*Arab J. Nucl. Sci. Appl., Vol. 56, 1, 41-48 (2023)*

ISSN 1110-0451

*Arab Journal of Nuclear Sciences and Applications*

*Web site: ajnsa.journals.ekb.eg*

(E S N S A)

# The Potential Effects of Cerium Oxide Nanoparticles Against Ferrous Sulfate-Induced Oxidative Stress in Male Albino Rats

**Amal. H. Mahmoud*, Manal .M Abbas and Hanan. A. Abd Elmonem**

*Biological Application Department, Nuclear Research Centre, Egyptian Atomic Energy Authority, Cairo*

ARTICLE INFO

*Article history:*

Received: 19th June 2022

Accepted: 20th Oct. 2022

*Keywords:*

*Cerium oxide nanoparticles;*
*Ferrous sulfate;*
*Oxidative stress;*
*Antioxidants;*
*Thyroxine.*

ABSTRACT

**Iron is a vitally essential element for the maintenance of health in the living organism, however iron overload can have harmful effects on health. Nanoparticles constitute a therapeutic approach for the remedy of many disorders. So the study was performed to examine the potential ameliorative effects of cerium oxide nanoparticles (CeONPs) against ferrous sulfate (FS) induced oxidative stress, hepatic, renal and thyroid functional disorders in rats. Twenty-eight male albino rats were equally assigned into four groups. Group1 received 1 ml saline (control), FS group rats received ferrous sulfate (30mg/Kg/day), CeONPs group (0.5mg/Kg/day) and the fourth group received CeONPs + FS (0.5mg/Kg + 30mg/Kg). Rats in all groups were injected intraperitoneally for two weeks (5 days/week). Results revealed that FS increased serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total bilirubin, total protein, creatinine, and urea. In addition, hepatic and renal levels of malondialdehyde (MDA) were increased while glutathione (GSH), superoxide dismutase (SOD) levels in the liver, kidney and serum thyroxine level were reduced. Treatment with CeONPs restored liver, kidney and thyroid functions to almost the normal levels and improved the oxidant – antioxidant status. So the study pointed to the ameliorative effect of CeONPs against Fs-induced disorders on oxidative stress, liver, kidney and thyroid function.**

## INTRODUCTION

Anemia especially iron deficiency anemia is one of the most widespread nutritional deficiencies in both developed and developing countries [1]. Iron deficiency results in weakness and learning dysfunction [2] and is involved in factors responsible for early neonatal arrival and their low weight [3].

On the other hand, unbound iron is a potent catalyst for lipid peroxidation and hydroxyl radical [4].In excessive iron status, iron is stored in liver its main store [5] as ferritin which cannot interact in the Fenton reaction [6].

Urinary excretion is one of the known pathways for iron elimination from the body. It was proposed that iron can cause renal failure [7] and it was involved in renal tubular damage due to the creation of hydroxyl ($OH^-$) radicals [8]. Ferrous sulfate is the prevalent supplement advised for anemia and reveals suitable absorption, but notable results showed that ferrous sulfate can exhibit unbalanced alterations in colon bacteria and increase systemic infection and divisive signals of the epithelium [9].

Recently, nanotechnology has developed new techniques to ameliorate and mitigate different diseases by decreasing ROS production. So many nanoparticles such as CeONPs are created for this advantage [10]. Cerium as a lanthanide has different applications in industry and Nano-medicine. Lately, cerium oxide ($CeO_2$) nanoparticles were examined for their potential of scavenging free radicals to prevent dangerous effects of chemical, biological and radiological attacks that enhance free radicals formation. The chemical structure of cerium oxide ($CeO_2$) nanoparticles facilitates its effect as an advisable and potent free radical scavenger inhibiting the production of reactive oxygen species (ROS) and decreasing cell death and apoptotic response [11].

Corresponding author: dr.amalhanafi.1@gmail.com
DOI: 10.21608/ajnsa.2022.145155.1604

Cerium oxide is a non-crystalline compound characterized by cerium atom with two oxidation states $4^+$ and $3^+$ [12]. The double oxidation state indicates that cerium oxide nanoparticle has oxygen vacancies [13], where reduction of $Ce^{4+}$ to $Ce^{3+}$ and loss of oxygen are followed by the formation of an oxygen vacancy. This crucial property of cerium oxide potentiates its scavenging ability and allowing them to play an active role as a catalyst in a broad range of reactions (e.g. enhancing the oxidation of carbon monoxide Co to $Co_2$) [14]. Also, in the biological background, cerium oxide nanoparticles can mimic enzymes antioxidants such as superoxide dismutase [15] and catalase [16]. Several studies have reported the antioxidant and beneficial effects of CeONPs in various conditions associated with high production of ROS such as diabetes [17], chronic inflammation, cardiac and kidney damage [18]; 19], cancer [20], and necrosis and apoptosis [21].

Considering the above, cerium oxide nanoparticles provide a new therapeutic antioxidant for reducing and eliminating ROS, so in the current study, cerium oxide nanoparticles were used to contradict the ferrous sulfate disorders on liver, kidney and thyroid organs in addition to the ferrous sulfate oxidant-antioxidant disequilibrium.

## MATERIAL AND METHODS

*Experimental animals*

Twenty-eight (28) adult male rats' average weights (180-190g) were derived from the Animal Breeding Unit of Biological Application Department at Nuclear Research Center, Atomic Energy Authority, Egypt. The animals were accommodated in cages under normal conditions of temperature (25 ± 2C°) and humidity with a light/dark cycle which was alternated every 12 hours, supplied with water and food *ad libitum.* The animal experiments were performed according to the Guidelines of the National Institute of Health care (NIH) Laboratory animals for the use of the Ethics Committee of Atomic Energy Authority approved the design of the experimental protocol. The animals were allowed to be acclimatized for two weeks before the beginning of the experiment, Afterward, the rats were allocated equally into four groups (7 rats in each group):

Group 1: Control group rats were intraperitoneally injected with 1 ml (0.9 % saline).

Group 2: rats were injected intraperitoneally (i.p.) with Ferrous sulfate ($FeSo_4$) 30mg / Kg B.wt. for two weeks (5days/week) 22].

Group 3: rats were injected with cerium oxide nanoparticles (CeONPs) (0.5mg/Kg B.wt.) for two week (5days/week) [23].

Group 4: rats were injected i.p. with $FeSo_4$ (30mg/Kg B.wt.) and CeONPs (0.5mg/gm B.wt.) for two weeks.

During the experimental period, rats were observed for any abnormal clinical symptoms and behavior.

*Blood and tissue sampling*

At the end of the experimental period (14 days), blood was gathered after rats' anesthetization from the heart, one half into heparinized tubes for estimating hematological indices, the other part of blood in test tubes to gain serum for biochemical parameters estimations, subsequently the liver and left kidney were quickly removed, washed using saline (NaCl 0.9%) then tissue was homogenized (1 gm of tissue in 10 ml cold buffer 50mM potassium phosphate, PH 7.5. 2mM EDTA). The homogenates were centrifuged at 4C° at 10,000Xg for 15 min. The resulted supernatants were collected and maintained at -80 for estimating lipid peroxidation indicator (MDA) levels and antioxidant markers glutathione and superoxide dismutase enzyme (SOD) activities according to the manufacturer's guide-line (Bio-diagnostics Co, Cairo Egypt).

*Hematological analysis*

Determination 0f Red blood cells count (RBCs), Hemoglobin (Hb), Hematocrit (HCT), and White blood cells count (WBCs) using auto hematological counter SweLab alpha.

*Biochemical analysis*

Serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), urea, and creatinine,total protein, albumin and total bilirubin were determined colorimetrically by commercial kits obtained from Biodiognostic Co. Egypt. Total iron binding capacity (TIBC) and serum iron(SI) levels were estimated by commercial kits.C-reactive protein (CRP) determined by Immuno- turbidimetry Assay using kits purchased from (Roche Diagnostics Gmbs, Mannheium, Germany) While serum level of thyroxine ($T_4$) was assessed by radioimmunoassay using kits obtained from DIA source immunoassay S.A. Rue du Bosquet, 2-B 1348Louvain-La-Neuve-Belgium. Determination of glutathione in blood was performed by the available kits (Biodiagonstic kits).

*Chemicals*

Cerium (IV) oxide nanopowder < 25 nm particle size (CAS Number: 1306-38-3) was purchased from Sigma-Aldrich Co., St.Louis USA. CeONPs were suspended in distilled water. Ferrous sulfate ($FeSo_4$) powder was obtained from NAT, Co., Laboratory chemical reagent, Egypt.

*Statistical analysis*

Data were analyzed using analysis of variance (ANOVA) test .Duncan's Range Test (DMRT) was prepared to compare between groups using SPSS. Software package V.20.0.

**RESULTS**

As presented in **Table (1)** FS treatment elevated levels of serum AST, ALT, ALP and serum total bilirubin as compared to the control group and CeONPs. Data showed that level of serum of total protein in rats treated with FS was significantly higher than those of control and CeONPs groups. Treatment with CeONPS significantly reversed changes in CeONPs + FS group.

**Table (2):** In FS treated group, kidney function markers (creatinine, urea) were significantly increased than the control group. I.P. injection of CeONPs lowered their elevations significantly in CeONPs + FS injected rats. CRP the inflammatory marker recorded a significant increase in FS group and CeONPs + FS groups as parallel to control group. Moreover, a significant decrease in CRP level in CeONPs group and CeONPs+FS group relative to FS group. The results revealed that FS decreased serum T4 levels significantly when compared to control group. CeONPs treatment increased T4 level compared to FS group.

**Table (3):** Showed changes in hepatic and renal MDA, GSH, and SOD activities. Data showed that FS-treated group exhibited substantial decrease in GSH and SOD in liver and kidney tissue relative to control. CeONPs treatment increased GSH and SOD activities in liver and kidney tissues in CeONPS +FS group with respect to the FS group. This indicates the effect of CeONPs in reducing oxidative damage caused by FS. Rats treated with FS showed a marked elevation in MDA concentration in liver and kidney tissues, which reduced and restored near to control group in CeONPs + FS group. Reduced glutathione level in blood decreased significantly in FS group. Meanwhile, intraperitoneal injection of CeONPs increased GSH level significantly in CeONPs + FS group compared to FS group.

**Table (4)** The Hematological parameters were illustrated in table (4) which showed a notable increase in, hemoglobin concentration, RBCs and hematocrit in Ferrous sulfate (FS) group in comparable to the control group. Meanwhile, significant decrease in hemoglobin content, RBC's count and hematocrit value in cerium oxide nanoparticles (CeONPs) treated group in comparison with control group, also a slight decrease in hematological indices in rats treated with both CeONPs +FS relative to control group. FS group treated rats showed significant decrease in WBC's count than control group. Meanwhile, a significant increase in WBC's count in CeONPs + FS group as compared to FS group was observed. Results in table (4) also, demonstrated that Ferrous sulfate treatment increased serum iron level with substantial decrease in TIBC. Conversely, in CeONPs treated group serum iron level lessened markedly with a high increase in TIBC. A remarkable increase in serum iron level and a decrease in TIBC in groups treated with CeONPs + FS compared to control group.

**Table (1): Effect of CeONPs on liver function tests (AST, ALT, ALP, Total bilirubin, Total protein and Albumin) in Ferrous Sulfate treated rats**

| Groups<br>Parameters | Control group | FS group | CeONPs group | CeONPs + FS group |
|---|---|---|---|---|
| AST (U/L) | $48 \pm 1.87^{b}$ | $58 \pm 1.65^{a}$ | $45 \pm 1.39^{b}$ | $55 \pm 1.11^{a}$ |
| ALT (U/L) | $211.57 \pm 4.24^{c}$ | $255.57 \pm 3.46^{a}$ | $202.42 \pm 5.49^{c}$ | $223.85 \pm 2.15^{b}$ |
| ALP (U/L) | $137.39 \pm 1.98^{b}$ | $209.23 \pm 2.44^{a}$ | $137.49 \pm 2.04^{b}$ | $143.54 \pm 2.05^{b}$ |
| Total bilirubin(U/L) | $0.88 \pm 0.02^{b}$ | $0.94 \pm 0.02^{a}$ | $0.86 \pm 0.02^{b}$ | $0.91 \pm 0.01^{ab}$ |
| Total protein (mg/dl ) | $6.82 \pm 0.12^{c}$ | $11.14 \pm 0.27^{a}$ | $7.18 \pm 0.25^{c}$ | $9.10 \pm 0.24^{b}$ |
| Albumin(mg/dl) | $4.03 \pm 0.16^{a}$ | $3.79 \pm 0.24^{a}$ | $4.11 \pm 0.14^{a}$ | $4.01 \pm 0.11^{a}$ |

Values are expressed as mean ± SE .N=7.
Means with different superscript letters in the same raw (a,b,c) are significantly different at ($p \leq 0.05$)

**Table (2): Effect of CeONPs on kidney function tests, CRP, and Thyroxin levels in Ferrous Sulfate treated rats**

| Groups<br>Parameters | Control<br>group | FS<br>group | CeONPs<br>group | CeONPs + FS<br>group |
|---|---|---|---|---|
| Creatinine (mg/dl) | $0.66 \pm 0.02^{c}$ | $0.80 \pm 0.01^{a}$ | $0.58 \pm 0.03^{d}$ | $0.72 \pm 0.02^{b}$ |
| Urea (mg/dl) | $43.4 \pm 1.61^{b}$ | $58.8 \pm 0.51^{a}$ | $39.8 \pm 1.1^{c}$ | $42.2 \pm 1.05^{bc}$ |
| CRP (mg/l ) | $4.22 \pm 0.09^{c}$ | $6.86 \pm 0.10^{a}$ | $3.50 \pm 0..08^{d}$ | $5.44 \pm 0.12^{b}$ |
| T4 | $89.52 \pm 2.78^{a}$ | $55.21 \pm 1.58^{c}$ | $88.5 \pm 2.31^{a}$ | $70.08 \pm 1.61^{b}$ |

Values are expressed as mean ± SE .N=7.

Means with different superscript letters in the same raw (a,b,c) are significantly different at ($p \leq 0.05$)

**Table (3): Effect of Cerium Oxide NPs on hepatic and renal oxidant- antioxidant status ( SOD ,GSH and MDA) levels and GSH level in blood in Ferrous Sulfate treated rates**

| Groups<br>Parameters | Control<br>group | FS<br>group | CeONPs<br>group | CeONPs + FS<br>group |
|---|---|---|---|---|
| GSH in Liver (mg/g tissue) | $25.41 \pm 0.89^{a}$ | $14.87 \pm 0.72^{c}$ | $25.31 \pm 0.74^{a}$ | $21.99 \pm 1.02^{b}$ |
| GSH in Kidney (mg/g tissue) | $18.97 \pm 0.96^{b}$ | $12.66 \pm 0.59^{d}$ | $22.35 \pm 0.83^{a}$ | $15.59 \pm 0.26^{c}$ |
| GSH in blood(mg/dl) | $59.57 \pm 1.53^{a}$ | $34.77 \pm 1.12^{c}$ | $61.42 \pm 2.19^{a}$ | $47.46 \pm 1.27^{b}$ |
| SOD in Liver (u/g tissue) | $304.06 \pm 4.29^{ab}$ | $207.09 \pm 5.43^{c}$ | $269.82 \pm 5.13^{b}$ | $258.60 \pm 2.99^{bd}$ |
| SOD in Kidney (uμ/g tissue) | $327.22 \pm 2.06^{a}$ | $233.31 \pm 6.8^{b}$ | $332.91 \pm 17.3^{a}$ | $232.45 \pm 6.12^{a}$ |
| MDA in Liver (nmol/g tissue) | $22.74 \pm 0.73^{c}$ | $28.25 \pm 0.78^{a}$ | $17.48 \pm 0.76^{d}$ | $25.06 \pm 0.54^{b}$ |
| MDA in Kidney (nmol/g tissue) | $44.43 \pm 1.06^{b}$ | $51.32 \pm 1.08^{a}$ | $28.42 \pm 0.79^{c}$ | $45.79 \pm 0.85^{b}$ |

Values are expressed as mean ± SE .N=7.

Means with different superscript letters in the same raw (a,b,c) are significantly different at ($p \leq 0.05$)

**Table (4): Effect of Cerium Oxide NPs on blood parameters, Serum Iron and Total Iron Binding Capacity in Ferrous Sulfate treated rates**

| Groups<br>Parameters | Control<br>group | FS<br>group | CeONPs<br>group | CeONPs + FS<br>group |
|---|---|---|---|---|
| Hb (g/L) | $13.66 \pm 0.14^{b}$ | $14.47 \pm 0.20^{a}$ | $9.23 \pm 0.12^{d}$ | $12.49 \pm 0.14^{c}$ |
| RBC's($x10^{6}$) | $4.74 \pm 0.11^{b}$ | $5.50 \pm 0.19^{a}$ | $2.78 \pm 0.10^{d}$ | $4.11 \pm 0.08^{c}$ |
| HCT (%) | $40.46 \pm 0.45^{b}$ | $43.13 \pm 0.64^{a}$ | $27.72 \pm 0.35^{d}$ | $37.67 \pm 0.37^{c}$ |
| WBC's($x10^{3}$) | $11.32 \pm 0.22^{a}$ | $9.71 \pm 0.18^{c}$ | $10.57 \pm 0.29^{a\,b}$ | $10.23 \pm 0.31^{c\,b}$ |
| Iron(μ/dl) | 69.0±0.72c | 88 ± 1.79a | 59 ± 1.85d | 76 ± 0.95b |
| TIBC (μ/dl) | 186.25 ± 4.55b | 146.63 ± 4.90d | 205.23 ± 4.78a | 160.40 ± 1.75c |

Values are expressed as mean ± SE .N=7.

Means with different superscript letters in the same raw (a,b,c) are significantly different at ($p \leq 0.05$)

## DISCUSSION

Iron deficiency is one of the most prevalent nutritional disorders leading to mild or severe anemia [24]. The front line of iron deficiency anemia treatment usually takes the form of oral drugs mainly consisting of ferrous iron which is commonly absorbed but causes several implications such as constipation gastric irritation and nausea [25]; [26]. Such symptoms indicated the instability of ferrous iron that oxidizes to ferric iron in the presence of oxygen, with the production of reactive oxygen species (ROS) that cause direct inflammation of the intestinal mucosa [27]. In patients suffering from iron deficiency anemia, the rate of iron absorption from a recommended dose of ferrous sulfate (FS) is about ~10% [28] in doses ranged from 10 mg in children to 30 mg in pregnant women [29].

In this study, FS exhibited elevation of hepatic enzymes activities where excessive iron deposition in the liver activates liver damage partly because of hepatocyte damage and partly because it induces oxidative stress and increases ROS production [30, 31]. Serum level of total bilirubin was increased in the FS group, which is in line with the results of previous studies which demonstrated that iron caused hepatic injury; elevate levels of serum total bilirubin [32].

CeONPs treatment markedly ameliorates the serum levels of AST, ALT, ALP and total bilirubin and protects liver against iron–induced toxicity in FS+ CeONPs group. This could be attributed to antioxidant activity of CeONPs. As mentioned earlier the double oxidation state of CeONPs potentiates its scavenging ability; in addition CeONPs can mimic enzyme antioxidants.

Iron overload is known to result in elevated serum creatinine and blood urea [33]. The present study showed that FS treatment induced renal damage and elevation in serum levels of creatinine and urea. Treatment with CeONPs probably through its antioxidative effect significantly reduced the FS-induced renal damage and kidney enzymes level decreased .Iron induces oxidative stress [34].

Oxidative stress arises due to an inequality of antioxidants and oxidants (ROS). Excessive production of ROS has potent cellular oxidizing potential and has harmful effects on different organ systems. SOD, GSH are presumed to be responsive indicators for hepatic and nephric oxidative stress factors. Glutathione (GSH) is the initial line of protection from oxidative stress effects and is an essential enzyme required for liver detoxifications, the decrease in GSH levels may be because of glutathione was consumed in the elimination of free radicals .Also, SOD stimulates the dismutations of superoxide radicals to hydrogen peroxide $H_2O_2$[35].

The results obtained in the current study demonstrated that FS promotes oxidative stress in the liver and kidney by decreasing SOD, GSH levels and increasing lipid peroxidation as proved by the increased levels of MDA, where the ROS resulted from FS can bind to protein and membrane lipids to produce MDA, causing cells damage. MDA is one of the primary reasons induced liver and intestine damage. CeONPs offer many active sites for the radical scavenging due to its large surface and the mixed valence states for unique redox chemistry, in which an auto regenerative reaction cycle continuous on the surface [$Ce^{3+} \rightarrow Ce^{4+} \rightarrow Ce^{3+}$] [36] by which CeONPs gain an unexpected free radical scavenging potential[37]. In the current study, significant restoration of glutathione and SOD in the CeONPs +FS group and a significant decrease in MDA level [38] showed that the antioxidant activity of CeONPs was through raising the antioxidant SOD level and lowering the lipid peroxidation marker MDA.

C-reactive protein (CRP) is a sensitive indicator of acute inflammation and tissue damage. FS treatment increases CRP level group. It is known that in the presence of oxygen, ferrous ions can be oxidized with generation of reactive oxygen species ROS, which intern cause inflammatory effects in the gut and at a systemic level [27]. CeONPs have high ability to eliminate free radicals, so decreasing CRP level in CeONPs +FS treated rats.

The present results showed a decrease in thyroid gland function in FS treated rats this revealed by the significant decrease in the level of T4. This may be attributed to the reason that FS caused thyroid injury through increasing oxidative stress by disturbing the oxidant and antioxidant balance.

Hemoglobin is the principal component of red blood cells that convey oxygen to different parts of the body to be utilized by tissue [39].As iron is the main element of hemoglobin constitution, FS supplementation increases Hb content notably which was demonstrated in FS treated rats [40]. In CeONPs group Hb content was significantly lower than normal rats due to decreased iron level (table4).Where low serum iron consequently decreases iron in blood circulation leading to decreased Hb content and consequently RBC᾽s and HCT value[41].

Balance of iron occurs through the different steps that start with its uptake by intestine, iron transfer to other cells and its distribution to blood. It is allocated by transferrin (protein carrier) and deposited in many organs commonly liver and spleen as ferritin [42]. TIBC is the extreme quantity of iron necessary to combined with transferrin in blood. High doses of received or supplemented iron, increases iron level and reduce total iron binding capacity causing transferrin saturation to increase. In FS group iron is absorbed rapidly showed a high level of serum iron (SI) and low TIBC. CeONPs decreased SI, resulting in increased TIBC.

## CONCLUSION

In this study ferrous sulfate (FS) can induce damage in the kidney and liver partly due to its direct effect on hepatocytes & nephrons and mainly through its oxidative stress .Meanwhile, CeONPs activate the antioxidant enzyme system against oxidative stress caused by FS, and attenuated lipid peroxidation (MDA) in liver and kidney. However CeONPs have a negative effect on hematological parameters and the mechanism whereby CeONPs exert this effect will be further studied.

## REFERENCES

[1] Scholl, T. O. and Hediger, M. L. (1994). Anemia and iron-deficiency anemia: compilation of data on pregnancy outcome. *The American journal of clinical nutrition*, ***59***(2), 492S-501S.

[2] Barooti, E., Rezazadehkermani, M., Sadeghirad, B., Motaghipisheh, S., Tayeri, S., Arabi, M., Salahi, S. and Haghdoost, A.A. (2010). Prevalence of iron deficiency anemia among Iranian pregnant women; a systematic review and meta-analysis. *Journal of reproduction & infertility*, **11**(1), p.17.

[3] Low, M., Farrell, A., Biggs, B. A., and Pasricha, S. R. (2013). Effects of daily iron supplementation in primary-school–aged children: systematic review and meta-analysis of randomized controlled trials. *Cmaj*, **185(**17), E791-E802..

[4] Poprac, P., Jomova, K., Simunkova, M., Kollar, V., Rhodes, C. J., and Valko, M. (2017). Targeting free radicals in oxidative stress-related human diseases. *Trends in pharmacological sciences*, **38**(7), 592-607.

[5] Papanastasiou, D. A., Vayenas, D. V., Vassilopoulos, A., and Repanti, M. (2000). Concentration of iron and distribution of iron and transferrin after experimental iron overload in rat tissues in vivo: study of the liver, the spleen, the central nervous system and other organs. *Pathology-Research and Practice*, **196**(1), 47-54.

[6] Anderson, G. J., and Frazer, D. M. (2005, November). Hepatic iron metabolism. In *Seminars in liver disease* (Vol. 25, No. 04, pp. 420-432). Copyright© 2005 by Thieme Medical Publishers, Inc., 333 Seventh Avenue, New York, NY 10001, USA..

[7] Zager, R. A., Johnson, A. C., and Hanson, S. Y. (2004). Parenteral iron nephrotoxicity: potential mechanisms and consequences. *Kidney international*, **66**(1), 144-156.

[8] Madhusudhan, K., and Oberoi, R. (2011). Renal iron deposition in aplastic anemia: Magnetic resonance imaging appearance. *Indian Journal of Nephrology*, **21**(2), 134.

[9] Werner, T., Wagner, S.J., Martínez, I., Walter, J., Chang, J.S., Clavel, T., Kisling, S., Schuemann, K. and Haller, D.(2011). Depletion of luminal iron alters the gut microbiota and prevents Crohn's disease-like ileitis. *Gut*, **60**(3),pp.325-333..

[10] Moridi, H., Hosseini, S.A., Shateri, H., Kheiripour, N., Kaki, A., Hatami, M. and Ranjbar, A.(2018). Protective effect of cerium oxide nanoparticle on sperm quality and oxidative damage in malathion-induced testicular toxicity in rats: An experimental study. *International journal of reproductive biomedicine*, **16**(4), p.261.

[11] Patil, S., Sandberg, A., Heckert, E., Self, W. and Seal, S.(2007). Protein adsorption and cellular uptake of cerium oxide nanoparticles as a function of zeta potential. *Biomaterials*, **28**(31), pp.4600-4607. [12] Chung D. (2003). Nanoparticles have health benefits too. New Scientist.179:2410–2416.7.

[13] Zhang, F., Wang, P., Koberstein, J., Khalid, S. and Chan, S.W.(2004). Cerium oxidation state in ceria nanoparticles studied with X-ray photoelectron spectroscopy and absorption near edge spectroscopy. *Surface Science*, **563**(1-3), pp.74-82.

[14] Montini, T., Melchionna, M., Monai, M. and Fornasiero, P.(2016). Fundamentals and catalytic applications of CeO2-based materials. *Chemical reviews*, **116**(10), pp.5987-6041.

[15] Heckert, E.G., Karakoti, A.S., Seal, S. and Self, W.T.(2008). The role of cerium redox state in the SOD mimetic activity of nanoceria. *Biomaterials*, **29**(18), pp.2705-2709.

[16] Pirmohamed, T., Dowding, J.M., Singh, S., Wasserman, B., Heckert, E., Karakoti, A.S., King, J.E., Seal, S. and Self, W.T.(2010). Nanoceria exhibit redox state-dependent catalase mimetic activity. *Chemical communications*, **46**(16), pp.2736-2738.

[17] Khurana, A., Tekula, S. and Godugu, C.(2018). Nanoceria suppresses multiple low doses of streptozotocin-induced Type 1 diabetes by inhibition of Nrf2/NF-κB pathway and reduction of apoptosis. *Nanomedicine*, **13**(15), pp.1905-1922..

[18] Pagliari, F., Mandoli, C., Forte, G., Magnani, E., Pagliari, S., Nardone, G., Licoccia, S., Minieri, M., Di Nardo, P. and Traversa, E.(2012). Cerium oxide nanoparticles protect cardiac progenitor cells from oxidative stress. *ACS nano*, **6**(5), pp.3767-3775.

[19] Manne, N.D., Arvapalli, R., Nepal, N., Shokuhfar, T., Rice, K.M., Asano, S. and Blough, E.R.(2015). Cerium oxide nanoparticles attenuate acute kidney injury induced by intra-abdominal infection in Sprague–Dawley rats. *Journal of nanobiotechnology*, **13**(1), pp.1-11.

[20] Nourmohammadi, E., Khoshdel-Sarkarizi, H., Nedaeinia, R., Sadeghnia, H.R., Hasanzadeh, L., Darroudi, M. and Kazemi oskuee, R.(2019). Evaluation of anticancer effects of cerium oxide nanoparticles on mouse fibrosarcoma cell line. *Journal of cellular physiology*, **234(**4), pp.4987-4996.

[21] Chen, S., Hou, Y., Cheng, G., Zhang, C., Wang, S. and Zhang, J.( 2013). Cerium oxide nanoparticles protect endothelial cells from apoptosis induced by oxidative stress. *Biological trace element research*, **154**(1), pp.156-166..

[22] Gholampour, F. and Saki, N.(2019). Hepatic and renal protective effects of quercetin in ferrous sulfate-induced toxicity. *Gen Physiol Biophys*, **38**(1), pp.27-38.

[23] Hajinezhad, M.R., Shahri, S.H., Rahdar, A. and Zamanian, H.(2020). Effects of cerium oxide nanoparticles on biochemical parameters and histopathological changes in lead-intoxicated rats. *Disease and Diagnosis*, **9(**4), pp.134-139.

[24] Kassebaum, N.J., Jasrasaria, R., Naghavi, M., Wulf, S.K., Johns, N., Lozano, R., Regan, M., Weatherall, D., Chou, D.P., Eisele, T.P. and Flaxman, S.R.(2014). A systematic analysis of global anemia burden from 1990 to 2010. *Blood, The Journal of the American Society of Hematology*, **123(**5), pp.615-624.

[25] Tolkien, Z., Stecher, L., Mander, A.P., Pereira, D.I. and Powell, J.J.(2015). Ferrous sulfate supplementation causes significant gastrointestinal side-effects in adults: a systematic review and meta-analysis. *PloS one*, **10**(2), p.e0117383.

[26] Girelli, D., Ugolini, S., Busti, F., Marchi, G. and Castagna, A.(2018). Modern iron replacement therapy: clinical and pathophysiological insights. *International journal of hematology*, **107(**1), pp.16-30. [27] Toblli, J.E., Cao, G., Olivieri, L. and Angerosa, M.(2008). Comparative study of gastrointestinal tract and liver toxicity of ferrous sulfate, iron amino chelate and iron polymaltose complex in normal rats. *Pharmacology*, **82**(2), pp.127-137.

[28] McDonagh, T. and Macdougall, I.C.(2015). Iron therapy for the treatment of iron deficiency in chronic heart failure: intravenous or oral?. *European journal of heart failure*, **17**(3), pp.248-262.

[29] Alleyne, M., Horne, M.K. and Miller, J.L.(2008). Individualized treatment for iron-deficiency anemia in adults. *The American journal of medicine*, **121(**11), pp.943-948..

[30] Badria, F.A., Ibrahim, A.S., Badria, A.F. and Elmarakby, A.A.(2015). Curcumin attenuates iron accumulation and oxidative stress in the liver and spleen of chronic iron-overloaded rats. *PLoS One*, **10**(7), p.e0134156.

[31] İdilman, İ.S., Akata, D., Özmen, M.N. and Karçaaltıncaba, M.(2016). Different forms of iron accumulation in the liver on MRI. *Diagnostic and Interventional Radiology*, **22**(1), p.22.

[32] Das, A., Chaudhuri, D., Ghate, N.B., Panja, S., Chatterjee, A. and Mandal, N., 2015. Protective effect of Clerodendrum colebrookianum leaves against iron-induced oxidative stress and hepatotoxicity in Swiss albino mice.. *Indian J. Exp. Biol.* **53**, 281–291

[33] Kadkhodaee, M. and Gol, A.(2004). The role of nitric oxide in iron-induced rat renal injury. *Human & experimental toxicology*, **23**(11), pp.533-536.

[34] Li, Z., Geng, Y.N., Jiang, J.D. and Kong, W.J.(2014). Antioxidant and anti-inflammatory activities of berberine in the treatment of diabetes mellitus. *Evidence-based complementary and alternative medicine*, *2014*, 289264

[35] Tao, X., Xu, Z.R. and Wang, Y.Z.(2006). Effects of dietary fluoride levels on growth, serum indexes and antioxidant systems in growing pigs. *Turkish Journal of Veterinary and Animal Sciences*, **30**(1), pp.65-70.

[36] Das, M., Patil, S., Bhargava, N., Kang, J.F., Riedel, L.M., Seal, S. and Hickman, J.J.(2007). Auto-catalytic ceria nanoparticles offer neuroprotection to adult rat spinal cord neurons. *Biomaterials*, **28**(10), pp.1918-1925.

[37] Colon, J., Herrera, L., Smith, J., Patil, S., Komanski, C., Kupelian, P., Seal, S., Jenkins, D.W. and Baker, C.H.(2009). Protection from radiation-induced pneumonitis using cerium oxide nanoparticles. *Nanomedicine: Nanotechnology, Biology and Medicine*, **5**(2), pp.225-231.

[38] Oró, D., Yudina, T., Fernández-Varo, G., Casals, E., Reichenbach, V., Casals, G., de la Presa, B.G., Sandalinas, S., Carvajal, S., Puntes, V. and Jiménez, W.(2016). Cerium oxide nanoparticles reduce steatosis, portal hypertension and display anti-inflammatory properties in rats with liver fibrosis. *Journal of hepatology*, **64**(3), pp.691-698.

[39] Yip, R.(1998). Parvanta I. Cogswell ME Mcdonnell SM Bowman BA Grummer-Strawn LM Trowbridge FL Recommendations to prevent and control iron deficiency in the United States. *Morb. Mortal. Wkly. Rep*, **47**, pp.1-29.

[40] Priwitzerova, M., Pospisilova, D., Prchal, J.T., Indrak, K., Hlobilkova, A., Mihal, V., Ponka, P. and Divoky, V.(2004). Severe hypochromic microcytic anemia caused by a congenital defect of the iron transport pathway in erythroid cells. *Blood*, **103(**10), pp.3991-3992.

[41] Matsumoto, N., Ikeda, H., Shigefuku, R., Hattori, N., Watanabe, T., Matsunaga, K., Hiraishi, T., Tamura, T., Noguchi, Y., Fukuda, Y. and Ishii, T.(2016). Hemoglobin decrease with iron deficiency induced by daclatasvir plus asunaprevir combination therapy for chronic hepatitis C Virus genotype 1b. *Plos one*, **11**(3), p.e0151238.

[42] De-Regil, L.M., Jefferds, M.E.D., Sylvetsky, A.C. and Dowswell, T.(2011). Intermittent iron supplementation for improving nutrition and development in children under 12 years of age. *Cochrane Database of Systematic Reviews*, (12). CD009085.