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RESEARCH ARTICLE

Influence of beta-glucan on Genotoxic effects of silver nanoparticles on immunized mice

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Abstract

In order to investigate the influence of beta-glucan and augment immune response on genotoxicity of Silver-nanoparticles,40 white mice ,both sexes average age 8-10 weeks were randomly divided into 4 groups equally and treatment as following: 1st group was administrated with 20m\kg BW of Silvernanoparticles(Ag-NPs) 20nm in size, for 6 weeks,2nd group was immunized with o.3ml of whole sonicated Salmonella hader(protein concentration 2mg\ml .S\C two dose ,two weeks interval and at same time administrated with Ag-NPs as in 1st group,3rd group was administrated with Ag-NPs as in 1st group and at same time was immunized as in 2nd group and fed diet supplementing with 2g\kg diet of beta glucan,4th group was administrated orally with o,3ml of sterile saline for 6 weeks and served as control negative group.

At the end of the experiment ,all the animals were sacrificed and bone marrow samples were taken for demonstration of blastic,mitotic index .micronuclei and chromosomal aberration.

The result showed low ratio of blastic and mitotic index with high number of chromosomal aberration and micronuclei in animals treated with Ag-NPs ,and the ratio of blastic index and mitotic index in cells of bone marrow cells of immunized animals –Ag-NPs were higher than those value in 1st group but less in 3rd group, few to moderate or absent of chromosomal aberration and micronuclei were recorded in immunized animals with or without fed diet supplementing with beta –glucan and administrated Ag-NPS.

It was concluded that Ag-NPs have genotoxic effects and augment immune response with Beta-glucan could diminished these toxic effects .

Introduction

Nanotechnology is a rapidly growing industry that expressed huge scientific and economic potential.Nanomaterials are engineered materials have at least one dimension of 100 nanometeres or less(1-Andre et al.,2006)

Later on,these nanoparticles particulary silver-nanoparticle widely Commercial using for variable purposes include fillers, opacifiers, catalysts, semiconductors, cosmetics, microelectronics, and drug carriers ,wound-dressing, bandages, catheters, and other medical devices (2,3)(Hagen, *et al*.,2011;Lee *et al*.,2010),silver-based hydrogel (4-Thomas ,*et al*.,2007) .in addition they were used in , soaps, pastes , water disinfectants and infant products (5-Fauss,2008),as well as Ag-NPs expressed a broad antimicrobial activity which expressed less chance of drug resistance(Barbu *et al*.,2009;Ip *et al*.,2006)(7,8)

Later on ,several researches demonstrated that Ag-NPs could induce toxicity in animals and humans through different ways of exposure such as inhalation(9)(Sung,*et al.*,2009). Ingestion (10)(Kim, *et al*.,2010),hypodermal . injection (11)(Tang *et al.*,2009)

Ag-NPs specific physical and chemical properties such as less changes in their morphological, shape and size in the environment due to expressed long-term stability at room temperature in aqueous medium (12-Pinto *et al*., 2010),As a result of these physical and chemical properties, Ag-NPs cause significant cytotoxicity and genotoxicity that associate with induction of oxidative stress and inflammatory reaction (13,14)(Asharani *et al*., 2009a; *AshaRani et al*., 2009b).

Ag-NPs induced Chromosomal aberration as due to interaction silver ions with DNA that may be prevent cell divion and DNA replication (15)(Morones *et al.*, 2005). (16)(Hui *et al*.,2012) reported that Ag-np induced genotoxicity via double-strand breaks (DSBs) and they suggested that DNA-PKcs is responsible for the repair of Ag-NP induced DNA damage. (17)(Lin *et al*.,2005) revealed that beta-glucan can augmented IkB kinase,NF-KB activity and MAPK phosphorylation.
therefore ,more researches were required to determine the toxic effects of Ag-NPs on animals and humans, In Iraq, there are little researchs about the genotoxic effects of Silver nanoparticles, therefore the current study aimed to determine the genotoxical effects of silver nanoparticles and the role of bega-glucan in neutralized these effects in immunized mice mice

Materials and method

Silver nanoparticles

Silver nanoparticles The size Silver nanoparticles 20 nm and were purchased from china and manufacturer from Hongwu Nanometer ,and were at least 99.99% pure.

Toxic dose

Dose levels were selected based on previous observations in a 28-day oral toxicity study by (Young *et al*.,2010)(18)

Antigen preparation

Whole sonicated Salmonella hader were preparated according to (19Mitove etal 1992)

Experimental design

Fourty white mice both sexes,average age 8-10 weeks randomly divided into four groups equally and treated as following :

1-1st group was administration with 20mg\kg B.W of Silver nanoparticle(Ag-NPs) (2nm size) for 6 weeks.

2-2nd group was immunized with Whole sonicate Salmonella hadar ,two dose, two weeks intervals and at same time was administrated with Ag-NPs as in the 1st group.

3-3rd group was immunized as in 2nd group and at same time administrated with silver nanoparticles as in 1st group and fed diet supplemented with b2mg\kg fed beta-glucan for 6 weeks .

$^{4-4th}$ group was oral administrated with 0.3 ml of sterile normal saline and it was served as control negative group.

all animals will be sacrificed at end of the experiment (6weeks) and samples were taken from bone marrow cells to determine chromosomal aberration and blastic and mitotic index according to (20 Yaseen,1990).

Result and Discussion

The result showed low ratio of blastic index and mitotic index(6.6+0.48,11.6+0.4 respectively) in cells of bone marrow of animal administration with Ag-NPs for 6 weeks as comparing with those radio in control negative group (25.3+0.34;12+0.21 respectively) but these ratio was high in immunized (23.0+0.50, 12.50+0.47 respectively) and immunized animals fed diet supplement with beta-glucan(27.00+0.47,13.00+0.40 respectively post-treated with Ag-NPs for 6 weeks (table:1).also the present study investigated chromosomal aberration in bone marrow cells of animals administrated with Ag-NPs for 6 weeks ,these aberration include high number of chromatoid ring,gap,fragments and centromeric associated with large number of micronuclei(Fig:1-5), while few chromatoid ring ,moderate chromatoid fragments and few micronuclei were seen in immunized animals post-treatment with Ag-NPs(Fig:6-8).The immunized –fed diet supplementing with beta-glucan expressed few chromatoid ring and fragment(Fig:9) but the control negative group showed few chromatoid fragment (Fig:10) (table:2)

Table:1.shows ratio of blastic index ,and mitotic index in immunized animals exposure to Ag-NPs with or without fed diet supplementing with beta-glucan

Groups	BI	MI	N
G1 Toxin	6.6 ± 0.48 Dc	11.6 ± 0.4 Ab	47 ± 0.49Da
G2 Immune + toxin	23 ± 0.50 Bb	12.5 ± 0.47 Ac	64.5 ± 0.48 Ba
G3 Immune +B+ toxin	27 ± 0.47 Ab	13 ± 0.4 Ac	60 ± 0.5 Ca
G4	25.3+0.34	12+0.21	63.7+0.40

L.S.D : 1.4

Table:2.shows chromosomal aberration in cells of bone marrow

Group	Type of chromosomal abberation			
	Ring chromatoid	Break chromatoid	Gap chromatoid	micronuclei
G1	+++	+++	+++	+++
G2	+	++	-	+
G3	+	+	-	-
G4	-	+	-	-

+=few number ,++=moderate number, +++= large number

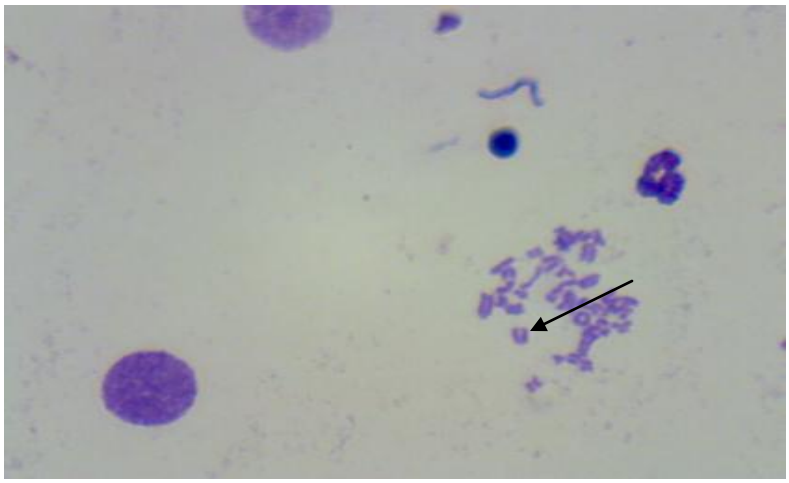

Fig:1 Shows large number of chromatoid rin ⟶ and fragment in bone marrow cells of animal treated with Ag-NPs for 6 weeks

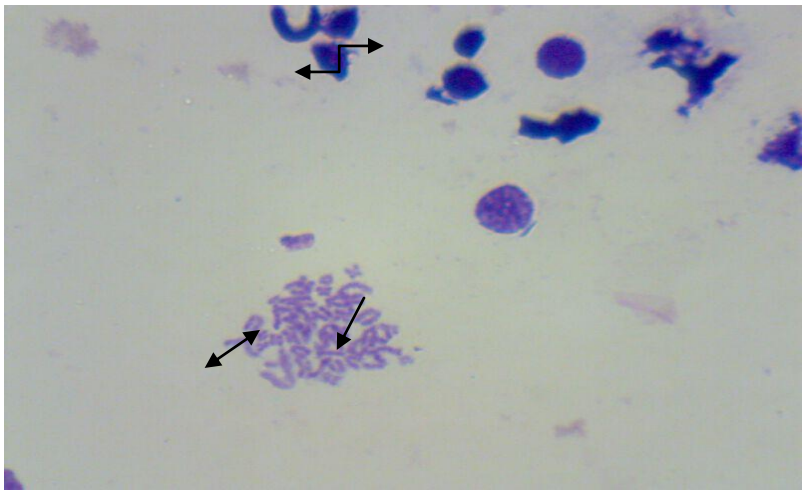

Fig:2. Shows large number of chromatoid gap ⟶ ,rin and fragment ⟷ as well as micronuclei ⇄ in bone marrow cells of animal treated with Ag-NPs for 6 weeks

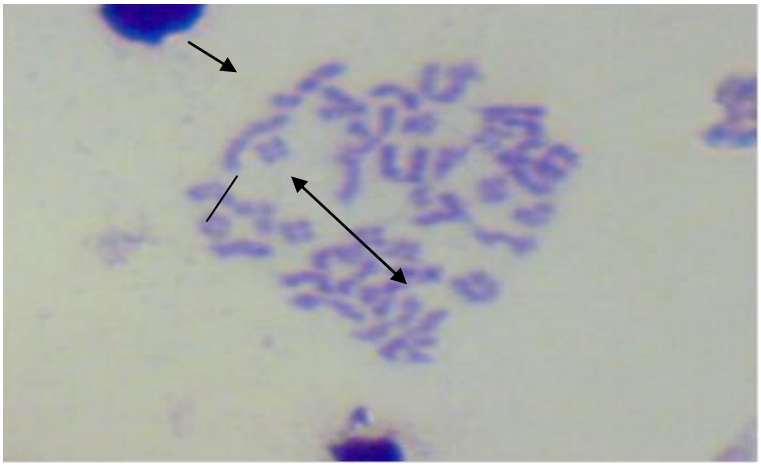

Fig:3.shows Chromatoid ring ,dicentric chromosome ⟷ chromatoid Gap ⟶ ,chromatoid fragment —— in bone marrow cells of treated animal with Ag.NPs

for 6 weeks

dicentric chromosomes

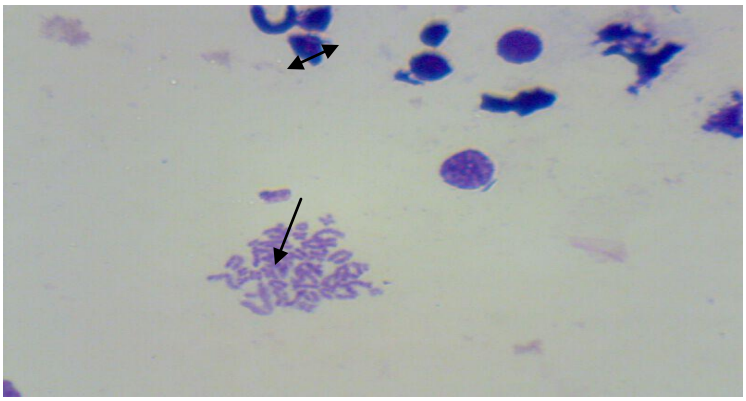

Fig:4. **Fig:7.shows large number of Chromatoid ring ⟶ in addition micronuclei ⟷ in bone marrow cells of treated animal with Ag.NPs**

for 6 weeks

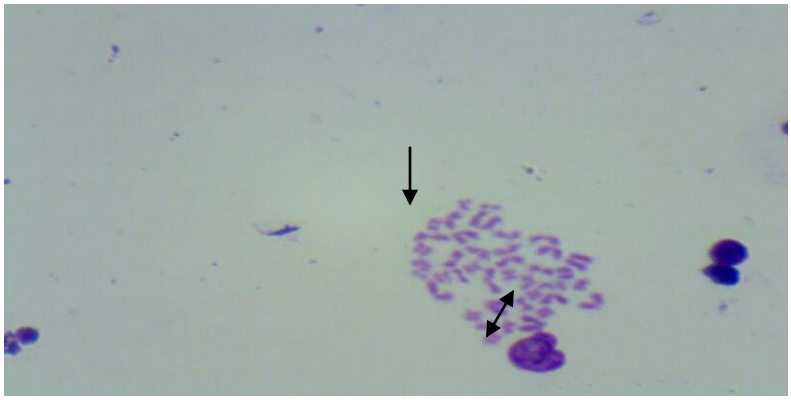

Fig:5.shows large number of chromatoid gap⟶ and fragment ⟷ with micronuclei in bone marrow cells of treated animal with Ag.NPs for 6 weeks

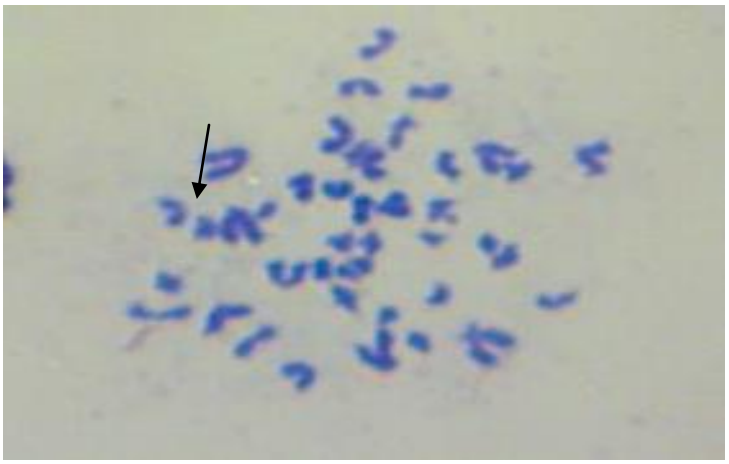

Fig:6.shows chromatid fragment ⟶ in chromosomal bone marrow cells of immunized animal at at 6 weeks post Ag-NPs adminstration

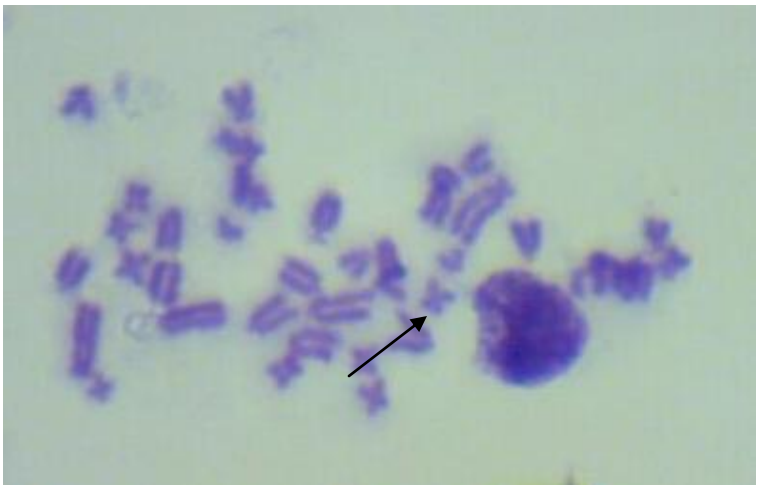

Fig:7..shows blastic cells ⟶ in chromosomal bone marrow cells of immunized animal at at 6 weeks post Ag-NPs adminstration

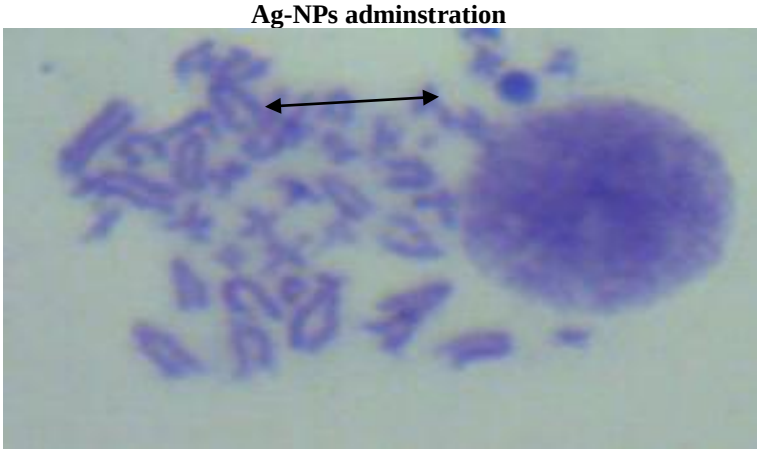

Fig:8..shows micronucle and chromatid ring ⟷ in bone marrow cells of immunized animal at at 6 weeks post Ag-NPs adminstration

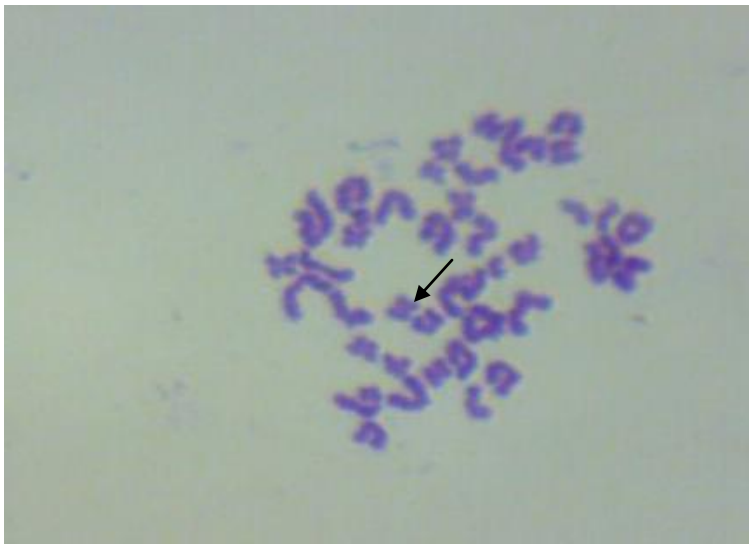

Fig:9 .shows ring chromatid ⟶in chromosomal bone marrow cell of immunized-betaglucan diet supplement at 6 weeks post-Ag-PNs administrated

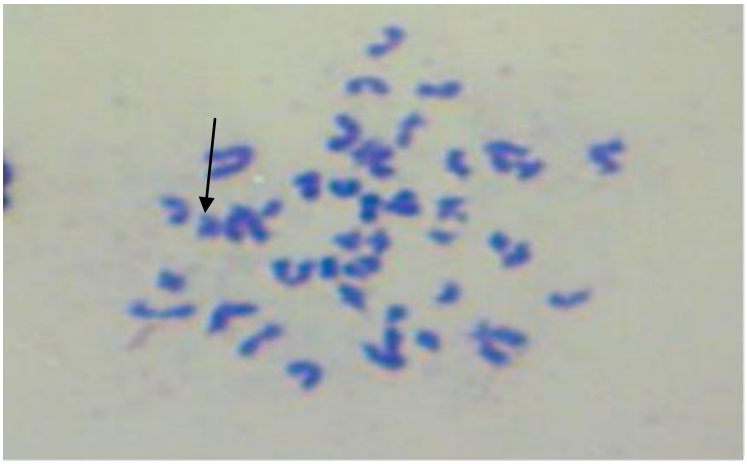

Fig:10. shows few chromatoid fragment ⟶in bone marrow cells of control negative group

The present study showed lower mitotic index and plastic index in bone marrow cells of animal exposure to Ag.NPs, these result may be indicated that these materials arrest cell division, these idea was agreement with (21)(Canman *et al.*,1994) and(22)(Boonstra and Post,2004) who explained that DNA damage associated with cell cycle arrest and irreversible damage that undergo apoptosis and accumulation of the cells in sub-G1 phase. Also(13) (AshaRani *et al*.,2009b) showed that Ag-NPs cause G2\M arrest in cells.

The present finding demonstrated high degree of chromosomal aberration in cells of bone marrow of animals treatment with Ag-NPs for 6 weeks as comparing with control negative group,these observation may indicated that Ag-NPs induced genotic effects these result was agreement with(23)(Ahamed *et al*.,2008) who showed that the Ag-NPs break DNA double strand and induced chromosomal aberration such as acentric and dicentric chromosomes, chromosomal fusions, and fragments(24)(Asharani *et al*.,2012) recorded DNA damage-signaling proteins, RAD51 and phosphorylated H2AX (γ-H2AX) when exposure to Ag-NPs

The cytotoxicity of the Ag-NPs in the present study may be due to their small size(20nm) that allow them to penetrate the cells membrance ,this idea was in consistent with (15)(Hui *et al*.,2012) and (12)(Asharani *et al*.,2009a) who suggested that Ag-NPS of 20nm can easily penetrated cell also toxicity of nanoparticles may be due to their negative surface charge that showed a high affinity for positive charge cell surface receptor or nuclear

receptor (2,25)(Patil *et al.*,2007;Asharani *et al*.,2010) therefore Ag.NPs can entered the nucleus and generated oxidative stress to the genomic materials(15) (Hui *et al*.,2012).

The current study showed that immunized animals administration with Ag-NPs expressed low blastic index as coparing with those values in control negative group ,these result may indicated that the Ag-NPs have immunotoxic effects ,these result was agreement with observation of(27) (Park *et al*.,2007) who reported that the Ag-NPs had cytotoxic effects on immune cells ,epithelial cells and cytokines production. Also (28) (Shin *et al.*,2007) demonstrated that Ag-NPs can induce toxic effects on immune cells and cytokines expression by monocytes.

However ,current finding showed that the blastic index and mitotic index in immunized –Ag-NP group were higher than those values in animals treated with Ag-NPs alone ,these result may be indicated that augment immune responses reduced genotoxic effects of Ag-NPs, also these idea was supported by finding few chromosomal aberration and micronuclei in these group. Cellular DNA and mitochondria damage were the main target of free radicals (29) (Ramzi *et al.*,1994).

(30)(Carlson *et al*.,2008) found that metal ion of silver can act as catalysts for metal reactive oxygen species that induced oxidative DNA damage, according to these evidence we suspected that ROS induced by Ag-NPs may be destructed the bases of the DNA and lead to different type of chromosomal aberration that were seen in the present study

Also (30) (Foldbjerg *et al*.,2011) suggested that the cytotoxic effects of Ag-NPs may be due to ROS production and (32)(Yang *et al*.,2012) showed that the genotoxic effects of Ag-NPs due direct result of NPs or due to interaction with Ag ions that release as a result of Ag-NPs –oxidize however, the genotoxicity of Ag-NPs Also recorded by (23)(Ahamed *et al.*,2008) and these toxicity may be associated with interaction of Ag-ions with phosphorus containing base (33)(Hatchtt *et al*.,1996) ,and ROS dependent DNA damage in vitro(31)(Foldbjerg *et al*.,2011) and in vivo (34)(Park *et al*.,2011).
On above basis, we suggested that the augment immune response may be either diminished oxidative stress induced by Ag-NP or prevented cellular interaction of Ag ions ,these investigation may supported idea
that mentioned by (13-Asharani ,et al.,2009) who demonstrated that the Ag-NPs induced chromosomal aberration either directly or through activation of catabolic enzymes and it causes cytoskeleton deformations and inhibit cell proliferation.

We postulated that the causes of high ratio of plastic index ,mitotic index and few chromosomal aberration in cells of bone marrow of immunized animals fed diet supplementing with beta-glucan post-treated with Ag-NPs, may be due to beta-glucan may act as antioxidant against Ag-NPs and therefore, they protected genomic material of DNA of bone marrow cells in the current study,these observation was in consistent with (35-Kriskova,2006) who demonstrated that beta-glucan of different origin act as a potent antioxidant and prevent damage by reactive oxygen species. Also (36)(Dmitry,2013) observed that the chitosan protect the cells from direct interaction with Ag-NPs.
Also beta-glucan activated protein kinase .DNA-PK play essential role in repair DNA damage(15)(Hui *et al*.,2012) reported that pharmacological inhibition of DNA-PKcs increased sensitivity to Ag-NP induced genotoxicity and cytoxicity also (37)(Bentle *et al*.,2007) investigated that DNA-PKc repaired double strand break (DSBs) as important component of(Non-homologous End-joining NHEJ)and impairment of these factor associated with DNA chemosensitivity .the present of micronuclei in the present study may indicated break in DNA these idea was agreement with (38)(Hande *et al*., 1996) who explained that Micronuclei originate from either lagging chromosomes or chromosome fragments which excluded from daughter nuclei following nuclear division prior to cytokinesis

However, the presence of micronuclei in the present study may be supported idea of genotoxicity of Ag-NPs these finding was in consistence with (39)(Kawata *et al*.,2009) who reported that 47.9% of HepG2 cell line expressed micronuclei post –exposure to 7-10nm of Ag-NPs.
Also (34)(Kim *et al*.,2011) showed that Ag-NPs stimulated DNA breakage and MN formation in dose-dependent manner, also (12)(Asharani *et al*.,2009) reported deposition of Ag-NPs inside the nucleus and they proposed that these deposition may lead to DNA damage, and effects on chromosomal morphology and segregation
Also (41)(Xul *et al*.,2012) reported micronuclei, nuclei disruption, chromatin concentration and cell apoptosis in rabbit reproductive organs tissues in sliver-nanoparticles –hydrogel administration through the vigina , however, the

chromosomal aberration in the current study may be given indicated that Ag-NPs were considered a potential carcinogenic risk factors and required a deep researches to understand their genotoxicity and carcinogenicity.

References

1-Andre,N.;Tian,X.;Lutz,M. and Ning,L.(2006). Toxic Potential of Materials at the Nanolevel. Science.311:622-627

2- Hagen Mikkelsen S, Hansen E, Christensen TB, Baun A, Foss Hansen S, Binderup M-L:**Survey on basic knowledge about exposure and potential environmental and health risks for selected nanomaterials.** Environmental Project No. 1370 2011

3- Lee J.H, Chae J.D, Kim D.G, Hong S.H, Lee W.M, Ki M: **Comparison of the efficacies of silver-containing dressing materials for treating a full-thickness rodent wound infected by methicillin-resistant Staphylococcus aureus.**

Korean J Lab Med 2010, **30:**20-27
Miljøprojekt 2011, **:**1-136

4- Thomas V, Yallapu M.M, Sreedhar B, Bajpai S.K: **A versatile strategy to fabricate hydrogel-silver nanocomposites and investigation of their antimicrobial activity.**

J Colloid Interface Sci 2007, **315:**389-395

.5- Fauss, E. 2008. The Silver Nanotechnology Commercial Inventory. University of Virginia. http://www.nanoproject org.

6- Barbu, E., Molnar, E., Tsibouklis, J., and Gorecki, D. C. (2009). The potential for nanoparticle-based drug delivery to the brain: overcoming the blood-brain barrier. Expert Opin. Drug Deliv. 6: 553–565

7- Ip, M., Lui, S. L., Poon, V. K., Lung, I., and Burd, A. (2006). Antimicrobial activities of silver dressings: an in vitro comparison. J. Med. Microbiol. 55: 59-63.

8-- Sung J.H, Ji J.H, Park J.D, Yoon J.U, Kim D.S, Jeon K.S, Song M.Y, Jeong J, Han B.S, Han J.H:**Subchronic inhalation toxicity of silver nanoparticles.**

Toxicol Sci 2009, **108:**452-461

9- Kim, YS, Song, MY, Park, JD, Song, KS, Ryu, HR, Chung, YH, Chang, HK, Lee, JH, Oh, KH, Kelman, BJ, Hwang, IK, Yu, IJ: Subchronic oral toxicity of silver nanoparticles. Part. Fibre Toxicol.. **7**, 20 (2010).

10- Tang J, Xiong L, Zhou G, Wang S, Wang J, Liu L, Jiage Li, Yuan F, Lu S, Wan Z, Chou L, Xi T:**Silver nanoparticles crossing through and distribution in the blood–brain barrier in vitro.**

J Nanosci Nanotechnol 2010, **10:**6313-6317

11-Pinto, V. V., Ferreira, M. J., Silva, R., Santos, H. A., Silva, F., and Pereira, C. M. (2010). Long time effect on the stability of silver nanoparticles in aqueous medium: effect of the synthesis and storage conditions. Colloids Surf. A Physicochem. Eng. Asp. 364: 19–25.

12-Asharani, P. V., Hande, M. P., and Valiyaveettil, S. (2009a). Anti-proliferative activity of silver nanoparticles. BMC Cell Biol. 10:1471-2121

13-AshaRani, P. V., Low Kah Mun, G., Hande, M. P., and Valiyaveettil, S. (2009b). Cytotoxicity and genotoxicity of silver nanoparticles in human cells. ACS Nano 3 : 279-290.

14- Morones, J.R., et al., The bactericidal effect of silver nanoparticles. Nanotechnology, 2005. **16**(10): p. 2346-2353.

15-Hui,K.L;Asharani,P.V. and Prakashm ,H.(2012).Enhanced genotoxicity of Silver nanoparticles in DNA repair deficient mammalian cells . Original Research ARTICLE.13:3389-3391.

16- Lin,Y.L;Liang,Y.C;Lee,SS, and Chiang,B.L.(2005).Polysaccharide purified from Ganonderma lucidum induced activation and maturation of human monocytes-derived dendritic cells by the NF-kappaB and p38 mitogen –activated protein kinase pathway.J.Leuko.Biol.78:533-543.

17- Asharani, P., Sethu, S., Lim, H. K., Balaji, G., Valiyaveettil, S., and Hande, M. P. (2012). Differential regulation of intracellular factors mediating cell cycle, DNA repair and inflammation following exposure to silver nanoparticles in human cells. Genome Integr. 3, 2.

18- Young S.K ,Moon, Y.S ,Jung , D.P, Kyng ,S.S and KooH.G (2010). Subchronic oral toxicity of silver nanoparticles . part fibre toxicol .2010

19-Mitove,I;Denches,V.and Linde,K(1992).Humoral and cell mediated immunity in mice after immunization with live oral vaccine of Salmonella typhimerium:autotrophic mutant with two attenuating markers.Vac:10:61

20-Yaseen,N.Y.(1990).Cytogenetic study on human colorectal cancer cells Ph.thesis,Univ,Sheffield.

21- Canman, C. E., Chen, C. Y., Lee, M. H., and Kastan, M. B. (1994). DNA damage responses: p53 induction, cell cycle perturbations, and apoptosis. Cold Spring Harb. Symp. Quant. Biol. 59: 277–286.

22-Boonstra, J., and Post, J. A. (2004). Molecular events associated with reactive oxygen species and cell cycle progression in mammalian cells. Gene 337, 1–13.

23- Ahamed, M., Karns, M., Goodson, M., Rowe, J., Hussain, S. M., Schlager, J. J., and Hong, Y. (2008). DNA damage response to different surface chemistry of silver nanoparticles in mammalian cells. Toxicol. Appl. Pharmacol. 233, 404–410.

24-Asharani, P., Sethu, S., Lim, H. K., Balaji, G., Valiyaveettil, S., and Hande, M. P. (2012). Differential regulation of intracellular factors mediating cell cycle, DNA repair and inflammation following exposure to silver nanoparticles in human cells. Genome Integr. 3, 2.

25-Patil, S., Sandberg, A., Heckert, E., Self, W., and Seal, S. (2007). Protein adsorption and cellular uptake of cerium oxide nanoparticles as a function of zeta potential. Biomaterials 28, 4600–4607

26-Asharani, P. V., Sethu, S., Vadukumpully, S., Zhong, S. P., Lim, C. T., Hande, M. P., and Valiyaveettil, S. (2010). Investigations on the structural damage in human erythrocytes exposed to silver, gold, and platinum nanoparticles. Adv. Funct. Mater. 20 :1233–1242

27- Park S, Lee Y.K, Jung M, Kim K.H, Chung N, Ahn E.K, Lim Y, Lee K.H: **Cellular toxicity of various inhalable metal nanoparticles on human alveolar epithelial cells.** Toxicol 2007, **19 Suppl 1:**59-65

28-- Shin, SH, Ye, MK, Kim, HS, Kang, HS: The effects of nano-silver on the proliferation and cytokine expression by peripheral blood mononuclear cells. Int. Immunopharmacol.. **7**, 1813–1818 (2007).

29-Ramze et al.,1994

30- Carlson, C., Hussain, S. M., Schrand, A. M., Braydich-Stolle, L. K., Hess, K. L., Jones, R. L., and Schlager, J. J. (2008). Unique cellular interaction of silver nanoparticles: size-dependent generation of reactive oxygen species. J. Phys. Chem. B 112, 13608–13619

31- Foldbjerg R, Dang D.A, Autrup H: **Cytotoxicity and genotoxicity of silver nanoparticles in the human lung cancer cell line, A549.**
Arch Toxicol 2011, **85:**743-750. PubMed Abstract

32-Yang X, Gondikas AP, Marinakos S.M, Auffan M, Liu J, Hsu-Kim H, Meyer JN: **Mechanism of silver nanoparticle toxicity is dependent on dissolved silver and surface coating in Caenorhabditis elegans.**
Environ Sci Technol 2012, **46:**1119-

33- Hatchett D.W, White H.S: **Electrochemistry of sulfur adlayers on the low-index faces of silver.** J Phys Chem 1996, **100:**9854-9859

34- Park M.V, Neigh A.M, Vermeulen J.P, de la Fonteyne L.J, Verharen H.W, Briede J.J, van Loveren H, de Jong W.H: **The effect of particle size on the cytotoxicity, inflammation, developmental toxicity and genotoxicity of silver nanoparticles.** Biomaterials 2011, **32:**9810-9817.

35-Krizkova,L.(2006).Antioxidant and antimutagenic activity of mannan neoglyoconjugatees :Mannan-human serum albumin and mannan-pencillinG acylase.Mutal .Res.606:72-79.

36-Dmitry S. Pesnya[a*] pages 275-281**Publishing models and article dates explained**
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37- Bentle, M. S., Reinicke, K. E., Dong, Y., Bey, E. A., and Boothman, D. A. (2007). Nonhomologous end joining is essential for cellular resistance to the novel antitumor agent, beta-lapachone. Cancer Res. 67: 6936–6945.
38- Hande, M. P., Boei, J., and Natarajan, A. T. (1996). Induction and persistence of cytogenetic damage in mouse splenocytes following whole-body X-irradiation analysed by fluorescence in situ hybridization.2. Micronuclei. Int. J. Radiat. Biol. 70:375–383

39- Kawata K, Osawa M, Okabe S: **In vitro Toxicity of silver nanoparticles at nanocytotoxic doses to HepG2 human hepatoma cells.**

Environ Sci Technol 2009, **43:**6046-6051

40- Kim J.S, Sung J.H, Ji J.H, Song K.S, Lee J.H, Kang C.S, Yu I.J: **In vivo genotoxicity of silver nanoparticles after 90-day silver nanoparticle inhalation exposure**

Saf Health Work 2011, **2:**34-38

41- Xu L, Chen L, Dong Z, Wang J, Wang Z, Shao A: **In vivo toxicity in reproductive organs of rabbit and in vitro cytotoxicity of silver nanoparticle based-hydrogel.**

Chi J Pharm Anal 2012, **2:**194-201.