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Research Article

Subchronic Effect of Silver Nanoparticles Following 28 Days of Repeated Oral Administration on Oxidative Stress, Inflammatory Biomarkers and DNA Fragmentation in Normal and Irradiated Rats

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Abstract: The present study was performed to evaluate the effect of 28 days subchronic oral administration of AgNPs in normal and irradiated rats (4 Gy). Oxidative stress biomarker (blood *glutathione*, serum *nitric oxide* and liver *MDA* as an index for lipid peroxidation), also some inflammatory parameters as *c-reactive protein* and *tumor necrosis factor alpha (TNF-α)* have been measured. Liver *DNA* fragmentation was examined to determine the damaging effect in liver tissues by gel electrophoresis technique. Results of the present study revealed that blood glutathione significantly increased in irradiated treated group irradiated with (4Gy) then treated with (42.599 mg/kg) (1/10 LD50 in irradiated animals) and in irradiated group (4Gy) as compared to normal control but the increase is more in the irradiated treated group. *NO* has been significantly increased in irradiated and irradiated treated group but with a lesser extent in irradiated treated group. All groups showed significant decrease in *CRP* as compared to normal control group. liver*DNA* fragmentation showed a damaging effect exhibited by AgNPs (26.878 mg/kg) (1/10 LD50 in normal animals) and irradiated treated group but to a lesser extent than AgNPs group, whereas vehicle Polyvinylpyrrolidone (PVP) and irradiation groups showed a damaging effects. Based on the present study we can conclude that AgNPs has a modulating effect in decreasing the elevated level of *NO* caused by irradiation. Meanwhile AgNPs potentiate the effect of irradiation on increasing blood *glutathione* level, decreases the damaging effect on liver *DNA* fragmentation caused by irradiation. These results can be promising in cancer patients receiving radiation therapy and treated with AgNPs, or those receiving AgNPs for any other medical purpose but such results awaits further investigations.

Keywords: CRP, gel electrophoresis, MDA, NO, PVP, TNF-alfa

INTRODUCTION

Ionizing Radiation (IR) consists of high energy electromagnetic radiations (X-Rays, gamma rays) and various types of atomic particles (electrons, protons, neutrons, alpha particles and other particulate radiation) which come from natural and manmade sources. Ionizing radiation is an important environmental risk factor for various cancers and also a major therapeutic agent for cancer treatment. exposure of mammalian cells to IR induces several types of damage to DNA, including double and single-strand breaks, base and sugar damage, as well as DNA-DNA and DNA-protein cross links (Barker *et al*., 2005).During radiotherapy ionizing radiation interact with biological systems to induce excessive free radicals or Reactive Oxygen Species (ROS) which attack various cellular components including DNA, proteins and membrane lipids, theraby leading to significant cell damage.

Nanoparticles have particle size in the range between 1and 100 nm and exhibit unique properties (Tjong and Chen, 2004). Silver Nanoparticles (AgNPs) are used in electronics, bio-sensing, clothing, food industry, paints, sunscreens, cosmetics and medical devices. They have been known to posses excellent free radical scavenging, antimicrobial and anti-inflammatory activities (Wong *et al*., 2009; Banerjee and Narendhirakannan, 2011). Due to their antibacterial activity, AgNPs are used commonly in medical products and devices, food storage materials, cosmetics, various health care products and industrial products. In medical applications, AgNPs have been used for silver based dressings (Lee *et al*., 2010; Elliott, 2010). Silver-coated catheters (Gabriel *et al*., 1996; Ahearn *et al*.,

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2000) silver based hydrogel (Wu *et al*., 2009; Thomas *et al*., 2007).

The results of several studies suggest that nanocrystalline silver specifically may play a role in altering or compressing the inflammatory events in wounds and facilitating the early phases of wound healing. Silver nanoparticles have also gained increasing interest in the field of nanomedicine due to their unique properties and obvious therapeutic potential in treating a variety of diseases, including retinal neovascularization (Bhattacharya and Mukherjee, 2008; Kalishwaralal *et al*., 2010) and acquired immunodeficiency syndrome due to Human Immunodeficiency Virus (HIV) (Lara *et al*., 2010; Sun *et al*., 2005). They inhibit Vascular Endothelial Growth Factor (VEGF)induced angiogenesis in bovine retinal endothelial cells), (Kalishwaralal *et al*., 2010). Silver oxide nanoparticles exhibit antitumor properties in transplanted Plisslymphosarcoma tumor models when administered by intravenous injection in the form of aqueous dispersions (Rutberg *et al*., 2008).

These broad applications, however, increase human exposure and thus the potential risk related to their short- and long-term toxicity. A large number of in vitro studies indicate that AgNPs are toxic to the mammalian cells derived from skin, liver, lung, brain, vascular system and reproductive organs. Interestingly, some studies have shown that this particle has the potential to induce genes associated with cell cycle progression, DNA damage and apoptosis in human cells at non-cytotoxic doses (Ahamed *et al*., 2010). In recent years, increasing data demonstrated that AgNPs could induce toxicity in vivo under a variety of exposure conditions like inhalation (Ji *et al*., 2007; Sung *et al*., 2009) orally (Kim *et al*., 2008; Kim *et al*., 2010) and via hypodermic injection (Tang *et al*., 2009).

The present study was performed in view of evaluating the subchronic effects of AgNPs after administration to normal and irradiated rats aiming to simulate the effect of exposing to both silver nanoparticles and ionizing radiation in human. That was done by measuring some Oxidative stress biomarkers such as [MDA, glutathione and NO] as well as certain inflammatory mediator were undertaken such as TNF-α, C-reactive proteins. The effect on DNA has been measured by liver DNA fragmentation test.

MATERIALS AND METHODS

Animals: White male wistar albino rats weighing (150-180)g used for the evaluation of the 28 days subchronic study. Animals were obtained from National Research Center, Cairo, Egypt. Rats were housed in plastic cages and were maintained under conventional laboratory conditions throughout the study. They were fed standard pellet chow (El-Nasr chemical Co., Abu Zaabal, Cairo and Egypt.) and water *ad libitum*. The animal treatment protocol has been approved by the animal care committee of the National centre for Radiation Research and Technology (NCRRT), Cairo, Egypt.

Drugs and chemicals: AgNPs were synthesized by chemical method at the national centre for radiation research and technology. AgNPs were administered orally in the 28 days study at a dose of 26.878 mg/kg and 42.599 mg/kg daily in AgNPs and irradiated treated AgNPs group respectively. Polyvinylepyrrolidone was purchased from Sigma-Aldrich chemical co. (U.S.A), to be orally administered as the vehicle control group in equivalent volume to the AgNPs and irradiated treated AgNPs groups.

- Ps in normal and irradiated (4Gy) rats.

The chosen doses in the present study were 26.878 mg/kg which corresponds to 1/10 LD50 in normal animals and 42.599 mg/kg which corresponds to 1/10 LD50 in irradiated (4Gy) animals.

Preparation of Silver nanoparticles: AgNPs were synthesized at Drug Radiation Research Dep.labs, at the national centre for radiation research and technology (NCRRT) according to a modified method of Mao *et al*. (2012) and El-Batal *et al*. (2013a, b). In a typical procedure, solution A was prepared by dissolving 0.05M of $AgNO_3$, 0.1 M glucose and 30mg/ml PVP(4000) in 700 mL deionized water .Solution B was prepared by adding 0.2 M NaOH in 300 mL deionized water. Then add solution B to solution A slowly for 20 min with continuous stirring. The reaction was maintained at 60C°and allowed to react for one hour. with adding of solution B to solution A immediately turned to bright yellow color indicating the formation of AgNPs after heating the solution changed to ruddy-brown color.

*The vehicle control was prepared as mentioned before without the addition of AgNO3.

Irradiation: Whole body gamma irradiation was performed at the national centre for radiation research and technology (NCRRT), Cairo, Egypt, using an AECL Gamma cell -40 biological indicator. Animals were irradiated at an acute single dose level of 4 Gy delivered at a dose rate of 0.758 rad/sec.

Characterization of silver nanoparticles:

UV-VIS spectral analysis: Preliminary characterization of the silver nanoparticles was carried out using UV–visible spectroscopy (JASCOJapan-

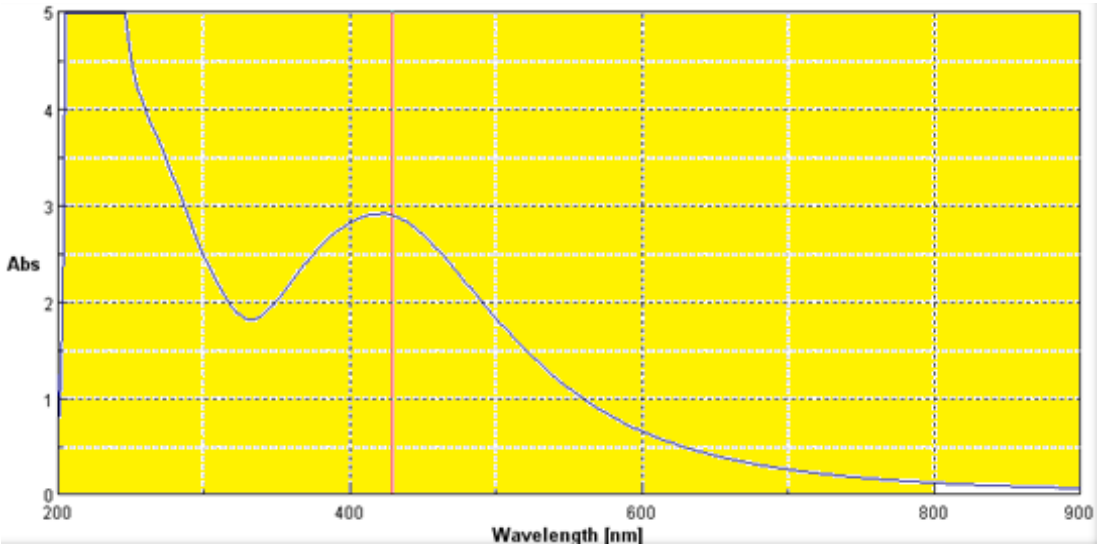

Fig. 1: UV-VIS spectral analysis of AgNPs

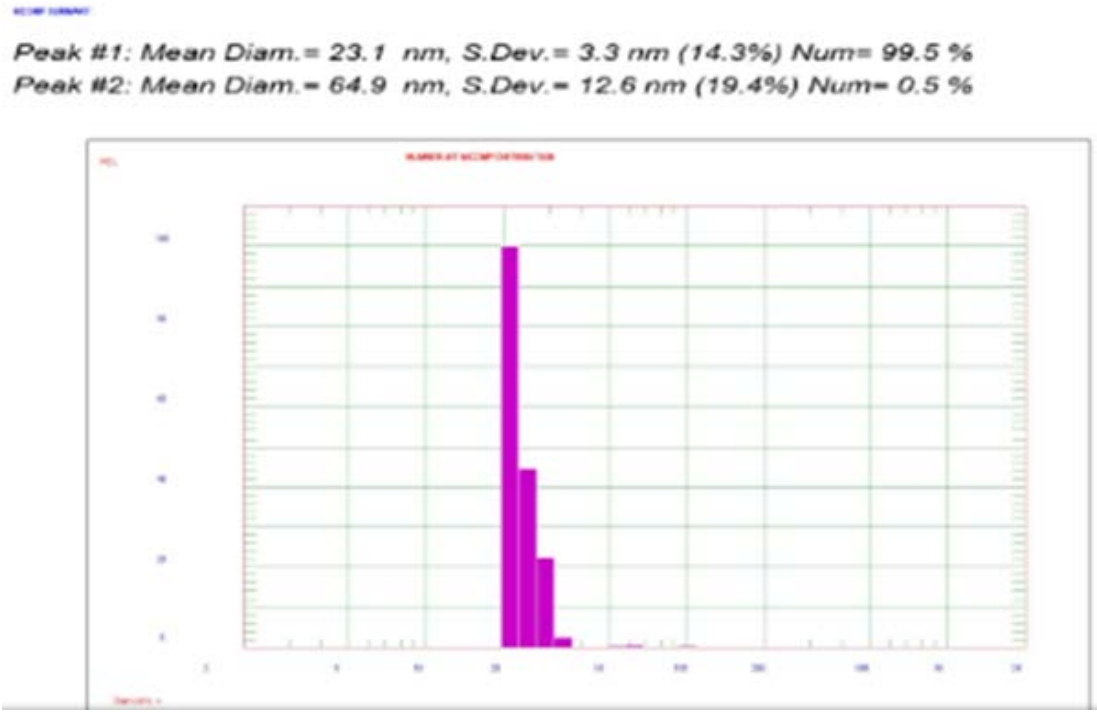

Fig. 2: Dynamic Light Scattering (DLS) of AgNPs showing the particle size diameter of AgNPs mean value is (23.1±3.3nm)

model V-560) at a resolution of 1 nm. Silver nanoparticles exhibit unique and tunable optical properties on account of their surface Plasmon resonance (SPR), dependent on shape, size and size distribution of the nanoparticles (Tripathy *et al*., 2010). The reduction of silver ions was monitored by measuring the UV-visible spectra of the solutions from 300 to 800 nm (Fig. 1).

Dynamic Light Scattering (DLS): Average particle size and size distribution were determined by the dynamic light scattering (DLS). Loeschner *et al*. (2011) technique (PSS-NICOMP 380-ZLS, USA) before measurements, the samples were diluted 10 times with deionized water. 250µl of suspension were transferred to a disposable low volume cuvette. After equilibration to a temperature of 25°C for 2 min, five measurements were performed using 12 runs of 10 s each (Fig. 2).

Transmission Electron Microscopy (TEM): The particle size and shape were observed by TEM nanoparticles (Tripathy *et al*., 2010) (JEOL electron

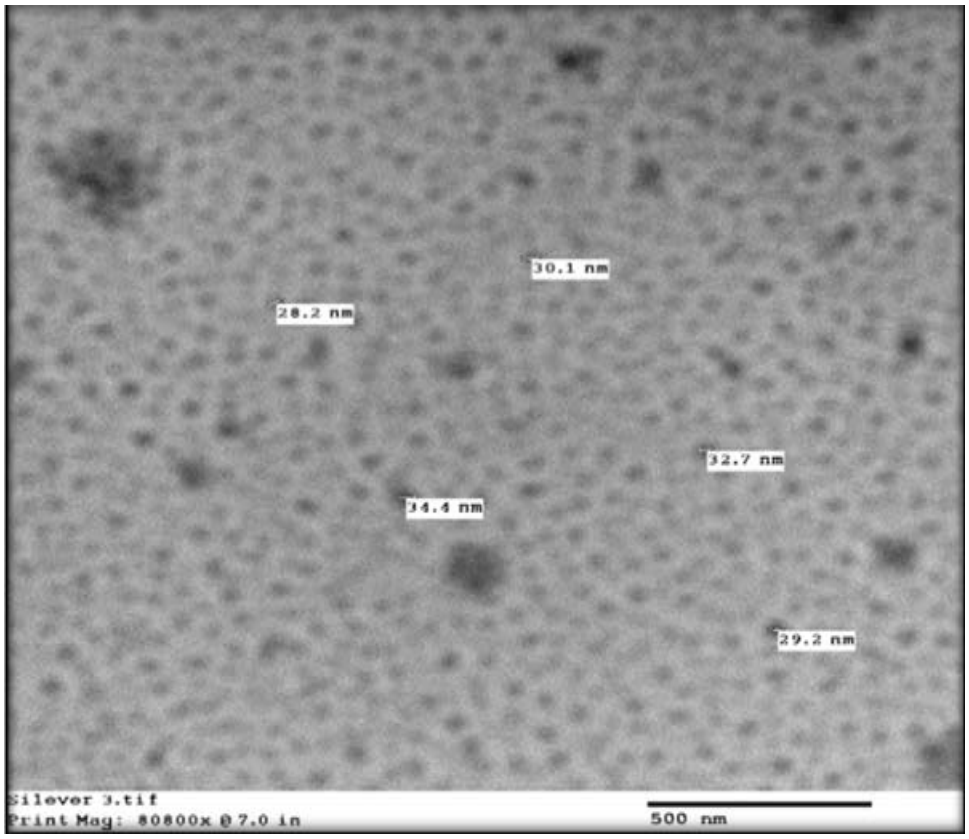

Fig. 3: Transmission Electron Microscopy (TEM) examination of AgNPs showed spherical shape of silver nanoparticles and good particle dispersion with average size at (30.92±2.56 nm)

microscope JEM-100 CX) operating at 80 kV accelerating voltage. The prepared Ag-NPS was diluted 10 times with deionized water. A drop of the suspension was dripped into coated copper grid and allowed to dry at room temperature (Fig. 3).

Experimental design: Rats were divided into 5 groups, each consists of 10 rats. The groups were: normal control received saline, vehicle control received Polyvinylpyrrolidone (PVP), silver nanoparticles group AgNPs received 26.878 mg/kg, irradiated group exposed to 4 Gy gamma irradiation, irradiated treated group exposed to 4 Gy gamma irradiation followed by 42.599 mg/kg AgNPs. Rats were exposed to irradiation at a single dose 24 hour before day 1 in the irradiated and irradiated treated groups. Saline, PVP, AgNPs were administered orally for 28 days. On the day 29 the rats were fasted over night then anasthestized by ether. Blood from the left ventricle were collected in non heparinzed tube and serum was collected by centrifugation (4000g for 15 min) for the biochemical evaluation.

Tissue sampling: Immediately after the animals were sacrificed liver of 8 animals in each group were excised washed with normal saline, blotted with filter paper, weighted and were placed in ice-cold saline (0.9 N NaCl) and homogenized. The homogenate was centrifuged at 2700 rpm for 15 mins then the supernatant was prepared for Lipid peroxide content determination the remaining 2 liver samples from each group were taken washed in normal saline and freezed for the estimation of liver DNA fragmentation test.

Biochemical estimation: Blood Glutathione was estimated using Glutathione reduced kits (biodiagonistic, Giza, Egypt). According to the method of Beutler *et al*. (1963) serum nitric oxide was estimated using nitric oxide kits (biodiagonistic, Giza, Egypt), according to the method of Montgomery and Dymock (1961). Serum Tumor necrosis factor alfa (TNF-α) was estimated by Elisa technique (glory science Co., ltd kit procedure) also. Serum C-reactive protein was estimated by Elisa technique according to C-reactive protein kit (Sunred kit procedure).

Lipid peroxidation content determination: It has been done according to the method of Yoshioka *et al*. (1979). The colorimetric determination of Thiobarbituric Acid Reactive Substances (TBARS) is based on the reaction of 1 molecule of malonaldialdehyde (MDA) with 2 molecules of

thiobarbituric acid (TBA) at low PH (2-3). The resultant pink pigment is extracted by n-butanol and the absorbance was measured at 535 nm using a spectrophotometer (UNICAM 8625, UV/VIS, England).

Qualitative evaluation of DNA fragmentation in liver tissue (agarose gel electrophoresis) technique: Liver tissue DNA were extracted in purified form according to Fermentas, genomic DNA purification kit (#K0721, #K0722). Agrose gel electrophoresis was carried out for the analysis of DNA fragmentation (Yokozawa and Dong, 2001).The resulting DNA was exposed to electrophoresis through a 2% agrose gel containing ethedinium bromide using TAE buffer (tris acetate EDTA) buffer equal quantity of DNA were loaded in each lane and a molecular DNA marker was used as a molecular mass standard. DNA fragmentation was visualized and photographed under ultraviolet illumination.

Statistical analysis: The values of the measured parameters were presented as mean±S.E.M. Comparisons between different treatments were carried out using one way Analysis of Variance (ANOVA) followed by Tukey-Kramer as post ANOVA multiple comparisons test. Differences were considered statistically significant when $p<0.05$.

RESULTS

Characteriztion of Silver nanoparticles: AgNPs absorbance were measured using UV analysis which reflects the occurance of the Ag in the nanoscale (Fig. 1).

Dynamic Light Scattering (DLS) examination of AgNPs revealed that the average mean value of the particle diameter is (23.1±3.3nm) (Fig. 2).

Examination of AgNPs using Transmission Electron Microscope (TEM) revealed spherical shape of silver nanoparticles and good particle dispersion with average size at (30.92±2.56 nm) (Fig. 3).

Subchronic effect of Silver nanoparticles on blood glutathione level in normal and irradiated rats: AgNPs (26.878 mg/kg) didn't significantly affect blood glutathione level as compared to vehicle control. Irradiation (4Gy) didn't significantly affect blood glutathione level as compared to vehicle control, but

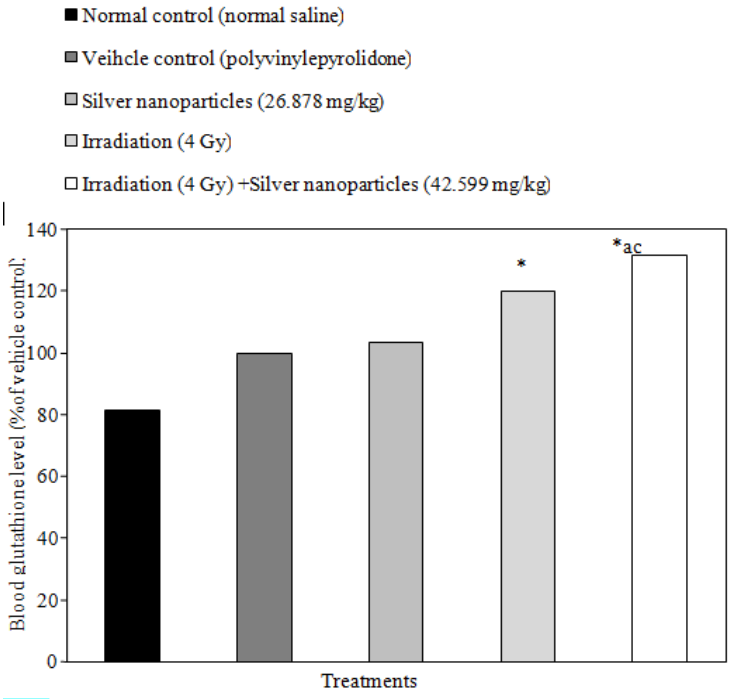

Fig. 4: Subchronic effect of Silver nanoparticles on blood glutathione level in normal and irradiated rats; All treatments AgNPs (26.878 mg/kg), AgNPs (42.599mg/kg, PVP were administered orally for 28 days; irradiation 4Gy was done before the start of the study in the irradiated and irradiated treated AgNPs group. N = 8 rats per group. Data was expressed as mean ±s.e.m. Statistical analysis was carried out using one way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparisons test; *: Significantly different from the normal control value at $p<0.05$; a: Significantly different from the vehicle control value at $p<0.5$, b: Significantly different from the irradiated control value at $p<0.05$, c: Significantly different from silver nanoparticles (26.878 mg/kg)value at $p<0.05$, d: Significantly different from irradiated+ silver nanoparticles (42.599mg/kg) value at $p<0.05$, 26.878 mg/kg = 1/10 LD50 of silver nanoparticles in normal mice; 42.599 mg/kg =1/10 LD50 of silver nanoparticles in irradiated (4Gy) mice

they showed significant increase as compared to the normal control. Combination of 4Gy irradiation and AgNPs (42.599 mg/kg) showed significant increase in blood glutathione level to 131.64% as compared to vehicle control, they also showed significant increase in blood glutathione level as compared to AgNPs (26.878 mg/kg) group . Our results reflect that (AgNPs) did not exert significant effect on blood GSH level however (AgNPs) potentiate the effect of irradiation on blood GSH as compared to vehicle control (Fig. 4)

Subchronic effect of Silver nanoparticles on serum nitric oxide level in normal and irradiated rats: Silver nanoparticles (26.878 mg/kg) didn't significantly affect serum nitric oxide level as compared to vehicle control. Irradiation (4Gy), showed significant increase in nitric oxide level to 1126.34% as compared to vehicle control, they also showed significant increase as compared to normal control, (AgNPs) and the combination group of irradiation (4Gy) and Silver nanoparticles (42.599 mg/kg). Combined treatment with irradiation (4Gy) and Silver nanoparticles (42.599 mg/kg) showed significant increase as compared to vehicle control. The increase in nitric oxide amounted to 925.57%. They also showed significant increase as compared to normal control and (AgNPs) treated groups .It could be concluded that (AgNPs) did not change serum NO as compared to the vehicle control. Irradiation significantly increase serum nitric oxide.

Combination of irradiation and (AgNPs) increase serum nitric oxide level but to a lesser extent than that observed with the irradiation group. Treatment with (AgNPs) attenuate the effect of irradiation on nitric oxide (Fig. 5).

Subchronic effect of Silver nanoparticles on liver lipid peroxidation content in normal and irradiated rats: Silver nanoparticles (26.878 mg/kg) didn't show any significant change in lipid peroxidation content in liver tissue as compared to vehicle control. Irradiation (4Gy) didn't show any significant alteration in lipid peroxidation content in liver tissue as compared to vehicle control as well. The combination of irradiation (4Gy) and Silver nanoparticles (42.599 mg/kg) didn't significantly affect lipid peroxidation content in liver tissue as compared to vehicle control.

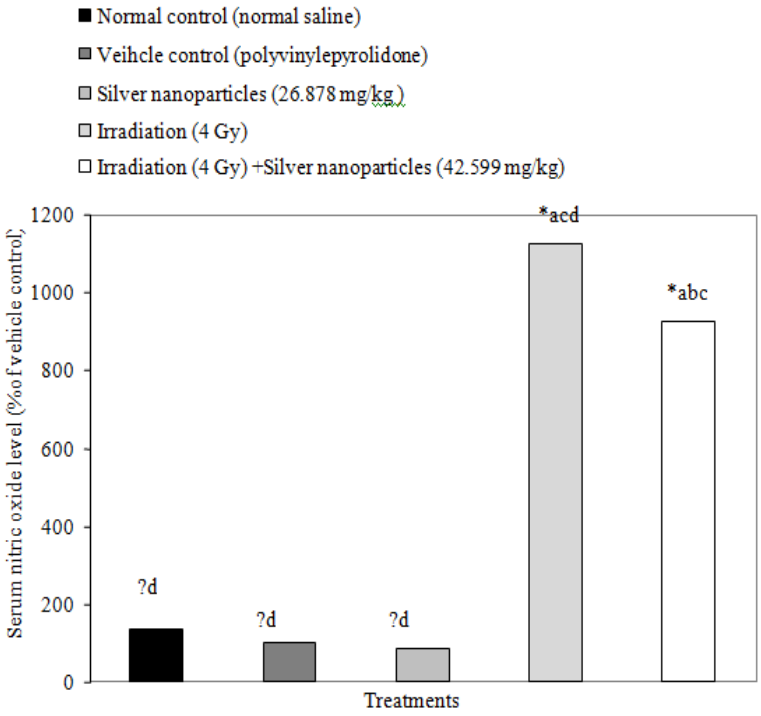

Fig. 5: Subchronic effect of Silver nanoparticles on serum nitric oxide level in normal and irradiated rats; All treatments AgNPs (26.878 mg/kg), AgNPs (42.599 mg/kg, PVP were administered orally for 28 days, irradiation 4Gy was done before the start of the study in the irradiated and irradiated treated AgNPsgroup. N = 8 rats per group. Data was expressed as mean±s.e.m. Statistical analysis was carried out using one way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparisons test; *: Significantly different from the normal control value at $p<0.05$, a: Significantly different from the vehicle control value at $p<0.05$, b: Significantly different from the irradiated control value at $p<0.05$, c: Significantly different from silver nanoparticles (26.878 mg/kg) value at $p<0.05$, d: Significantly different from irradiated+ silver nanoparticles (42.599 mg/kg) value at $p<0.05$; 26.878 mg/kg = 1/10 LD50 of silver nanoparticles in normal mice, 42.599 mg/kg = 1/10 LD50 of silver nanoparticles in irradiated (4Gy) mice.

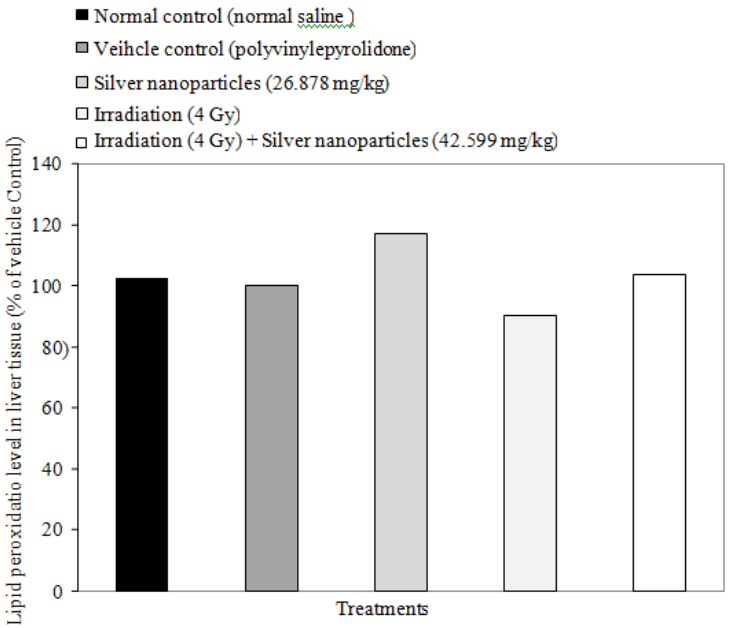

Fig. 6: Subchronic effect of Silver nanoparticles on liver lipid peroxidation content in normal and irradiated rats ; All treatments AgNPs (26.878 mg/kg), AgNPs (42.599 mg/kg, PVP were administered orally for 28 days, irradiation 4Gy was done before the start of the study in the irradiated and irradiated treated AgNPsgroup. N = 8 rats per group. Data was expressed as mean±s.e.m. Statistical analysis was carried out using one way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparisons test; *: Significantly different from the normal control value at $p<0.05$, a: Significantly different from the vehicle control value at $p<0.05$, b: Significantly different from the irradiated control value at $p<0.05$, c: Significantly different from silver nanoparticles (26.878 mg/kg)value at $p<0.05$, d: Significantly different from irradiated+silver nanoparticles (42.599 mg/kg) value at $p<0.05$; 26.878 mg/kg = 1/10 LD50 of silver nanoparticles in normal mice.42.599 mg/kg = 1/10 LD50 of silver nanoparticles in irradiated (4Gy) mice

Results revealed that (AgNPs), irradiation and their combination did not effect on lipid peroxidation content in liver tissues (Fig. 6).

Subchronic effect of Silver nanoparticles on serum tumor necrosis factor alfa level in normal and irradiated rats: Silver nanoparticles (26.878 mg/kg) didn't exert any significant change in serum tumornecrosis factor alfa(TNF-α) as compared to vehicle control. Irradiation (4Gy) didn't significantly change serum tumor necrosis factor alfa (TNF-α) as compared to vehicle control as well . Treatment with Irradiation (4Gy) and Silver nanoparticles (42.599mg/kg) didn't significantly affect serum tumor necrosis factor alfa as compared to vehicle control . The obtained results indicate that (AgNPs), irradiation and their combination did not alter serum tumor necrosis factor alfa as compared to vehicle control (Fig. 7).

Subchronic effect of Silver nanoparticles on C-reactive protein level in normal and irradiated rats: Silver nanoparticles (26.878 mg/kg), Irradition (4Gy) and the Combination of irradiation (4Gy) and Silver nanoparticles (42.599 mg/kg) didn't significantly affect serum C- reactive protein activity as compared to vehicle control. Vehicle, Silver nanoparticles (26.878 mg\kg), Irradiation (4Gy) and the Combination of irradiation (4Gy) and Silver nanoparticles (42.599 mg/kg) significantly decrease serum C- reactive protein activity as compared to normal control.

Data revealed that vehicle PVP decrease serum CRP level. AgNPs and irradiation both decrease serum C-RP as well. There was no interaction between irradiation and (AgNPs) on serum C-reactive protein the double treatment decrease serum CRP level also as compared to normal control (Fig. 8).

Subchronic effect of Silver nanoparticles on Qualitative evaluation of DNA fragmentation in liver tissue in normal and irradiated rats. (Gel electrophoresis technique):

Lane M = Molecular Weight Marker
Lane 1 = Normal control
Lane 2 = Irradiation 4 Gy
Lane 3 = Silver nanoparticles 26.878mg/kg
Lane 4 = Irradiation 4 Gy+Silver nanoparticles 42.599 mg/kg
Lane 5 = Vehicle polyvinylpyrrolidone

The separation of DNA fragments from liver tissue of male albino rat treated with AgNPs at a dose of 26.878 mg/kg, vehicle (PVP), Irradiation (4 Gy) followed by AgNPs 42.599 mg/kg, Irradiation (4Gy) and normal control treated with saline is expressed by

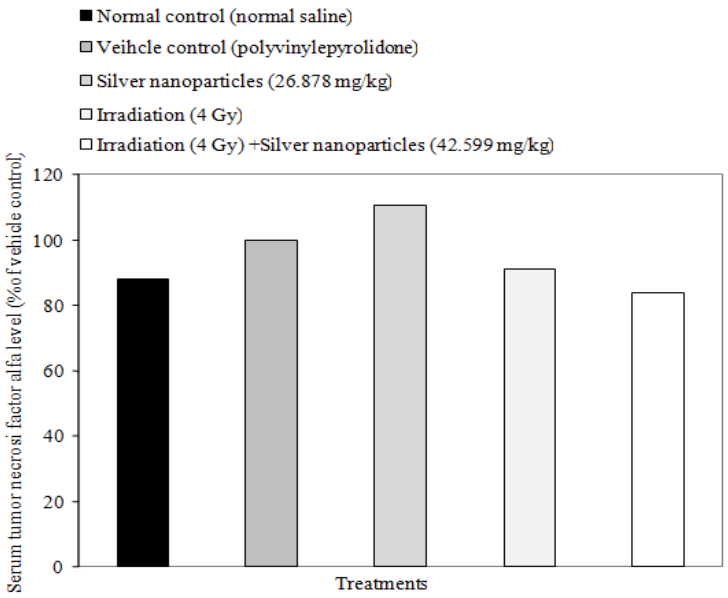

Fig. 7: Subchronic effect of Silver nanoparticles on serum tumor necrosis factor alfa level in normal and irradiated rats; All treatments AgNPs (26.878 mg/kg), AgNPs (42.599 mg/kg, PVP were administered orally for 28 days; irradiation 4Gy was done before the start of the study in the irradiated and irradiated treated AgNPs group. N = 8 rats per group. Data was expressed as mean±s.e.m. Statistical analysis was carried out using one way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparisons test; *: Significantly different from the normal control value at $p<0.05$, a: Significantly different from the vehicle control value at $p<0.05$, b: Significantly different from the irradiated control value at $p<0.05$, c: Significantly different from silver nanoparticles (26.878 mg/kg)value at $p<0.05$, d: Significantly different from irradiated+ silver nanoparticles (42.599 mg/kg) value at $p<0.05$; 26.878 mg/kg = 1/10 LD50 of silver nanoparticles in normal mice; 42.599 mg/kg =1/10 LD50 of silver nanoparticles in irradiated (4Gy) mice

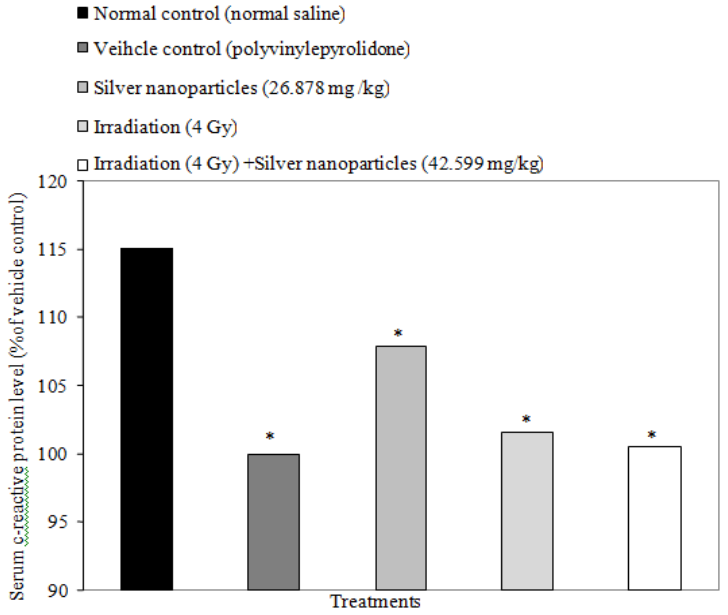

Fig. 8: Subchronic effect of Silver nanoparticles on serum C-reactive protein level in normal and irradiated rats; All treatments AgNPs (26.878 mg/kg), AgNPs (42.599 mg/kg, PVP were administered orally for 28 days, irradiation 4Gy was done before the start of the study in the irradiated and irradiated treated AgNPsgroup. N = 8 rats per group. Data was expressed as mean±s.e.m. Statistical analysis was carried out using one way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparisons test; *: Significantly different from the normal control value at $p<0.05$, a: Significantly different from the vehicle control value at $p<0.05$, b: Significantly different from the irradiated control value at $p<0.05$, c: Significantly different from silver nanoparticles (26.878 mg/kg) value at $p<0.05$, d: Significantly different from irradiated+ silver nanoparticles (42.599 mg/kg) value at $p<0.05$; 26.878 mg/kg = 1/10 LD50 of silver nanoparticles in normal mice., 42.599 mg/kg = 1/10 LD50 of silver nanoparticles in irradiated (4Gy) mice

Table 1: Rf values for each examined group indicating DNA fragmentation

Lanes:	Lane 1	Lane 2	Lane 3	Lane 4	Lane 5
Rows	(Rf.)	(Rf.)	(Rf.)	(Rf.)	(Rf.)
R1		0.0963		0.109	
R2	0.125	0.12	0.127	0.137	
R3					0.218
R4					
R5	0.338			0.337	0.341
R6		0.348	0.349		

R.F values of the 5 examined groups; Lane M molecular weight marker, Lane 1 Normal control, Lane 2 Irradiation 4 Gy; Lane 3 Silver nanoparticles 26.878 mg/kg, Lane 4 Irradiation 4 Gy+Silver nanoparticles 42.599 mg/kg, Lane 5 Vehicle polyvinylpyrrolidone

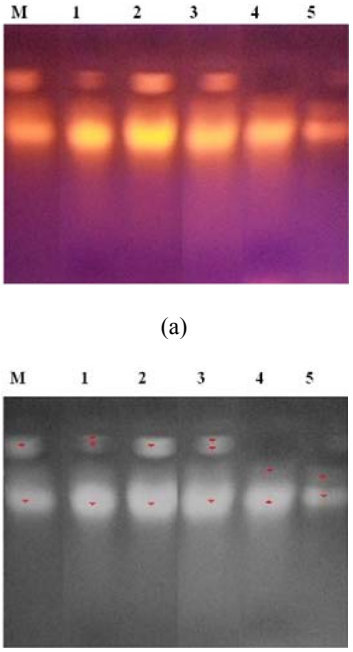

Fig. 9: (a) Subchronic effect of Silver nanoparticles on Qualitative evaluation of DNA fragmentation in liver tissue in normal and (b) irradiated rats; Lane M molecular weight marker, Lane 1 Normal control, Lane 2 Irradiation 4 Gy, Lane 3 Silver nanoparticles 26.878 mg/kg, Lane 4 Irradiation 4 Gy+Silver nanoparticles 42.599 mg/kg, Lane 5 Vehicle polyvinylpyrrolidone

the R.F value (Table 1) and graphically illustrated by electrophoresis gel image, molecular weight-optical density chart (Fig. 9A and B) respectively.

DNA gel electrophoresis in liver tissue of control sample didn't show any band which indicates the absence of DNA fragmentation. (Lane 1), despite the presence of slight release of DNA resulting from handling and processing of sample which indicated by the R.F value (0.338).

Irradiated (4Gy) group showed slight smear band along the lane which indicates partial DNA fragmentation and small release of DNA indicated by 3 bands of fragments. The last one appeared at R.f., value (0.348) which is relatively more than the R.F of the normal control.

Treatment with AgNPs at a dose of 26.878 mg/kg affected the DNA stability as shown by clear flourscent bandalong the lane and release the DNA smear molecules at R.F (0.349). (Lane 3).

Irradiation at a dose of (4Gy) followed by AgNPs treatment at a dose of 42.599 mg\kg (Lane 4) showed clear flourscent smear along the lane but less dense than the AgNPs band (lane 3), although it showed DNA fragmentation and slight release of DNA smear indicated by 3 bands of fragments the last one appeared at R.f value (0.337).

Vehicle control (PVP) showed slight effect on liver rat exhibited by slight smear band and release of DNA at R.F(0.341) (Lane 5).

It could be concluded that AgNPs exhibit a damaging effect on liver DNA which can be attenuated when combined with irradiation. Combination with irradiation cause a DNA damaging effect but to a laser extent than that of AgNPs alone.

In addition, the damaging effect of the combination of irradiation and AgNPs is less than that of the irradiated group. Alone although they both exhibit 3 bands of fragments Vehicle control showed a slight effect on liver DNA.

DISCUSSION

In the present study rats were subchronically evaluated after 28 days of oral administration of AgNPs in normal and after irradiation (4 Gy) at a dose level of 26.878 mg/kg and 42.599 mg/kg respectively. The chosen doses in the present study were 26.878 mg/kg which corresponds to 1/10 LD50 in normal animals and 42.599 mg/kg which corresponds to 1/10 LD50 in irradiated (4Gy) animals. These doses are according to the results of a previous study (Amin *et al*., 2015).

Finding of the present study revealed that AgNPs didn't significantly affect blood glutathione as compared to vehicle control.

Irradiation didn't significantly affect blood glutathione as compared to the vehicle control, although the irradiated group showed significant increase in glutathione level as compared to normal control group. In contrast to our result, Sarkar *et al*. (1983) showed that Blood GSH in rats exposed to 4 Gy remained unaltered.

The combination of irradiation and AgNPs 42.592 mg\kg significantly increase blood glutathione level as

compared to vehicle control. In agreement to our results, Genter *et al*. (2012) found that GSH levels in blood were higher 7 days after AgNPs exposure at the high dose of the AgNPs group and that change is a dose dependent. They deemed that GSH represents a reserve to restore oxidized molecules to their reduced state; a likely explanation for this increase in GSH is a physiological response to counteract the toxic effects of an oxidizing agent this explanation is in agreement to that of Genter *et al*. (2012).

The increase in glutathione by AgNPs is a dose dependent and can be explained in accordance of Rahman *et al*. (2009) they suggest, that the changes occur on genes expression in the mouse brain after exposure to 25nm AgNPs that Fmo2 gene was up-regulated significantly This increase in Fmo2was 95-fold with 500 mg/kg Ag-25 nanoparticles; however, it was only a 4-fold increase with 1000 mg/kg. It has been reported that the Fmo gene is involved in the oxidative metabolism of various xenobiotics and catalyzes the oxidation of reduced glutathione (GSH) to glutathione disulfide (Cereda *et al*., 2006). Up-regulation of Fmo2 by Ag-25 nanoparticles may disturb GSSG/GSH balance (the balance in the reduced and oxidized forms of the glutathione).

On the other hand Morones *et al*. (2005)who suggested that silver nanoparticles may interact with protein and enzymes with thiol groups like glutathione within cells which are substantial components of the cells antioxidant defense mechanism. While the same mechanism will cause deactivation of glutathione (Chen *et al*., 2008).

From the findings of our results it can be concluded that AgNPs 42.599 mg/kg increased blood GSH and potentiate the effect of irradiation (4Gy) on blood glutathione level.

Our results further indicate that AgNPs, irradiation and their combination have no effect on lipid peroxidation content in liver tissue. This result is in accordance with Scown *et al*. (2010) in their study rainbow trout (Oncorhynchusmykiss) were exposed via the water to commercial silver particles they found that there were no effects of AgNPs on lipid peroxidation in any of the tissues analyzed for any of the silver particles tested and they explained that due to the low uptake rates. AgNPs were found to be the most highly concentrated within gill tissues.

Ramachandran and Nair (2011) performed anti - lipid peroxidation assay using goat liver homogenate and DPPH scavenging test measure the radicals cavenging activity of antioxidants against free radicals like the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical, this test established the anti - oxidant potency of the silver nanoparticles.

In contrast to our results Piao *et al*. (2011) had found that concerning cytotoxicity of AgNPs in human liver cells, AgNPs treated cells showed increase in lipid peroxidation content compared to control cells. The difference in the results may be due to difference between the effect of AgNPs on animals and human cells. These results suggest that AgNPs damage cellular components via oxidative stress. The increased level of hepatic MDA, a byproduct of cellular lipid peroxidation, that AgNPs induced oxyradicals in the liver tissues. This explanation is in accordance to that of Choi *et al*. (2011).

Data of this study revealed that irradiation significantly increase serum nitric oxide levels. This is in accordance to Gorbunov *et al*. (2000).That showed that whole body gamma irradiation of rats enhanced the formation of NO. The enhancement of NO production was attributed to expression of the inducible nitric oxide synthase (Ibuki and Goto, 1997). NO have been suggested to be involved in acute radiation response in tissues such as liver, intestine, colon and brain (Chi *et al*., 2006).

NO plays an important role in inflammation and carcinogenesis and has now implicated as an important signaling molecule under normal physiological condition. Increased NO results in increased nitration of proteins at tyrosine, which can cause protein dysfunction or alteration in signal transduction pathways (Narangand Krishna, 2005).

On the other hand, ionizing radiation has been confirmed to potentiate NO production in microphages. The increase of NO production in irradiated macrophages contributed to tumoricidal activity with the activation mechanisms differing between high dose and low dose of irradiation. High dose of irradiation activate macrophage directly, whereas low dose irradiation acts indirectly through interaction with neighboring cells and the paracrine induction of cytokines (Ibuki and Goto, 2004).

The present study showed that AgNPs has no effect on serum NO as compared to the vehicle control. Combination of irradiation and AgNPs increase serum nitric oxide but to a lesser extent than irradiatied group. It may be concluded that AgNPs) decrease the effect of irradiation on serum nitric oxide.

Accordingly it may be recomeneded to use such combination in radiotherpy, AsVascular Endothelial Growth Factor **(**VEGF) induces vascular endothelial migration, proliferation and capillary-like network formation *in vitro* and vasculogenesis and angiogenesis *in vivo*(Ferrara, 1999). Addition of broad-spectrum (Nitric oxide synthase) NOS inhibitors blocks these VEGF functions *in vitro.* So according to our findings it can be postulated that AgNPs may exhibits antioxidant effect through NOS inhibition which results in blocking of VEGF activity.

In accordance to our findings Ramachandran and Nair (2011) tested the effectiveness of AgNPs on tumor

growth delay and its feasibility as an adjuvant in radiotherapy. AgNPs administration aided in significant tumor growth delay and this antitumor effect was more prominent in conjunction with gamma radiation treatment (4 Gy).

Inflammation is a physiological process in response to tissue damage resulting from microbial pathogen infection, chemical irritation and/or wounding. The relation between inflammation and atherosclerosis, diabetes, cancer, arthritis and Alzheimer's disease has been well substantiated (Maiti and Agrawal, 2007; Ohshima and Bartsch, 1994; Stevens *et al*., 2005; Virani *et al*., 2008). Ionizing radiation has been shown to exaggerate the inflammatory responses and to enhance the release of inflammatory mediators in experimental animals (el-Ghazaly *et al*., 1985, 1986; el-Ghazaly and Khayyal, 1995; Khayyal *et al*., 1995) and humans (Cole, 1993; FitzGerald *et al*., 2006), a process which probably involves the local vascular system, the immune system and various cells within the injured tissue.

The present study found that AgNPs, irradiation and their combination have no effect on serum tumor necrosis factor alfa as compared to vehicle control. In contrast Małaczewska *et al*. (2011) found enhanced production of the major proinflammatory cytokines TNF-alfa which initiate the inflammatory cascade.

On the other hand AgNPs was shown to lower the serum level of proinflammatory cytokine expression of TNF-alfa (Wright *et al*., 2002; Bhol and Schecter, 2007; Tian *et al*., 2007; Nadworny *et al*., 2008). Bhol and Schetchter (2007) revealed that, administration of AgNPs orally significantly reduced inflammatory changes, partly through suppression of (TNF-alfa).

In our study Silver nanoparticles (26.878 mg/kg), Irradiated rats (4Gy) and the Combination of irradiation 4Gy and Silver nanoparticles (42.599 mg/kg) significantly decrease serum c-reactive protein activity as compared to normal control .This effect on serum c-reactive protein may be explained by the anti-inflammatory effect of both AgNPs and 4(Gy) irradiation.

In accordance to our results other investigators (Wright *et al*., 2002; Bholand Schecter, 2007; Tian *et al*., 2007; Nadworny*et al*., 2008) revealed that AgNPs were shown to lower the serum level of acute phase proteins involved in the inflammatory process. C-Reactive Protein (CRP) is a protein found in the blood, the levels of which rise in response to inflammation which mean that C-reactive protein is an acute-phase protein.

Maneewattanapinyo *et al*. (2011) suggested that the releasing of Ag+during the preparation process of AgNPs may lead to vary in toxicity. Colloidal AgNPs treatment mediated biological effects is independent on Ag+. Some investigators suggest that Ag+releasing from AgNPs in the aqueous state might induce inflammatory response and stimulate metallic detoxification processes (Ahamed *et al*., 2010; Lubick, 2008; Miura and Shinohara, 2009; Park *et al*., 2010).

Ramachandran and Nair (2011) claimed that the anti inflammatory, antioxidant and radio protecting properties of AgNPs must have aided in a faster recovery of normal tissues inirradiated animals without interfering with the delaying effects of radiation on tumor growth. They also revealed that AgNPs alleviated the extent of acute and chronic inflammation in different paw edema models in mice through the radical scavenging and anti-inflammatory activities of AgNPs

Małaczewska *et al*. (2011) in their study on AgNPs in experimental endotoxemia had found that after 7 days of use, AgNPs enhanced the phagocytic activity and doses of 2.5 ppm stimulated the mitogenic response of splenocytes. While after 14 days of administration AgNPs lowered the phagocytic activity regardless the dose applied. These may be an explanation for the absence of proinflammatory mediators in our study after 28 days of continuous oral administration of AgNPs in the two doses 26.878 mg/kg and 42.599 mg/kg respectively.

Results of the present study revealed that AgNPs 26.878 mg/kg exhibit a damaging effect on liver DNA, which attenuated when combined with 4 (Gy) irradiation. Combination with irradiation causes a damaging effect also but to a lesser extent than AgNPs alone. In addition, the damaging effect of the combination of irradiation and AgNPs 42.599 mg/kg is less than that of the irradiated group alone although they are both exhibit three bands as demonstrated in the RF table. Meanwhile, vehicle control showed a slight effect on liver DNA.

The effect of irradiation on liver DNA is in agreement with the findings of Ibuki and Goto (1997) they have suggested that DNA strand breaks caused by hydroxyl radicals formed inside the cells by gamma irradiation plays an important role in enhancement of nitric oxide production but lipid peroxidation has a little effect

An increase in the DNA damage after gamma irradiation has been observed in previous studies (Hosseinimehr *et al*., 2002; Hosseinimehr *et al*., 2003).This is may be due to an excessive generation of reactive oxygen species or due to reduction of vasodilators like nitric oxide which in turn can increased by increased amount of ROS.

DNA damaging effect exhibited by AgNPs in our findings is consistent with the findings of Tiwari *et al*. (2011) who found damage in the DNA strand recorded with the AgNPs treatment at the high dose AgNPs (40 mg/kg) group.

In addition, our study revealed that the damaging effect on liver DNA exhibited by AgNPs can be

attenuated when combined with irradiation (4 Gy). Combination of AgNPs with irradiation causes a damaging effect on DNA liver tissue also but to a laser extent than that of AgNPs alone. The damaging effect of the combination of irradiation and AgNPs is less than that of the irradiated group alone.

Our results are in accordance to that of Chandrasekharan *et al*. (2011a) they have found that Oral administration of AgNPs one hour prior to radiation exposure reduced the radiation induced damage. Exposure of mice to whole body gamma irradiation resulted in formation of micronuclei in blood reticulocytes and chromosomal aberrations in bone marrow cells while AgNPs administration resulted in reduction in micronucleus formation and chromosomal aberrations indicating radioprotection.

Also in agreement to our results Chandrasekharan *et al*. (2011b) illustrated that cellular DNA was found to be protected from radiation-induced strand breaks in various tissues (spleen cells, bone marrow cells and blood leucocytes) of animals. Besides, AgNPs could enhance the rate of repair of cellular DNA in blood leucocytes and bone marrow cells when administered immediately after radiation exposure.

In conclusion, This study shows that AgNPs exhibit some oxidative stress and DNA damaging effects in liver tissues. Anti-inflammatory effect of AgNPs may be suggested as well. AgNPs attenuate the damaging effect of gamma radiation. Thus results suggest the possibility of using AgNPs as an adjuvant in cancer radiotherapy to protect normal tissues from radiation damages.

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