EFFECT OF LEAD ON MALE REPRODUCTION IN EXPERIMENTAL ANIMAL MODEL

Muralidhar C[1], Vijay Prasad S[2], Sridhar I[3]

[1]*Medical Officer, Under DMHO, Medak, Telangana*
[2]*Assistant Professor, Dept. of Pharmacology, Dr Vithalrao Vikhe Patil Foundation's Medical College, Ahmednagar, Maharashtra*
[3]*Assistant Professor, Dept. of Pharmacology, Government Medical College, Nizamabad, Telangana.*

ABSTRACT

Introduction: In early 1960's, there is a first evidence of the toxic effects ionizing radiation on elevated oxygen levels in aerobes and proposed that oxygen toxicity is due to free radical formation. An alteration between oxidants and antioxidants in favour of the oxidants, potentially leading to damage is termed "oxidative stress". Lead and cadmium do not have any detectable beneficial biological roles rather it produces detrimental effects on biochemical, physiological and behavioral dysfunctions. Even a little lead poisoning can cause serious health problems, and at very high levels, it can be fatal. Mainly it affects the heamopoeitc system, Liver, Kidney, Cardiovascular system and reproductive system. **Methodology:** Experimental rats, injected intraperitoneally with lead acetate for 15 days at the dosage of 50, 100 mg/kg/day body weight and compared to control rats injected with deionized distilled water instead. At the end of study testis were removed and right testis was used for testicular antioxidant Malandealdehyde (MDA) levels estimation by Thiobarbituric acid reactive substance assay and left testis was used for histopathological analysis. Unpaired t test and ANOVA was used for statistical analysis. **Results** : The MDA (nmole /gm tissue) levels in control, lead 50mg, lead 100mg groups were 12.16±0.4, 17.06±0.16 and 18.11±0.13. Histopathology examination Lumen showing decreased sperm count and maturation. Some of the lumens showing absence sperm maturation. **Conclusion:** Study on lead-exposed rat testis have shown that reduction of spermatogenesis formation and sperm maturation. Increased MDA levels indicate that it may be due to oxidative stress. The toxicity of lead was noted at level ≥50mg/kg.

Keywords: Lead, Lipid peroxidation, Male reproduction, Testicular histology

INTRODUCTION

Infertility is defined as one year of unprotected intercourse without pregnancy [1-3]. It affects approximately 15% -30% of all couples trying to conceive [1, 4,5]. Of all the cases of human infertility 20% are due to male factor [6]. Several studies have examined the relationship between stress and sexual behavior in male rats. Increasing evidence suggests that the cumulative damage caused by reactive oxygen species contribute to numerous diseases [7].

In the late 1950's, free radicals and antioxidants were almost unheard of in the clinical and biological sciences but chemists had known about them for years in the context of radiation, polymer and combustion technology. In early 1960's, there is a first evidence of the toxic effects ionizing radiation on elevated oxygen levels in aerobes and proposed that oxygen toxicity is due to free radical formation [8].

Various forms of physical, psychological, environmental and heavy metals are believed to produce free radicals. Recently, oxidative stress has become the focus of interest as a potential cause of male infertility. Many industrial chemicals are known to have a negative impact on human reproduction, particularly exposures to heavy metals such as aluminum, Cd^{2+}, Fe^{2+}, Chromium (Cr^{2+}), Ni^{2+} and Pb^{2+} [9].

Several studies have examined the relationship between stress and sexual behavior in male rats. Increasing evidence suggests that the cumulative damage caused by reactive oxygen species contribute to numerous diseases [7].

Normally, equilibrium exists between reactive oxygen species (ROS) production and antioxidant scavenging activities in the male reproductive organs [10]. Testicular membranes are rich in polyunsaturated fatty acids and thus susceptible to peroxidation injury, it leads to decrease sperm production and maturation [11].

Lead and cadmium do not have any detectable beneficial biological roles rather it produces detrimental effects on biochemical, physiological and behavioral dysfunctions. Even a little lead poisoning can cause serious health problems, and at very high levels, it can

DOI: 10.5455/ijcbr.2017.34.13

eISSN: 2395-0471
pISSN: 2521-0394

Correspondence: Sangishetti Vijay Prasad. Department of Pharmacology, Dr Vithalrao Vikhe Patil Foundation's Medical College, Ahmednagar, MHS-414111. Email: vijayfarmac@gmail.com

be fatal [12]. Mainly it affects the heamopoeitc system, Liver, Kidney, Cardiovascular system and reproductive system.

To better understand the disease progression experimental animal models are required [13]. Rats were enable us to obtain answers in a short period of time, since 10 days in the life of a rat are approximately equivalent to 1year of human life [14, 15].

The threshold level has been difficult to establish due to the selection of the exposure indicator and the reproductive endpoints. So in the preset study was undertook to study the effect of lead in testicular tissue antioxidant stasus and histological changes

MATERIALS AND METHODS

Study design: An experimental animal based study

Ethics approval: The study was approved by the institutional animal ethics committee

Animal: Adult male albino rats weighing 220 – 250 g and aged 10-12 weeks old were obtained from authorized animal breading centre. The animals were kept in wire bottomed cages in a room under standard condition of illumination with a 12 - h light-dark cycle at 25 ± 1° C. They were provided with tap water and balanced diet ad libitum. The study was approved by the IAEC authorities and it followed the CPCSEA rules on animal protection.

Sample size: In each group n=6

Drug preparation: Lead was obtained from the authentic distributor. Drugs were dissolved in isotonic saline solution and injected intra peritoneally

Grouping: Rats were randomly divided into 3 groups

Group 1: Control received normal saline

Group 2: Received lead 50mg/kg

Group 3: Received lead 100mg/kg

Methodology : Experimental rats (Group 2,3) injected intraperitoneally with lead acetate for 15 days at the dosage of 50, 100 mg/kg/day body weight and compared to control rats injected with normal saline.

Animal sacrifice, collection and preparation of samples: At the end of the study each animal was sacrificed by cervical dislocation.

Tissue preparation: The testicular tissue was transferred into 10% w/v of Phosphate buffer (pH 7.4). The tissue was homogenized using a manual homogenizer. The broken cells debris were removed by centrifugation 3,000rpm for 10min. The obtained supernatant were divided into aliquots and stored in -80^{o}c. The level of Lipid peroxidation (MDA) was measured by Thiobarbituric acid reactive substance assay (TBARS) by Burge Aust [16].

Histopathological examination: Tissue specimens from testes of all experimental rats were collected at the end of the study and fixed in neutral buffered formalin, processed by conventional method, embedded in paraffin, sectioned at 4-5um and stained by Haematoxylin and Eosin [17].

Statistical analysis: To analysis unpaired t test and ANOVA was applied.

RESULTS

Group	**Testicular MDA levels (nmole /gm tissue)**
Control	12.2±0.41
Lead 50mg/kg/day	17.01±0.15***
Lead 100mg/kg/day	*18.09±0.1****$*

*Comparison with normal group, $ comparison with low doses vs High dose lead

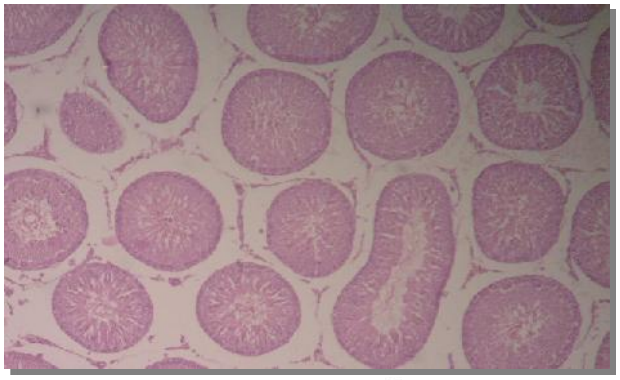

Figure 1. Normal rat testis (10x)

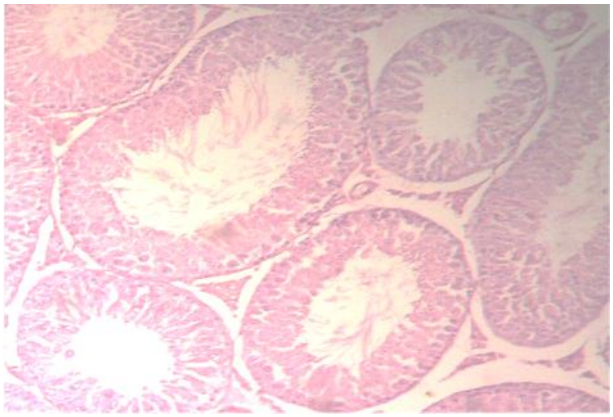

Figure 2. Lead induced toxicity (10x)

[Lumen showing decreased sperm count and maturation (blue arrow). Some of the lumens showing absence sperm maturation (red arrow)]

There are a number of mechanisms by which exposure to lead may reduce male fertility.

DISCUSSION

In the present study MDA levels were increased. This probably reflects the increased in lipid oxidation due to either increased production of free radicals or decreased antioxidant defense mechanism or both.

Histo-pathological results: Microscopically testes of stress group marked necrosis of spermatogoneal cells ling seminiferous tubles associated with incomplete spermatogenesis and Azo Spermia. Degeneration of germ cells lining seminiferous tubles.

There are a number of mechanisms by which exposure to lead may reduce male fertility [18]. The lead con-

centration in the tissue could affect hormone receptor kinetics, enzyme activities and hormone secretion. Although environmental exposure to lead may impair spermatogenesis, as shown in several animal studies [19]

Direct toxic effects on sperm and gonads have been observed in animal tests. Lead and other cations (mercury, copper) may cause a partial replacement of zinc which is essential for sperm head chromatin stabilization it may lead to decreased fertility or DNA damage in the fertilization process [20].

Male rats exposed with lead PbB 15–23 µg/dl have been observed in genomic expression in 2-cell embryos fathered by male rats. Interestingly, fertility was reduced only at a higher PbB level (27–60 µg/dl). It suggest an effect on the regulation of gene transcription or translation rather than direct genetic damage to the male germ cell [21].

CONCLUSION

Study on lead-exposed rat testis have shown that reduction of spermatogenesis formation and sperm maturation. Increased MDA levels indicate that it may be due to oxidative stress. The toxicity of lead was noted at level ≥50mg/kg.

Source of funding: Nil

Conflict of interest: Nil

REFERENCES

1. Sunil B Yadav, Adinath N. Suryakar, Anil D. Huddedar, Puspha P. Durgawale, Pramod S. Shukla. Antioxidant treatment a new therapeutic approach to reversible male infertility. Biomedical Research. 2006;17(3):175-178
2. Jaiswar Shyam Pyari, Sachan R, Singh RK, Agarwal Monica: Free radicals in female infertility. J Obstetrics Gynecology India. 2006;56(1):64-67
3. Ghasem Saki, Fakher Rahim, Ozra Alah Vaysi. E□ect of forced swimming stress on in vivo fertilization capacity of rat and subsequence o□spring quality. J Hum Reprod Sci. 2010;3(1):32-34
4. Shima Toghyani, Gholam R Dashti, Nasim Roudbari, Shaila Rouzbehani, Ramesh Monajemi. Lithium carbonate inducing disorders in three parameters of rat sperm. Advanced biomedical Research. 2013;2:55
5. Kartikeya Makker, Ashok Agareal, Rakesh Sharma. Oxidative stress & male fertility. Indian J Med Res. 2009;129:357-367
6. Rekha DK, Nayanatara AK, Ramaswamy, Sheila RP, Ramesh Bhat, Venkappa SM. Infertility in male wistar rats induced by cadmium chloride: Role of ascorbid acid. Journal of Chinese clinical medicine. 2009;4(11):616-21
7. Lobo V, Patil A, Phatak A, Chandra N. Free radicals, antioxidants and functional foods: Impact on human health. Pharmacogn Rev. 2010;4(8):1-20
8. Gutteridge JMC, Halliwell B. Free radical and antioxidants in the year 2000. A historical look to the future. Ann. NY. Acad. Sci. 2000;899:136-147
9. Susan Beno□, Asha Jacob, Ian Hurley. Male infertility and enivormental exposure to lead and cadmium. Human reproduction update 2000;6(2):107-221
10. Vijayprasad S, Ghongane BB, Nayak BB. E□ect of vitamin C on male fertility in rats subjected to forced swimming stress. J Clin Diagn Res. 2014 Jul;8 (7):HC05-8.
11. Ashok Agarwal, Gurpriya Virk, Chloe Ong, Stefan S du Plessis. E□ect of Oxidative Stress on Male Reproduction. World J Mens Health. 2014;32(1): 1–17.
12. Monisha Jaishankar, Tenzin Tseten, Naresh Anbalagan, Blessy B. Mathew, Krishnamurthy N. Beeregowda. Toxicity, mechanism and health e□ects of some heavy metals. Interdiscip Toxicol. 2014; 7(2): 60–72.
13. Nitish Bhatia, Partha Pratim Maiti, Abhinit Choudhary, Atul Tuli, Daljit Masih, Mohd. Masih Uzzaman Khan, Tasneem Ara, and Amteshwar Singh Jaggi. Animal models of stress. IJPSR. 2011;2 (5):1147-1155
14. Nayanatara AK, Vinodini NA, Ahemed B, Ramaswamy CR, Shabarianth Ramesh Bhat. Role of ascorbic acid in monosodium glutamsate mediated e□ect on testicular weight, sperm morphology and sperm count in rat testis. Journal of Chinese clinical medicine. 2008;3(1):1-5
15. John AR, Roman SD. Antioxidant Systems and Oxidative Stress in the Testes Oxidative Medicine and Cellular Longevity. 2008;1(1):15-24
16. Saalu LC, Akunna GG, Ajayi JO. Modulating Role of Bitter Leaf on Spermatogenic and Steroidogenesis Functions of the Rat Testis. American Journal of Biochemistry and Molecular Biology. 2013;3:314-321
17. Zheng-Wei Yang, Ling-Shu Kong, Yang Guo, Jin-Qi Yin, Nathaniel Mills. Histological changes of the testis and epididymis in adult rats as a result of Leydig cell destruction after ethane dimethane sulfonate treatment: a morphometric study. Asian J Androl 2006; 8 (3): 289–299
18. Sallmén M. Exposure to lead and male fertility. Int J Occup Med Environ Health. 2001;14(3):219-22.

19. Beno S, Jacob A, Hurley IR: Male infertility and environmental exposure to lead and cadmium. Hum Reprod Update. 2000, 6: 107-121..

20. Markku Sallmén. Exposure to lead and male fertility. IJOMEH. 2001; 14(3):219—222

21. Gandley R, Anderson L, Silbergeld EK. Lead: Male-mediated effects on reproduction and development in the rat. Environ Res 1999; 80: 355–63

How to Cite this article: Muralidhar C, Vijay Prasad S, Sridhar I. Effect of lead on male reproduction in experimental animal model. *Int j. clin. biomed. Res.* 2017;3(4): 60-63