

Research Article

Evaluation of Acute and Subchronic Toxicity of Silver Nanoparticles in Normal and Irradiated Animals

¹Yara M. Amin, ¹Asrar M. Hawas, ¹A.I. El-Batal, ¹Seham H.M. Hassan and ²Mostafa E. Elsayed

¹National Centre for Radiation Research and Technology, Atomic Energy Authority,

²Department of Pharmacology and Toxicology, Faculty of Pharmacy, Cairo University, 11562, Egypt

Abstract: The present study was performed to evaluate the subchronic toxicity of Silvernano particles (AgNPs) at a diameter 23.1 ± 3.3 nm on normal and irradiated rats (4Gy) following 28 days of oral administration as compared to vehicle control. In the term of LD50 the lethality of AgNPs in normal and irradiated mice was undertaken. The LD50 of AgNPs in normal and irradiated (4Gy) mice is 268.781 mg/kg (ppm) and 425.990 mg/kg (ppm), respectively. Toxicity was reduced in irradiated mice as compared to normal mice. The selected doses used in the subchronic study were orally administered for 28 days. The doses corresponds to 1/10 LD50 which are 26.878 and 42.599 mg/kg in normal and irradiated rats, respectively. Following the 28 days liver and kidney function tests, changes in body weight, organ weight/body weight ratio (ow/bw), histopathological study and AgNPs distribution in liver, kidney, lung, testes were taken as a criteria for evaluation of the toxicity in normal and irradiated rats. Data showed significant decrease in serum urea and AST levels in irradiated group and a significant increase in serum GGT in AgNPs group. AgNPs significantly increased body weight. There was accumulation of AgNPs in both AgNPs and irradiated treated AgNPs groups. In descending order AgNPs were found in lung>liver>kidney>testes. In irradiated treated AgNPs group AgNPs were found in descending order in kidney>testes> lung>liver. Concentration of AgNPs in kidney and testes in the group of combination of irradiation (4Gy) and AgNPs (42.599 mg/kg) is significantly higher than that of AgNPs (26.878 mg/kg) group. Changes in ow/bw ratio has been shown in testes in both irradiated and irradiated treated AgNPs groups. Histopathological findings showed damage in liver, kidney and lung in both AgNPs and irradiated treated AgNPs groups. The damage in the liver in irradiated treated is less than that of AgNPs group. That may be explained that irradiation decrease the toxic effect of AgNPs on liver. Both irradiated and irradiated treated groups showed severe damage on testes that is due to the damaging effect of irradiation on gonads .and that can be confirmed by the decrease in tw/bw ratio. Irradiation showed a damaging effect on the other organs as well.

Keywords: AST, ALT, GGT, histopathological study, LD50, PVP

INTRODUCTION

Radiation is a process in which energetic particles or energetic waves travel through a medium or space. There are two distinct types of radiation. Namely ionizing, Non-ionizing radiation. Ionizing radiation is capable of depositing enough localized energy in living cells to disrupt the atoms and molecules on which it impinges, producing ions and free radicals in the process and giving rise in turn to biochemical changes that may lead to injury (Shapiro, 1972).

Nanotechnology, as defined by the United States (US) Nanotechnology Initiative, is the understanding and control of matter at dimensions of roughly 1-100 nanometers. As the need for the development of new medicines is pressing, the nanoscale functions of the biological components of living cells; nanotechnology has been applied to diverse medical fields such as oncology and cardiovascular medicine. As a result, an

improved understanding of the potential risks, comprising of exposure (which may be to free particles or due to particle release from composites) and hazard assessments, associated with exposure to nanomaterials is necessary and in particular, nanomaterial characteristics that are responsible for detrimentally affecting human health (Maynard *et al.*, 2006).

Metallic silver has also been used for surgical prosthesis and splints, fungicides and coinage. Soluble silver compounds, such as silver salts, have been used for treating mental illness, epilepsy, nicotine addiction, gastroenteritis, stomatitis (Alidaee *et al.*, 2005, Tanweer and Hanif, 2008) and sexually transmitted diseases, including syphilis and gonorrhoea (Gougeon *et al.*, 1996).

In recent years, the safety aspects of the oral route of silver nanoparticles took a great concern because of increase their applications in:

Corresponding Author: Yara M. Amin, National Centre for Radiation Research and Technology, Atomic Energy Authority, Egypt

This work is licensed under a Creative Commons Attribution 4.0 International License (URL: <http://creativecommons.org/licenses/by/4.0/>).

- Contaminated food
- Occupational exposures to metallic silver dust, silveroxide and silver nitrate aerosols.
- Drinking water including use of silver : copper filters in water purification.
- Silver nitrate or colloidal silver therapies in oral hygiene and gastrointestinal infection, (v) silver acetate antismoking therapies.

The present study was performed to evaluate the acute and subchronic effects of AgNPs in normal and irradiated animals (4Gy). Acute toxicity was determined through performing the lethal dose 50 (LD50). Subchronic toxicity of AgNPs was undertaken through determination of serum ALT, AST, GGT, urea, creatinine. Changes in body weight ,organ weight to body weight ratios, distribution of silver through several organs liver, kidney, lung, testes and histopathological examination for these organs has been carried as well.

MATERIAL AND METHODS

Animals: White male swiss albino mice weighing (20-25) g used for the determination of the LD50. 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*. All procedures in this study were carried out according to guidelines of Ethics Committee of Faculty of Pharmacy, Cairo University.

Drugs and chemicals: Silver nanoparticles were synthesized by chemical method. AgNPs were administered via intraperitoneal route in the LD50 experiment in several doses and were administered orally in the 28 days subchronic study in a dose of 26.878 and 42.599 mg/kg. Polyvinylpyrrolidone was purchased from Sigma-Aldrich chemical co. (U.S.A) to be orally administered as the vehicle control group in equivalent volume to that in AgNPs and irradiated treated AgNPs group.

Preparation of Silver nanoparticles: AgNPs was synthesized at Drug Radiation Research Dep.labs , at the National Centre for Radiation Research and Technology (NCRRT) by Prof.Dr Ahmed El-Batal according to the methods of (Mao *et al.*, 2012, El-Batal *et al.*, 2013^a and El-Batal *et al.*, 2013^b). In a typical procedure, solution A was prepared by dissolving 0.05M of AgNO₃, 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

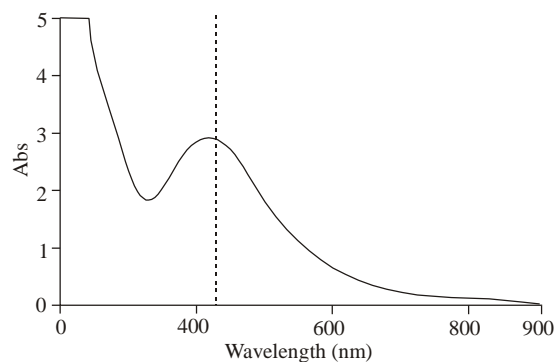


Fig. 1: UV-VIS spectral analysis of AgNPs

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 (4Gy) 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-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 measuringthe 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 10times 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

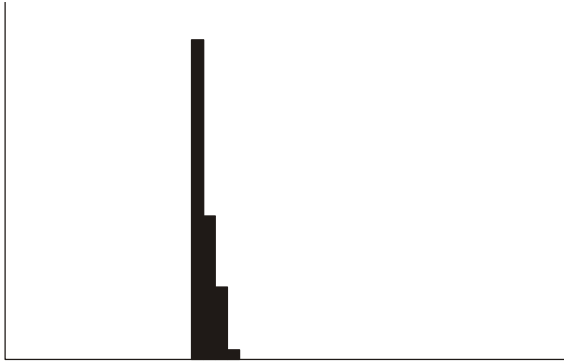


Fig. 2: Dynamic light scattering (DLS) of AgNPs revealed that the particle diameter mean value is $(23.1 \pm 3.3 \text{ nm})$

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:

Determination of LD₅₀: The Lethal Dose fifty (LD₅₀) of the drug AgNPs was determined by intraperitoneal injection to native and irradiated albino mice according to Sperman and Karber method (Finney, 1964).

Determination of subchronic toxicity: rats were divided into 5 groups, each consists of 10 rats. the groups are: normal control received saline, vehicle

control received polyvinylpyrrolidone (PVP), silver nanoparticles AgNPs group received 1/10 LD₅₀ in normal mice 26.878 mg/kg, irradiated group exposed to gamma irradiation (4Gy), irradiated treated group exposed to gamma irradiation (4Gy) followed by 1/10 LD₅₀ in irradiated mice 42.599 mg/kg. Rats were exposed to irradiation in a single dose 24 h 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 anesthetized by ether blood from the left ventricle was collected in non heparinized tube and serum was separated by centrifugation (4000g for 15 min) for the biochemical evaluation.

Animal's observations and effect on body weight: Animals were weighed weekly to evaluate the change on body weight.

Biochemical estimation: Serum Alanine Amino Transaminase (ALT) and Aspartate Amino Transaminase (AST) were estimated using ALT and AST kits (biodiagnostic, Giza, Egypt) Reitman and Frankel (1957) serum gamma glutamyl transferase was estimated using GGT kit (spectrum) kinetic colorimetric according to (Szasc, 1974) method. Serum urea was estimated according to (stanbio enzymatic urea nitrogen kit procedure No.2050) (Henry *et al.*, 1974). Serum creatinine was estimated according to creatinine kit (biodiagnostic Giza, Egypt) by colorimetric kinetic method (Bartles *et al.*, 1972).

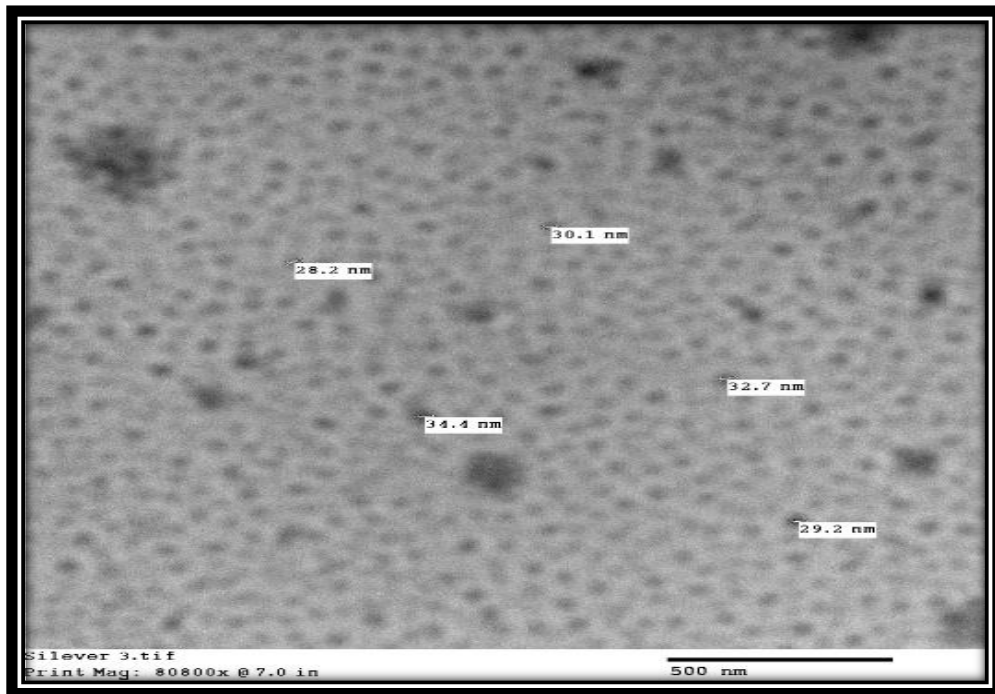


Fig. 3: Transmission Electron Microscopy (TEM) examination of AgNPs showed spherical shape of silver nanoparticles and good particle dispersion with average size at $(30.92 \pm 2.56 \text{ nm})$

Organ weight/body weight ratio: After collecting blood samples the rats were sacrificed by cervical dislocation liver, kidney, lung, testes were removed carefully, weighed to estimate organ weight/body weight ratio.

After weighing the organs they were divided into 2 samples from each group for the histopathological study and the rest of organs for determination of the tissue silver content.

Histopathology: Tissue specimens for the examined organs liver, kidney, lung, testes from all groups were collected and fixed in neutral buffer formalin, processed by conventional method, embedded in paraffin, sectioned at 4-5µm and stained by Haematoxylin and Eosin (Bancroft *et al.*, 1996).

Determination of tissue silver:

Sample preparation:

Tissue samples: Tissue samples were prepared by washing thoroughly by deionized water. The weighted samples were digested in concentrated pure nitric acid (65%), (S.G.1.42) and hydrogen peroxide in 1:4 ratios (IAEA, 1980). Sample digestion is carried out with acids at elevated temperature and pressures by using microwaves, Sample preparation labstation, MLS-1200 MEGA, Sample is then converted to soluble matter in deionized water to appropriate concentration. Silver was detected quantitatively using standard curve method (Kingston and Jassie, 1988).

Instrumentation: Silver then were estimated quantitatively by using SOLAR System Unicam 939 Atomic Absorption Spectrometer equipped with a deuterium background correction, fitted with GFTV accessory a SOLAR GF90. Silver concentration in the original sample could be determined by the calibration curve method using standard stock solutions 1000µg/ml. The element concentration in the original sample could be determined from the following equation.

$$C_2 \text{ microgram/gram} = C_1 \text{ microgram} \times D / \text{sample weight}$$

C1 = Silver concentration in solution

C2 = Silver concentration in sample

D = Dilution factor

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

Characterization of Silver nanoparticles: AgNPs absorbance were measured using UV analysis Fig. 1.

Dynamic Light Scattering (DLS) examination of AgNPs revealed that the average mean value of the particle diameter is $(23.1 \pm 3.3 \text{ nm})$ Fig. 2.

Examination of AgNPs using transmission electron microscope (TEM) revealed spherical shape of silver nanoparticles and good particle dispersion with average size at $(30.92 \pm 2.56 \text{ nm})$ Fig. 3.

The median lethal dose 50 LD50: The LD50 of Silver nanoparticles in normal and irradiated (4Gy) mice is 268.781, 425.990 mg/kg, respectively.

Toxicity of Silver nanoparticles was reduced 1.584 fold in irradiated (4Gy) mice as compared to its toxicity in normal mice (Fig. 1).

Subchronic effect of Silver nanoparticles on serum urea and serum creatinine levels in normal and irradiated rats: AgNPs (26.878 mg/kg) didn't significantly affect serum urea and creatinine level as compared to vehicle control.

Irradiated rats (4Gy) decreased serum urea level significantly to 71.76% of the vehicle control while they didn't significantly affect serum creatinine level. Combination of irradiation (4Gy) and AgNPs (42.599 mg/kg) showed no significant change in both serum urea and creatinine as compared to vehicle control. AgNPs has no effect on urea and creatinine levels. AgNPs antagonize the effect of irradiation on urea level as compared to vehicle control (Fig. 4a, b).

Subchronic effect of Silver nanoparticles on serum Alanine Amino Transferase, Aspartat Amino Transferase and Gamma glutamyltransferase levels in normal and irradiated rats.: AgNPs (26.878 mg/dl) didn't significantly affect serum ALT, serum AST level as compared to vehicle control although

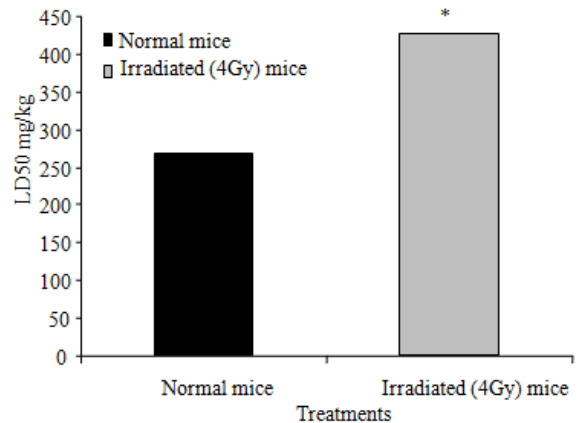


Fig. 4: LD50 of Silver nanoparticles in normal male mice as compared to the LD50 of Silver nanoparticles in irradiated male mice in a dose of (4Gy)

AgNPs showed significant increase inGGT level to 261.13% as compared to vehicle control and also showed significant increase as compared to normal control. Irradiation (4Gy) showed no significant change in serumAL, and serumGGT levels although they showed significant decrease in serum AST to 86.68% as compared to vehicle control. Both serum ALT, serum AST level in the (4Gy) irradiated group showed significant decrease as compared to normal control. Combination of irradiation (4Gy) and AgNPs (42.599 mg/kg) showed no significant change in serum ALT, serum AST and serumGGT level as compared to vehicle controlalthough, they show significant decrease as compared to AgNPs group. Irradiation antagonizes the effect of AgNPson serum GGT. AgNPs antagonize the effect of irradiation on serum AST. There is no interaction between irradiation and AgNPs on serum ALT. However they antagonize the effect of irradiation on ALT as compared to normal control (Fig. 5a to c).

Subchronic effect of Silver nanoparticles on the change of body weight throughout 28 days in normal and irradiated rats: Body weight in the normal control group at 0 times was 129 gm. The

values of the body weights were increased to 142, 144.8 gm, 156.8 and 163.4 gm on the 1st, 2nd, 3rd and 4th week, respectively. Therefore results of the body weight revealed significant increase on the 3rd and 4th week.

Body weight in the vehicle control group at 0 time was 112.8 gm. The demonstrated body weights were increased to 118.6gm, 128.2gm, 148.4gm and 161gm at the 1st, 2nd, 3rd and 4th week, respectively. Thus the body weight of the vehicle control showed significant increase on the 2nd, 3rd and 4th week as compared to the initial value.

Body weight of the AgNPs treated group significantly increased on the 4th week, while body weight in both irradiated and irradiated treated AgNPs group significantly increased on the 3rd and 4th week as compared to the initial value.

In general all the examined groups exhibited increase of the body weight at the end of the experimentation time (4th week) as compared to the initial value. The increase in the body weight was in descending order of the following vehicle control group>irradiated treated AgNPs group>AgNPs group >normal control group>irradiated group (Fig. 6).

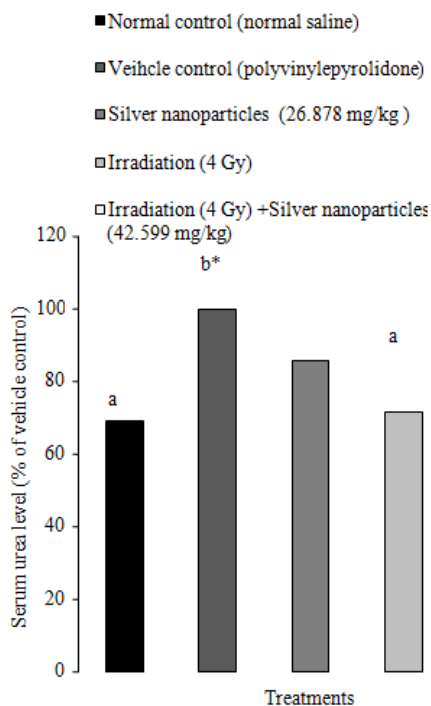


Fig. 5a: Subchronic effect of Silver nanoparticles on serum urea level in normal and irradiated rats; All treatments AgNPs (26.878mg/kg), AgNPs (42.599mg/kg), PVP were administered orally for 28 days, irradiation (4Gy) was done before the start of the study in the irradiated and irradiated treated AgNPs 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 <0.05; 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 (42.599mg/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

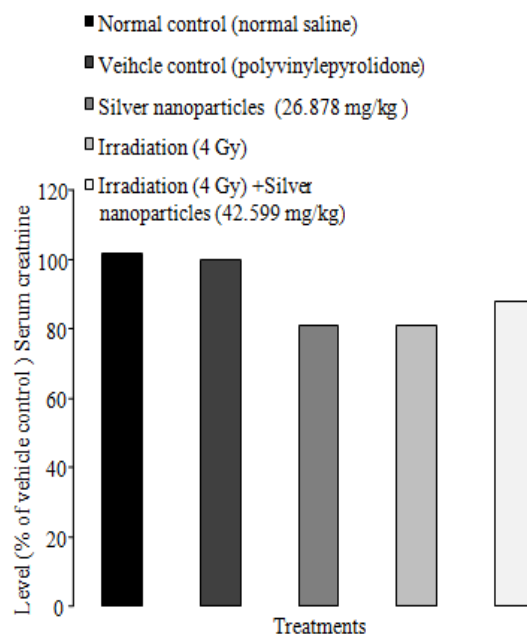


Fig. 5b: Subchronic effect of Silver nanoparticles on serum creatinine level in normal and irradiated rats; All treatments AgNPs (26.878mg/kg), AgNPs (42.599mg/kg, PVP were administered orally for 28 days, irradiation (4Gy) was done before the start of the study in the irradiated and irradiated treated 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 $P < 0.05$; *: Significantly different from the vehicle control value at $P < 0.05$; *: Significantly different from the irradiated control value at $P < 0.05$; *: Significantly different from silver nanoparticles (26.878 mg/kg) value at $P < 0.05$; *: 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

Subchronic effect of Silver nanoparticles on the ratio of organ weight to total body weight in normal and irradiated rats: AgNPs (26.878 mg/kg) didn't significantly affect the ratio of liver, kidney, lung, testis weight to total body weight as compared to vehicle control. Irradiation (4Gy) didn't significantly affect the ratio of liver, kidney, lung weight to total body weight as compared to vehicle control. However they showed significant decrease in the ratio of testis weight to total body weight as compared to vehicle control. Also it showed significant decrease in liver weight/body weight ratio as compared to normal control. The Combination of (4Gy) irradiation and AgNPs (42.599 mg/kg) didn't significantly affect the ratio of liver, kidney, lung, weight to total body weight as compared to vehicle control, while they showed significant decrease in the ratio of testis weight to total body weight as compared to vehicle control. Liver weight/body weight in irradiated and vehicle group showed significant decrease as compared to normal control. It could be concluded that there is no interaction between irradiation and AgNPs in liver, kidney, lung weight/body weight ratio as compared to vehicle control. Irradiation showed a decrease in testis weight /body weight ratio and AgNPs when combined with irradiation also decreased testes weight /body weight ratio (Fig. 7a to d).

Organ distribution of silver nanoparticles in normal rats after subchronic treatment: Concentration of AgNPs microgram per gram tissue in organ tissues liver, kidney, lung, testes is below limit of detection (2 ng/g) wet tissue (Loeschner *et al.*, 2011) in vehicle control, normal control and (4Gy) irradiated groups.

The organ distribution pattern is different in AgNPs (26.878 mg/kg) group and the group of combination of irradiation (4Gy) and AgNPs (42.599 mg/kg). Silver concentration was listed in a descending order in AgNPs (26.878) mg/kg group as the following: lung, liver, kidney, testes. While in the group of combination of irradiation 4Gy and AgNPs (42.599 mg/kg) silver concentration was listed in a descending order as the following: kidney, testes, lung and liver.

Concentration of AgNPs microgram per gram tissue in, kidney, testes in the group of combination of irradiation (4Gy) and AgNPs (42.599 mg/kg) is significantly higher than that of AgNPs (26.878 mg/kg) group.

It could be concluded that silver accumulation in the examined organs is dose dependent and the target organs are lung and liver in the AgNPs group. Irradiation increases the accumulation of silver in testes and kidney rather than lung and liver.

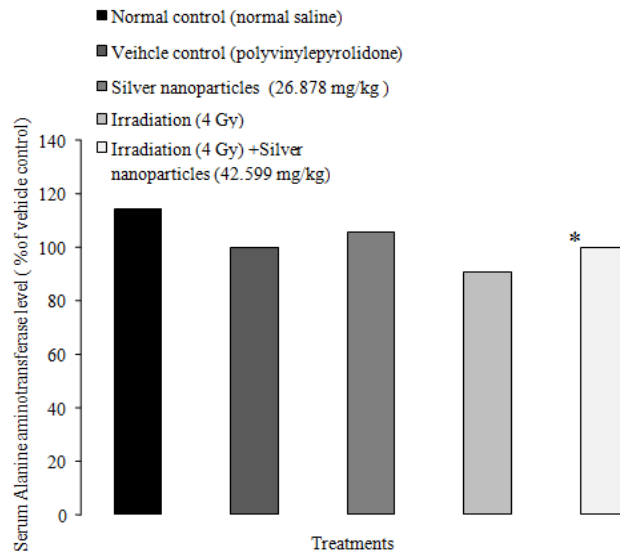


Fig. 6a: Subchronic effect of Silver nanoparticles on serum Alanine amino transferase level in normal and irradiated rats; All treatments AgNPs (26.878mg/kg), AgNPs (42.599mg/kg, PVP were administered orally for 28 days , irradiation (4Gy) was done before the start of the study in the irradiated and 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 $P < 0.05$; ^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 (42.599mg/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

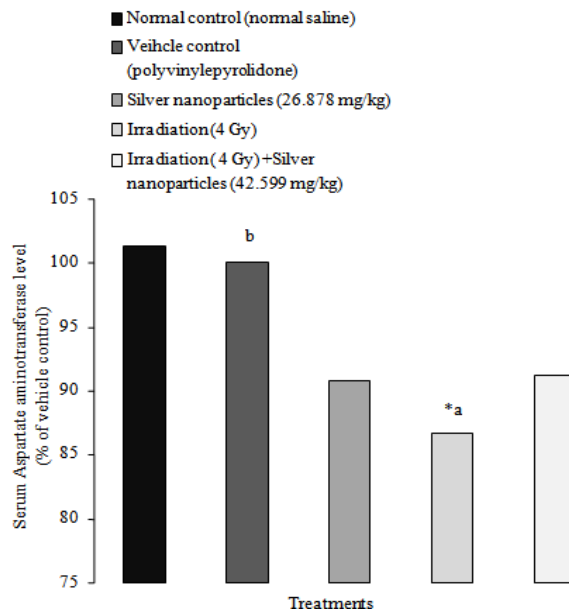


Fig. 6b: Subchronic effect of Silver nanoparticles on serum aspartate amino transferase level in normal and irradiated rats; All treatments AgNPs (26.878mg/kg), AgNPs (42.599mg/kg, PVP were administered orally for 28 days , irradiation (4Gy) was done before the start of the study in the irradiated and 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 $P < 0.05$; ^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 (42.599mg/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

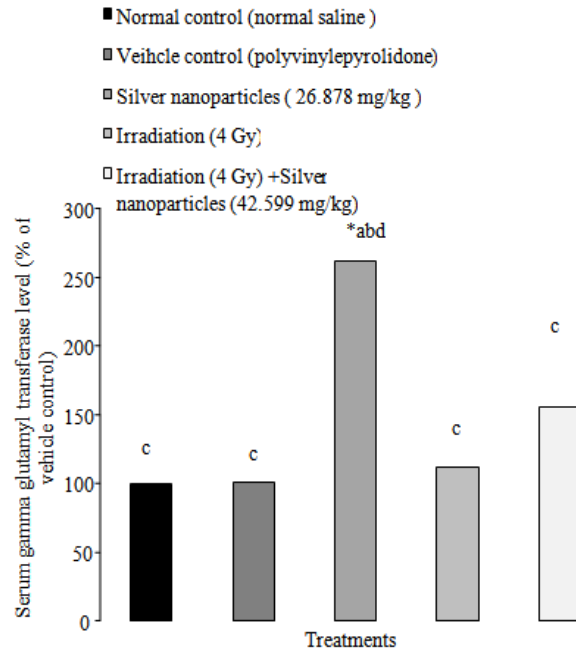


Fig. 6c: Subchronic effect of Silver nanoparticles on serum gamma glutamyl transferase level in normal and irradiated rats; All treatments AgNPs (26.878mg/kg), AgNPs (42.599mg/kg), PVP were administered orally for 28 days, irradiation (4Gy) was done before the start of the study in the irradiated and 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 $p < 0.05$; ^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 (42.599mg/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

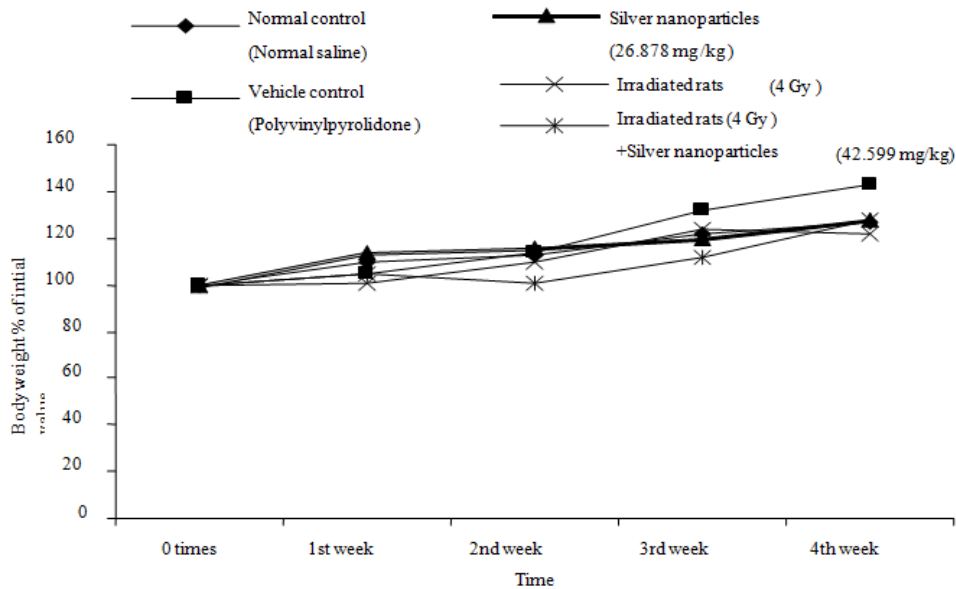


Fig. 7: Subchronic effect of Silver nanoparticles on the change in body weight normal and irradiated rats after 28 days; All treatments AgNPs (26.878mg/kg), AgNPs (42.599mg/kg), PVP were administered orally for 28 days, irradiation (4Gy) was done before the start of the study in the irradiated and irradiated 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; 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

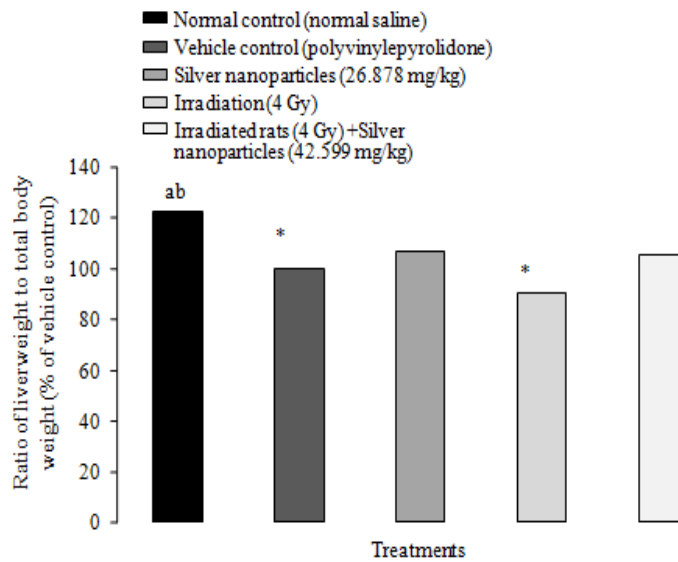


Fig. 8a: Subchronic effect of Silver nanoparticles on the ratio of liver weight to total body weight in normal and irradiated rats; All treatments AgNPs (26.878mg/kg), AgNPs (42.599mg/kg, PVP were administered orally for 28 days, irradiation (4Gy) was done before the start of the study in the irradiated 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 $p < 0.05$; °: Significantly different from the vehicle control value at $p < 0.05$; ˆ: Significantly different from the irradiated control value at $p < 0.05$; ˆ: 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

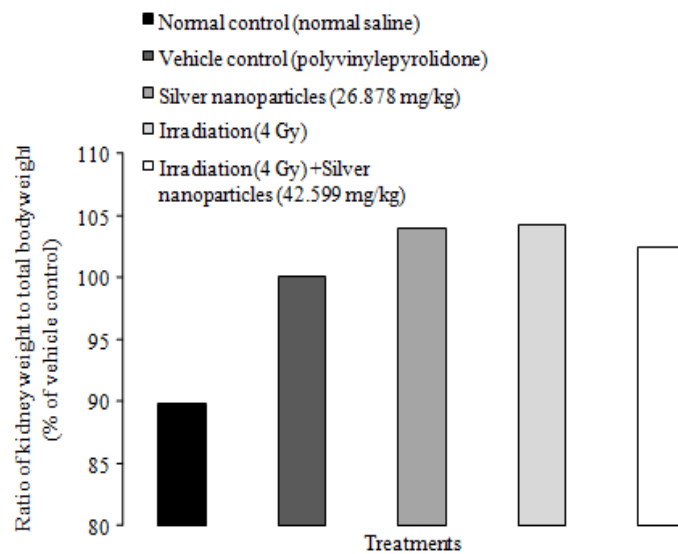


Fig. 8b: Subchronic effect of Silver nanoparticles on the ratio of kidney weight to total body weight in normal and irradiated rats All treatments AgNPs (26.878mg/kg), AgNPs (42.599mg/kg, PVP were administered orally for 28 days, irradiation (4Gy) was done before the start of the study in the irradiated 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 $P < 0.05$; °: Significantly different from the vehicle control value at $P < 0.05$.; ˆ: Significantly different from the irradiated control value at $P < 0.05$; ˆ: 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

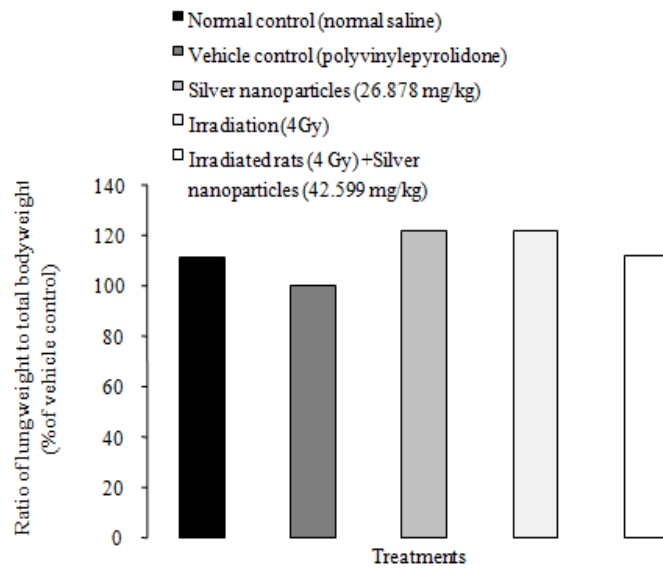


Fig. 8c: Subchronic effect of Silver nanoparticles on the ratio of lung weight to total body weight in normal and irradiated rats; All treatments AgNPs (26.878mg/kg), AgNPs (42.599mg/kg, PVP were administered orally for 28 days, irradiation (4Gy) was done before the start of the study in the irradiated 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 $p < 0.05$; ^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 (42.599mg/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

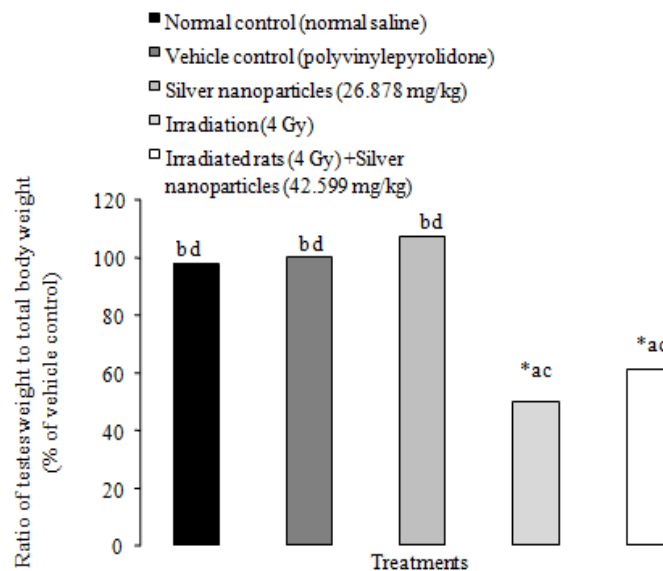


Fig. 8d: Subchronic effect of Silver nanoparticles on the ratio of testes weight to total body weight in normal and irradiated rats All treatments AgNPs (26.878mg/kg), AgNPs (42.599mg/kg, PVP were administered orally for 28 days , irradiation (4Gy) was done before the start of the study in the irradiated 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 $p < 0.05$; ^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 (42.599mg/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

Histopathological examination:

- Liver:** Normal control group that received saline showed normal histological structure of hepatic lobule as showed in the Fig. 8, the central vein is surrounded by normal hepatocytes. Vehicle control group that received polyvinylepyrrolidone showed mild histological changes including kupffer cell activation. AgNPs (26.878 mg/kg) mg/kg group showed severe changes including cytoplasmic vacuolization of hepatocytes and focal hepatic hemorrhage. Irradiation (4Gy) group showed moderate changes including fatty changes of hepatocytes and pyknosis of hepatocytic nuclei. Irradiation (4Gy) followed by AgNPs 42.599 mg/kg group showed moderate changes demonstrated by fatty changes of hepatocytes and cytoplasmic vacuolization. Both Irradiated and Irradiated treated with AgNPs groups showed slight improvement as compared to that received AgNPs alone (Fig. 9).
- Kidney:** Histological examination of kidney specimens in normal group revealed normal kidney tissues demonstrated in normal renal parenchyma and normal renal tubules.

The kidney sections of rats treated orally with vehicle (polyvinylepyrrolidone) showed mild changes demonstrated by congestion of renal blood vessels. Kidney of rats treated with AgNPs 26.878 mg/kg showed severe changes exhibited by dilatation and congestion of renal blood vessel and the presence of protein casts in the lumen of the renal tubule as well as vacuolization of the epithelial lining of renal tubule. The observed degree of damage in the drug group is significantly more than that of the vehicle group. Irradiation of rats at a single dose of (4Gy) showed moderate changes demonstrated by cytoplasmic vacuolization of the epithelial lining cells of renal tubule. On the other hand kidney of group exposed to a single dose of (4Gy) followed by of AgNPs 42.599 mg/kg showed severe changes exhibited by hypertrophy and vacuolization of glomerular tubes and focal renal hemorrhage .demonstrated kidney damage in the group receiving the combination of single dose of (4Gy) followed by AgNPs 42.599 mg/kg is significantly more than that irradiated group (Fig. 10).

- Lung:** Normal control group that received saline showed normal histological structure of lung tissues. Lung sections of rats treated with vehicle (polyvinylepyrrolidone) showed severe changes

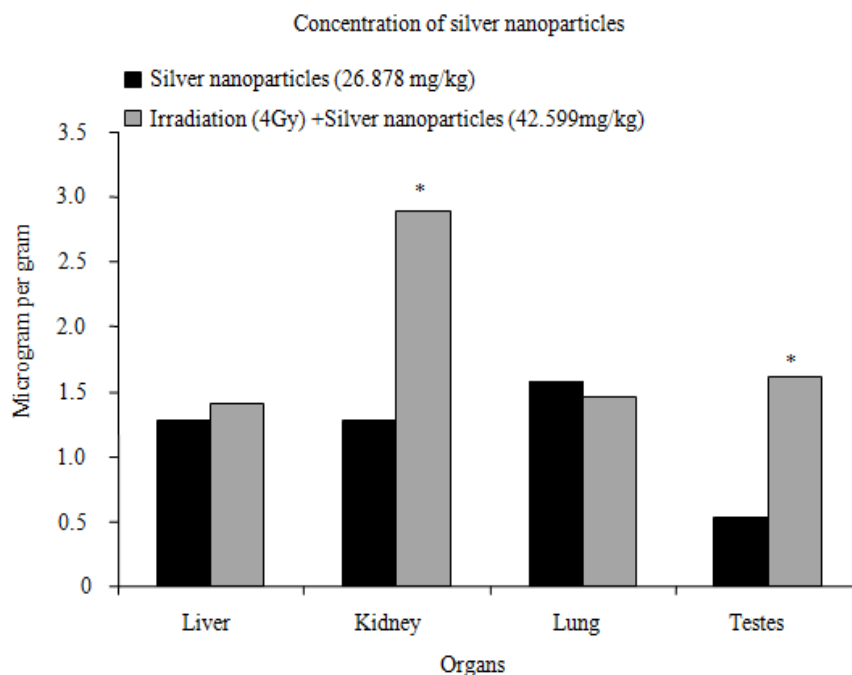


Fig. 9: Organ distribution of silver nanoparticles in normal and irradiated rats after subchronic treatment; All treatments AgNPs (26.878mg/kg), AgNPs (42.599mg/kg, PVP were administered orally for 28 days, irradiation (4Gy) was done before the start of the study in the irradiated and irradiated treated 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 silver nanoparticles (26.878 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

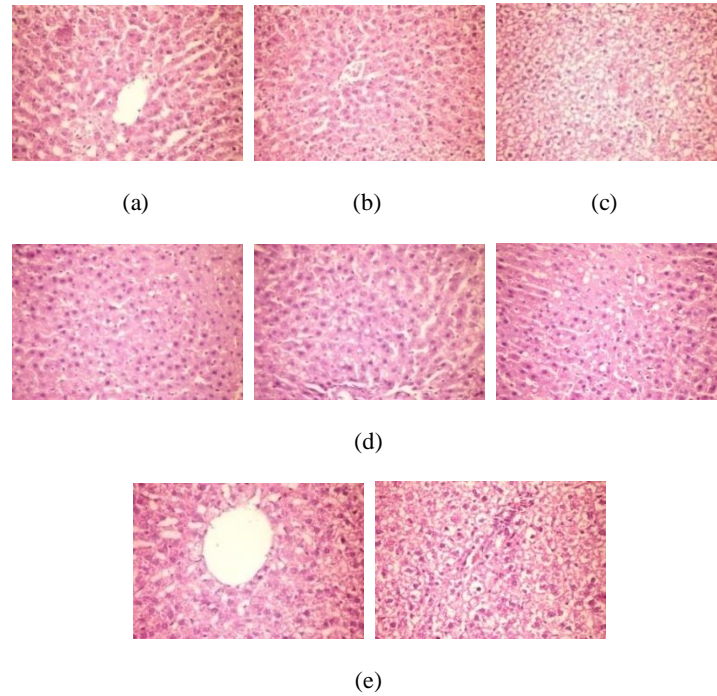


Fig. 10: H&E staining of rat liver (Fig. 10); (a): A showed normal histological structure of hepatic lobule (-); (B): showed mild histological changes including kupffer cell activation(+); (c): showed severe changes including cytoplasmic vacuolization of hepatocytes and focal hepatic hemorrhage(+++); (d): Showed moderate changes including fatty changes of hepatocytes and pyknosis of hepatocytic nuclei (++) ; (e): Showed moderate changes demonstrated by fatty changes of hepatocytes and cytoplasmic vacuolization (++)

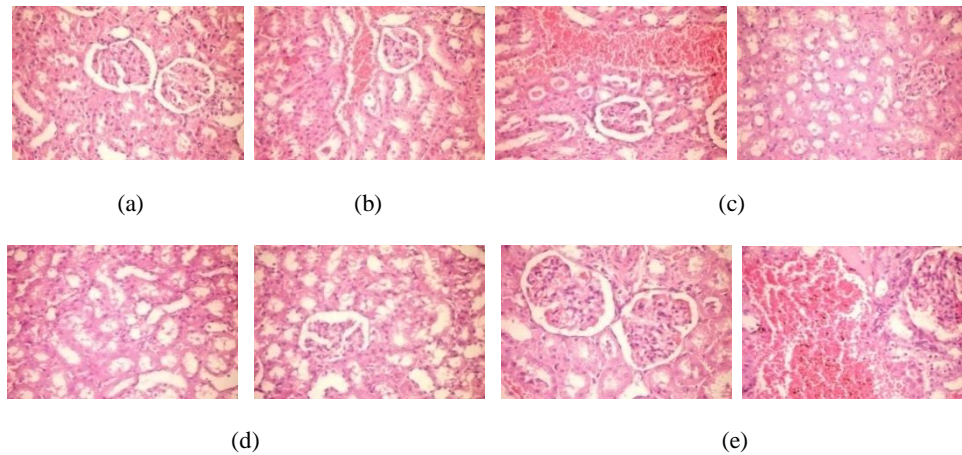


Fig. 11: H&E staining of rat kidney (Fig. 11); (a): Normal kidney tissues demonstrated in normal renal parenchyma and normal renal tubules.(-); (B): showed mild changes demonstrated by congestion of renal blood vessels (+); (C): showed severe changes exhibited by dilatation and congestion of renal blood vessel and the presence of protein casts in the lumen of the renal tubule as well as vacuolization of the epithelial lining of renal tubule (+++); (D): showed moderate changes demonstrated by cytoplasmic vacuolization of the epithelial lining cells of renal tubule (++) .; (e): showed severe changes exhibited by hypertrophy and vacuolization of glomerular tubes and focal renal hemorrhage

indicated by interstitial pneumonia. AgNPs 26.878 mg/kg showed severe changes including thickening of interstitial tissues and focal interstitial pneumonia. Lung of rats exposed to a single dose of gamma irradiation (4Gy) showed severe changes as illustrated by interstitial pneumonia .Irradiation of rats at a single dose of (4Gy) followed byAgNPs 42.599 mg/kg showed severe changes exhibited by interstitial pneumonia and the figure shows massive cell infiltration as well. Lung of rats in the combination group of irradiation (4Gy) followed by AgNPs 42.599 mg/kg showed no improvement than that of the other groups irradiated group, silvernanoparticle group or even the vehicle group (Fig. 11).

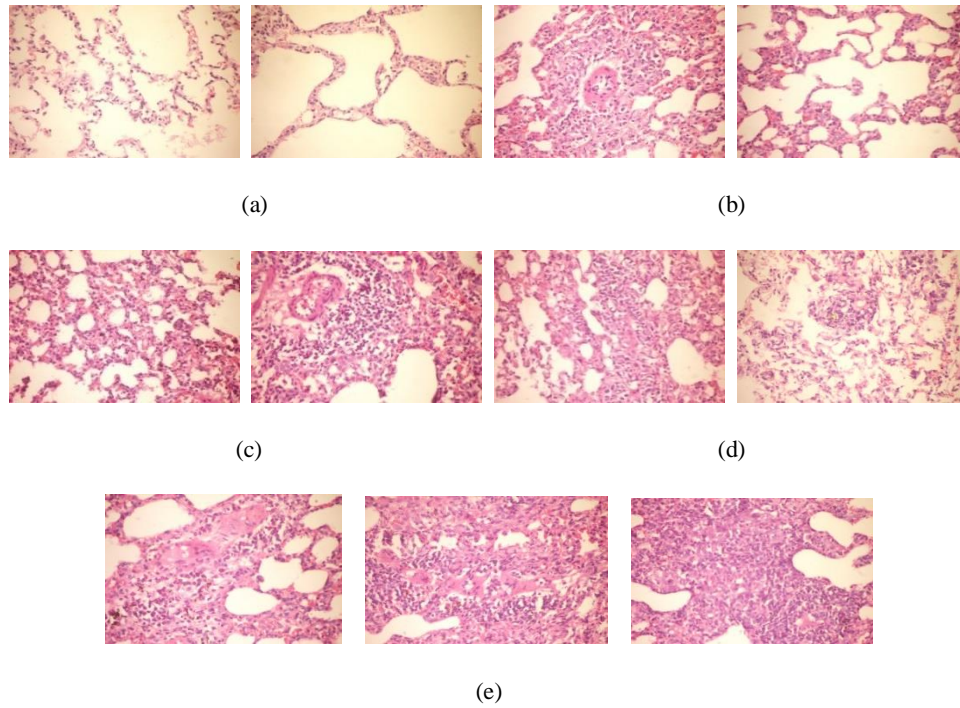


Fig. 12: H&E staining of rat Lung (Fig. 12); (a): saline showed normal histological structure of lung tissues(-);(b): showed severe changes indicated by interstitial pneumonia(+++); (c): showed severe changes including thickening of interstitial tissues and focal interstitial pneumonia (+++).; (d): showed severe changes as illustrated by interstitial pneumonia (+++); (e): showed severe changes exhibited by interstitial pneumonia and massive cell infiltration (+++)

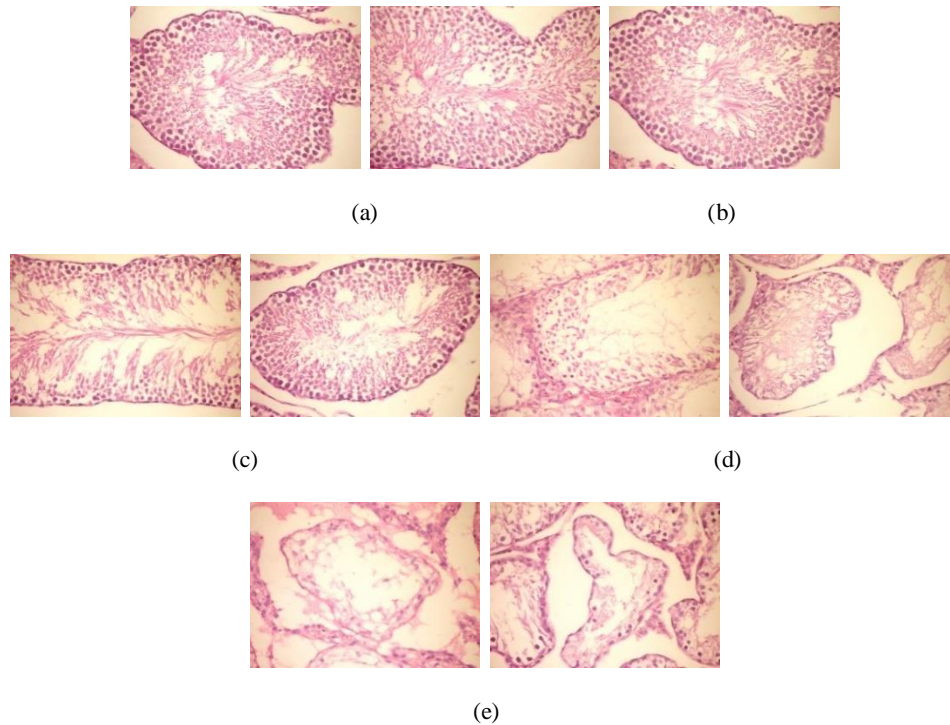


Fig. 13: H&E staining of rat Testis (10); (a): showed normal histological structure of of testis as represented by normal seminiferous tubules (-); (b):showed no histological changes than that of the normal control group (-); (b); (c): showed no histological changes (-); (d): showed severe changes demonstrated by testicular degeneration and marked necrosis of spermatogoneal cells lining seminiferous tubules (+++).; (e): showed severe changes demonstrated by testicular degeneration and complete absence of spermatogoneal cells lining seminiferous tubules (+++)

- **Testes:** Normal control group that received saline showed normal histological structure of testis as represented by normal semiferous tubules. Vehicle control group that received polyvinylepyrrolidone showed no histological changes than that of the normal control group. AgNPs 26.878 mg/kg as well as the vehicle group showed no histological changes. Irradiation of rats at a dose level of (4Gy) showed severe changes demonstrated by testicular degeneration and marked necrosis of spermatogoneal cells lining semiferous tubules. Irradiation of rats of (4Gy) followed by AgNPs 42.599 mg/kg showed severe changes demonstrated by testicular degeneration and complete absence of spermatogoneal cells lining semiferous tubules. The combination group of irradiation 4Gy and AgNPs 42.599 mg/kg showed no improvement than the irradiated group (Fig. 12 and 13).

Histopathological examination: Demonstrated in liver, kidney, lung, testes Where A) normal control received saline. B) vehicle control received PVP.C) AgNPs (26.878mg/kg). D) irradiated rats (4Gy). E) irradiated rats (4Gy) then treated AgNPs (42.599 mg/kg. (-) indicates no changes. (+) indicates mild changes (++) indicates moderate changes (+++) indicates severe changes.

DISCUSSION

Finding of the acute toxicity had evaluated The LD50 of AgNPs in normal and irradiated mice (4Gy). The LD50 of AgNPs in normal and irradiated mice (4Gy) is 268.781 mg/Kg, 425.990 mg/Kg respectively. It has been shown that the toxicity of AgNPs was reduced 1.582 fold in irradiated mice 4Gy as compared to its toxicity in normal mice. In accordance to our results (Ramachandran and Nair, 2011) revealed that AgNPs administration bestowed survival advantage to the mice exposed to lethal dose of gamma radiation.

In agreement to our results (Chandrasekharan *et al.*, 2011) 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.

In contrast to our result (Ordzhonikidze *et al.*, 2010) estimated the dose of silver nanoparticles that causes 50% lethality is 1.9×10^{-6} mg per gram of body mass the difference in results is may be due to the different doses and several dilutions used.

Results of the subchronic study showed that AgNPs (26.878mg/kg). Given for 28 days didn't affect

serum urea, creatinine, ALT, AST levels. Similar results have been reported by (Kim *et al.*, 2008, 2011). The results are in agreement with data reported by (Kim *et al.*, 2010) as well.

AgNPs (26.878 mg/kg). Showed significant increase in serum gamma glutamyltransferase level. These results are in agreement with the data obtained by (Tiwari *et al.*, 2011). In contrast to our results (Kim *et al.*, 2010) showed no significant change on serum gamma glutamyltransferase exhibited by AgNPs. The difference of the results could be attributed to difference in the doses as well as the duration of administration.

Results of the present study revealed that AgNPs has no effect on kidney function biomarkers. AgNPs has relatively safe effects on kidney and liver functions.

Irradiation (4Gy) decreased serum urea but it didn't affect serum creatinine level. Irradiation significantly decreases serum AST but it didn't affect serum ALT and serum GGT.

The combination of irradiation (4Gy) and AgNPs 42.599 mg/kg didn't affect serum urea, creatinine, ALT, AST. It is concluded that AgNPs normalized the effect of irradiation on urea and AST levels. Irradiation decreases the effect of AgNPs on serum GGT level.

That is mean that the combination of irradiation (4Gy) and AgNPs 42.599 mg/kg has a beneficial effects on liver and kidney functions.

The present study showed that AgNPs didn't affect the ratios of liver, kidney, lung, testis to total body weight similar effect has been obtained with (Kim *et al.*, 2010).

Irradiation (4Gy) and irradiation treated AgNPs group also showed significant decrease in the ratio of testis weight to total body weight. That is can be explained by the damaging effect of irradiation on the gonads. Direct irradiation to the testis will, in lower doses, affect the germinal epithelium (Ogilvy-Stuart and Shalet, 1993). Results are confirmed with the histopathological findings of the present study which showed severe changes demonstrated by testicular degeneration and marked necrosis of spermatogoneal cells lining semiferous tubules.

The findings of our study revealed that AgNPs showed significant increase in body weight. Combination of both irradiation and AgNPs also showed significant increase in body weight. The increase in the body weight was in descending order of the following vehicle control group > irradiated treated AgNPs group > AgNPs group > normal control group > irradiated group. In accordance to our results (Ramachandran and Nair, 2011) Found that AgNPs treatment delayed the loss in body weight caused by irradiation when combined with (4Gy) gamma irradiation.

On the other hand our findings are in contrast to that of Kim *et al.*, 2008 which found that AgNPs did not show any significant changes in body weight.

Histopathological examination our results showed severe damage caused by AgNPs on liver. Irradiation (4Gy) and the combination of irradiation (4Gy) and AgNPs 42.599 mg/kg showed moderate changes which can be explained that combining irradiation with AgNPs showed slight improvement as compared to that received AgNPs 26.878 mg/kg only. This improvement can be confirmed by the study results on normalization serum AST and GGT levels in the group of combination of irradiation (4Gy) and AgNPs.

In accordance to our results (Sung *et al.*, 2009, Kim *et al.*, 2007) evaluated Liver toxicity by histopathology included bile-duct hyperplasia and increased foci (Furchner *et al.*, 1986). Suggested that the damage in liver could be attributed that the absorption of silver after oral administration. AgNPs have been shown to be subjected to a first-pass effect through the liver, resulting in excretion into the bile.

Kidney of rats treated with silver nanoparticles 26.878 mg/kg and in the group of combination of (4Gy) irradiation and Silver nanoparticles (42.599 mg/kg) showed severe damage as well. In accordance to our results (Creasey and Moffat 1973, Danscher, 1981, Ham and Tange, 1972, Moffat and Creasey, 1972) revealed that the damage in liver and kidney is may be attributed that the uncleaned silver that has been shown to be deposited in the renal glomerular basement membrane mesangium (Day *et al.*, 1976), Kupffer cells and sinusoid endothelium cells in the liver (Danscher, 1981). Also the damage in liver and kidney was in accordance to the results of (Kim *et al.*, 2010).

Lung of rats treated with AgNPs 26.878 mg/kg and in the the group of combination of irradiation (4Gy) and AgNPs (42.599 mg/kg) showed severe changes. This results are in accordance to that of (Hyun *et al.*, 2008) the lungs samples in high doses group indicated foamy macrophages in the alveolar. In contrast to our results (Kim *et al.*, 2010) showed that histopathologic examination of lung tissue did not show any treatment-related effects.

Results had found that irradiation exhibit moderate damaging effect on liver, kidney and severe damage in lung.

Organ distribution pattern is different in AgNPs (26.878 mg/dl) group and the group of combination of irradiation 4Gy and Ag NPs (42.599 mg/Kg). The highest silver concentration listed in a decreasing order in AgNPs group were found in lung > liver > kidney > testes. While the highest silver concentration in the combination irradiation (4 Gy) and AgNPs (42.599 mg/Kg) listed in a decreasing order were found in kidney > testes > lung > liver. Contrast to our results (Loeschner *et al.*, 2011) listed the highest silver concentrations in decreasing order, were found in small intestine, stomach, kidney and liver tissues of the exposed animals. In accordance to our findings (Sung *et al.*, 2009) revealed that the target organs for silver nanoparticles were shown to be the liver and lungs in a

90-day inhalation study. Kim *et al.* (2007) found that the target organ is the liver in a 28-day oral toxicity study AgNPs microgram deposition in kidney and testes in the group of combination of irradiation 4Gy and AgNPs (42.599 mg/kg) is significantly higher than that of AgNPs (26.878 mg/kg) group.

Hypothesized that the toxic effects of silver are proportional to free silver ions, but it is unclear how this relates to silver nanoparticles. AgNPs in the blood, indicate that the orally absorbed silver from nanoparticles is able to enter the blood circulation and be distributed to other organs. Since similar effects have not been previously reported for soluble silver, there is a possibility that these effects are due to particles as opposed to ionized silver. Common treatment-related endpoints and distribution were found in different studies indicating that distribution and toxicity do not appear to be dependent on particle size in the tested range or route of administration. In previous reports, the target organs for silver nanoparticles were shown to be the liver in a 28-day oral toxicity study (Kim *et al.*, 2007) and the liver and lungs in a 90-day inhalation study (Sung *et al.*, 2009).

It can be suggested that the damage illustrated in the histopathological examination in liver, kidney, lung may be related to AgNPs deposition in the tissue. These effects may be due to particle size since gold nanoparticles (which are not likely to be ionized) show similar effects (KEMTI (Korea Environment and Merchandise Testing Institute), 2009). Our very limited data makes it tempting to hypothesize that effects from silver nanoparticles are not only due to ionization of silver from the surface of silver nanoparticles, but may originate (at least in part) from direct effects of nanoparticles.

It can be concluded from our study that the LD50 of AgNPs via the intraperitoneal route in normal and (4Gy) irradiated mice is 268.781 (ppm) and 425.990 mg/kg (ppm), respectively. Also it can be concluded that the toxic effect exhibited by AgNPs or irradiation (4Gy) can be decreased to a certain extent in the combination. This was revealed through the increased LD50 value, antagonism of liver and kidney function tests. AgNPs antagonized the effect of irradiation on lw/bw as compared to the normal control, changes in total body weight, decrease the histopathological liver damage.

AgNPs were suggested to be useful as a radioprotector during different radiation exposure in whole body radiation from body weight loss and mortality.

REFERENCES

- Alidaee, M.R., A. Taheri, P. Mansoori and S.Z. Ghodsi, 2005. Silver nitrate cautery in aphthous stomatitis: A randomized controlled trial. *Br. J. Dermatol.*, 153: 521-525.

- Bartles, H., M. Bohmer and C. Herili, 1972. Clin. Chem. Acta, 37: 193.
- Bancroft, J.D., A. Stevens and D.R. Turner, 1996. Theory and practice of histological techniques. 4th Edn. Churchill, Living Stone, New York.
- Chandrasekharan, D.K., P.K. Khanna and C.K. Nair, 2011. Cellular radioprotecting potential of glyzyrrhizic acid. Silver Nanoparticle and Their Complex, 2011.
- Creasey, M. and D.B. Moffat, 1973. The deposition of ingested silver in the rat kidney at different ages. *Experientia*, 29: 326-327.
- Danschler, G., 1981. Light and electron microscopic localization of silver in biological tissue. *Histochemistry*, 71: 177-186.
- Day, W.A., J.S. Hunt, A.P. McGiven, 1976. Silver deposition in mouse glomeruli. *Pathology*, 8: 201-204.
- ^aEl-Batal, A.I., M.A. Amin, M. Mona, K. Shehata and M.A. Merehan, 2013. Synthesis of silver nanoparticles by bacillus stearothermophilus using gamma radiation and their antimicrobial activity. *World Appl. Sci. J.*, 22(1): 01-16.
- ^bEl-Batal, A, I. El-Baz, A.F. Abo Mosalam, F.M. and A.A. Tayel, 2013. Gamma irradiation induced silver nanoparticles synthesis by *Monascus purpureus*. *J. Chem. Pharmaceutical Res.*, 5(8): 1-15.
- Finney, D.J., 1964. In statistical method in biological assay. Charles Criffin and Company Limited, London, pp: 528.
- Furchner, J.E., C.R. Richmond and G.A. Drake, 1968. Comparative metabolism of radionuclides in mammals-IV. Retention of silver-110 m in the mouse, rat, monkey and dog. *Health Phys.*, 15: 505-514.
- Gougeon, M.L., H. Lecoeur, A. Dulioust, M.G. Enouf, M. Crouvoiser and *et al.*, 1996. Programmed cell death in peripheral lymphocytes from HIV-infected persons: Increased susceptibility to apoptosis of CD4 and CD8 T cells correlates with lymphocyte activation and with disease progression. *J. Immunol.*, 156: 3509-3520.
- Ham, K.N. and J.D. Tange, 1972. Silver deposition in rat glomerular basement membrane. *Aust. J. Exp. Biol. Med. Sci.*, 50: 423-434.
- Henry, J.P., Todd, Sanford and Davidson, 1974. Clinical diagnosis and measurements by laboratory methods. 16 Edn., W.B., Saunders and Co., Philadelphia PA, pp: 260.
- Hyun, J.S., B.S. Lee, H.Y. Ryu, J.H. Sung, K.H. Chung, I.J. Yu, 2008. Effects of repeated silver nanoparticles exposure on the histological structure and mucins of nasal respiratory mucosa in rats. *Toxicol. Lett.*, 182: 24-28.
- IAEA, 1980. Elemental analysis of biological materials, international Atomic energy Agency. IAEA, Veinna Technical Report Series No. 197: 379.
- KEMTI (Korea Environment and Merchandise Testing Institute), 2009. Subchronic inhalation toxicity of gold nanoparticles. A Report Submitted to the Korean Food and Drug Administration, Seoul, Korea 2009.
- Kim, T., M. Kim and H. Park, 2011. Size-dependent cellular toxicity of silver nanoparticles. *J. Biomed. Mater. Res.*, 100A(4).
- Kim, Y.S., M.Y. Song, J.D. Park, K.S. Song, H.R. Ryu, Y.H. Chung, H.K. Chang and *et al.*, 2010. Subchronic oral toxicity of silver nanoparticles. *Particle Fibre Toxicol.*, 2010: 7-20.
- Kim, Y.S., J.S. Kim, H.S. Cho, D.S. Rha, J.M. Kim, J.D. Park, B.S. Choi and *et al.*, 2008. Twenty-eight-day oral toxicity, genotoxicity and gender-related tissue distribution of silver nanoparticles in sprague-dawley rats. *Inhalat. Toxicol.*, 20(6): 575-583.
- Kim, J.S., E. Kuk, K.N. Yu, J.H. Kim, S.J. Park, H.J. Lee and *et al.*, 2007. Antimicrobial effects of silver nanoparticles. *Nanomedicine*, 3: 95-101.
- Kingston, H.M. and L.B. Jassei, 1988. Introduction to microwave sample preparation ACS. Washington, DC, pp: 126-130.
- Loeschner, K., N. Hadrup, K. Qvortrup, A. Larsen, X. Gao, U. Vogel and A. Mortensen, 2011. Distribution of silver in rats following 28 days of repeated oral exposure to silver nanoparticles or silver acetate. *Part Fibre Toxicol.*, 8: 18.
- Mao, A., X. Jin, X. Gu, X. Wei and G. Yong, 2012. Rapid green synthesis and surface enhanced Raman scattering effect of single crystal silver nanocubes. *J. Molecular Struct.*, 1021: 158-161.
- Maynard, A.D., R.J. Aitken, T. Butz, V. Colvin, K. Donaldson, G. Oberdörster *et al.*, 2006. Safe handling in nanotechnology. *Nature*, 444: 267-269.
- Moffat, D.B. and M. Creasey, 1972. The distribution of ingested silver in the kidney of the rat and of the rabbit. *Acta Anat.*, 83: 346-355.
- Ordzhonikidze, C.G., L.K. Ramaiyya, E.K. Egorova and A.V. Rubanovich, 2009. Genotoxic effects of silver nanoparticles on mice in vivo. *Acta Naturae*, pp: 2075-8251.
- Ogilvy-Stuart, A.L. and S.M. Shalet, 1993. Effect of radiation on the human reproductive system. *Environ. Health Perspect.*, 101(2): 109-116.
- Ramachandran, L. and C.K.K. Nair, 2011. Therapeutic Potentials of Silver Nanoparticle Complex of α -Lipoic Acid. Intech, 2011.
- Reitman, A. and S. Frankel, 1957. *Amer. J. Clin. Path.*, 28: 56.
- Shapiro, J., 1972. A guide for Scientists and physicians. Radiation Protection Cambridge, Harvard University Press, Cambridge.
- Szasc, G., 1974. Presjin *J. Pclin. Chem. Cline. Biochem.*, 12: 224.

- Sung, J.H., J.H. Ji, J.D. Park, J.U. Yoon, D.S. Kim, K.S. Jeon and *et al.*, 2009. Subchronic inhalation toxicity of silver nanoparticles. *Toxicol Sci.*, 108(2):452-461.
- Tanweer, F. and J. Hanif, 2008. Silver nitrate cauterisation does concentration matter. *Clin. Otolaryngol.*, 33: 503-504.
- Tiwari, D.K., T. Jin and J. Behari, 2011. Dose-dependent in-vivo toxicity assessment of silver nanoparticle in Wistar rats. *Epub.*, 21(1): 13-24.
- Tripathy, M., A. Chrsse, N. Karam C. Prathna and M. Amitaya, 2010. *Nanopart. Res.*, 12: 237-246.