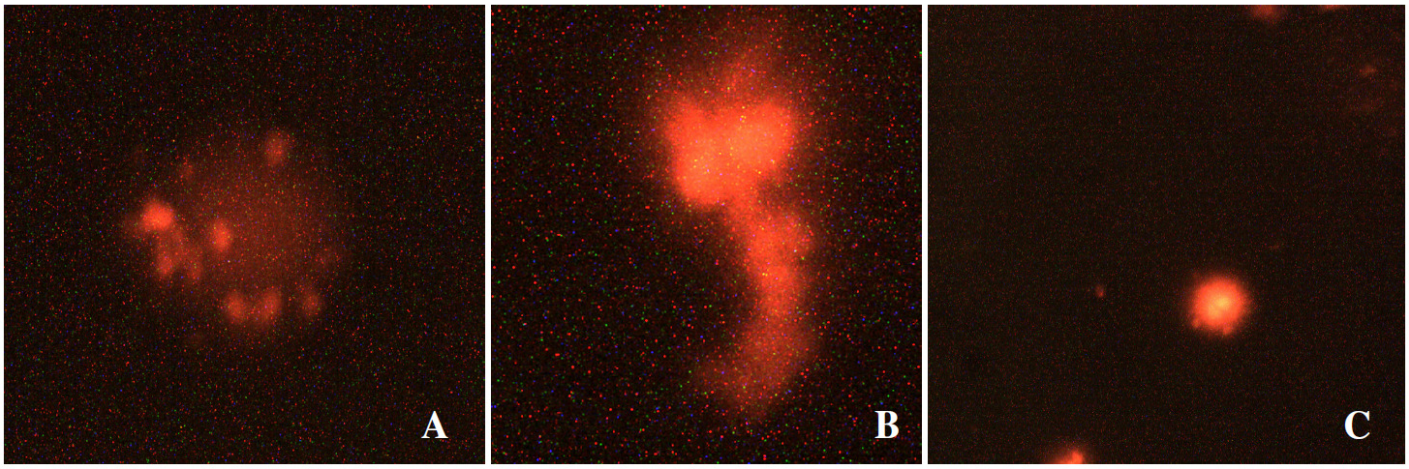

Fig. 1: Comet assay results in female rat peripheral blood lymphocytes examined by florescent microscope (400X) of (A) Brucella infected group And (B) Brucella infected group (C) control group showed fluorescent sphere without any DNA damage no tail. (Ethedium bromide stain).

Histopathology:

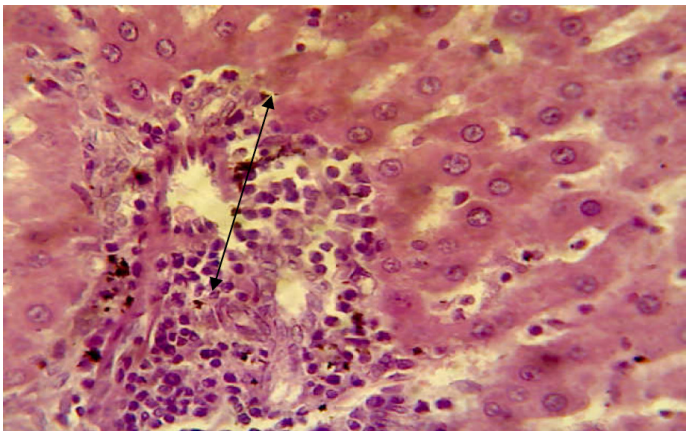

Fig. 1 : Section in the liver animal at 4 weeks post infection shows inflammatory cells infiltration in the portal area ↔ (H & E stain 400X)

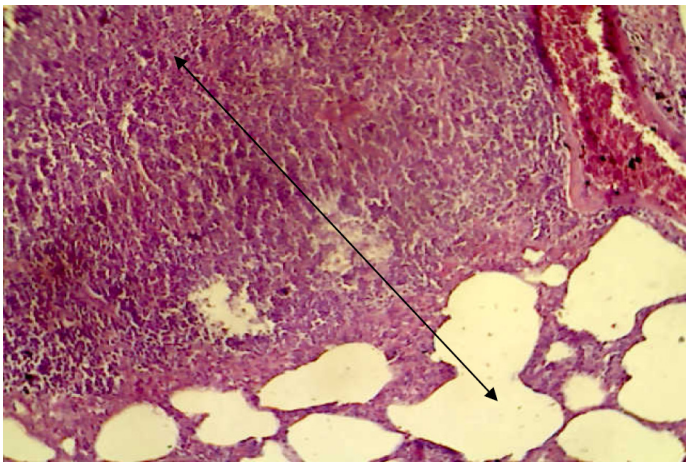

Fig. 2 : Section in the lung animal at 4 weeks post infection shows marked inflammatory cells infiltration in the interstitial tissue with emphysema ↔ (H & E stain 400X)

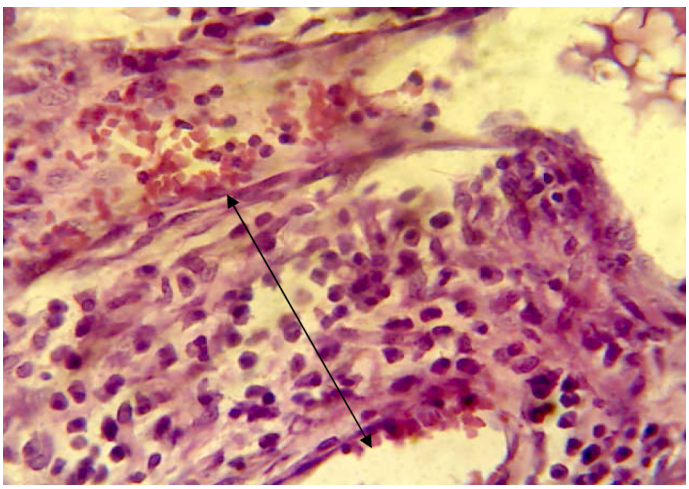

Fig. 3 : Section in the uterus animal at 4 weeks post infection shows neutrophils infiltration in the endometrium in congested blood vessels ↔ (H & E stain 400X)

Discussion

MDA oxidative stress

The organic oxidative stress compound containing the basic consist from the nominal formula $CH_2(CHO)_2$ is malondialdehyde (MDA). Malondialdehyde characterized by several properties like, a colorless liquid, is a highly reactive compound that exists much like the MDA. This is a common feature and characteristic phenomenon and marker represent for oxidative stress (Kumawat *et al*., 2014).

In an organism the product free radicals produce the cycle degradation of lipid peroxidation. The final cell product is Malondialdehyde (MDA) represent for one of the cells 'final products result from for the peroxidation and degradation of polyunsaturated fatty acids. The increase in free radicals revealed overproduction of MDA. The patients with cancer increase the level of malondialdehyde and this is generally referred to relationship between the cancer and increase measurement of oxidative stress and antioxidant status. the detection measurement Malondialdehyde (MDA) content in the studies was refer to a lipid peroxidation marker and detection in oxidative stress and redox signaling pathways; In particular, some studies occur in the plant on abiotic and biotic stress. And there is several publication for malondialdehyde from the past decade (Gaweł *et al*., 2004).

Based on table (1), the fifth group (control positive group) showed that level of MDA at the (30) day was higher value (45.80±1.65a) as compared with other groups at ($p<0.05$). Then the third group (that treated with gold nanoparticles only) showed (33.34±0.58) as MDA level at the day (30) post immunization at ($p<0.05$). Following by the first group (that treat by immunized with Rev-1 only) showed level of MDA was (23.34±0.52) at ($p<0.05$), Then the second group (that treated immunized with Rev-1 + gold nanoparticles) showed level of MDA was (13.57±0.89) at ($p<0.05$). While the fourth group (that treated with immunized with Rev-1 + gold nanoparticles + Freunds complete adjuvant) showed level of MDA was (5.48±0.51) at ($p<0.05$). While the sixth group (control negative) showed lower value in MDA level (0.38±0.10) compared with other groups at ($p<0.05$)as table (1).

There are Many studies for bacterial infection have shown and detect that will be increase of MDA, anywhere *Bacillus thuringiensis* ssp. infection has an impact. When the Infection with galleries can detect the increase the levels of serum superoxide dismutase concentration (SOD), S-transferase (GST), catalase (CAT), and malondialdehyde (MDA), glutathione. And these studies and this hypothesis of that bacterial infection reveal to express high level of oxidative stress rates, including MDA (Dubovskiy *et al*., 2008) (Lorente *et al*., 2013).

Increasing of Malondialdehyde in serum is occurring in stress status, Malondialdehyde is indicator in infection, radiation, toxicity and inflammation, therefore the results agree with our results (Yman, 2006).

In different biological sample Given very wide broad of differences in concentrations of malondialdehyde (MDA), and this result detect the MDA for oxidative stress used as biomarker for different studies in clinical investigations. Increasing of the malondialdehyde indicate oxidative stress such as inflammation, bacterial infection and associated high level of free radicals (Khoubnasabjafari *et al*., 2015) (Cherian *et al*., 2019) MDA is become higher and increase in serum, urine, and nasal fluid and saliva samples in oxidative stress (Cui *et al*., 2018).

While sixth group (G6) (non-treated group) showed lower concentration of MDA in serum because the animals of this group were not subjected to stress or inflammation, so no

increase in Malondialdehyde level was seen, and this is similar to our findings and supported it.

DNA damage

DNA damage is detect and significantly refer to different from in mutation, although they are both forms defect of DNA. The defect and an irregular chemical structure of DNA lead to damage of DNA and this damage also occur by mutation is a shift of DNA with the regular base pair sequence. The destruction and change in the structure of genetic material result from DNA damage and that lead to prevent of DNA from proper function and reduce the efficiency of replication (Giglia-Mari *et al*., 2011).

The mutation and damage of DNA result from varying biological defect. The DNA replication can repair the damage occur in DNA sequence, such repair is not 100 percent successful. In non-replicative cells, such as in the adult the cells in the brains or muscles doesn't replication, and in the adult unrepaired DNA damage accumulates, and cause aging. (See also the aging theory of DNA damage.) Errors occur in cell when replicating past damage in the DNA template strand or during repair of DNA damage, for example cells lining the colon. These mistakes of DNA replication can cause mutations of DNA or epigenetic alterations. All these forms of modifications of DNA mutation may be repeated and passed to cells functional subsequent generations. In the gene occur different alterations and change function or regulation of gene expression and these change possibly contribute to progression to cancer (Chatterjee and Walker, 2017).

There are numerous contain of checkpoints in the cell cycle that used to detect ensure the cell is in good shape and have to advance make towards mitosis. The three major and important checkpoints are at G1/s, G2/m, and anaphase-regulating used for progression at the spindle assembly to control level. Theses Checkpoints G1 and G2 include the important screening for damaged to DNA. (Lara-Gonzalez *et al*., 2012) The cell cycle is more effective to DNA damage during the S process more than any other part of the cell cycle. G2 checkpoint tests act as for completeness of damaged DNA and DNA replication. DNA damage is an alteration represent in DNA's chemical structure, such as a breakdown of DNA strand, and a base missing to DNA's backbone; or a chemically modified and changed in the base, including 8-OHdG. Also damage to DNA can occur during either naturally condition, or by environmental factors. The DNA damage response represent as is a complex signal transduction pathway observed and which recognizes when DNA i damaged and initiates the cellular response effect from the damage (Martin, 2008).

The result showed that the lower level of DNA damage is occur in the fourth group (that treated immunized with Rev-1, gold nanoparticles and Freunds complete adjuvent) as compared with the others groups (first, second and third group) at ($p<0.05$). The fifth group (control positive) showed higher level of DNA damage was (54.06±0.41) as compared with other groups at ($p<0.05$) as table (2).

Among bacterial toxins, these bacterial genotoxins are special and unique properties because their molecular target to effect is DNA. The result of bacterial intoxication or infection is the induction of DNA damage and breaks which result in irreversible cell cycle arrest or death of the target cells if not the cell is properly repaired. The number of Gram-negative bacteria produced toxin such as Salmonella Typhi and Escherichia coli these toxin lead to cell damage. (Grasso and Frisan, 2015).

Living organisms are constantly exposed to a multiple of harmful DNA agents that can affect their health cell. Damage of DNA usually occurs in bacterial infections, however. DNA damage mechanisms are often caused by different factors including radiation, bacterial infection and toxicity (Deplanche *et al*., 2019; Sahan *et al*., 2018; Žgur-Bertok, 2013).

There are several factor which cause to DNA damage related with the cell genome continually subjected to factors include exogenous and endogenous damage factors. Unless restored, malignant transformation of the cell occur by deleterious mutation result from of DNA. In the current study showed the DNA damage, apoptosis and delay in secretory phenotype of the cell cycle. All the reason effect on cells have developed an advanced and delay in efficient monitoring method, accordingly. All results in previous study support our results and agree with (Roos and Kaina, 2012; Pearl *et al*., 2015).

Histopathology

The main features of pathological changes in the examined organs induced by *Brucella melitensis* infection of no immunized animals, in the current study, are suppurative inflammation, these result was coincident with result of (Al-Khafaji1 and Al-Sultany, 2020) However, the main pathological lesions in the examin organs were neutrophils and macrophages infiltration in the 5th group ,these result may indicated chronic Brucella infection since, during acute infection, Brucella can attract neutrophils that engulf these pathogen (Colotta *et al*., 1992) and neutrophils can produced chemokines that attract the macrophages (Sabroe *et al*., 2005) (Sabroe *et al*., 2002), neutrophils act to kill the pathogens by the respiratory burst (Nauseef, 2007), however, Brucella can resisted killing by neutrophils and cause death of these cells by LPS (Braude, 1951) (Ackermann *et al*., 1988), the severe lesions in the 5th group post infection associated with high levels of MDA and DNA damage may due to *Br*-LPS-induced PMN cell death correlates with increase of ROS mediated by NADPH oxidase that induced oxidative tissue damage and DNA break down in addition to . necrosis or cause apoptosis (Fadeel *et al*., 1998), it was reported that DNA damage by oxygen radicals is a well-known phenomenon in a variety of cells, including PMNs (Geering and Simon, 2011)

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