Synthesis, Characterization and Biocompatibility of Holmium Zirconate/Zinc Sulphide Nanocomposite in Albino Mice in a Gender Specific Manner

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Summary: Aim of this study was to report the biocompatibility of Holmium zirconate, zinc sulphide and holmium zirconate/zinc sulphide ($HO_2Zr_2O_7$/ZnS) nanocomposite in albino mice. Holmium zirconate, zinc sulphide and holmium zirconate/zinc sulphide ($Ho_2Zr_2O_7$/ZnS) nanocomposite were synthesized by, normal microemulsion, chemical co-precipitation and two step synthesis method, respectively. The synthesized materials were characterized by X-ray diffraction (XRD) for the confirmation of phase while scanning electron microscopy (SEM) was used morphological analysis. The composition and the particle size distribution were confirmed by energy dispersive spectroscopy and particle size analysis respectively. Seven week old mice were divided into two groups in a gender specific manner: control group that were intraperitoneally injected with saline solution and treated group were administered with 50 mg/ml solvent/Kg body weight of Holmium zirconate/zinc sulphide nanocomposite for 22 days. A series of neurological tests, blood cell count, selected serum parameters and biomarkers of oxidative stress were analysed in vital organs of both treatments. It was observed that nanocomposite treated female mice remained mobile ($P = 0.05$) for longer time while both male ($P = 0.03$) and female ($P = 0.02$) mice had more rotations than saline treated mice during open field test. Nanocomposite treated male had reduced stretch attend reflex during light dark box test. Blood and serum parameters remained unaffected ($P > 0.05$) when compared between nanocomposite treated and untreated mice of both genders. Malondialdehyde concentration was significantly elevated ($P = 0.04$) in liver of male while superoxide dismutase concentrations were significantly reduced ($P = 0.05$) in brain of female albino mice treated with nanocomposite than their respective control groups.

Keywords: Nanocomposite; Holmium zirconate; Albino mice; Behavior; Hematology; Biomarkers of oxidative stress.

Introduction

The usage of nano-materials as composites have gained importance in science and technology owing to their superior properties like mechanical, thermal, physical, chemical, electrical conductivity, optical, photoluminescence and other novel attributes [1]. Recently, nano-composites have been introduced, due to their structural characteristics like machinery parts, coatings in scratch-resistant and flame-retardant cables [2]. In the periodic table group IIIB, there are seventeen elements that are known as rare earth elements (REEs) and they have numerous industrial and medicinal applications [3]. This extensive use of REEs has led to their accumulation in the environment and has led to REEs enrichment in human and animal bodies through the food chain [4]. It has been established that REEs concentrations beyond safe limits can cause acute and chronic toxicity in living systems and can damage the nervous and reproductive systems of animals [5]. In recent years, the most important materials for research are the nanocomposites formed by the doping of zinc sulphide (ZnS) nanoparticles with transition metal ions like Manganese, Chromium, silver and rare earth metals like lanthanum and zirconium. [6,7]. These nanocomposite are used as thermal barrier coatings (TBCs) as they have low thermal conductivity with higher melting points and higher thermal expansion coefficients [8, 9]. Holmium Zirconate/Zinc Sulphide ($HO_2Zr_2O_7$/ZnS) used in present study is a nanocomposite that is used as used as semiconductor, oxidation catalysts, for immobilization of radioactive waste, as fuel cells ion conductors and as high permittivity dielectrics [10, 11]. It also commonly used in telecommunication, data recording and microwave devices [12]. Despite of their extensive utilization, very little information is available in literature about their biocompatibility. Aim of present study was to report the effect of intraperitoneal injection of Holmium Zirconate/Zinc Sulphide nanocomposite on behavior, blood chemistry and biomarkers of oxidative stress from liver and brain of albino mice in a gender specific manner under sub chronic experimental conditions.

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Experimental

Synthesis of Holmium Zirconate nanoparticles

Holmium zirconate was prepared by normal microemulsion method. Briefly, stoichiometric proportions of Holmium nitrate Ho $(NO_3)_3$ and Zirconyl chloride ($ZrOCl_2$) were dissolved and mixed in deionized water. The aqueous solution of Cetyltrimethl ammonium bromide (CTAB) with the molar ratio between metal and surfactant as 1:1.5 was added in to the mixture and stirred. This solution was heated up to 343K and 2M Ammonium hydroxide Solution (NH_4OH) was added slowly as precipitating agent with constant magnetic stirring. The ammonia solution was added until the pH of the solution reached to 11-12. The precipitates of $Ho_2Zr_2O_7$ were obtained and the sample was stirred for further three hours in order to control the crystallite size and the homogeneity of the samples. The $Ho_2Zr_2O_7$ precipitates were dried at 373K in an oven and finally annealed at temperature of 1123K for 8 hours to obtain the required phase.

Synthesis of zinc sulphide

The zinc sulphide was synthesized by the simple and economic co-precipitation method. The required amount of zinc nitrate ($Zn(NO_3)_2$) was dissolved in deionized water to prepare 0.1M solution. This solution was stirred on hotplate and added 0.1M sodium sulphide solution. The precipitate was formed. These precipitates were washed several time with deionized water and finally with ethanol. These were dried in vacuum oven at 343K. The powdered sample was finally stored in desiccator for further characterization.

Synthesis of $Ho_2Zr_2O_7$/ZnS nanocomposite

The $Ho_2Zr_2O_7$/ZnS nanocomposite was synthesized by two step method. The 0.1M solution of zinc nitrate was prepared in deionized water and the weighed amount of prepared holmium zirconate nanoparticles were also added in the zinc nitrate solution. The mixture was stirred vigorously and 0.1M solution of sodium sulphide was added drop wise in this mixture. The mixture was washed with deionized water and centrifuged. The powder obtained was dried in a vacuum oven at 343K and stored in desiccator for further characterization.

Characterization of $Ho_2Zr_2O_7$ and ZnS and their nanocomposite

The crystalline nature, phase and the purity of the synthesized materials ($Ho_2Zr_2O_{7and}$ ZnS and their nanocomposite) was confirmed by the X-ray diffraction (XRD) analysis. For this purpose, X-ray diffractometer (Bruker D-8) was used. Hitachi S4800 scanning electron microscope (SEM) was used to investigate the surface morphology of the synthesized materials. The elemental composition of the synthesize materials was carried out by the energy dispersive X-ray (EDX) analysis. The average crystallite size was estimated by using the Scherer's formula.

Experimental Animals and Design

Albino mice (seven week old) were used as experimental animals and were maintained at the animal house at Bio Park of Bahauddin Zakariya University, Multan. Standard food and water was provided *ad libitum*. Conditions for maintaining the subjects were same as described elsewhere [13].

Mice were divided into two groups. First group intraperitoneally received 50 mg/Kg body weight of Holmium Zirconate Zinc Sulphide nanocomposite for 22 days. While control group intraperitoneally received saline (0.9 % NaCl) solution for same duration. Both groups consisted up of 14 mice with equal distribution of males and females. Rota rod, open field, light and dark and Morris water maize tests were carried out after 14 days of dose supplementation in both experimental treatments to access the neurological performance.

Body Weight Analysis

During the whole experimental duration, body weight was recorded on daily basis to demonstrate the effect of Holmium Zirconate/Zinc Sulphide nanocomposite treatment on body weight of albino mouse.

Rota Rod Test

Rota rod test was performed to report neuro muscular coordination as previously reported by Iqbal *et al*. [13].

Light and Dark Transition Test

The light/dark box test was performed to analyse the exploratory and locomotory behavior of nanocomposite treated and untreated animals following Zahra *et al*. [14].

Open Field Test

Open filed test was conducted to report exploratory and anxiety related behaviours in mice following Zahra *et al*. [14].

Morris Water Maze (MWM)

Morris water maze test was conducted to report the effect of nanocomposite on spatial learning of mice following Iqbal *et al.* [13].

Blood and serum collection

At the end of dose supplementation experiment, mice were anaesthetized with isoflurane and blood was sampled from retro-orbital sinus and/or through direct cardiac puncture and one part was used for complete blood counting by using hematology analyzer SYSMEX, 21 (Japan). While the second part was centrifuged at 13000 RPM for ten minutes and the extracted serum was used for the estimation of creatinine, cholesterol, low density lipoprotein, high density lipoprotein and triglycerides by using diagnostic kits.

Determination of biomarkers of oxidative stress from liver and brain

Liver and brain were surgically removed, following animal sacrifice under anaesthesia, rinsed in saline solution and stored at -20°C until concentrations of superoxide dismutase [15], lipid peroxidation [16] and Catalase activity [17] was determined in both organs from all treated groups.

Statistical Analysis

Statistical package Minitab (version 16, USA) was used for data analysis. All data were expressed as Mean ± Standard error of mean. Two sample student's t-test was applied to compare all studied parameters of complete blood count, serum and biomarkers of oxidative stress between Holmium Zirconate/Zinc Sulphide nanocomposite treated and untreated albino mice of both genders. Significance level was set at $P < 0.05$.

Result and Discussion

Structural analysis of Holmium Zirconate/Zinc Sulphide nanocomposite

XRD pattern for the $Ho_2Zr_2O_7$/ZnS composite is shown in Fig. 1A. The peaks assigned with symbol * are related to the holmium zirconate while that of represented by # are for ZnS. These peaks are well matched with the standard patterns ICSD 00-022-0332 and ICSD 00-001-0792 for holmium zirconate and zinc sulphide, respectively. Both the materials are crystallized into cubic phase. The presence of peaks for both the materials indicates that the composite has been successfully formed.

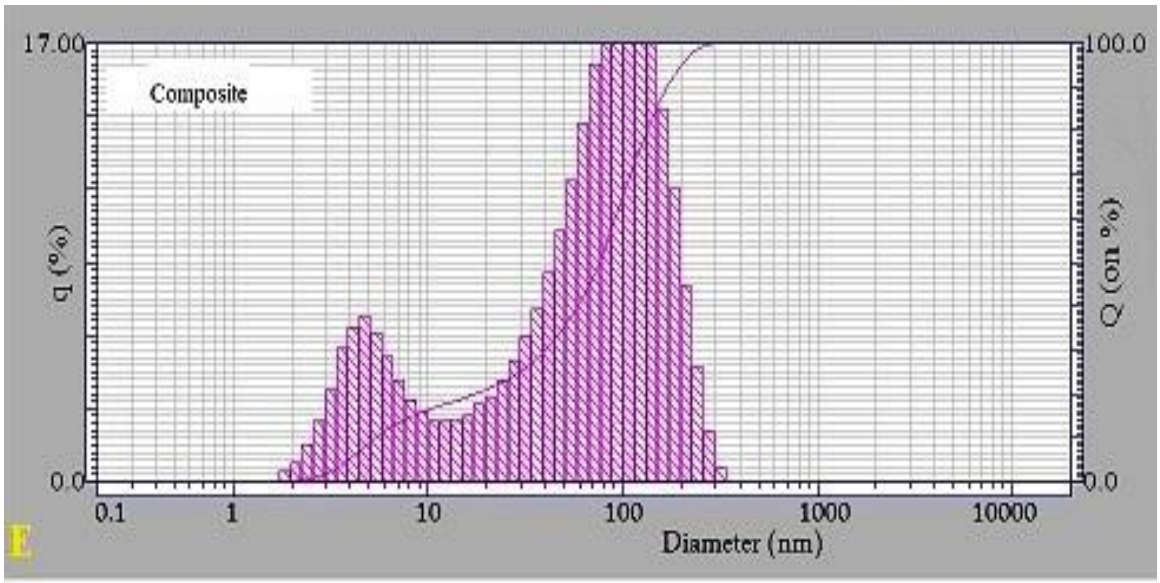
Morphological and Compositional analysis of Ho2Zr2O7/ZnS nanocomposite

The surface morphology and the particle size of the synthesized materials i.e. ZnS and its composite with holmium zirconate was determined by using scanning electron microscopy (SEM). The SEM images for both the materials are shown in Fig 1B and C. It is clear from the Fig 1B that the zinc sulphide has the sheet like morphology with clear boundaries and the distribution of these sheets is homogenous. The size of these sheet ranges from 50 to 150nm. The small round shape particles of holmium zirconate are decorated on the surface of ZnS sheets as shown in Fig 1C.

The composition of the composite was confirmed by energy dispersive spectroscopic (EDS) analysis. The EDS spectra for the $Ho_2Zr_2O_7$/ZnS nanocomposite are shown in Fig 1D. All the peaks in the spectrum are related to the elements present in the material. There is no peak for any other element which confirms that the composite is pure. The percentage for each element i.e. weight and atomic percent is given in supplementary Table-1. The particle size distribution was confirmed by particle size analyzer and is shown in Fig 1E. The particle size distribution was found to lie in the range of 5-150nm, 10-100nm and 5-200nm, for ZnS, $Ho_2Zr_2O_7$ and their composite, respectively. The particle size distribution is in agreement with that SEM analysis.

Body Weight Analysis

Analysis of the data indicated that change in body weight varied non significantly ($P > 0.05$) when compared between Holmium Zirconate/Zinc Sulphide nanocomposite and saline treated albino mice of both genders at all the studied time points (Fig. 2).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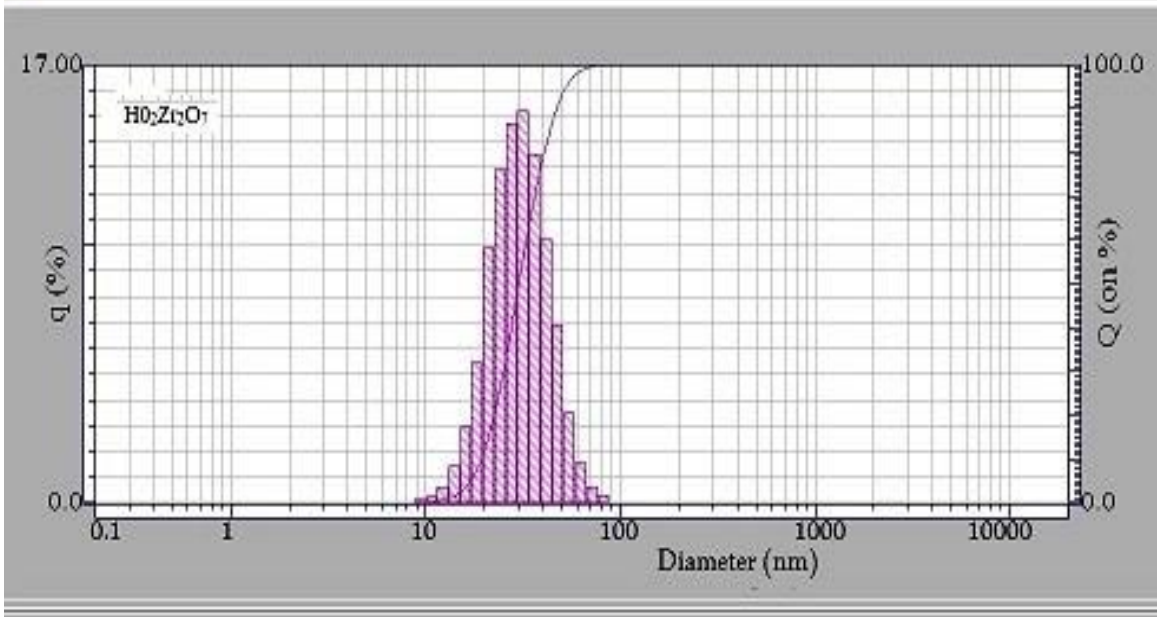
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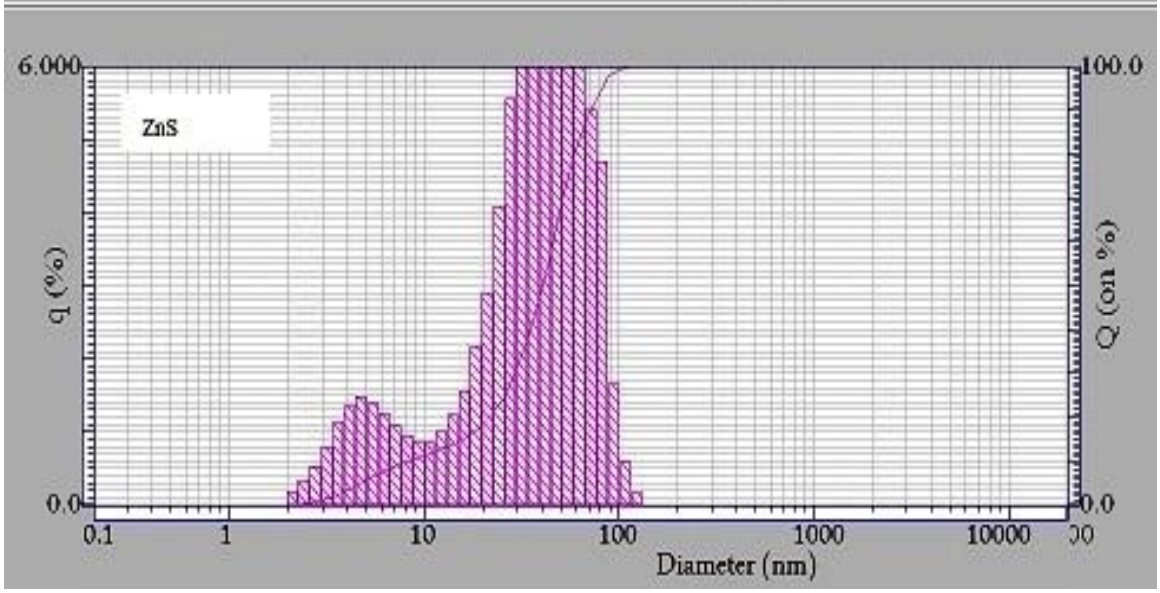
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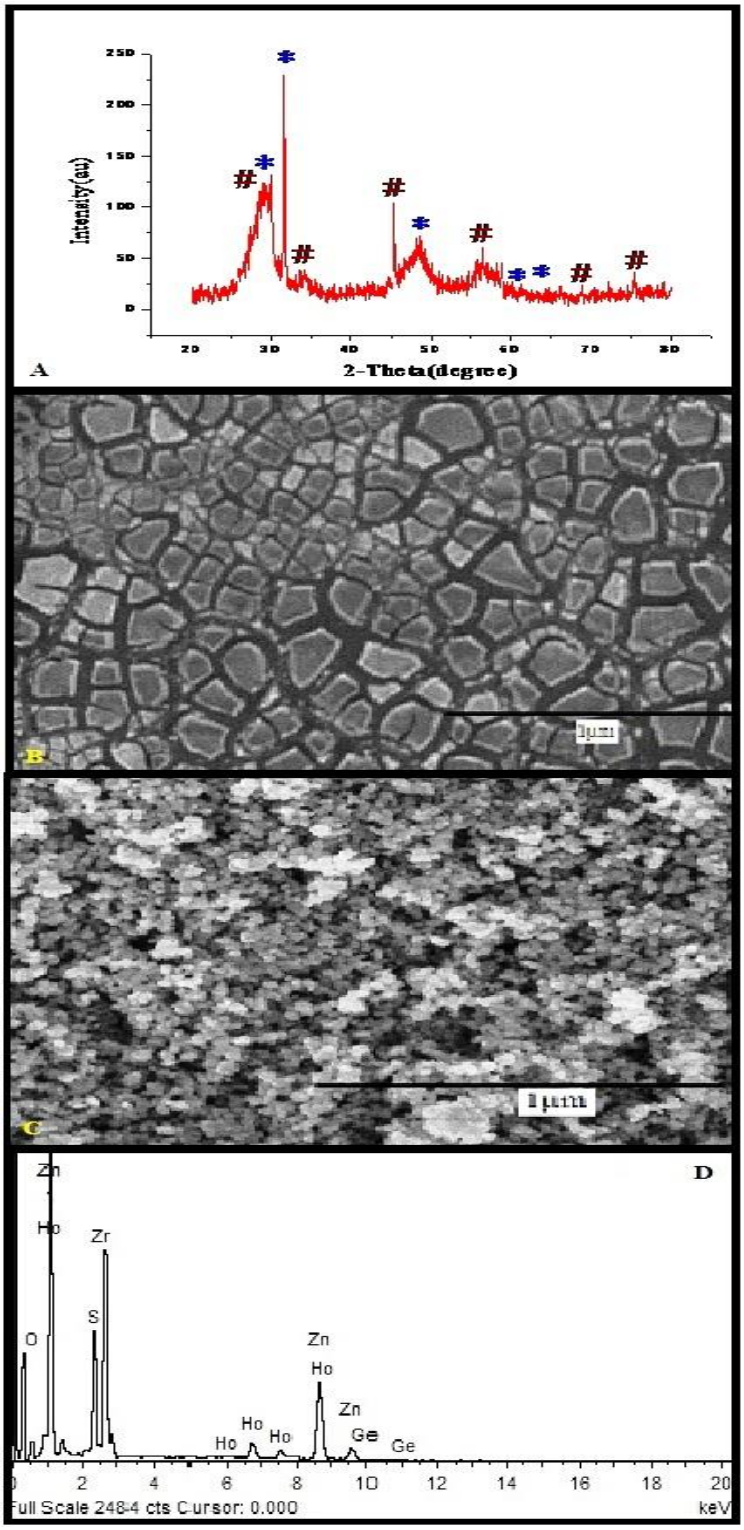

Fig. 1: (A) X ray diffraction (XRD) pattern for $Ho_2Zr_2O_7$/ZnS nanocomposite. Scanning electron micrograph (SEM) images of (B) ZnS and (C) $Ho_2Zr_2O_7$/ZnS nanocomposite. (D) Energy-dispersive X-ray spectroscopy (EDS) spectrum for $Ho_2Zr_2O_7$/ZnS nanocomposite. (E) Particle size distribution of ZnS, $Ho_2Zr_2O_7$ and $Ho_2Zr_2O_7$/ZnS nanocomposite.

Table-1: Comparison of various studied parameters of light and dark box test between Holmium Zirconate Zinc Sulphide nanocomposite nanoparticles (50 mg/ ml of solvent/ Kg body weight) and saline treated albino mice. N = 7 for each treatment. All values are expressed as mean ± standard error of Mean. P-value represents the results of 2 sample t-test calculated for each studied parameter.
P > 0.05 = Non -significant; P ≤ 0.05 = least significant (*)

Parameters	Saline treated control female mice	Holmium Zirconate Zinc Sulphide treated female mice	P-value	Saline treated control male mice	Holmium Zirconate Zinc Sulphide treated male mice	P-value
Transition frequency	13.29 ± 2.5	20.43 ± 2.5	0.06	19.1 ± 4.4	12.3 ± 3.2	0.2
Rearing frequency	9.86 ± 2.2	6.14 ± 1.2	0.2	5.1 ± 0.6	3.7 ± 1.2	0.3
Stretch attend frequency	43.3 ± 7.1	41 ± 4.3	0.8	33.0 ± 2.2	29.0 ± 4.8	0.05*
Time in dark (sec)	199.3 ± 19	200 ± 11	1	193.0 ± 21.2	199.7 ± 33.8	0.9
Time in light (sec)	100.7 ± 19	100 ± 11	1	109.8 ± 22.6	100.3 ± 33.7	0.8

Rota Rod Test

Analysis of data revealed that rota rod test performance varied non- significantly (P > 0.05) when compared between albino mice treated with Holmium Zirconate/Zinc Sulphide nanocomposite and their saline treated control group (Fig. 3).

Light and dark box test

Analysis of data revealed that all the studied parameters of light and dark box test varied non-significantly (P > 0.05) when compared between Holmium Zirconate/Zinc Sulphide nanocomposite and untreated albino mice (Table-1).

Open field

Analysis of the results indicated that Holmium Zirconate/Zinc Sulphide nanocomposite treated female mice remained mobile (P = 0.05) for longer time while both male (P = 0.03) and female (P = 0.02) mice had more rotations than saline treated mice. All other studied parameters varied non-significantly (P > 0.05) when compared between two treatments for both genders (Table-2).

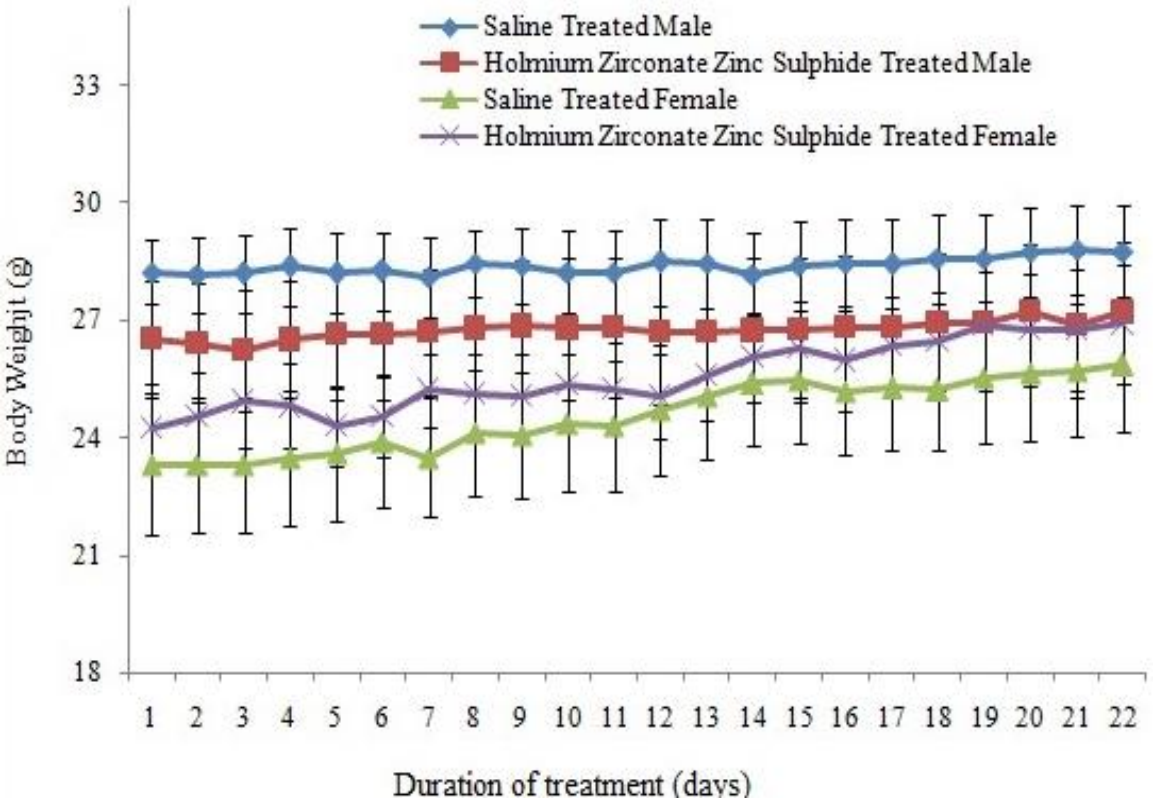

Fig. 2: Comparison of body weight between Holmium Zirconate Zinc Sulphide nanocomposite (50 mg/ ml of solvent/ Kg body weight) and saline treated albino mice for 22 consecutive days. N = 7 for each treatment. All values are expressed as mean ± standard error of Mean. P > 0.05 = non significant at all studied time points.

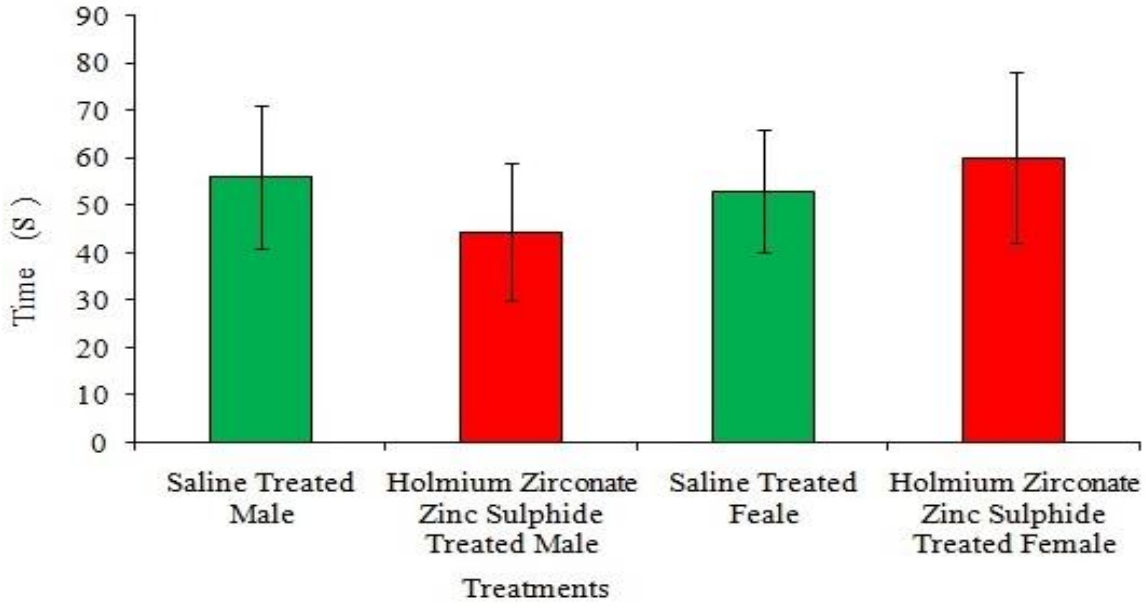

Fig. 3: Comparison of rota rod test performance between Holmium Zirconate Zinc Sulphide nanocomposite nanoparticles (50 mg/ ml of solvent/ Kg body weight) and saline treated albino mice. N = 7 for each treatment. All values are expressed as mean ± standard error of Mean. P-value represents the results of 2 sample t-test calculated for studied parameter.

Table-2: Comparison of various studied parameters of open field between Holmium Zirconate Zinc Sulphide nanocomposite nanoparticles (50 mg/ ml of solvent/ Kg body weight) and saline treated albino mice. N = 7 for each treatment. All values are expressed as mean ± standard error of Mean. P-value represents the results of 2 sample t-test calculated for each studied parameter.

Parameters	Saline treated control female mice	Holmium Zirconate Zinc Sulphide treated female mice	P-value	Saline treated control male mice	Holmium Zirconate Zinc Sulphide treated male mice	P-value
Distance (m)	**19.6 ± 2.5**	**25.5 ± 2**	**0.09**	**20.4 ± 2.3**	**17.1 ± 1.6**	**0.3**
Mean Speed (m/s)	**0.03 ± 0.004**	**0.04 ± 0.003**	**0.09**	**0.03 ± 0.004**	**0.03 ± 0.003**	**0.2**
Time mobile (sec)	**466.9 ± 24**	**525 ± 10**	**0.05***	**468.4 ± 28.0**	**456.1 ± 20.0**	**0.7**
Time immobile (sec)	**131 ± 24**	**75 ± 10**	**0.05***	**131.6 ± 28.0**	**143.9 ± 20.0**	**0.7**
Mobile episodes	**26.6 ± 2.2**	**20.7 ± 2.1**	**0.07**	**26.7 ± 4.3**	**29.0 ± 3.5**	**0.7**
Immobile episodes	**26 ± 2.3**	**20 ± 2.2**	**0.08**	**25.7 ± 4.3**	**28.1 ± 3.5**	**0.7**
Rotations	**24.4 ±1.7**	**30.6 ±1.7**	**0.02***	**22.1 ± 4.0**	**26.1 ± 2.3**	**0.4**
Clockwise rotations	**10.71 ± 1.1**	**15.43 ± 2.4**	**0.1**	**13.9 ± 2.7**	**13.1 ± 2.5**	**0.9**
Anti-clockwise rotation	**13.71±1.9**	**15.14±2.4**	**0.7**	**8.3 ±1.4**	**13.0 ± 1.3**	**0.03***

P > 0.05 = Non -significant; P ≤ 0.05 = least significant (*)

Morris Water Maze test

Analysis of data revealed that all the studied parameters varied non-significantly (P > 0.05) when compared between Holmium Zirconate/Zinc Sulphide nanocomposite and saline treated albino mice during all the four training days (Data not shown here).

Analysis of results indicated that all the studied parameters of probe trial varied non-significantly (P > 0.05) when compared between albino mice treated with Holmium Zirconate/Zinc Sulphide nanocomposite and their control group (Data not shown here).

Complete Blood Count Analysis

Upon comparison of complete blood count parameters of albino mice treated with Holmium Zirconate/Zinc Sulphide nanocomposite with their saline treated control, it was observed that all studied parameters varied non significantly (P > 0.05) between the two experimental treatments for both genders (Table-3).

Table-3: Comparison of studied complete blood count parameters between Holmium Zirconate Zinc Sulphide nanocomposite (50 mg/ml of solvent/Kg body weight) and saline treated albino mice of both gender. N = 7 for each treatment. All values are expressed as mean ± standard error of mean. P value indicates the results of 2-sample t-tests calculated for each parameter.

Studied parameters	Saline treated control male mice	Holmium Zirconate Zinc Sulphide treated male mice	P value	Saline treated control female mice	Holmium Zirconate Zinc Sulphide treated female mice	P value
White blood cells $\times 10^3$ µl	4.5 ± 3.4	4.83 ± 2.6	0.8	4.21 ± 1.1	6.5 ± 1.4	0.6
Lymphocytes $\times 10^3$ µl	3.1 ± 1.8	3.2 ± 1.7	0.9	3.27 ± 0.76	5.3 ± 1.2	0.4
Monocytes $\times 10^3$ µl	0.2 ± 0.2	0.26 ± 0.2	0.6	0.18 ± 0.15	0.3 ± 0.07	0.8
Granulocytes	1.26 ± 1.6	1.37 ± 0.8	0.9	0.8 ± 0.31	0.9 ± 0.2	0.6
Lymphocytes (%)	73.7 ± 11.9	67.0 ±11.6	0.3	77.8 ±5.89	80.3 ± 2.2	0.4
Monocytes (%)	4.71 ± 2.4	4.63 ± 2.6	1	4.06 ± 1.89	5.06 ± 0.8	0.5
Granulocytes (%)	21.6 ± 11.2	28.3 ±11.1	0.3	18.2 ± 6.34	14.9 ± 1.8	0.5
Red blood cells $\times 10^3$ µl	5.07 ± 1.4	5.52 ± 1.8	0.6	6.58 ± 1.82	7.4 ± 0.34	0.1
Hemoglobin (g/dl)	9.23 ± 2.4	9.99 ± 3	0.6	12.2 ± 2.3	12.1 ± 0.46	0.8
hematocrit $\times 10^3$ µl	20.1 ± 4.7	22.3 ± 7.7	0.5	30.6 ± 9.2	33.4 ± 1.7	0.9
Mean corpuscular volume (µm³)	39.9 ± 1.9	40.5 ± 2.9	0.7	46.4 ± 2.43	45.2 ± 0.9	0.9
Mean cell hemoglobin (pg)	18.3 ± 1.06	18.4 ± 1.6	0.9	19.34 ± 3.32	16.5 ± 0.3	0.7
Mean cell hemoglobin concentration(g/dl)	45.9 ± 2.86	45.5 ± 3.7	0.9	41.9 ± 8.14	36.5 ± 0.9	0.7
Red cell distribution width (fL)	20.8 ± 2.4	19.2 ± 1.9	0.2	16.2 ± 2.24	18.2 ± 1	0.7
Red cell distribution width - SD (fL)	32.4 ± 4.6	29.8 ± 2	0.2	29.1 ± 2.7	30.2 ± 1.5	0.9
Platelets $\times 10^3$µl	532 ± 389	424 ± 167	0.5	259 ± 215	416 ± 36	0.1
Mean platelet volume (µm³)	9.04 ± 1.8	9.09 ± 1.6	1	7.31 ± 0.9	7.4 ± 0.4	0.8
Platelets (%)	0.45 ± 0.3	0.38 ± 0.2	0.6	0.2 ± 0.12	0.31 ± 0.04	0.9
Platelet distribution width (%)	23.7 ± 5.8	24.1 ± 7.8	0.9	22.5 ± 9.7	22.3 ± 1.1	0.6

P > 0.05 = Non significant

Table-4: Comparison of various studied serum parameters between Holmium Zirconate Zinc Sulphide nanocomposite (50 mg/ml of solvent/Kg body weight) and saline treated albino mice of both gender. N = 7 for each treatment. All values are expressed as mean ± standard error of mean. P value indicates the results of 2-sample t-tests calculated for each parameter.

Parameters	Saline treated control male mice	Holmium Zirconate Zinc Sulphide treated male mice	P value	Saline treated control female mice	Holmium Zirconate Zinc Sulphide treated female mice	P value
Triglyceride (mg/dL)	598 ± 537	794 ± 793	0.6	264 ± 177	249 ± 148	0.9
Cholesterol (mg/dL)	339 ± 165	324.4±55.4	0.8	450 ± 173	412 ± 158	0.7
High density lipoprotein (mg/dL)	191 ± 309	279 ± 524	0.7	80.3 ± 34.6	65.5 ± 31.6	0.4
low density lipoprotein (mg/dL)	264 ± 207	160.6 ± 58	0.4	317 ± 162	297 ± 139	0.8
Creatinine (mg/dL)	3.83 ± 5.7	1.78 ± 1.4	0.4	16.6 ± 16.4	6.53 ± 8.49	0.3

P > 0.05 = Non significant

Serum Biochemical Profile Analysis

When the studied serum parameters were compared between Holmium Zirconate/Zinc Sulphide nanocomposite treated and untreated mice, it was observed that all the parameters varied non significantly (P > 0.05) between the two treatments for both genders (Table-4).

Biomarkers of oxidative stress analysis

Data analysis revealed that Malondialdehyde was the only biomarkers of oxidative stress that varied significantly (P = 0.04) in liver in Holmium Zirconate/Zinc Sulphide nanocomposite treated male mice than their control group. While all other studied parameters from liver and brain varied non-significantly (P > 0.05) when compared between the two treatment groups in both genders (Table-5).

Nanotechnology, a multidimensional field, has been evolved in past few decades and performing important role in industry, pharmacology, agriculture and environment but at the same time it is becoming a potential source for human exposure to nanoparticles (NPs) [18, 19]. They can enter in human body by various routs, through ingestion, inhalation and dermal penetration and can disturb the normal physiology. Some commercially used nanomaterials release toxic ions has adverse effects on human health [20]. As Holmium zirconate/zinc sulphide nanocomposite is part of various materials that are part of everyday life, it was worth studying their biocompatibility in a model animal, albino mouse.

It has been reported that the particles of smaller size have higher dissolution rate as compared to larger particles in the gastric fluid. After

administration, larger particles with a diameter higher than 200 nm are easily sequestered by the spleen and eventually removed by the cells of the phagocyte system, resulting in decreased nano material concentrations in blood. Small particles with diameters less than 10 nm are rapidly removed through extravasations and renal clearance. Particles with a diameter ranging from 10 to 100 nm are optimal for intravenous injection and have the most prolonged blood circulation times and offer the most effective distribution in targeted tissues [4]. In the present investigation, ZnS had particle size ranging between 5-150 nm, $Ho_2Zr_2O_7$ particle size was between 10-100 nm while the composite particle size was 5 to 200 nm. All three have particle size ranges within the optimal size range for effective distribution in living systems as discussed above and these smaller particles can play a major role to effect physiology of the subjects due to their higher dissolution (Fig. 1).

Analysis of body weight data revealed that there was no significant change in body weight when compared between nanocomposite treated and untreated mice of both genders during present study (Fig. 2). Our results are in agreement with those of Aftab *et al*. [21] who had reported that oral treatment of 75 mg/ml solvent/Kg body weight of Lanthanum Zirconate NPs did not affect the body weight of albino mice of both genders as compared to control groups, Khosa *et al*. [4] had also reported that oral treatment of variable doses of neodymium zirconate zinc sulfide nanocomposite treatment did not affect the change in body weight of albino mice of both genders at all studied time points.

Analysis of neurological test data indicated that most of the studied parameters of rota rod, light dar box and Morris water maze test performance remained unaffected in mice upon treatment with Holmium Zirconate/Zinc Sulphide nanocomposite but nanocomposite treated female mice remained mobile ($P = 0.05$) for longer time than control group (Table 1). These results are in agreement with those of Khosa *et al*. [4] who had reported that male mice treated with 20mg/ml saline/kg bodyweight of neodymium zirconate zinc sulfide nanocomposite had significantly increased time mobile during open field test while rota rod test performance remained unaffected as compared to control group.

Blood is multipurpose connective tissue involved in the transport of gasses and metabolites [22]. It has been documented that both cell count and serum are affected by environmental factors, stress and nutritional deficiencies and are used as health indicator and are used for the disease diagnosis [23]. Analysis of our results indicated that all studied parameters of complete blood count and serum biochemical profile remained unaffected when compared between nanocomposite treated and untreated albino mice of both genders (Table 3, 4). These results are contradictory to Aftab *et al*. [21] who had reported significant decrease in white blood cells, lymphocytes count and serum cholesterol in mice treated with 75 mg/ml solvent/Kg body weight of Lanthanum Zirconate NPs as compared to control group. These differences in results are probably due to different nature of applied rare earth metal based compounds in two studies,

There are many studies that have reported that NPs application results in reactive oxygen species (ROS) generation leading to oxidative stress and cell death [24-27]. Lipid peroxidation of membranes is also indicator of oxidative stress and Malondialdehyde (MDA) is an end product of this process [28]. Our results revealed significant alterations ($P = 0.04$) in MDA levels in liver of Holmium Zirconate/Zinc Sulphide treated male mice as compared to that of control indicating increased lipid peroxidation of membranes in liver cells that may lead to disturbed liver physiology (Table 5). These observations are in line with those reported by Niki *et al*. [29] that rare earth ions when enters in the liver they accumulate and finally turns into metastable hydrogen oxide particles causing toxicity. Superoxide dismutase generates H_2O_2 from super oxide free radicals that are more toxic than oxygen derived free radicals and are detoxify by catalase and reduced glutathione. Brain is a high energy demanding organ and hence it is vulnerable to be damaged by oxidative stress [30]. Our result indicated that Holmium Zirconate/Zinc Sulphide nanocomposite administration resulted in a-significant decrease ($P = 0.05$) in SOD activity in the brain of female albino mice indicating a change in brain physiology in a gender specific manner (Table-5).

In conclusion, our results indicated that treatment with 50 mg/ml/solvent/Kg body weight of Holmium zirconate zinc sulphide nanocomposite for 22 days did not affected most of the studied behavioral (except open field) tests, hematological and serological parameters in albino mice of both genders. However, analysis of markers of oxidative stress in liver and brain has indicated that the applied dose of Holmium zirconate/zinc sulphide has disturbed the Malondialdehyde in liver and Superoxide dismutase levels in brain of male and female albino mice respectively. It is recommended

that their effects in living systems should be explored further under variable experimental conditions.

Conflict of Interest

Authors declare that they do not have conflict of interest of any sort with anyone.

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