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Mercury Selenide Nanoparticles Induced Immunological Alterations in Male Wistar Rat

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Abstract -Mercury is one of the most detrimental pollutants throughout the globe and its presence in ecosystem is both natural and anthropogenic. Its toxicity is well evident for public health disaster in Minamata Bay, Japan (1950) and in Iraq (1972). The aim of the present study was to determine the immunotoxic responses in wistar rats under stress of Mercury Selenide Nanoparticles (HgSe NPs) which were synthesized by sonochemical method based on the reaction between $HgCl_2$, $SeCl_4$ and Hydrazine hydrate ($N_2H_4H_2O$) in water , in presence of Triethanolamine (TEA), the capping agent and characterized by XRD (X-ray Diffraction), TEM (Transmission Electron Microscopy), SEM (Scanning Electron Microscopy) and SAED (Selective Area Electron Diffraction) patterns because these patterns determined the phase, purity, size and morphology of the NPs. The ratio of Hg to Se was 57:43, indicating that the particles are rich in mercury and the size of NPs were estimated by Debye-Scherrer equation. The immunotoxic responses of HgSe NPs were assessed in four sets of rats, which include one acute (1d) and three for sub-acute (5, 10 and 15ds), sets comprised of six rats each. The controls were run simultaneously. The effect of oral administration of HgSe NPs at different dose i.e. 800 ppm for acute and 160, 80, and 53 ppm for sub-acute (5, 10 and 15ds), respectively were studied on immunological parameters which include Lymphocytes, Monocytes, Neutrophils, Platelets, IgG and T-Cell Markers (CD3, CD4, CD8 and CD4/CD8 ratio) and which revealed significant enhancement in Lymphocytes, Neutrophils, CD3 and T Cell-Markers besides exhibited significant reduction in IgG, Monocytes, Platelets, CD4 and CD4/CD8 ratio on account of HgSe NPs after acute and sub-acute intoxication. Alterations in above immunological parameters depend upon the physicochemical properties and mechanism of action HgSe NPs which include ROS, free radical generation and oxidative stress.

Keywords: Mercury, HgSe NPs, Sonochemical method, Immunological parameters, oxidative stress.

1. Introduction

Mercury is a heavy metal of known toxicity, noted for inducing public health disaster in Minamata Bay, Japan (1950) (EHD, 2002) and in Iraq (1972) (Clarkson, 1981). Mercury, ranked third (Arsenic – Ist and lead – IInd) in a list of hazardous substances by the ATSDR (2001). Its presence in ecosystem is both natural and anthropogenic. The major natural sources of mercury are degassing from the earth's crust, emissions from volcanoes and evaporation from water bodies, whereas the anthropogenic include metal smelting, coal burning, mining and industrialization (Boylan *et al.,* 2003). Approximately, 10,000 tons mercury originates from degassing of earth's crust and 20,000 tons/year is added by anthropogenic activities (Hansen and Dasher, 1997). It is further estimated that the mercury emissions will keep on increasing at a rate of 5% a year (Zhang *et al.,* 2002). Most of the mercury production is limited to Spain, Kyrgyzstan, China and Tajikistan (Hylander and Meili, 2003) where, a variety of adverse health effects including neurological, renal, respiratory, immune, dermatologic, reproductive and developmental sequelle have been in vogue (Risher-John and Amler-Sherlita, 2005). Due to wide use of mercury in agriculture, industrial, medical and other fields, its exposure could not be avoided and thus has been considered as an occupational hazard for dental staff (Rowland and Baird, 1994), chloralkali factory workers and gold miners (Grandjean *et al.,* 1999). Exposure to mercury promotes the reactive oxygen species (ROS)

formation such as hydrogen peroxides, these ROS enhances the subsequent iron and copper induced production of lipid peroxides and highly reactive hydroxyl radicals (Hussain *et al.,* 1999). Detrimental effects caused by free radicals occur when there is an imbalance between free radical production and radical scavenging capacity of antioxidant system in favour of former (Garg *et al*., 2005).

In the present investigation, the nano-size particles of mercury selenide (HgSe) synthesized by sonochemical method based on the reaction between $HgCl_2$, $SeCl_4$ and Hydrazine hydrate ($N_2H_4H_2O$) in water , in presence of Triethanolamine (TEA) (Zare *et al.,* 2012) was considered for experimentation. Nanoparticles can modify the physico-chemical properties of the material as well as create the opportunity for increased uptake and interaction with biological tissues through inhalation, ingestion, and injection. This combination of effects can generate adverse biological effects in living cells. Nanoparticles are able to cross biological membranes and access cells, tissues, and organs. Nanoparticles may or may not exhibit size-related properties that differ significantly from those observed in fine particles or bulk materials. Size is therefore a key factor in determining the potential toxicity of a particle. Besides, other properties of nanoparticles that influence toxicity include: chemical composition, shape, surface structure, surface charge, aggregation, solubility and the presence or absence of functional groups of other chemicals (Shinde *et al.,* 2012).

Contrary to mercury, Selenium (Se) is an essential nutrient to human body and it plays an important role in maintaining human immunity system and reducing the risk of cancer (Wallace *et al.,* 2009). Selenium has been shown to provide protection against the toxic effects of Hg, As and possibly Pb (Jonnalgadda *et al.*,1993). This antagonism of Se and Hg was first reported by Pelletier (1985). Selenium is an antioxidant that helps protect the body by scavenging free radicals that harm cells. It helps activate prostaglandins that are important in regulating blood pressure and dealing with inflammation, and is vital in the production of the enzyme, glutathione peroxidase, which helps in detoxifying the body.

Considering all these facts present study is designed to find out toxic effects of HgSe NPs on immunotoxic responses in wistar rats.

2. Materials and Methods

2. 1. Experimental Animal

Adult healthy male rats selected for experimentation were procured from inbred colony of animal house of Zoology Department, Dr. B. R. Ambedkar University, Agra. The mean weight of rats for experimentation was 110±10gm which were maintained in polypropylene cages at the temperature 27±1^{o}C and a photoperiod of 12 hours/day. The rats were fed with standard pellet diet and provided water *ad libitum.*

2. 2. Experimental Chemical

Mercury Selenide nanoparticles (HgSe NPS) were synthesized by sonochemical method (Suslick, 1990) based on the reaction between $HgCl_2$, $SeCl_4$ and hydrazine hydrate ($N_2H_4H_2O$) in water, in presence of triethanolamine (TEA), capping agent. Sonochemical method provided an ideal atmosphere for the preparation nanoparticles.

2. 2. 1. Synthesis Of Hgse Nanoparticles

Firstly, 0.1 aqueous solutions of $HgCl_2$ and $SeCl_4$ were prepared separately. 0.5 ml of TEA was then added into the $HgCl_2$ solution and mixed with $SeCl_4$ solution. 2 ml of hydrazine hydrate was then added drop-wise, at a rate of one drop/second, and black precipitate was obtained. The solution was then irradiated with ultrasonic for 50 minutes under air atmosphere. The temperature was controlled at 25^{0}C using a thermometer in solution. The precipitates were centrifuged, washed with deionized water and ethanol in sequence for 4-5 times after cooling to temperature. The material was then dried at 70^{0}C in an oven.

2. 2. 2. Characterization Of Hgse Nanoparticles

HgSe, a semimetal, characterized by high electron mobility, large electron concentration, and a variation of band gap with temperature (Ren *et al.,* 1994) possesses a unique combination of

properties; which made it as a candidate material for detailed investigations of solid state phenomena (Hankare *et al.*, 2001) whereas electrical properties lead to the wide applications in optoelectronic technology including photoconductive photovoltaic, IR detector, IR emitter, tunable lasers and thermoelectric coolers (Singh and Mishra, 1999). HgSe nanostructures were characterized by XRD (X-Ray Diffraction) fig.1, TEM (Transmission Electron Microscopy) fig.2, SEM (Scanning Electron Microscopy), XPS (X-ray Photoelectron Spectroscopy) and SAED (Selective Area Electron diffraction) patterns fig.3 (Zare *et al.*,2012), because these patterns determined the phase, purity, size and morphology of the products. The ratio of Hg to Se was 57:43, indicating that the particles are rich in mercury and the size of particles were estimated by Debye-Scherrer equation, i.e.

$$D = K\lambda/\beta\cos\theta \quad (1)$$

Where,
K = Scherrer constant (accounted for the shape of the particle and its value is 0.9)
λ = Wavelength of light used for the diffraction
β = "Full width at half maximum" of the sharp peaks
θ = angle measured

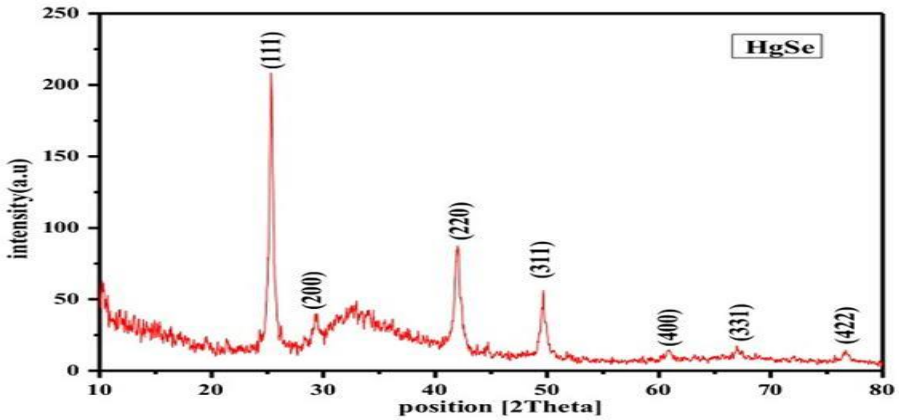


Fig. 1. XRD pattern of HgSe NPs

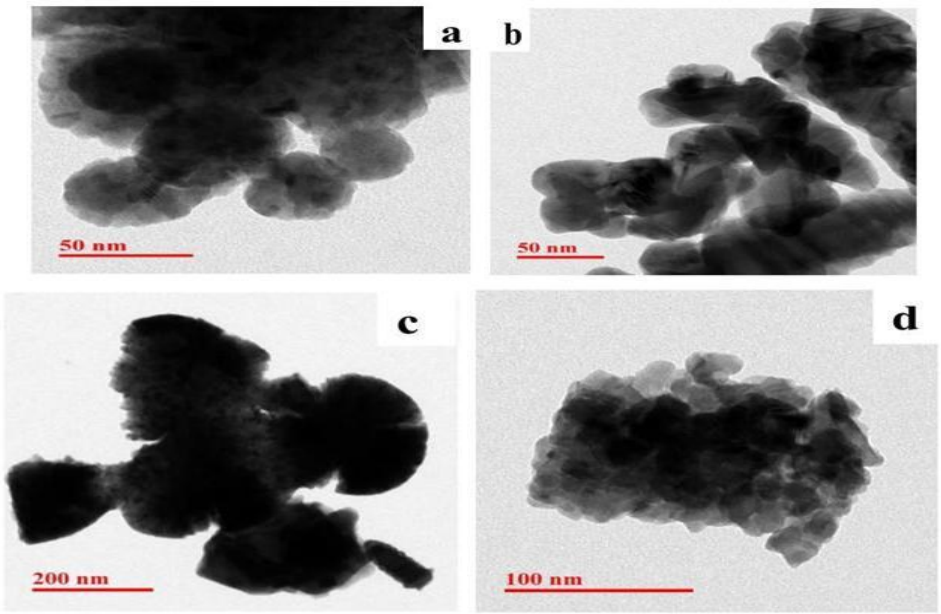


Fig. 2. TEM picture of HgSe prepared in the presence of 0.5ml of TEA and 2ml of hydrazine for 50 min. depicting size and morphologies of the particles

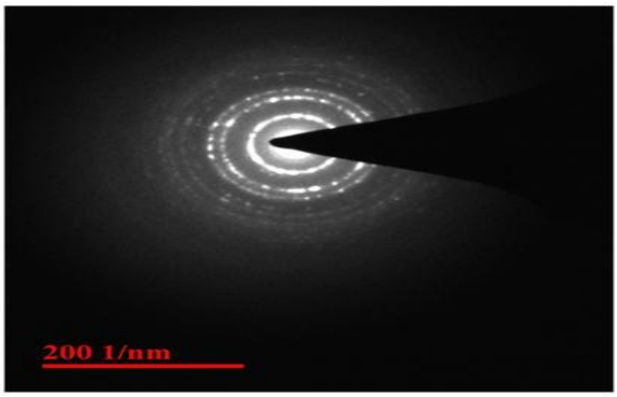
- 

Fig. 3. Selective area electron diffraction (SAED) pattern of HgSe

2. 3. Experimental Protocol

All the wistar rats were randomly divided into four sets, one for acute (1 day) and three for sub-acute (5, 10 and 15 days) comprised of six rats each. Each set were further divided into two groups, one group served as control group given distilled water only and other served as treated group given mercury nanoparticles orally per Os *vide infra.*

2. 4. Parameters Tested

The immunological parameters *viz.* Lymphocytes, monocytes, neutrophils and platelets were analysed by Autohematology analyzer (BC-2800 VET Mindray), IgG by Immunoturbidimetry method (Melik and Fogler, 1982) whereas, T-cell markers (CD3, CD4, CD8 and CD4/CD8 ratio) were analysed by Flow cytometry method (Mitnacht *et al.*, 1998).

2. 5. Statistical Analysis

Data have been expressed as mean and standard error. Student "t" test was used to find out significance at the levels $P < 0.05$, $P < 0.01$ and $P < 0.001$(Fisher and Yates, 1950).

3. Results

Lymphocytes percentage was significantly ($P<0.05$) increased in acute and sub-acute (5ds) sets, while it was non-significantly increased in subacute (10 and 15 ds) sets (Table 1).

Significant reduction ($P<0.01$) and ($P<0.05$) in monocytes percentage has been observed after acute and sub-acute (5ds) intoxication of HgSe NPs and non-significant reduction was found in sub-acute (10and 15 ds) sets (Table 1). HgSe NPs non-significantly ($P>0.05$) decreased Neutrophils percentage in acute set while significantly ($P<0.05$) decreased or increased in sub-acute (5 and 10ds) sets and non-significantly increased in sub-acute (15ds) set (Table 1). Platelets were significantly ($P<0.001$) decreased after acute and sub-acute (5 and 10ds) intoxication and significantly increased after 15 days treatment (Table 1).T-Cell Marker CD3 percentage was significantly ($P< 0.05$) increased in acute & sub–acute (5ds) sets, while non-significantly increase after 10 and 15 ds intoxication. CD4 and CD8 percentage were significantly decreased after 1 and 5 days treatment while non significantly decreased after 10 and 15 days treatment duration, however CD4/CD8 ratio exhibited reduction in both sets (Table 1). IgG was significantly decreased ($P<0.01$) ($P<0.05$) in acute and sub-acute (5ds) sets, while non-significantly decreased after 10 and 15 days treatment duration (Table 1).

4. Discussion

In the present study enhancement in lymphocyte percentage due to inflammation under influence of HgSe NPs has been observed. *Silva et al.* (1999), also studied the production of white blood cells to fight against incoming nanoparticles affirms the present findings. Further, it can be anticipated that the nanoparticles bring about inflammation in lymph nodes, which inturn involve other cells to in inflammatory reactions. Perhaps due to these increased cell divisions transition in lymphocytes from S to G phase gets increased.

Table. 1. Effect of HgSe NPs on immunological parameters of wistar rats in different experimental sets.

Parameters	Acute		Sub-acute					
			5 – Days		10 – Days		15 – DAYS	
	Control	Treated	Control	Treated	Control	Treated	Control	Treated
Lymphocytes (%)	36.67 ±2.027	42.67 ± 1.763[a]	36 ± 2.516	49.67 ± 3.179[a]	35 ± 1.154	37 ± 1.732	36 ± 2.309	38.33 ± 2.027

Monocytes (%)	2.33 ±0.333	0.33 ± 0.333[b]	2.67 ± 0.333	1.33 ± 0.333[a]	2.67 ± 0.333	1.67 ± 0.333	3 ± 0	2.67 ± 0.333
Neutrophils (%)	56.67 ± 2.403	55 ± 1.732	55 ± 1.154	46 ± 3.464[a]	54.33 ± 1.855	62 ± 1.154[a]	54 ± 2.309	62 ± 1.154
Platelets x 10^3	606.33 ± 21.88	96.33 ± 0.88[c]	585.67 ± 3.480	219 ± 22.67[c]	604 ± 8.717	215 ± 23.21[c]	605 ± 5.77	700 ± 26.62[a]
CD3 (%)	74.33 ± 2.603	83.67 ± 2.027[a]	75 ± 1.732	81.67 ± 2.027[a]	74 ± 3.785	77.33 ± 4.055	75.67 ± 3.479	77 ± 2.645
CD4 (%)	61 ± 2.943	44.67 ± 3.318[a]	60.33 ±1.763	39.33 ± 2.333[a]	60 ± 4.564	51.67 ± 2.027	58 ± 1.154	54 ± 2.309
CD8 (%)	36 ± 2.309	48 ± 3.785[a]	34 ± 2.08	44. 67 ± 2.403[a]	33.33 ± 1.763	36.67 ± 2.027	32.7 ± 1.686	35.67 ± 2.027
CD4/CD8	1.69	0.93	1.77	0.88	1.80	1.39	1.77	1.51

a ($P < 0.05$), b ($P < 0.01$), c ($P < 0.001$)

Decrease in percentage of monocytes and neutrophils (representative of phagocytic cells) indicate apoptosis of both due to the oxidative stress induced by nanoparticles. These apoptotic reactions could result from mitochondrial perturbations and the release of proapoptotic factors (Nel *et al.,* 2006), as mitochondria are redox active organelles, there is every possibility of altering ROS production and thereby overloading or interfering with antioxidant defenses (Jain *et al.,* 2011), Several types of nanoparticles, target mitochondria directly (Xiao *et al.,* 2003; Oberdorster *et al*., 2005). Mercury increases apoptosis of monocytes and reduces its phagocytic ability (Crinnion, 2000) while, monocytes exhibit more vulnerability to the toxic effect of $HgCl_2$ than T and B lymphocytes and toxic events have been proposed to be associated with programmed cell death (Shenker *et al.,* 1992). Contrary to the enhancement of Lymphocytes (%), percentages of monocytes registered non-significant decline after 10 and 15 days treatment respectively along with enhancement in neutrophils percentage from day 10 to 15 due to dose concentration of HgSe nanoparticles within these days, because in these days, Selenium exert its effect on mercury by binding with it in the body and reduces the toxicity of mercuric chloride (Crinnion, 2000). Monocytes and Neutrophils function as phagocytes in the immune system. Phagocytosis plays a key role in non-specific and specific immune responses of the immune system and represents the first line of defence of the immune system against invading agents or xenobiotics (Van Oss, 1987).

The present study shows significant decrease in platelets after acute (1day) and sub-acute (5 and 10 days) treatments due apoptosis and disintegration of platelets induced by HgSe NPs. Further, a

relative increase in platelets was found after 15 days treatment due to antioxidative properties of Selenium, because in low concentration, Selenium counteracts the effect of mercury and exerts their effect in the body of animal. Platelets participate in the interaction between Xenobiotics and host-defence. They are part of the innate and adaptive immune system, and play a role in the initiation of inflammation by interacting with leucocytes.

Since the immune system is the interplay of T and B cells responses which have been supplicated by specific markers, the T-Cell markers (CD3, CD4 and CD8) which are used to identify and investigate the cell surface molecules which provide targets for immunophenotyping of cells. These markers play a central role in cell mediated immunity and can be distinguished from other lymphocytes, by the presence of T-cell receptor (TCR) on the cell surface. There are several types of markers namely CD3, CD4 and CD8 which are found on T-cells, with distinct functions.

An enhancement in the percentages of CD3 and CD8 markers after acute and subacute treatment of HgSe NPs indicates higher signal transduction by CD3 marker and increased concentration of xenobiotic substance as recognised by the marker CD8. These enhancement in markers are indicative of onset of T-cell functionality in defense which in the present study is HgSe NPs. Higher exposure to metallic mercury increased the number of CD8 cells (Moszczynski *et al.,* 1995). CD3, a T- Cell specific marker is a transmembrane protein found on T-lymphocytes that function in signal transduction, is necessary to differentiate T-cells from other populations. HgSe NPs, which act as antigen, stimulate the T-cell receptor (TCR). Probably TCR-CD3 complex interaction plays an important role in mediating cell recognition events. The CD3 count measures all T- Cells, which is essentially for CD4 and CD8 cells. The CD8 markers detect and try to ward-off negative cell activities which in the present study are these NPS.

The reduction in percentage of CD4 marker and in CD4/CD8 ratio after acute and subacute treatment is an outcome of HgSe NPs. CD4 makers; perhaps act as an alarming bell of the immune system against invasion of the nanoparticle induced changes. CD4 markers are commonly known as T-helper cells. Decrease in CD4 (%) is the result of opportunistic attack. When CD4 (%) is low, the majority of CD3 cells get generated by CD8 cells. Thus, low ratio of CD4/CD8 (<1) in the present investigation indicates the imbalanced immune system. CD4/CD8 ratio reflects healthy immune system if the values are above 1 up to 6.

IgG, the most important and sophisticated immunoglobulin, is first immunoglobulin that gets affected if any antigen (xenobiotic substance) enters the body (Roitt, 1996).

Present study shows reduction in IgG level after acute and sub-acute treatment of HgSe nanoparticles compared to control can be correlated with the T-helper Cell maker (CD4) because IgG requires ‘help’ from T-helper cell. Thus, impaired T-Cell functions seem to be a cause of reduced IgG level (Vandebriel *et al.,* 2014). Reduction in IgG level suggests suppression of the functional immune system, leading to reduced resistance to xenobiotic substance.

The possible mechanism regarding reduction in IgG is that Mercury Selenide nanoparticles decrease the proliferation capacity of T Cells and T-Cell dependent antibody responses which affect B-Cells, are functionally compromised by the reduced proliferative responses to orally administration of Hg nanoparticles resulting in decreased production of serum antibodies (IgG).

5. Conclusion

Alterations in aforesaid immunological parameters have been on the basis of dose concentration of HgSe NPs, NPs mechanism and their physicochemical properties. During the high dose concentration, nano size HgSe particles enhance the generation of free radicals, cause oxidative stress leading to the destruction of cell membranes and cell organelles through apoptosis or programmed cell death. But as their dose concentration is low and treatment duration is increased then the effect of HgSe NPs is decreased due to antagonistic behaviour of mercury and selenium in the body because in longer treatment duration, selenium binds mercury and counteracts its toxic effect.

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