Microemulsion-based synthesis of strontium hexaferrite cobalt iron oxide nanoparticles and their biocompatibility in albino mice

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ABSTRACT
A number of strontium-based iron oxides are available for the industrial use. Among them, strontium hexaferrite cobalt iron oxide (Sr₂Co₂Fe₁₂O₂₂) is preferred over others due to its better magnetic and electrical properties than others. This material is also extensively used for the absorption of microwave radiations. This study was conducted to account for the synthesis and characterization of Sr₂Co₂Fe₁₂O₂₂ nanoparticles and to report their biocompatibility in albino mice. Nanoparticles were synthesised by normal microemulsion, XRD analysis confirmed the single and scanning electron microscopy (SEM) revealed average particle size ranged between 30 and 50 nm. Nine-week-old male mice were intraperitoneally administered with 50 mg/mL of solvent/kg body weight of strontium hexaferrite cobalt iron oxide nanoparticles for 22 days. Control group was maintained in parallel. A series of neurological tests (rota rod, light and dark box, open field and Morris water maze) were conducted in both groups. Blood samples were collected from direct cardiac puncture, and parameters of complete blood count, serum biochemical parameters and antioxidant were determined in liver and brain tissues of all subjects. Analysis of result revealed that all studied neurological test performances varied nonsignificantly (P > 0.05) between the two treatments except clockwise rotations during open field test that were significantly reduced (P = 0.05) in Strontium hexaferrite Cobalt Iron Oxide nanoparticle-treated male albino mice than in the control group. All studied complete blood count and serum parameters varied nonsignificantly (P > 0.05) between two treatments. It was observed that superoxide dismutase concentration was significantly higher (P = 0.05) in the liver of nanoparticle-treated male mice. In conclusion, we are reporting that applied dose of strontium hexaferrite cobalt iron oxide nanoparticles is affecting the exploratory behaviour and antioxidant metabolites of male albino mice.
1. Introduction

Compounds having iron oxide as their major component are called ferrites and are generally used as insulating ferromagnetic ceramic compounds due to their unique properties such as electrical sensitivity, corrosion resistance and chemical and mechanical stabilities [1,2]. Their magnetic properties are attributed to their hexagonal crystal structure [3,4]. Strontium hexaferrite cobalt iron oxide (Sr₂Co₂Fe₁₂O₂₂) nanoparticles belong to Y-type ferrites [5]. The high electrical resistivity, decrease in coercivity and increase in saturation magnetization are the prosperities due to which these materials are used in manufacturing microwave, recording devices [6] telecommunications, magneto-optical and permanent magnets [7]. Hexaferrite nanoparticles have capability to bind with proteins, antibody, enzymes or drugs and targeted to tumour or tissues in the presence of external magnetic field and are used in cancer therapy [8]. Despite their extensive utilization, limited information is available in literature regarding the biocompatibility of strontium hexaferrite cobalt iron oxide. This study was designed to account for the synthesis and characterization of strontium hexaferrite cobalt iron oxide nanoparticles and to report their effects on behaviour, blood chemistry and antioxidant metabolites of liver and brain of albino mice in a gender-specific manner.

2. Materials and methodology

2.1. Synthesis of strontium hexaferrite cobalt iron oxide nanoparticles

The regents used for the synthesis of Y-type hexaferrite were Fe(NO₃)₃·9H₂O (97%; Riedel-de Haen, China), Co(NO₃)₂·6H₂O (>99%; Merck, USA), Sr(NO₃)₂ (99%; Merck), cetyltrimethyl ammonium bromide (CTAB) (97%; Merck) as a surfactant, NH₃ (35%; Fisher Scientific) as a precipitating agent and methanol (99%; Merck) as a washing agent. The Y-type hexaferrite sample with nominal composition Sr₂Co₂Fe₁₂O₂₂ was prepared by the normal microemulsion method. The required amounts of metal salts i.e. iron nitrate, cobalt nitrate and strontium nitrate were dissolved in the deionised water to form the solution of required molarities and mixed in a baker. CTAB was also added in metals solution in 1:15 ratio (metals:CTAB). The solution was stirred on the magnetic hot plate by maintaining the temperature at 333 K. The ammonia solution (2 M) was added drop wise to form the precipitates. After that, these precipitates were washed with deionised water until the pH of the mixture became neutral and, finally, the precipitates were washed with methanol to remove the water insoluble impurities. The precipitates were then dried in an oven at 373 K. Final annealing was carried out at 1323 K for 8 h, using box furnace (Heyaius, Hanau, Germany).

2.2. Characterization of strontium hexaferrite cobalt iron oxide

The crystalline phase of the synthesised material was investigated by using X-ray diffractometer (X’pert PRO PAN Analytical Diffractometer) which uses CuKα as a source of radiation (λ = 1.54056 Å), while the CuKβ was filtered using Ni filter. The elemental composition of the synthesize Y-type hexaferrite was carried out by the energy dispersive X-ray (EDX) analysis. The surface morphology was done by scanning electron microscopy (SEM). The crystallites’ sizes and lattice parameters (lattice constants and cell volume) were calculated from XRD data. The average crystallite size (D) was estimated by using the following Scherer’s formula:
\[ D = \frac{k\lambda}{\beta \cos \theta} \]  

where \( \lambda \) represents the wavelength of incident X-rays, \( \beta \) stands for the full width half maxima, \( \theta \) is the Bragg angle and the value of shape factor \( K \) is 0.89 for hexaferrites’ materials.

The lattice constants \((a \text{ and } c)\) and the cell volume \((V_{\text{cell}})\) were calculated by using the following relations:

\[
\frac{1}{d^2} = \frac{4}{3} \left( \frac{h^2 + hk + k^2}{a^2} \right) + \frac{l^2}{c^2} 
\]

\[ V_{\text{cell}} = 0.866a^2c \]

where \(d\) is the spacing between the two consecutive planes and \(hkl\) are the Miller Indices.

### 2.3. Experimental animals and design

Adult albino mice (9-week old) were used as experimental animals. Mice were kept individually in cages and provided with standard mice diet and water ad libitum. Room temperature was maintained at 22 ± 1 °C. The room was illuminated with artificial light from 8 am to 6 pm. All the experimental protocol and animal handling procedures were approved by the Ethical Committee of Institute of Pure and Applied Biology at Bahauddin Zakariya University Multan. Mice of both genders were divided into two groups. Treated group \((N=8 \text{ for male and female mice})\) received 50 mg/kg body weight of strontium hexaferrite cobalt iron oxide nanoparticles for 22 days through intraperitoneal injection, while control mice \((N=8 \text{ for male and female mice})\) intraperitoneally received saline (0.9% NaCl) solution for 22 days. A series of neurological tests such as rota rod, light and dark, open field and Morris water maize were carried out after 14 days of dose supplementation in both experimental treatments. Dose was supplemented 30 min before the conduction of each test. Throughout the experiment, the changes in weight for each mouse were recorded to demonstrate the effect of experiment material on body weight of each mouse.

### 2.4. Rota rod test

Rota rod apparatus is used to test the balance and neuromuscular coordination of an animal. Rota rod test was performed by using a locally manufactured apparatus comprising of rotating drum with the acceleration of 40 rpm. During experimentation, each mouse received three training trials followed by three experimental trials. Mean time spent on rotating drum was compared between the control and strontium hexaferrite cobalt iron oxide treated groups following Allahyar et al. [9]

### 2.5. Open field test

Open field test is used to assess locomotory and exploratory behaviours of an animal Iqbal et al. [10] A computational tracking system, ANY-maze (Stoeling, Wood Dale, IL, USA) connected with video camera (XPod-058, Shanghai, China), was used to detect the behaviour of mice in the open field chamber \((40 \text{ cm} \times 40 \text{ cm} \times 70 \text{ cm})\). Each mouse was released in the corner of the open field box for 10 min of test duration. Maximum speed \((m)\), mean speed \((m/s)\), time mobile and time immobile \((s)\), mobile episodes, immobile
episodes and rotations (clockwise and anticlockwise) were noted following Allahyar et al. [9]

### 2.6. Light/dark box test

The light/dark box test equipment was having an area of \((45\, \text{cm} \times 27\, \text{cm} \times 27\, \text{cm})\) made up of plywood and consisted of one-third dark safe chamber \((18\, \text{cm} \times 27\, \text{cm})\) and two-third light aversive chamber \((27\, \text{cm} \times 27\, \text{cm})\) with the light intensity of 200 W. The two chambers were connected by an opening \((7.5\, \text{cm} \times 7.5\, \text{cm})\) located in the center of the dividing wall adjacent to floor. The floor was divided into \(9\, \text{cm} \times 9\, \text{cm}\) squares and was covered with Plexiglas. A mouse was placed in the centre of light chamber keeping its snout towards opening in the wall. Time spent in each chamber, transition frequency, rearing, stretch attended, defecation and urination were counted over a 5 min test following Zahra et al. [11]

### 2.7. Morris water maze

Morris water maze (MWM) consists of a circular pool of a diameter of 122 cm and a depth of 76 cm. In the pool, mice were trained to swim to find a platform concealed \((1.5\, \text{cm})\) under water surface. In order to identify the position of concealed platform, distal extra-maze cues of different colors and dimensions were attached to the room walls. During the whole experiment, both colors and dimension of visual cues were kept constant. Water temperature was maintained at \(21 \pm 1\, ^\circ\text{C}\). The circular pool was partitioned into four quadrants (compass locations: NE, NW, SW and SE) by a computerised tracking/image analyzer system (video camcorder Addlink Software; Scientific, Barcelona, Spain) coupled to computational tracking system, ANY-maze. The middle of the southeast quadrant was selected as a place for concealed platform where it remained during the whole experiment.

In the spatial acquisition phase, mice were subjected to 16 training trials: four training trials per day and four training days with an intertrial interval of 30 min. Mice were released (randomly) in the circular pool from the four compass locations for swimming. For 120 s, mice were allowed to search the platform by swimming. Then, the mice were allowed to stay on the platform for another 30 s after failure of subject to find the concealed platform within 120 s.

In acclimatization training session of first training day in the MWM, each mouse was manually placed on the platform and was subsequently guided back to the platform after the swimming of 30 s. Parameters such as average time, distance travelled to reach the hidden platform, time immobile during the trial, time mobile and mean speed were recorded. After the abovementioned phase of acquisition, each mouse was subjected to probe trial. Probe trials were administered by the removal of platform. Animals were released from the north start point to swim freely for 60 s. The mouse swimming path was tracked and analysed for the proportion of swimming time and/or path length spent to reach platform, time mobile, rest time during the trial, total latency, mean visit to platform and average speed as followed by Kipnis et al. [12].

### 2.8. Blood and serum collection

Following the intraperitoneal injections for 22 days, mice were anaesthetised and blood was collected either from retro-orbital sinus or through direct cardiac puncture and
divided into two parts. One part was used for the study of complete blood count parameters by using haematology analyzer SYSMEX, 21 (Japan), while the second part was centrifuged at 13000 rpm for 10 min, and the extracted serum was used for the estimation of cholesterol, creatinine, low-density lipoprotein, high-density lipoprotein and triglycerides by using diagnostic kits.

2.9. Determination of antioxidant metabolites in liver and brain

Following sacrifice, liver and brain were surgically removed, rinsed in isotonic saline solution and stored immediately at −20°C. In both organs, concentrations of superoxide dismutase were calculated following Chidambara et al. [13], estimation of lipid peroxidation was carried out as described by Haider et al. [14] and catalase activity was determined following Lateef and Qureshi [15].

2.10. Statistical analysis

Statistical package Minitab (version 16; PA, USA) was used for data analysis. All data were expressed as mean ± standard error of mean. Significance level was set at $P < 0.05$. Two sample Student’s $t$-test was applied to compare all studied parameters of behaviour, complete blood count, serum and antioxidant profile between strontium hexaferrite cobalt iron oxide nanoparticle-treated and untreated albino mice of both genders.

3. Results

3.1. Structural analysis of strontium hexaferrite cobalt iron oxide nanoparticles

The XRD pattern for the synthesised Y-type strontium hexaferrite cobalt iron oxide nanoparticles is shown in Figure 1(A). All the peaks in the pattern were well matched with the standard pattern (ICSD-01-072-0750), which indicates that the material was crystallised into rhombohedral single phase. The peaks were appeared at $2\theta = 22.6, 24.7, 28.5, 30.8, 31.9, 32.7, 33.7, 35.1, 35.6, 37.4, 39.3, 40.9, 42.1, 43.2, 45.9, 50.08, 52.7, 54.8, 56.02, 57.3, 59.02, 60.8, 62.4, 64.1, 64.4, 66.7 and 68.6°$, which were related to the Miller indices $107, 0012, 0112, 110, 1013, 116, 0114, 021, 119, 1101, 1112, 0210, 1114, 128, 1211, 2113, 1214, 039, 0222, 2023, 1028, 0030, 226, 2026$ and $2212$, respectively. The calculated lattice parameters such as lattice constants ($a$ and $c$) and cell volume were found to be $5.92$ and $43.48$ Å and $1319.6$ Å³, respectively, which was also in agreement with the standard pattern indicating that the synthesised material was in pure single phase. The average crystallite size was also calculated by using Equation (1), and it was found to be around $40$ nm (Figure 1(A)).

3.2. Morphological and compositional analysis of strontium hexaferrite cobalt iron oxide nanoparticles

The SEM image of Sr$_2$Co$_2$Fe$_{12}$O$_{22}$ is shown in Figure 1(B). The particles were in hexagonal plate-like shape with clear boundaries. The particle size calculated from SEM image was found to be $30–50$ nm range, which is in good agreement with that of calculated from XRD data (Figure 1(B)).

The composition of the synthesize sample was determined by EDX, and the pattern is shown in Figure 1(C). All the peaks in the pattern were related to the Fe, Co and Sr, which
confirm that there was no other element in the materials. The experimental composition for Sr, Co and Fe was found to be 1.97, 1.93 and 11.91, respectively. This indicates that the theoretical and experimental compositions are in good agreement with each other.

### 3.3. Rota rod test

Rota rod test results indicated that mice injected with 50 mg/mL of solvent/kg body weight strontium hexaferrite cobalt iron oxide nanoparticles spent more time on rota rod apparatus than mice injected with saline water but difference in rota rod test performance did not reached statistical significance for both male ($P = 0.7$) and female ($P = 0.4$) albino mice.

### 3.4. Light/dark box test

Analysis of the data revealed that all the studied parameters of light/dark box test varied nonsignificantly ($P > 0.05$) when compared between strontium hexaferrite cobalt iron oxide nanoparticle-treated and saline water-treated albino mice (Table 1).
3.5. Open field test

Results of open field test indicated that all studied parameters of open field test varied nonsignificantly when compared between strontium hexaferrite cobalt iron oxide nanoparticle- and saline-treated albino mice as compared to their respective control groups except clockwise rotations that were significantly reduced (\(P = 0.05\)) in strontium hexaferrite cobalt iron oxide nanoparticle-treated male albino mice than the control group (Table 2).

Table 1. Comparison of various studied parameters of light/dark box between 50 mg/mL of solvent/kg body weight strontium hexaferrite cobalt iron oxide (50 mg/mL of solvent/kg body weight) and saline treated adult albino mice.

| Parameters                  | Saline-treated male group | Strontium hexaferrite cobalt iron oxide-treated male group | Saline-treated female group | Strontium hexaferrite cobalt iron oxide-treated female group | \(P\) value |
|-----------------------------|---------------------------|------------------------------------------------------------|-----------------------------|------------------------------------------------------------|------------|
| Transition frequency        | 19.7 ± 2.3                | 14.3 ± 1.5                                                 | 14.7 ± 2.3                  | 15.7 ± 1.7                                                 | 0.7        |
| Rearing frequency           | 9.3 ± 1.4                 | 5.5 ± 1.4                                                 | 5.6 ± 1.7                   | 2.5 ± 0.8                                                 | 0.1        |
| Stretch attend frequency    | 39.1 ± 5.1                | 32.1 ± 5.2                                                 | 37.3 ± 6.8                  | 26.3 ± 4.8                                                 | 0.2        |
| Time in dark (s)            | 157.3 ± 13.4              | 152.8 ± 24.9                                              | 176.4 ± 22                  | 168.1 ± 16                                                 | 0.8        |
| Time in light (s)           | 142.6 ± 13.4              | 147.1 ± 24.9                                              | 123.6 ± 22                  | 133.1 ± 15                                                 | 0.7        |
| Urination                   | 5 ± 0.3                   | 2 ± 0.4                                                  | 0.7 ± 0.3                   | 0.3 ± 0.2                                                 | 0.4        |
| Defecation                  | 7 ± 0.4                   | 7 ± 0.3                                                  | 2.67 ± 1.2                  | 2.00 ± 1.3                                                 | 0.4        |

All values are expressed as mean ± standard error of mean. \(P\) value represents the results of two-sample \(t\)-test calculated for each parameter. \(P > 0.05 = \) nonsignificant.

Table 2. Comparison of studied parameters of open field test between saline and 50 mg/mL of solvent/kg of body weight strontium hexaferrite cobalt iron oxide nanoparticles treated albino mice.

| Parameters                  | Saline-treated male group | Strontium hexaferrite cobalt iron oxide-treated male group | Saline-treated female group | Strontium hexaferrite cobalt iron oxide-treated female group | \(P\) value |
|-----------------------------|---------------------------|------------------------------------------------------------|-----------------------------|------------------------------------------------------------|------------|
| Distance (m)                | 24.4 ± 1.9                | 19.8 ± 2.4                                                 | 20.0 ± 2.5                  | 21.5 ± 1.1                                                 | 0.6        |
| Mean speed (m/s)            | 0.04 ± 0.003              | 0.03 ± 0.004                                              | 0.03 ± 0.004                | 0.03 ± 0.001                                              | 0.6        |
| Time mobile (s)             | 479.1 ± 14.7              | 426.9 ± 30.7                                              | 431 ± 37                    | 455.5 ± 9.8                                               | 0.5        |
| Time immobile (s)           | 120.8 ± 14.7              | 173 ± 30.7                                                | 169 ± 37                    | 144.3 ± 9.8                                               | 0.5        |
| Mobile episodes             | 25.3 ± 2.8                | 31.2 ± 4.09                                               | 31.7 ± 2                    | 30.6 ± 2                                                  | 0.6        |
| Immobile episodes           | 24.7 ± 2.9                | 30.3 ± 4.1                                               | 31. ± 2.1                   | 30.1 ± 2.1                                               | 0.7        |
| Max speed (m/s)             | 0.29 ± 0.03               | 0.23 ± 0.01                                               | 0.20 ± 0.02                 | 0.24 ± 0.02                                               | 0.3        |
| Rotations                   | 30.3 ± 1.5                | 25.3 ± 2.2                                               | 25.7 ± 2.5                  | 27.8 ± 1.8                                               | 0.5        |
| Clockwise rotations         | 16.8 ± 1.4                | 12.3 ± 1.3                                               | 14.2 ± 2                    | 14.0 ± 1.2                                               | 0.9        |
| Anticlockwise rotation      | 13.7 ± 1.3                | 13 ± 1.0                                                | 11.5 ± 1.5                  | 13 ± 1.4                                                  | 0.2        |

Values are expressed as mean ± standard error of mean for all treatments. \(P\) value represents the results of two-sample \(t\)-test calculated for each parameter. \(P > 0.05 = \) nonsignificant. 

\(^4P < 0.05 = \) least significant.
3.6. Morris water maze test

3.6.1. Acquisition phase test

Analysis of the results revealed that strontium hexaferrite cobalt iron oxide nanoparticle-treated male albino mice covered more distance on training days 2 ($P = 0.03$) and 4 ($P = 0.04$) to reach the platform area than saline-treated control group, while this parameter during training days 1 and 3 and all other studied parameters during the acquisition phase varied nonsignificantly ($P > 0.05$) when compared between two experimental treatments (data not shown here).

3.6.2. Probe trial

Analysis of results revealed that all studied parameters during probe trials varied nonsignificantly ($P > 0.05$) when compared between 50 mg/mL of solvent/kg body weight strontium hexaferrite cobalt iron oxide-treated and untreated albino mice (data not shown here).

3.7. Body weight analysis

Analysis of the data indicated that body weight varied nonsignificantly ($P > 0.05$) at all the studied time points (22 consecutive days) when compared between 50 mg/mL of solvent/kg body weight of strontium hexaferrite cobalt iron oxide nanoparticle-treated and untreated albino mice (data not shown here).

Table 3 Comparison of studied complete blood count parameters between Strontium Hexaferrite Cobalt Iron Oxide nanoparticles (50 mg/ml of solvent/Kg body weight) ($N = 16$) and saline treated albino mice ($N = 16$) of both gender. 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 | Hexaferrite strontium cobalt iron oxide treated male mice | $P$ value | Saline treated control female mice | Hexaferrite strontium cobalt iron oxide treated female mice | $P$ value |
|--------------------|----------------------------------|----------------------------------------------------------|-----------|-----------------------------------|----------------------------------------------------------|-----------|
| WBC $\times 10^3$ μl | 3.6 ± 0.7 | 3.4 ± 0.7 | 0.8 | 5.5 ± 0.7 | 6.5 ± 1.4 | 0.6 |
| LYM $\times 10^3$ μl | 2.9 ± 0.6 | 2.9 ± 0.5 | 0.8 | 4.2 ± 0.7 | 5.3 ± 1.2 | 0.4 |
| MON $\times 10^3$ μl | 0.2 ± 0.05 | 0.2 ± 0.05 | 0.9 | 0.3 ± 0.06 | 0.3 ± 0.07 | 0.8 |
| Granulocytes | 0.5 ± 0.1 | 0.5 ± 0.08 | 0.9 | 1.01 ± 0.2 | 0.9 ± 0.2 | 0.6 |
| LYM % | 81.3 ± 3.1 | 79.3 ± 1.7 | 0.6 | 74.7 ± 6.6 | 80.3 ± 2.2 | 0.4 |
| MON % | 4.5 ± 0.83 | 4.6 ± 0.7 | 0.9 | 6.1 ± 1.1 | 5.06 ± 0.8 | 0.5 |
| Granulocytes (%) | 14.2 ± 2.7 | 16.0 ± 1.3 | 0.6 | 19.2 ± 5.7 | 14.9 ± 1.8 | 0.5 |
| RBC $\times 10^6$ μl | 7.0 ± 0.47 | 6.6 ± 0.9 | 0.7 | 7.4 ± 0.4 | 7.4 ± 0.34 | 0.1 |
| HGB (g/dl) | 12.2 ± 0.52 | 11.0 ± 1.1 | 0.4 | 11.9 ± 0.5 | 12.1 ± 0.46 | 0.8 |
| HCT $\times 10^6$ μl | 31.4 ± 2.2 | 28.9 ± 3.7 | 0.6 | 32.9 ± 2.5 | 33.4 ± 1.7 | 0.9 |
| MCV (μm$^3$) | 45.1 ± 1.3 | 44.1 ± 0.9 | 0.5 | 45.5 ± 1.6 | 45.2 ± 0.9 | 0.9 |
| MCH (pg) | 17.8 ± 0.9 | 17.7 ± 1.2 | 0.9 | 16.2 ± 0.4 | 16.5 ± 0.3 | 0.7 |
| MCHC (g/dl) | 39.5 ± 1.6 | 40.1 ± 2.3 | 0.8 | 35.9 ± 1.1 | 36.5 ± 0.9 | 0.7 |
| RDW (fk) | 16.7 ± 0.9 | 17.0 ± 1.2 | 0.8 | 18.7 ± 0.8 | 18.2 ± 1 | 0.7 |
| RDW-SD (fk) | 28.8 ± 0.8 | 29.3 ± 1.3 | 0.6 | 29.9 ± 0.9 | 30.2 ± 1.5 | 0.9 |
| PLT $\times 10^3$ μl | 354 ± 67 | 322 ± 70 | 0.7 | 419 ± 55 | 416 ± 36 | 0.1 |
| MPV (μm$^3$) | 7.7 ± 0.7 | 7.6 ± 0.4 | 0.9 | 7.6 ± 0.8 | 7.4 ± 0.4 | 0.8 |
| PLT (%) | 0.26 ± 0.04 | 0.25 ± 0.06 | 0.9 | 0.32 ± 0.05 | 0.31 ± 0.04 | 0.9 |
| PDW (%) | 23.5 ± 3.7 | 24.6 ± 3.6 | 0.8 | 21.2 ± 1.8 | 22.3 ± 1.1 | 0.6 |

Where WBC = White blood cells; LYM = Lymphocytes; HGB = Hemoglobin; HCT = hematocrit; MCH = Mean cell hemoglobin; MCHC = Mean corpuscular hemoglobin concentration; RDW = Red cell distribution width; PLT = Platelets; MPV = Mean platelet volume; PDW = Platelet distribution width. $P > 0.05$ = Non significant.
3.8. Effect of strontium hexaferrite cobalt iron oxide nanoparticles on the complete blood count of albino mice

Upon comparison of complete blood count parameters of albino mice treated with hexaferrite strontium cobalt iron oxide with saline-treated control, it was observed that all studied parameters varied nonsignificantly ($P > 0.05$) between the two experimental treatments for both genders (Table 3).

3.9. Effect of strontium hexaferrite cobalt iron oxide nanoparticles on serum biochemical profile of albino mice

When the studied serum parameters were compared between strontium hexaferrite cobalt iron oxide nanoparticle-treated and untreated mice, it was observed that all the parameters varied nonsignificantly ($P > 0.05$) between the two treatments for both genders (Table 4).

3.10. Effect of strontium hexaferrite cobalt iron oxide nanoparticles on antioxidants profile of albino mice

Data analysis revealed that superoxide dismutase concentration in liver was significantly higher ($P = 0.005$) in strontium hexaferrite cobalt iron oxide nanoparticle-treated mice.

Table 4 Comparison of various studied serum parameters between Strontium Hexaferrite Cobalt Iron Oxide nanoparticles (50 mg/ml of solvent/Kg body weight) ($N = 16$) and saline treated albino mice ($N = 16$) of both gender. 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 | Hexaferrite strontium cobalt iron oxide treated male mice | $P$ value | Saline treated control female mice | Hexaferrite strontium cobalt iron oxide treated female mice | $P$ value |
|------------------------|----------------------------------|----------------------------------------------------------|----------|------------------------------------|----------------------------------------------------------|----------|
| Triglyceride (mg/dL)   | 298 ± 47                         | 231.8 ± 30                                               | 0.3      | 161 ± 42                           | 231.8 ± 22                                               | 0.2      |
| Cholesterol (mg/dL)    | 321.5 ± 17                       | 279.0 ± 16                                               | 0.09     | 287 ± 63                           | 241 ± 42                                                 | 0.6      |
| HDL (mg/dL)            | 191 ± 309                        | 279 ± 524                                                | 0.7      | 67.1 ± 16                          | 54.9 ± 20                                                | 0.6      |
| LDL (mg/dL)            | 205.7 ± 25                       | 184.9 ± 17                                               | 0.5      | 178 ± 55                           | 148 ± 50                                                 | 0.6      |
| Creatinine (mg/dL)     | 0.8 ± 0.3                        | 1.09 ± 0.3                                               | 0.5      | 0.9 ± 0.2                          | 3.7 ± 1.8                                                 | 0.2      |

Where HDL = High density lipoprotein; LDL = low density lipoprotein.
$P > 0.05$ = Non significant.

Table 5. Comparison of studied antioxidant metabolites of liver and brain in albino mice treated with strontium hexaferrite cobalt iron oxide nanoparticles (50 mg/mL of solvent/kg body weight) ($N = 16$) with their saline-treated control mice ($N = 16$) of both gender.

| Organ | Metabolite            | Saline-treated control male mice | Strontium hexaferrite cobalt iron oxide-treated male mice | $P$ value | Saline-treated control female mice | Strontium hexaferrite cobalt iron oxide-treated female mice | $P$ value |
|-------|-----------------------|----------------------------------|----------------------------------------------------------|----------|------------------------------------|----------------------------------------------------------|----------|
| Brain | Superoxide dismutase (U/g) | 0.6 ± 0.1                       | 0.7 ± 0.1                                               | 0.6      | 0.8 ± 0.2                           | 0.4 ± 0.09                                               | 0.1      |
|       | Catalase (mg/dL)      | 36.0 ± 5.0                       | 33.5 ± 1.3                                              | 0.6      | 46 ± 5.1                            | 40.3 ± 4.6                                               | 0.4      |
|       | Malonaldehyde (pmol/g) | 99.9 ± 10                        | 90.7 ± 9.2                                              | 0.5      | 245 ± 53                            | 204.5 ± 31                                               | 0.5      |
| Liver | Superoxide dismutase (U/g) | 0.2 ± 0.03                      | 0.5 ± 0.08                                              | 0.005*   | 0.6 ± 0.1                            | 0.9 ± 0.2                                               | 0.3      |
|       | Catalase (mg/dL)      | 14.9 ± 4.1                       | 13.8 ± 1.7                                              | 0.7      | 20.6 ± 2.7                          | 31.9 ± 6.5                                               | 0.1      |
|       | Malonaldehyde (pmol/g) | 71.3 ± 4.1                       | 66.2 ± 1.7                                              | 0.8      | 160 ± 38                            | 193 ± 48                                                 | 0.6      |

All values are expressed as mean ± standard error of mean. $P$ value indicates the results of two-sample $t$-tests calculated for each parameter. $P > 0.05$ = nonsignificant.

$*P < 0.05$ = significant.
than their control group. While all other studied parameters from liver and brain varied nonsignificantly \( (P > 0.05) \) when compared between the two treatment groups in both genders (Table 5).

4. Discussion

Nanotechnology is the area of active research in these days due to its wide range of applications in medicine, environmental protection, industry and consumer products [16]. Nanoparticles (NPs) can enter in human body by various routes, through ingestion, inhalation, injection and dermal penetration and can induce toxicity on environment and human either deliberately or accidently [17]. NPs are small in size and have large surface area due to which they can easily cross barriers and enter in circulatory system, transported to organs and tissues lead to oxidative damage [18]. Pathways by which nanoparticles’ uptake may occur include hemolysis and thrombogenicity. These pathways include numerous activities such as reduced number of blood cells, antimitotic properties and increasing the number of cells involved in the immune processes [19]. They also lead to the formation of free radicals due to oxidative stress. These free radicals interact with lipids, proteins and nucleic acids changing the cell signalling and transcription [20].

Despite the large scale industrial use of strontium hexaferrite cobalt iron oxide nanoparticle, limited information is available in literature regarding their biocompatibility in living systems. It has been reported that the particles of smaller size have higher dissolution rate as compared to agglomerated (larger particles) in the gastric fluid and the animals are more sensitive to smaller particles as compared larger one [21]. Internalization of magnetic nanoparticles strongly depends upon the size of the particles. 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 nanoparticles 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. These particles are small enough to evade the RES of the body as well as to penetrate small capillaries of the tissues and offer the most effective distribution in targeted tissues [22]. In the present investigation, some of the particles agglomerate into larger particles as indicated by the SEM image (Figure 1). The nonagglomerated (smaller) particles play a major toxic role due to their higher dissolution. During the present study, we applied the nonagglomerated, fine nanoparticles to demonstrate their biocompatibility in albino mice and our results indicated that the crystalline structure of nanoparticles was around 40 nm in size and nanoparticles of this size can be effectively distributed to blood capillaries [21].

Analysis of neurological test data indicated that the studied parameters of rota rod, light/dark box and MWM test performance varied non significantly \( (P > 0.05) \) when compared between saline-treated and strontium hexaferrite cobalt iron oxide nanoparticle-treated albino mice of both genders indicating that either these nanoparticles are unable to cross the blood–brain barrier effectively or the receptors may not be present everywhere in the central nervous system. Our open field test results showed that all studied parameters varied nonsignificantly when compared between NP-treated and untreated albino mice except clockwise rotations that were significantly reduced in strontium hexaferrite cobalt iron oxide nanoparticle-treated male mice than saline-treated control group (Table 2). These results indicate that cerebellum part of male brain has potentially the
receptor for strontium hexaferrite cobalt iron oxide nanoparticles as this part is reported to regulate the locomotory and exploratory behaviours resulting in improved performance than in the control group.

Blood has special importance in the living system and involves in the transport of carbon dioxide, oxygen and various metabolites [23]. Hematological parameters are affected by stress, environmental factors and nutritional deficiencies and hence can be used as good health indicator and are used on regular basis for the diagnosis of various diseases [24]. Gaharwar and Paulraj [25] had reported a significant decrease in red blood cells in rat that were exposed to either 15 or 30 mg/kg iron oxide. They had suggested that these changes in red blood cells are due to abnormalities induced by iron oxide in hemopoietic system that is responsible for erythrocyte production. Our results revealed that total red blood cell count was lower in strontium hexaferrite cobalt iron oxide-treated mice as compared to saline-treated male albino mice, although the difference did not reached statistical significance (P = 0.7) (Table 3). The difference in the two studies is probably due to the nature of chemicals (as we used NPs containing iron oxide as a component while the mentioned study used iron oxide only) and due to the different concentrations of the applied chemicals.

Our results revealed decrease in white blood cells (P = 0.8) and lymphocytes (P = 0.8) in NP-treated male mice (Table 3). Tabish et al. [26] had reported decreased white blood cells, lymphocytes and platelets' concentration in rabbits following a 10 mg/kg body weight implantation of cobalt iron oxide and suggested that nanoparticles at high doses can disturb the immune system in animals through affecting white blood cell production.

Nanoparticles induce oxidative stress that leads to the formation of reactive oxygen species that can disturb the antioxidant system that is consists of enzymes superoxide dismutase (SOD), catalase and nonenzymes such as vitamins E and C and glutathione [27]. Oxidative stress also causes lipid peroxidation of membranes. Superoxide dismutase generates H2O2 from super oxide free radicals that are more toxic than oxygen-derived free radicals and are detoxify by catalase and reduced glutathione [28]. Gahrawar and Pulraj [25] had reported a reduction in SOD and increased lipid peroxidation in iron oxide nanoparticle-treated rats as compared to control. In contrast, our results revealed increased SOD activity (P = 0.005) and reduced lipid peroxidation (P = 0.8) in liver and brain of strontium hexaferrite cobalt iron oxide-treated male mice as compared to saline-treated control group (Table 5). Increased level of SOD in liver during present study indicates increased H2O2 production in liver of the strontium hexaferrite cobalt iron oxide nanoparticle-treated male mice. These differences in results are probably due to different experimental designs, animals model used, applied dosage and exposure time.

Our results revealed lower value of catalase enzyme (P = 0.7) in strontium hexaferrite cobalt iron oxide-treated male mice as compared to control treatment. Our results are in agreement with Gahrawar and Pulraj [25] who had reported reduced catalase activity in liver and kidney of iron oxide nanoparticle-treated rat.

5. Conclusion

In conclusion, our results indicated that 50 mg/mL of solvent/kg body weight of strontium hexaferrite cobalt iron oxide nanoparticles did not affected the studied neurological, hematological and serological parameters of albino mice of both genders, when compared with saline-treated control mice. Analysis of antioxidant metabolites in liver has indicated that the applied dose of strontium hexaferrite cobalt iron oxide can disturb the H2O2-associated metabolic pathways that resulted in increased SOD concentrations in male
mice. As these nanomaterials are part of our everyday life, so it is recommended that their effects in living systems should be explored under variable dose and exposure time conditions to get a broader vision regarding their biocompatibility.

Disclosure statement

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

Notes on contributors

Furhan Iqbal has designed and supervised the project.

Sana Akhtar and Sehrish Saba has performed behavioral experiments.

Sauma Rehman, Ahmad Hassan and Noreen Samad performed hematological tests.

Muhammad Amir, Mudassar Akram, Maryam Ijaz and Kiran Asif did the antioxidant analysis and MNA synthesize and characterize the nano material.

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