

EFFECT OF A SINGLE DOSE OF PLGA NANOPARTICLES ENCAPSULATED PEPTIDE ON SOME PRODUCTIVE AND PHYSIOLOGICAL ASPECTS

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ABSTRACT : This study aims to evaluate the productive and physiological effect of polylactide-co-glycolide (PLGA) –peptide in broilers. A total of ninety eight day old, unsexed broiler (Rose) chicks were divided randomly into seven groups (2 replicate in each group) as follows : G1-control, G2 - chicks received traditional vaccine (Volvac[®] IBD MLV), G3 – Chicks Received PLGA nanoparticles only, G4, G5, G6 and G7 – chicks were received prepared vaccine at 160, 80, 40 and 20 µg of peptide loaded PLGA respectively.. Productive parameter for broilers were calculated. Coagulated blood samples for broilers were collected at day 42 for determination of some physiological parameters. Results show a significant increase in body weight gain accompanied by best feed conversion ratio in G6 (PLGA+40 µg peptide). The same group (G6) and (G7) reveal higher glutathione reductase (GSH) concentration in serum with lower malondialdehyde (MDA) concentration as compared to control (G1) and other treated groups. Concerning the enzymes, the data represent an increase in ALT with a decrease in AST and ALP in serum of broilers that received PLGA alone (G3) and that received 160 µg peptide loaded PLGA (G4).

Key words : Productive, physiological parameters, PLGA, broilers.

INTRODUCTION

The field of polymer nanoparticle (PNP) is rapidly growing and playing a necessary role in a varied field of area extending from medicine to biotechnology, conducting material to sensors, electronics and so forth (Rao J P and Geckeler K E 2011; Hameed Anaheed Hamdi 2018). Polymers are the most common materials for erecting nanoparticle-based drug carriers. In 1979 and for the first time the polymer polyal kyl cyanoacry latnano particle was studied as absorption of anticancer drugs (Bolhassani A *et al*, 2014). Biodegradable nanoparticles (NPs) (diameter: 10 to 1000 nm) are acquisition increased attention because of their ability to avail as a viable carrier for delivery of drugs and vaccine, which serve as excellent carriers by enhancing solubility, increasing half-life for clearance and targeting drug to specific sites in the body and have been proved to enhance the oral bioavailability of oral inactive antibiotics (Rauta P R, 2016).

A different type of synthetic polymers are used in preparing nanoparticles, such as poly (d,l-lactide-co-glycolide) (PLG) (Thomas C *et al*, 2011), poly (d, l-lactic-coglycolic acid) (PLGA) (Lu J-M *et al*, 2009), poly (ethylene glycol) (PEG), poly (g-glutamic acid) (g-PGA)

Akagi T *et al*, 2012) and polystyrene (Zhao K *et al*, 2014). PLG and PLGA nanoparticles are the most extensively investigated because of their biocompatibility and biodegradability (Danhier F *et al*, 2012). These nanoparticles entrap antigen for delivery to certain cells or sustain antigen release because of their slow degradation rate (Manish M *et al*, 2013).

Poly lactide-co-glycolide (PLGA) is a synthetic copolymer of lactic acid (α-hydroxypropanoic acid) and glycolic acid (hydroxyl acetic acid) and one of the best regularly degradable polymers. PLGA has generated great interest because of its astonishing biodegradability, biocompatibility, and mechanical strength (Stevanovic M *et al*, 2008). Thus the experiment design to evaluate the effect of PLGA as adjuvant on productive parameters and some physiological parameters in broilers.

MATERIALS AND METHODS

PLG nanoparticles encapsulating peptide were prepared by solvent evaporation method (McCall R and Sirianni R, 2013; Kumar V, 2014), with some modification, which can be summarized: 200 mg of PLGA dissolved in 2 ml of DMSO and left at room temperature overnight, PVA prepared by dissolving 2gr in 100 ml of diH₂O. The

PLGA solution added drop wise to PVA and left overnight on stirrer then the product solution centrifugal and wash 3X and the precipitate collected and lyophilized for 72hr.

In this study, 98 one day old, broiler chick (Ross-308) taken from a commercial hatchery, Suhoor Al-Khairat \ Babil province were used, randomly divided equally into 7 groups (14 chick) fed on an ordinary diet, starter from day 1 to 20 (22 % crude protein, 2926 K Cal / Kg) and finisher from day 21 to 42 (19 % crude protein, 3109 K cal / Kg) *ad libitum*. At day 19 the broiler chick administered orally with distilled water (G1), Traditional vaccine(G2), PLGA NPs (G3), PLGA+160µg peptide(G4), PLGA+80µg peptide(G5), PLGA+40µg peptide(G6) and PLGA+20µg peptide(G7).

The following parameters were detected

Body Weight, Feed Consumption and Feed Conversion Ratio (FCR)

At the beginning and end of exp. the chicks weighted individuals using digital balance and body weight gain mean were calculated for each group. Feed intake (F.C.) was measured according to Al-Zubaidi (Al-Zubaidi S S, 1986) by weighting the remaining fees at the end of exp. and subtracted from total quantity offered at the beginning of exp.. The F.C. Mean for each group was calculated by dividing the total quantity of feed intake on the number of chicks in the group. According to Al-Zubaidi (14), FRC calculated by using the following equation:

$$\text{FRC} = \text{Feed intake (GM)} / \text{body weight (GM)}.$$

Liver Function Tests (ALT (GPT); AST (GOT) and ALP

The liver function tests were done according to the direction of kits manufactured company (Mindray company) and NCCLS (SAS, 2010).

Serum Malondialdehyde (MDA) level and Glutathion Reductase

Detection lipid peroxidation is essential to estimate oxidative stress, Lipid peroxidation forms Malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE), as a natural bi-products. So measuring the MDA is one of the most accepted assays for oxidative damage. By using Cohesion Biosciences kit the MDA concentration in serum was detected and at wavelength 532 nm. Glutathione reductase is a flavoprotein that catalyzes the NADPH-dependent reduction of oxidized glutathione (GSSG) to glutathione (GSH). This enzyme is essential for the GSH redox cycle, which maintains adequate levels of reduced cellular GSH. A high GSH/GSSG ratio is essential for protection against oxidative stress. By using Cohesion Biosciences kit the oxidation of NADPH to NADP⁺ is

accompanied by a decrease in absorbance at 340 nm.

Statistical Analysis

Statistical analysis of data was performed using SAS (Statistical Analysis System - version 9.1). One-way ANOVA and least significant differences (LSD) post hoc test were performed to assess significant differences among means. ($P < 0.05$) was considered statistically significant (Al-Hyali H M and Al-ubory K H, 2001).

RESULTS AND DISCUSSION

Body Weight, Feed Consumption and Feed Conversion Ratio

The body weight data of broilers at the end of the experimental period are presented and figure (1). There is a significant ($p \leq 0.05$) increase in mean body weight (BW) of G6 (NPs+40 µg. Peptide) which record the highest weight (3110 ± 33.16 gm) while G4 (NPs+160 µg. Peptide) record the lowest BW. (2810 ± 70.71 gm). At the meantime, there is no significant ($p \leq 0.05$) difference among other groups (3040 ± 67.82 gm; 3030 ± 94.33 gm; 2950 ± 44.72 gm; 2900 ± 70.71 gm; and 2850 ± 70.71 gm for G5, G1, G7, G3 and G2 respectively.

In food consumption, there are arithmetic, but not significant ($p \leq 0.05$) differences among all experimental groups during the experiment period (figure 2).

FCR data figure (3) showed a significant increase ($p \leq 0.05$) in G4, which reported 1.42 ± 0.03 while G6 had the lowest value (1.29 ± 0.02). The rest groups of the experiment (G1, G2, G3, G5, and G7) show accounting, but not significant differences ($p \leq 0.05$).

Liver Function Tests

In figure (4), there is a significant ($p \leq 0.05$) increase in ALT value in a group of broilers received PLGA nanoparticle (G3) and PLGA+160µg(G4) compared to control and groups that received ordinary vaccine and those received PLGA loaded with other concentrations of peptide and all these groups show a significant ($p \leq 0.05$) increase in ALT value compared with control (G1). On the other hand, the mean values of AST(5) in broilers serum reveals a lower significant ($p \leq 0.05$) values in AST in broilers serum of G2, G3 and G4 groups in comparison with control and all other treated groups. Moreover, the table reveals a significant ($p \leq 0.05$) decrease in ALP values in serum of all treated groups (G2, G3, G4, G5 and G7) compared with G1 and G6 (Lu J-M *et al*, 2009).

Oxidant and antioxidant enzymes (serum MDA and Glutathione Reductase concentration)

Figures (7) and figure(8) represent the mean value of serum MDA and glutathione reductase activity in all groups respectively. There is a significant ($p \leq 0.05$)

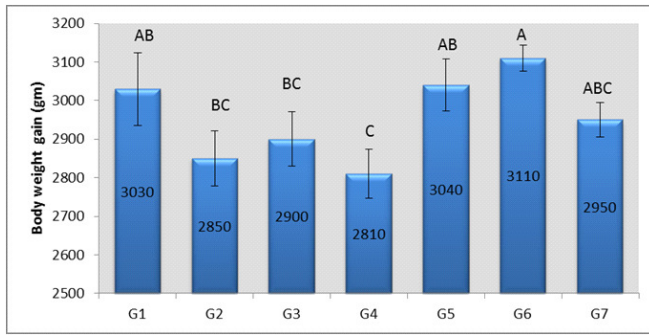


Figure 1 : Effect Of PLGA Nps And PLGA Loaded With Different Concentration Of Peptide On Body Weight gain (gm) In Broilers.

G1=received diH2O, G2= ordinary vaccine,G3=PLGA nanoparticle,G4= PLGA+160 µg peptide, G5=PLGA+80 µg peptide,G6=PLGA+40 µg peptide, G7=PLGA+20 µg peptide. N=14

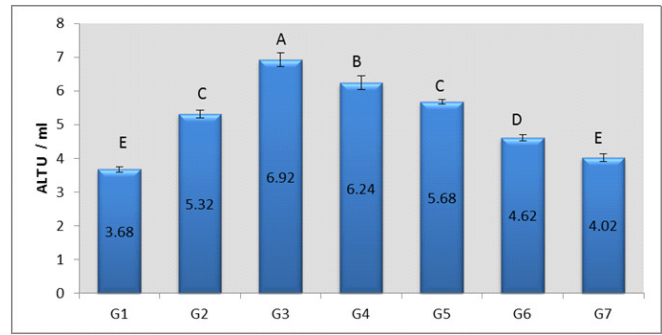


Figure 4 : Effect Of PLGA Nps And PLGA Loaded With Different Concentration Of Peptide On Serum Concentration Of Alanine Transaminase (ALT) In Broilers.

LSD: 0.4008 The numbers represented as Mean±S E.G1=received diH2O, G2= ordinary vaccine,G3=PLGA nanoparticle,G4= PLGA+160 µg peptide, G5=PLGA+80 µg peptide, G6=PLGA+40 µg peptide, G7=PLGA+20 µg peptide.

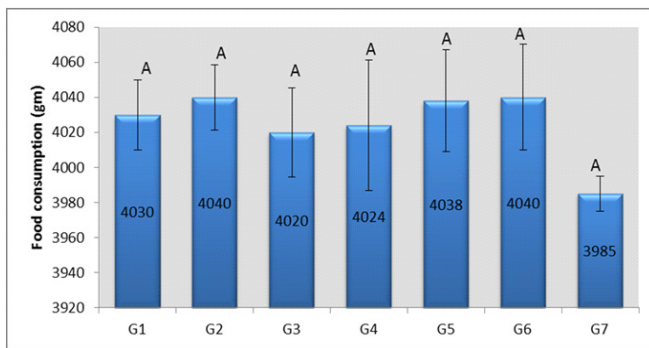


Figure 2 : Effect Of PLGA Nps And PLGA Loaded With Different Concentration Of Peptide On Feed Consumption (gm) In Broilers.

LSD: 74.523 The numbers represented as Mean±S E. G1=received diH2O, G2= ordinary vaccine,G3=PLGA nanoparticle,G4= PLGA+160 µg peptide, G5=PLGA+80 µg peptide,G6=PLGA+40 µg peptide, G7=PLGA+20 µg peptide.N=14

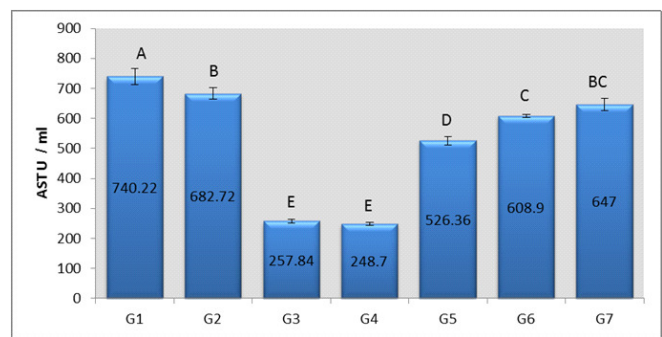


Figure 5 : Effect Of PLGA Nps And PLGA Loaded With Different Concentration Of Peptide On Serum Concentration Of Aspartate Transaminase (AST) In Broilers.

LSD: 46.598 The numbers represented as Mean±S E.G1=received diH2O, G2= ordinary vaccine,G3=PLGA nanoparticle,G4= PLGA+160 µg peptide, G5=PLGA+80 µg peptide, G6=PLGA+40 µg peptide, G7=PLGA+20 µg peptide

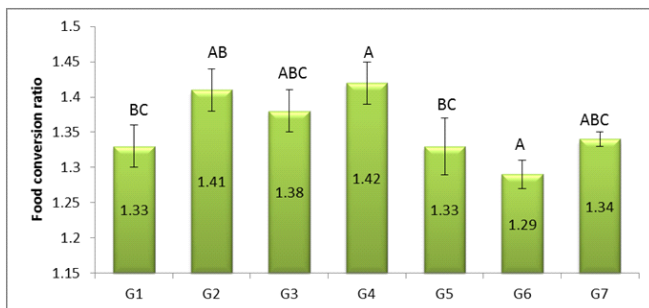


Figure 3 : Effect Of PLGA Nps And PLGA Loaded With Different Concentration Of Peptide On Feed Conversion Ratio In Broilers.

LSD: 0.0918 The numbers represented as Mean±S E. G1=received diH2O, G2= ordinary vaccine,G3=PLGA nanoparticle,G4= PLGA+160 µg peptide, G5=PLGA+80 µg peptide,G6=PLGA+40 µg peptide, G7=PLGA+20 µg peptide.N=14.

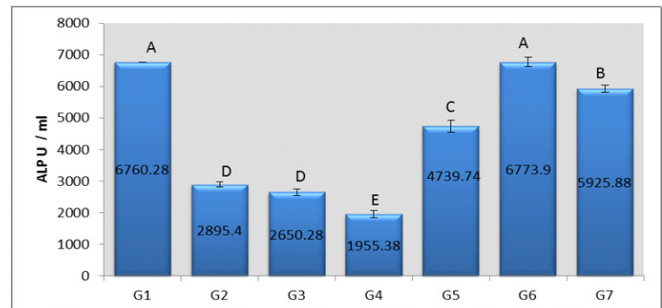


Figure 6 : Effect Of PLGA Nps And PLGA Loaded With Different Concentration Of Peptide On Serum Concentration Of Alkaline Phosphatase In Broilers.

LSD: 385.74. The numbers represented as Mean±S E.G1=received diH2O, G2= ordinary vaccine,G3=PLGA nanoparticle,G4= PLGA+160 µg peptide, G5=PLGA+80 µg peptide, G6=PLGA+40 µg peptide, G7=PLGA+20 µg peptide

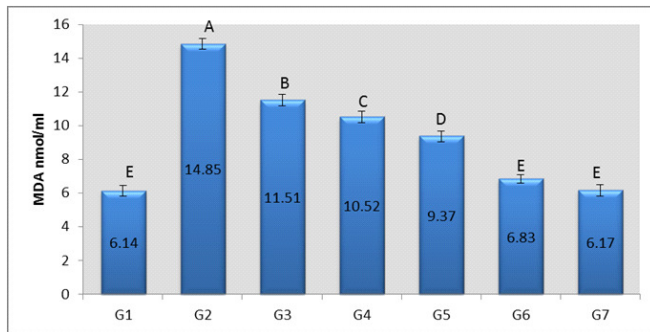


Figure 7 : Effect Of PLGA Nps And PLGA Loaded With Different Concentration Of Peptide On Serum Concentration Of MDA (Nmol/ml) In Broilers.

LSD: 0.9437. The numbers represented as Mean \pm S E. G1=received diH₂O, G2= ordinary vaccine, G3=PLGA nanoparticle, G4= PLGA+160 μ g peptide, G5=PLGA+80 μ g peptide, G6=PLGA+40 μ g peptide, G7=PLGA+20 μ g peptide

increase in MDA level (14.85 ± 0.33 nmol/ml) accompanied with a significant ($p \leq 0.05$) decrease in glutathione reductase activity (1.10 ± 0.05 U/ml) in serum of broilers received ordinary vaccine (G2) compared with control and all other groups received PLGA Nps and PLGA loaded with peptide. On the other side, the figures show a lower significant ($p \leq 0.05$) values in MDA (6.83 ± 0.25 and 6.17 ± 0.33) for G6 and G7 sequentially in comparison to G3 (11.51 ± 0.33), G4 (10.52 ± 0.34) and G5 (9.37 ± 0.33) with higher significant ($p \leq 0.05$) levels in glutathione reductase activity (2.40 ± 0.12 and 1.89 ± 0.08) in broilers of G7 and G6 respectively in comparison to G3 (1.45 ± 0.09), G4 (1.38 ± 0.09) and G5 (1.60 ± 0.15) sequentially.

Productive parameters

The goal of poultry industry is obtain the higher body weight in short time with low cost. The significant increase in the body weight of broilers at the end of the experiment specially in G6 which received PLGA+40 μ g of peptide, compared to those received ordinary vaccine (G2) may be attributed to the improvement of immunity which reflected in improved productivity traits in poultry and this is consistent with finding of Al-Hyali and Al-Jubory (Razooqi R H *et al*, 2018) who attribute the body weight increasing to the effect of IBD vaccine on the thyroid gland and thyroxine releasing which has important role in cell metabolism and growth hormone. Also Razooqi *et al* (Engineer C *et al*, 2011) show that use of polymeric NPs lead to increase body weight in broilers. Moreover, the increase in the body weight may be due to the effect of PLGA nanoparticles (adjuvant) itself when degraded inside the body to its monomer compounds (lactic acid and glycolic acid) (Mani-López E *et al*, 2012). These two acids play a role in the body

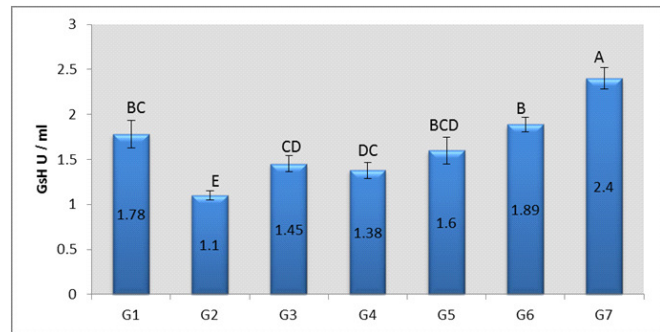


Figure 8 : Effect Of PLGA Nps And PLGA Loaded With Different Concentration Of Peptide On Serum Concentration Of Glutathione Reductase (U/ ml) In Broilers.

LSD: 0.3309 The numbers represented as Mean \pm S E. G1=received diH₂O, G2= ordinary vaccine, G3=PLGA nanoparticle, G4= PLGA+160 μ g peptide, G5=PLGA+80 μ g peptide, G6=PLGA+40 μ g peptide, G7=PLGA+20 μ g peptide

weight improvement either by decrease the PH of the gut and inhibition of the pathogenic bacterial growth or by increasing nutrient retention.

These reasons are consistent with the findings of Mani-Lopez *et al* (Kim J W *et al*, 2015) and Kim *et al* (Kil DY *et al*, 2011). On the other hand, these acids may increase the activity of digestive enzymes (pancreatic enzymes), that lead to improve the digestion of protein and other nutrients (Pryde S E *et al*, 2002). Also the current result may be assigned to the peptide sequence (antigenic region) which loaded on the PLGA nanoparticles, this peptide composed of several amino acids, some of them are essential a.a, which in addition to stimulate immune system, they may enter in the gluconeogenesis by TCA cycle and protein building (Tkachenko H *et al*, 2016).

Feed intake in our experiment reveals there was non-significant differences among other groups, and this could be explained by the better hygiene and management under which all birds were reared and that matches with findings of Razooqi *et al* (Engineer C *et al*, 2011).

The lower FCR is better and means a lower amount of food is needed to obtain higher body weight. Therefore, broilers of G6 which subjected to 40 μ g peptide loaded on PLGA showed the highest body weight accompanied by lower FCR.

Liver function enzymes

Transaminases like AST and ALT play significant roles in amino acid and protein metabolism, and they might be released into the plasma following tissue damage and dysfunction (Thrall M A *et al*, 2004). Aspartate aminotransferase (AST) activity has been reported in the liver, skeletal muscle, heart muscle, brain and kidney.

Increases of plasma AST activity in birds are suggested when such activity is greater than 275 IU/L and it considered to be markedly increased when the activity is greater than 800 IU/L while for Alanine aminotransferase (ALT) activity in most species of normal birds ranged from 19 to 50 IU/L (Senanayake S S *et al*, 2015).

Therefore, depending on such information, the results of current experiment confirm a normal serum ALT and AST levels in all treated and control groups of broilers. Moreover, Alkaline phosphatase activity in birds primarily results from osteoblastic activity. Therefore, increases in the plasma ALP activity are suggestive of skeletal growth, healing fractures and pre-ovulation condition in hens (Senanayake S S *et al*, 2015). The ALP enzyme activity also increased with age and it was higher during the growth of birds from 15 to 25 days of age and reached a plateau from the age of 25 to 40 days.

Alkaline phosphatase ALP is an enzyme responsible for dephosphorylation of a substrate, therefore, it is produced in all types of tissues in the body, but it gets activated in alkaline pH. Thus, elevated levels of ALP can be mostly seen in liver damages and might be as a by-product in active bone formation (Fu C *et al*, 2017) also Fu *et al* (Krucinska I *et al*, 2017) mention that the high reactivity of ALP represented the osteogenic differentiation of the cells so when graphene oxide (GO) (bone tissue engineering) loaded on PLGA Nps the mechanical properties, ALP activity, calcium deposition and osteogenic gene expression became obviously stronger than those of free GO. While Krucinska *et al* (Surai P F, 2015) observed no significant differences in AST, ALT and ALP values in rabbits received PLGA Nps loaded with insulin-like growth factor (IGF1).

The findings of the present experiment confirm that the broilers involved in the study possess a normal healthy condition with no damage in their tissues. All broilers received ordinary vaccine or those have vaccine loaded with nanoparticles showed lower values of ALP compared with control. The increase in the level of liver enzymes even if within the normal limits recorded by Thrall *et al* (Senanayake S S *et al*, 2015) may be due to the increase in weight recorded in the birds of the experiment, which resulted in an increase in liver activity to produce enzymes and the protein industry that led to weight gain.

The elevation observed in the ALP enzyme may be due to the need to build a strong skeleton that will tolerate the increase in weight in animals.

Oxidant and antioxidant enzyme

Oxidative stress, defined as an imbalanced condition between oxidants formation and elimination by cellular

antioxidant system, has been regarded as one of the most pesky issues in the modern poultry industry (Gessner D K *et al*, 2017 and Lee M T *et al*, 2019), owing to the fact that the source of these oxidants varies, from normal metabolism like byproducts of mitochondrial respiration and other physiological metabolism in tissues (Mishra B and Jha R, 2019), to an externally triggered form like immune response- activated superoxide radicals from polymorphonuclear leukocytes and other phagocytes, and radiation exposure-produced hydroxyl radicals (Gessner D K *et al*, 2017). Developing of the oxidative stress tends to cause damage to lipids, DNA, proteins and other cell constituents, resulting in severe disruption of cell integrity or even tissue damage (Mishra B and Jha R, 2019 and Ibrahim W *et al*, 2017)

The significant decrement in MDA level, in serum of groups received PLGA NPs and PLGA loaded with different concentration of peptide at the end of the experiment, with significant elevation, in glutathione reductase activity in same manners as showed in figures (Akagi T *et al*, 2012 and Zhao K *et al*, 2014) respectively in compared with G2 which received traditional vaccine, indicate that polymeric nanoparticles have little or no toxicity and do not cause increase level of free radicals in compared to traditional vaccine.

The MDA is used as a biomarker of oxidative stress. MDA may cause damage of proteins and lipids which present in cell membrane (Risom L *et al*, 2005). The key factors involved in NP-induced ROS include prooxidant functional groups on the reactive surface of NP, active redox cycling on the surface of NP due to transition metal-based NP and particle-cell interactions (Khanna P *et al*, 2015). While according to Khanna *et al* (Astete C E *et al*, 2011) polymeric nanoparticles such as Chitosan and PLGA reported no toxicity due to ROS because they decrease cellular damage and reducing ROS formation by interaction with NPs and preventing formation of hydroxyl radicals and allowing the cell's antioxidant defense mechanisms to neutralize ROS before they become toxic (Yu M *et al*, 2012 and Zhao K *et al*, 2014). These findings are documented by the results of the current study.

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