Impact of Bone Marrow Mesenchymal Stem Cells on Different Body Organs of Albino Rats Exposed To Cadmium Chloride Toxicity: A Systematic Review

Elham H. Ahmed [1], Mohammed E. Grawish[2], Mohamed Abdulrahman[3], Menatalla M. Elhindawy[4], Mohamad E. Helal[2]

[1]Assistant Lecturer, Faculty of Dentistry, Mansoura University, Egypt, [2]Professor of Oral Biology, Faculty of Dentistry, Mansoura University, Egypt [3]Assistant Professor of Oral Biology, Faculty of Dentistry, Mansoura University, Egypt, [4]Lecturer of Oral Biology, Faculty of Dentistry, Mansoura University, Egypt

Abstract

Background and Objective: Cadmium chloride (Cd cl_2) is a highly toxic heavy metal that causes severe degenerative effects to different body organs. Bone marrow mesenchymal stem cells (BMMSCs) may have a protective effect upon $CdCl_2$ induced structural and functional changes in the rat's organs. This systematic review aimed to test the regenerative effects of bone marrow-derived mesenchymal stem cells on different body organs of albino rats exposed to cadmium chloride toxicity.

Methods: Experimental animal studies were identified by the following electronic databases: PubMed, Scopus, Ovid Medline, KoreaMed, Google Scholar, Latin American and Caribbean Health Sciences Literature (LILACS) and Central Cochrane Library. The literature search was done from January 2010 to 2020.

Conclusion: From the selected articles, using stem cell therapy enhances the regeneration process in $CdCl_2$ toxicity of different body organs.

Keywords*: Cadmium chloride; bone marrow stem cells; rats; systematic review.*

Introduction

Civilization advances and increased environmental pollution exaggerate the effects of different xenobiotics as heavy metals on the function of the living organism [1-3]. Heavy metals are defined as trace naturally occurring metallic elements that found throughout the crust of the earth and have high density when compared to water [4]. Human exposure to these metals has been increased dramatically due to their excessive use in industry, agriculture and technological applications [5]. Cadmium is one of the most abundant and toxic heavy metals. It is the 17th most toxic metal and its concentration in earth crust reach about 0.1 mg/kg. It accumulates in body throughout the life. Human exposed to Cd by inhalation and ingestion from plants leading to acute and chronic intoxications. Cd remains in soils and sediments for many years. Plants take up this metal which gets accumulated in them and concentrates along the food chain, reaching the human body [6, 7]. Once absorbed, Cd is rapidly cleared from the blood and concentrates in various tissues. Chronic exposure to inorganic Cd results in accumulation of the metal mainly in the liver and kidneys, as well as in other tissues and organs causing many metabolic and histological changes, membrane damage, altered gene expression and apoptosis [8]. Mesenchymal stem cells are excellent option for cell therapy because they are easily accessible, cells can expand to clinical scales in a relatively short time and

Corresponding author:
Elham H. Ahmed
Assistant Lecturer, Faculty of Dentistry, Mansoura University, Egypt Postal Adress: Dikerness, Dakahlia, Egypt, Mobile: +201018777245
e-mail: elhamhahmed2017@yahoo.com

can be preserved with minimal loss of potency [9]. The regenerative potential of MSCs has been widely studied as it included in treating tissue lesions caused by ionizing radiation and clinical radiotherapy. Animal studies and early clinical experiences suggested a role for MSCs in the regeneration of these tissue injuries by differentiating into functional parenchymal cells and creating a nurturing microenvironment for other cells [10]. According to PICO, the research question for this systematic review was that 'dose BMMSCs reduce cadmium toxicity in rat's different body organs. The population was rats; the intervention was use of stem cells to treat cadmium induced toxicity; the comparator was control positive groups that receive cadmium without stem cell treatment; the outcome was the therapeutic effects of stem cells on toxicity induced by Cd in different body organs.

Methods

Protocol development and eligibility criteria: The question was formulated using the PICO format for the research question construction. The methodology of this SR was designed on the basis of the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) [11].

Inclusion criteria: All the studies involved in this SR should have the following criteria: **(1)** Experimental animals exposed to Cd were only rats, **(2)** The toxicity was induced by cadmium chloride only, **(3)** Treatment option was BMMSCs, **(4)** Affected structures were different body organs, **(5)** All included studies must contain control group that receive cadmium chloride with no treatment, **(6)** The main evaluation is based on the histopathological and/or laboratory evaluation tests and **(7)** The outcomes were degenerative changes of Cd toxicity in different body organs and regenerative effects of BMMSCs.

Exclusion criteria were **(1)** Animals other than rats, **(2)** Therapeutic modalities other than BMMSCs, **(3)** Toxicity induced by heavy metals other than Cd toxicity, **(4)** Studies with no control group, **(5)** Reviews, **(6)** Book and book chapter, **(7)** Case reports, (**8)** Clinical studies, **(9)** Studies on human and **(10)** In vitro studies.

Information sources and search strategy: The PROSPERO and the Cochrane Database of SRs were searched in 10 August 2020 and no existing reviews were found dealing with the effect of BMMSCs on Cd-induced toxicity in different body organs. To obtain eligible studies related to our criteria, comprehensive search of peer reviewed literature, published up to 2020, was performed of the electronic databases PubMed, Scopus, Ovid Medline, koreaMed, Google Scholar, Latin American and Caribbean Health Sciences Literature (LILACS) and Central Cochrane Library. The literature search was done from 2010 to 2020. The population was rats; the intervention was use of stem cells to treat cadmium induced toxicity; the comparator was control positive groups that receive cadmium without stem cell treatment; the outcome was the therapeutic effects of stem cells on toxicity induced by Cd in different body organs. As mentioned before, the research question for this systematic review was that 'dose BMMSCs reduce cadmium toxicity in rat's different body organs. All searches were performed independently by two authors (EH and ME) to identify published articles related to the focused question.

Searching keywords: Search word/term was performed as following: stem cells and cadmium toxicity **or** bone marrow stem cells and cadmium chloride and rats **or** stem cells and cadmium and salivary glands and rats **or** "cadmium chloride" toxicity and bone marrow stem cells in rats.

Study selection and data collection: Two authors (EH&MM) screened all the titles and abstracts, unrelated studies at this stage were excluded. All the potentially related studies were evaluated independently and the relevant studies were extracted by each author. Disagreements between the two authors about study inclusion or exclusion criteria were solved by third author (MEH). The data of interest from the selected studies was tabulated and the following data were obtained: authors and their country, journal (publication year), number of study groups, number of rats used in the study, gender and weight, type of stem cells and their concentration, duration of the study and outcomes. Fields for which information not found in a publication were entered as "unknown.

Assessment of risk of bias (ROB) in included studies: To assess the quality of the articles, two authors (EH&MEG) independently used the SYRCLE's risk

of bias (RoB) tool [17]. They assessed selection bias (sequence generation, baseline characteristics, allocation concealment), performance bias (random housing, blinding of the operators), detection bias (random outcome assessment, blinding of outcome assessor), attrition bias (incomplete outcome data, selective outcome reporting) and other sources of bias. Each item was assessed to be low, high, or unclear RoB. "Unclear" means either lack of information or uncertainty over the possibility of bias ***Table 1*** Any disagreements in the assessment were also resolved by discussion with a third author (MA).

Statistical analysis: Degree of chance – adjusted agreement (kappa coefficient value) was used to determine the inter-reviewer reliability.

Table 1: risk of bias assessment using syrcle's risk of bias tool for animal studies

Author	Selection bias			Performance bias		Detection bias		Attrition bias			Overall risk
	Sequence generation	Baseline characterisrics	Allocation sequence concealment	Random housing	Blinding of operator	Random of outcome assessment	Blinding of outcome assessor	Incomplete outcome data	Selective outcome reporting	Other potential sources of bias	
Elbaghdady H A M et al [12]	High risk	Low risk	High risk	Low risk	High risk	Unclear risk	Unclear risk	Unclear risk	Low risk	Low risk	High risk
Elbaghdady HAM et al [13]	Low risk	Low risk	High risk	Unclear	High risk	Unclear risk	Unclear risk	Unclear risk	Low risk	Low risk	High risk
Wang YJ et al [14]	Low risk	Low risk	High risk	Low risk	High risk	Unclear risk	Unclear risk	Unclear risk	Low risk	Low risk	Low risk
Abdel Latif H et al [15]	High risk	Low risk	High risk	Low risk	High risk	Unclear risk	Unclear risk	Low risk	Low risk	Low risk	High risk
Hussein YM et al [16]	Low risk	Low risk	High risk	Low risk	High risk	Unclear risk	Unclear risk	Unclear risk	Low risk	Low risk	High risk

Results

The total articles identified from the search through PubMed, Scopus, Ovid Medline, koreamed, Google Scholar, LILACS and Central Cochrane Library were 1967 articles. After filtering, 1259 were excluded, in addition to 43 duplicates were removed. After the eligibility criteria were applied on the remaining 665 articles, five unduplicated studies were included in this review. A flow chart for the selection process is presented in ***figure1***

Study characteristics: According to the publication date, the included five studies were divided into one performed in *Saudi Arabia* at 2015 [16], one in china at 2017 [14], two in Egypt at 2018 [12, 13, 15]. All studies used cadmium chloride to induce toxicity with different concentrations ranging from, 0.4-5 mg/kg body weight. One study induce cadmium toxicity by intraperiotonial (IP) injection of 0.4mg/kg body weight five times per week for five weeks [14], two studies used single IP dose of 2 mg/kg body weight [12,13], one study inject cadmium IP with the dose of 1 ml/kg saline (solvent of Cd) for 3 days 2020 [15] and the remaining study used Cd orally with the dose of 5 mg/kg body wt/day) for 30 days [16]. All studies concentrated on testis as an organ of interest [13-16]. except one study had taken liver, kidney and testis as organs for the study [12]. According to the treatment used, BMMSCs used in all studies with concentration ranged from 1×10^6 in three studies [12,15,16], 1×10^7 cells in one study [14] and 2×10^6 in one study [13]. Four

studies injected BMMSCs intravenously (via retro-orbital, penile vein, portal vein and via tail vein) [12, 13, 14, 15] and one study via intratesticular injection [16].

Results of risk of bias within the study: The overall RoB for the included studies was low risk in one study [14] and high risk in four studies [12, 13, 15, 16] due to the lack of random allocation concealment of the rats in the study groups, unmentioned blindness of investigator and assessor in most of the studies, in addition to unmentioned information about random housing of animals, if there have the same housing conditions or not. In addition, the reporting of the outcome for the most of studies was not mentioned, if there were any damaged or missing samples or not.

Study of low risk of bias: Wang et al [14] used $Cdcl_2$ to induce damage in testis of rats with dose of 0.4 mg/kg body weight by intraperiotonial injection 5 times per week for five weeks. Five weeks following Cd exposure; 5×10^6 BMMSCs were transplanted into rats by retro-orbital injections on two consecutive days (1×10^7 BMSCs in total) and sacrificing 2 weeks after cell transplantation. H&E stain showed that testes appeared to have varying degrees of injury as atrophy, endothelial cell swelling and reduced layers of mature cells. The apoptosis rate markedly decreased, body weights were higher in stem cell group than Cd group and greater Cd accumulation was detected when compared with the control group. Histologically, there was a significant improvement in the pathological changes caused by Cd in model group including more cell layers and regularly arranged spermatogenic cells layers. The status of rats improved significantly compared with the model rats.

Studies of high risk of bias: Elbaghdady et al [12] used Cd to induce testicular toxicity; each rat received 2 mg/kg IP Cd dissolved in normal saline. Sperms collection, counting and viability were assessed. A lesser weight gain was recorded in Cd-group as compared to the controls. Histologically; necrosis, marked interstitial fibrosis and infiltration of mononuclear inflammatory cells are the main obvious changes. Testis showed an overall atrophy thus; the study supposed that Cd impairs the reproductive capacity caused by the severe testicular damage. BMMSCs used to treat Cd-induced toxicity in testis. Each rat received 2 mg/kg intra-peritoneal $Cdcl_2$ dissolved in normal saline then the first dose of 1×10^6 stem cells in 0.2 ml DMEM injected intravenously via penile vein. After one week, the second dose of 1×10^6 SCs was given. Results showed that there was an overall reduction in the severity of $Cdcl_2$-induced pathological damage after BMMSCs treatment. Higher body weight gain and higher sperm count and sperm were recorded in comparison with the Cd-group. **Elbaghdady et al [13]** also used Cd to induce hepato-renal and testicular toxicity. 2 mg/kg $Cdcl_2$ dissolved in normal saline was injected IP for each rat. When compared to the control group, the rats treated with $Cdcl_2$ showed a great decrease in the total number, motility and viability of sperm. There were high levels of abnormal sperm morphology. Lipid peroxidation and oxidative stress were associated with the increase in testicular necrosis and lowered sperm count and viability. Significant abnormalities in all measured biochemical analyses were documented. Rats showed marked decrease in total protein levels and significant increases in serum uric acid and creatinine levels and this may be due to kidney damage caused by $Cdcl_2$ exposure. After Cd treatment, the rats received two successive doses of MSCs separated by one week, each dose of 1×10^6 cells/ rat suspended in 0.2 ml DMEM via intravenous injection into the portal veins. Results showed a statistically significant increase in sperm total count, viability and motility in comparison with the controls. Improvement in all biochemical measures were recorded to the extent that they reached the normal values. The microscopic examination of liver sections of stem cells treated animals showed marked reduction in the hepatic vacuolation, mild leukocytic infiltration and significant decrease in cell degeneration. **Abdel Latif et al [15]** used $Cdcl_2$ to induce toxicity in their study. $CdCl_2$ was dissolved in normal saline and injected intra-peritoneally, each rat received1 mg/ kg for consecutive three days. Rats were received BMMSCs injection once via tail vein, at a dose of 1×10^6 dissolute in 0.5 ml of PBS after Cd treatment for 3 days and remained for 4 weeks. Sections of the stem cell-treated group showed normal architecture of tubules, minimal fluid exudates and vacuoles. Serum levels of testosterone showed marked increase (137%) while MDA and NO levels were decreased as related to non-treated group. Moreover, there were 154% significant increases in the mean values SOD and 138% significant increments in the mean values of GSH in relation to that of non –treated group. BAX level recorded significant decrease, however Bcl-2 levels

were decreased. **Hussein et al [16]** induced toxicity to rats using Cd at the dose of 5 mg/kg body weight dissolved in water daily via oral administration for 30 days then blood samples were collected from retro-orbital plexus and used for detection of sex hormones using ELISA, liver and kidney functions and total antioxidant capacity. Oxidative stress parameters **(**MDA and NO) contents and antioxidant parameters **{**reduced glutathione (GSH), Superoxide dismutase (SOD) activity and CAT activity} were estimated. Results revealed that there was marked decrease in the body weight after 4 weeks of Cd ingestion with gradual and sustained significant decrease in the weights of left and right testis. There was a significant decrease in serum levels of testosterone and LH and marked elevation in FSH, prolactin serum levels, MDA, NO, CAT, ALT, AST, ALP, creatinin and urea levels in relation to control values, while total protein and albumin were not affected. Decrease in sperm count and sperm motility was recorded. BMMSCs were used to regenerate damaging effects of Cd. 24 hours after the last dose of $cdcl_2$, animals were received a single intratesticular injection of rat BMMSCs containing 1×10^6 cells then left for 30 days. Results revealed that treatment of cd-exposed rats with stem cells caused body weight gain, reversed or fixed all injuries caused by $cdcl_2$ and retained to control levels. Significant improvement in the sperm count and motility so, they indicated that BMMSCs may play an important role in recovering the testicular function of adult rats.

Discussion

This systematic review aimed to assess the therapeutic and protective effects of BMMSCs as a treatment option for Cd induced toxicity, so the experimental animal chosen for this review was the albino rats, as they provide an appropriate model to study naturally or experimental occurring cadmium toxicity to different body organs. All the included studies were published in the last 10 years. Heavy metals are metallic elements that have high density compared to water [18]. There is an increasing ecological and global public health concerns related to environmental contamination by these metals and exposure of human has increased as a result of their frequent use in many industrial, technological and agricultural applications [5]. Industrial progress has brought human into close contact with several injurious chemicals, including heavy metals such as cadmium, lead and mercury. Cd is a highly toxic metal with widespread exposure to human causing tissue damage that lack effective treatment [14]. It presents at low levels but human activity as tobacco smoking has greatly increased its level. Exposure to this metal can occurs in the workplace and in the environment as it is utilized in a number of industrial practices and is considered as a contaminant of the environment and dietary products [19]. Cadmium accumulation occurs mainly in the soft tissues as the liver, kidney and testes [20, 21]. This metal acts as a catalyst so its toxicity associated with oxidative tissue damage. Cd increases the production of ROS as a result of its inhibitory effects on mitochondrial electron transport [22]. The present review aimed to investigate the effect of injected BMSCs to decrease the hazardous effects of $Cdcl_2$ which induced changes in some biochemical and histological parameters in different body organs of rats.it was concluded that BMMSCs have the ability to improve and recover the cellular damage induced by Cd exposure in testis, liver and kidney of rats. It was not possible to perform a direct comparison between the selected studies due to different variations between them in number of stem cells implanted, concentration of Cd, route of toxicity induction, period of the experiment and the organs of interest, so quantitive meta-analysis of the data could not be carried out. Irrespective of all of that difference, the majority of the studies demonstrated a statistically significant improvement in the outcome measures between the groups used stem cells as a treatment for Cd induced toxicity. After all of that, the present review has some limitations. Firstly, studies selected should be written in English, but this under represents the studies written by any language other than English. Secondly, the finding of most of the included studies don't based on follow up periods even short follow up periods as long follow up period will be difficult to be done in animal studies. Thirdly, the toxicity induction in these studies has different concentrations, periods and organs of concern. Also stem cells used of different numbers and routs of administration that may affect the regeneration.

Conclusion

The finding from this systematic review demonstrates that Cd induces great injury to different body organs. Using stem cell therapy enhances and improves the regeneration process in Cd toxicity of

different body organs so they can be used as a therapeutic potential to overcome harmful effects of such toxicity.

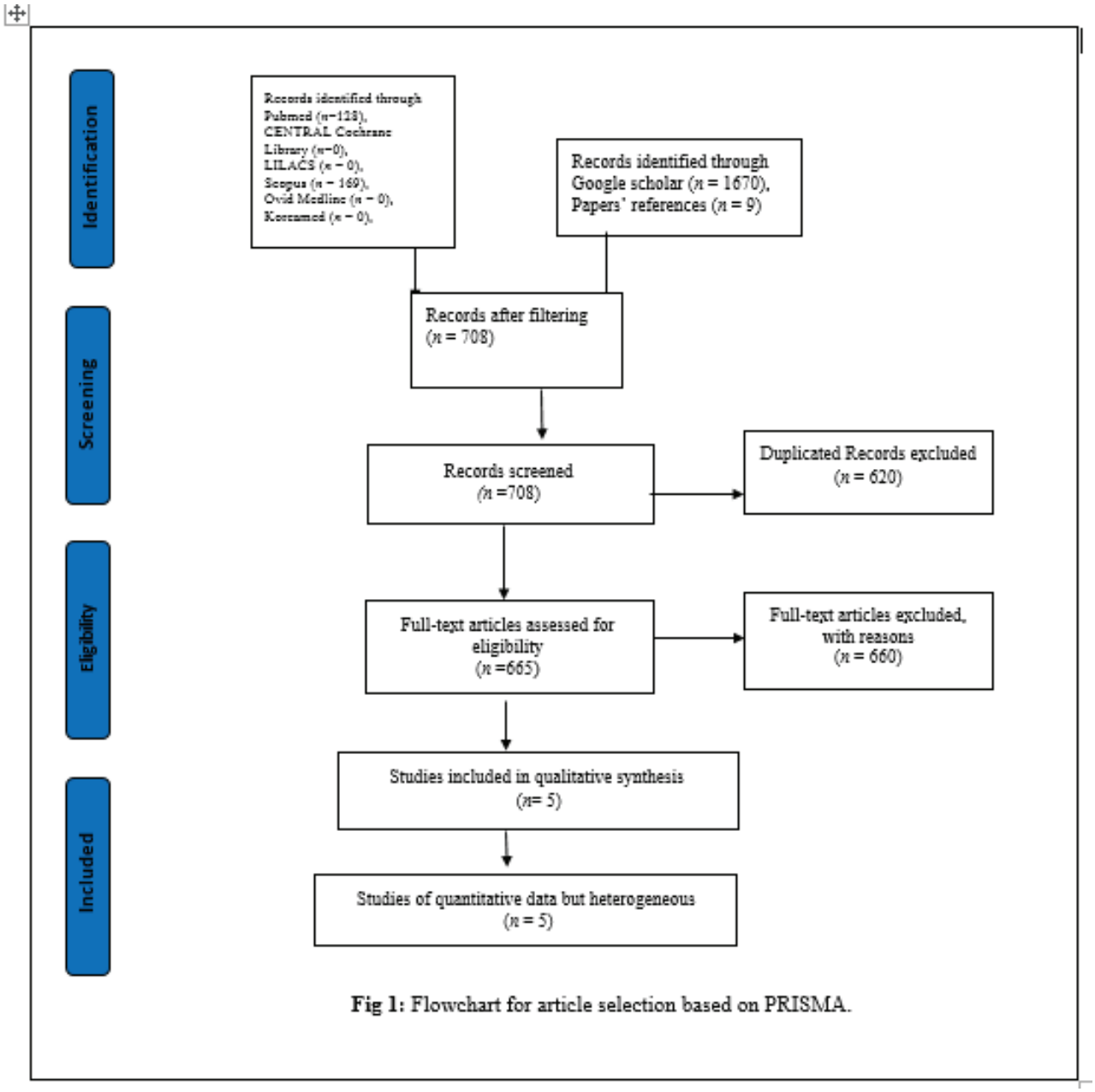

Fig 1: Flowchart for article selection based on PRISMA.

Fig 1: Flowchart for article selection based on PRISMA.

Ethical Clearance- Taken from faculty of dentistry ethical committee, Mansoura, Egypt

Source of Funding- Self.

Conflict of Interest -Nil.

References

1. Moniuszko-Jakoniuk J, Brzóska MM. Environment pollution and Health. Lek Wojsk. 1999;75,7-8: 419-26.
2. Kulikowska E, Moniuszko-Jakoniuk J, Miniuk K. Role of zinc in physiologic and pathologic processes in the organism. Pol Tyg Lek. 1991 Jun;46(24-26):470–3.
3. Waszkiel D, Dąbrowska E, Stokowska W. Influence of the work environment of the dentition glassblowers. Czas Stomatol. 1998;51(1):13–7.
4. Ferguson JE. The Heavy Elements, In: Chemistry, Environmental Impact and Health Effects,

Oxford, Pergamon Press. 1990;8:211–212.

5. Bradl H. Heavy Metals in the Environment: Origin, Interaction and Remediation. London: Academic Press. 2002; 6: 1-282.
6. WHO/FAO/IAEA. World Health Organization. Switzerland: Geneva; Trace Elements in Human Nutrition and Health. 1996.
7. Mutlu A, Lee BK, Park GH, Yu BG, Lee CH. Long-term concentrations of airborne cadmium in metropolitan cities in Korea and potential health risks. Atmos Environ. 2012;47:164–73.
8. Waisberg M, Joseph P, Hale B, Beyersmann D. Molecular and cellular mechanisms of cadmium carcinogenesis. Toxicology. 2003 Nov;192(2-3):95–117.
9. Parekkadan B, Milwid JM. Mesenchymal stem cells as therapeutics. Annu Rev Biomed Eng. 2010 Aug;12(1):87–117.
10. Nicolay NH, Lopez Perez R, Debus J, Huber PE. Mesenchymal stem cells – A new hope for radiotherapy-induced tissue damage? Cancer Lett. 2015 Oct;366(2):133–40.
11. Moher D, Liberati A, Tetzlaff J, Altman DG; PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. BMJ. 2009 Jul;339 jul21 1:b2535.
12. Elbaghdady HA, Alwaili MA, El-Demerdash RS. Amelioration of cadmium-induced testes' damage in rats by the bone marrow mesenchymal stem cells. Ecotoxicol Environ Saf. 2018 Feb;148:763–9.
13. Elbaghdady HA, Alwaili MA, El-Demerdash RS. Regenerative potential of bone marrow mesenchymal stem cells on cadmium chloride-induced hepato-renal injury and testicular dysfunction in sprague dawley rats. Ecotoxicol Environ Saf. 2018 Nov;164(164):41–9.
14. Wang YJ, Yan J, Zou XL, Guo KJ, Zhao Y, Meng CY, et al. Bone marrow mesenchymal stem cells repair cadmium-induced rat testis injury by inhibiting mitochondrial apoptosis. Chem Biol Interact. 2017 Jun;271:39–47.
15. Hussein YM, Hussein RM, Amin AI, Mohamed AS, Hussein HS. Evaluation of Mesenchymal Stem Cells and Vitamin E in Treatment of Infertile Male Albino Rats. Int. J. of Multidisciplinary and Current research. 2015; 3: 932-951.
16. Abdel Latif H, Abdel Khalek R, AbdelGalil W, AbdAllah H, Fawzy A, AbdelFattah S. Nanocurcumin versus mesenchymal stem cells in ameliorating the deleterious effects in the cadmium-induced testicular injury: A crosstalk between oxidative and apoptotic markers. Andrologia. 2020 Jul;00:e13760.
17. OHAT. Risk of Bias Tool. 2015
18. Ferguson JE. The Heavy Elements, In: Chemistry, Environmental Impact and Health Effects, Oxford, Pergamon Press. 1990;8:211–212.
19. Salvatori F, Talassi CB, Salzgeber SA, Spinosa HS, Bernardi MM. Embryotoxic and long-term effects of cadmium exposure during embryogenesis in rats. Neurotoxicol. Teratol. 2004;26: 673-680.
20. World Health Organization. Environmental Health Criteria, Cadmium. IPCS, Geneva, 1992.
21. Yang H, ShuY. Cadmium transporters in the kidney and cadmium-induced nephrotoxicity. Int. J. Mol. Sci. 2015; 16 (1):1484–1494.
22. Filipič M, Fatur T, Vudrag M. Molecular mechanisms of cadmium induced mutagenicity. *Human and Experimental Toxicology*. 2006; 25 (2):67–77.