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ORIGIONAL PAPER

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Vitamin E is Protective Against Lead Toxicity of the Reproductive System in Male Rats

Abstract. This study examined the reproductive toxicity of lead in male rats exposed to 2% lead acetate in drinking water for six weeks and the protective activity of vitamin E. In lead- treated males, the weights of the body, vas deferens, seminal vesicle and prostate gland decreased significantly by 45%, 33%, 35% and 42% respectively, while the weight of the testes did not change significantly. Moreover, the levels of plasma LH and testosterone were significantly decreased in the rats given lead. Decreased sperm motility and sperm count with increased sperm abnormalities were found in rats exposed to lead. Some histological changes were observed in the testes of treated rats.Vitamin E administration at dose level of 10mg/100g resulted in a reduction of the deleterious effects of lead.

Keywords: Lead, Vitamin E, Gonads, Sperm, LH, Testosterone.

الدور الوقائي لفيتامين (هـ) في الحد من التأثير الضار للرصاص على المناسل الذكرية في الجرذان

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المستخلص: يهتم هذا البحث بدراسة التأثير الضار للرصاص على المناسل الذكرية للجرذان المعالجة بخلات الرصاص (2%) في مياه الشرب لمدة ستة أسابيع، وكذلك دراسة التأثير الوقائي لفيتامين (هـ). تسببت معاملة الحيوانات بخلات الرصاص في نقص أوزان الجسم، الوعاء الناقل، الحويصلة المنوية وغدة البروستاتا. بينما لم يتغير وزن الخصى تغيراً معنوياً. وأظهرت النتائج نقصاً معنوياً في هرموني التستوستيرون، والهرمون اللوتيني في بلازما الدم. وكذلك حركة وعدد الحيامن، نتيجة معالجة الجرذان بخلات الرصاص. وزادت نسبة الحيامن غير الطبيعية، نتيجة لخلات الرصاص. أوضحت النتائج أيضاً بعض التغييرات النسيجية في خصى الجرذان المعاملة بالرصاص. وبالمقابل فقد قلل فيتامين (هـ)، عند جرعة تكافيء 10 ملجم/100 جم، من هذه التأثيرات الضارة للرصاص.

كلمات مدخلية: رصاص، فيتامين (هـ)، مناسل، حويصلة منوية، هرمون.

Introduction

Lead, a ubiquitous environmental contaminant, continues to represent a serious health concern. The reports differ in their conclusions about a possible effect of lead on serum and intratesticular testosterone levels. Sokol *et al.* (1985) reported a significant decrease in serum and intratesticular testosterone levels in Wistar rats after 30 days of exposure to lead (0.1 and 0.3% of lead acetate in the drinking water). On the contrary, Johansson and Wide (1986) did not find significant variations in plasma testosterone levels of mice exposed to lead during 3 months (0.1% of lead chloride in the drinking water).

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Some studies performed with human populations occupationally exposed to lead showed that this metal did not produce any significant change in the endocrine testicular function (Cullen *et al.* 1984). Others reported that the workers of a lead smelter showed a significant decrease in their serum testosterone level and a decreased response of LH following gonadotropin-releasing hormone administration (Braunstein *et al.* 1978). In lead exposed males, decreased sperm counts have been demonstrated (McGregor and Mason, 1990). It is reported that when lead levels in the blood of adult males reached 50(g/100ml, testicular damage occurred and spermatogenesis was inhibited (Hildebrand *et al.* 1973).

Vitamin E (α–tocopherol), which is a critical component of the antioxidant system in all tissues, (Machlin and Bendich, 1987) is a free radical scavenger that has received attention as a potential therapeutic agent to prevent or reduce clinical disease states thought to be associated with excess free radical production (Horwitt, 1991). Increasing the dietary intake of antioxidants may provide

protection against cellular degeneration by oxidants and free radicals (Pauling, 1970). Vitamin E may be a useful antidote for iron toxicoses and that iron-induced depletion of vitamin E may play a role in the pathogenesis of iron toxicity (Omara and Blakley, 1993). Treatment with α-tocopherol after thermal skin injury prevented the vitamin E reduction and peroxidative membrane damage of erythrocytes and improved their deformability (Bekyarova and Yankova, 1998). Topical application of α-tocopherol, the most prominent naturally occurring form of vitamin E, inhibits ultraviolet induced carcinogenesis and DNA damage (McVean and Liebler, 1999).

This study evaluates the protective effect of vitamin E against toxicity of lead on the male reproductive system.

Materials and methods

• Animals

Thirty male Wister rats were used in this investigation. The average weight of the rats was 150±4.6g. The animals were maintained under standard laboratory conditions (12h light, temperature 23°C). They were fed dry ration ad lib. The rats were randomly divided into three groups of 10 animals each.

• Administration of lead acetate and vitamin E

The animals were treated daily for six weeks as follows:

Group (1) served as control and was given tap water,

Group (2) was treated with 2% lead acetate in drinking water (Bjorklund *et al.* 1981) and

Group (3) was administered orally 10mg/100g Bwt (1ml fluid/rat) of vitamin E (Paget and Barnes, 1964) and exposed to 2% lead acetate in drinking water.

Both lead acetate and vitamin E were obtained from Merck (Darmstadt, Germany).

• Body and reproductive organ weight

The weight of the body was recorded 2, 4, and 6 weeks post treatment in the three groups. At the end of the experiment, the animals were sacrificed and the weights of the testes, vas deferens, epididymis, seminal vesicle and prostate gland were recorded.

Hormone assay

Serum LH and testosterone were assayed at the end of the experiment by enzyme immunoassay (Bio Merieux, France). The assay is based on a sandwich technique using two monoclonal antibodies.

• Evaluation of sperm

One epididymis was removed after six weeks of treatment, cleaned of fat, dissected in 10ml of normal saline (0.9% NaCl) and incubated at 37°C. Sperms were counted using a hemocytometer (Neubauer) and percentage of sperm motility was determined. Smears were prepared from the suspension, stained with 1% eosin solution and examined for sperm abnormalities (Baloch and Cohen, 1964).

• Histological study

The testes of different groups were fixed in 10% formol saline. Paraffin wax sections of testes were stained with hematoxylin and eosin and examined under the light microscope.

• Statistical analysis

Statistical difference was calculated by using the one way analysis of variance and least significant difference (LSD) range test.

Results

The body weight (Table 1) of the rats subjected to lead only showed a significant decrease ($p<0.01$) of 17%, 32% and 45% after two, four, and six weeks of treatment, respectively. On the other hand, the body weight significantly decreased ($p<0.01$) by 14% in the animals administered vitamin E and lead in combination after six weeks. The values of the vitamin E and lead group were significantly higher than those of the lead group.

Table 1: Effect of lead singly and in combination with vitamin E on body weight (g) of male rats

Treatment	Time in week						
	0	2	% of change^	4	% of change	6	% of change^
Control	145.10±1.81	208.80 ±5.49	-	300.60±10.88	-	382.80 ±10.69	-
Lead	153.90±1.88	172.50**±3.41	-17.39	205.10**±2.81	-31.77	263.80** ±4.98	-45.11
Lead & vitamin E	155.60±2.49	210.16••±5.11	+0.65	290.60••±4.61	-3.33	330.50••••±8.12	-13.66

Each value is mean of 10 replicates + S.E.
**: Significant difference from control at p<0.01.
••: Significant difference from lead group at p<0.01.
^: The percent of change is relative to control value.

Table 2: Weights (g) of testes and accessory sex organs relative to body weight of male rats treated with lead singly and in combination with vitamin E for 6 weeks

Treatment	Organ				
	Testis	Epididymis	Vasa deferentia	Seminal vesicle	Prostate gland
Control	1.26 ±0.02	0.23 ±0.007	0.06 ±0.005	0.46 ±0.01	0.33 ±0.01
Lead	1.27 ±0.03	0.24 ±0.01	0.04**±0.002	0.30** ±0.01	0.19** ±0.01
Lead and vitamin E	1.25 ±0.05	0.24 ±0.01	0.05 ±0.004	0.37••±0.002	0.24•• ±0.02

Each value is mean of 10 replicates + S.E.
*,**: Significant difference from control at p<0.05 and p<0.01 respectively.
•,•• : Significant difference from lead group at p<0.05 and p<0.01 respectively.

The results as shown in Table 2 above indicate a significant decrease (p<0.01) of 33% in vasa deferentia weight of rats given lead only by as compared to control, while the weight of vasa deferentia did not change significantly in the rats given lead with vitamin E. The weights of the seminal vesicle and prostate gland decreased significantly in all treated groups. The weights of the seminal vesicle and prostate gland of rats treated with lead alone declined by 35% and 42% respectively relative to the control value, while they decreased by 20% and 27% in rats given vitamin E and lead in combination. The weights of these two organs of rats given lead and vitamin E in combination were significantly higher then those of rats treated with lead only.

Table (3) shows that treatment of rats with lead alone for six weeks caused a significant decrease in serum LH (p<0.01) and testosterone (p<0.05) levels by 15% and 9%, respectively. The level of the two hormones did not change significantly in the rats given lead and vitamin E in combination. Both the LH and testosterone levels of lead and vitamin E group were significantly higher than those of the lead group.

Table 3: Serum LH and testosterone levels (mlU/ml) of male rats treated with lead singly and in combination with vitamin E for 6 weeks.

Treatment	Hormone LH(mlU/ml)	Testosterone (mlU/ml)
Control	6.68 ±0.09	3.84 ±0.04
Lead	5.68 ±0.13**	3.50* ±0.11
Lead and vitamin E	6.50 ±0.10••	3.86• ±0.07

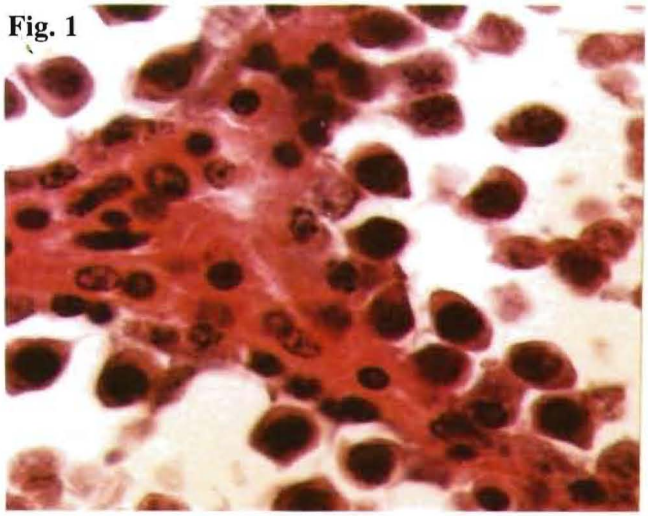
Each value is mean of 10 replicates ± S.E.
*,**: Significant difference from control at p<0.05 and p<0.01 respectively.
•,••: Significant difference from lead group at p<0.05 and p<0.01 respectively

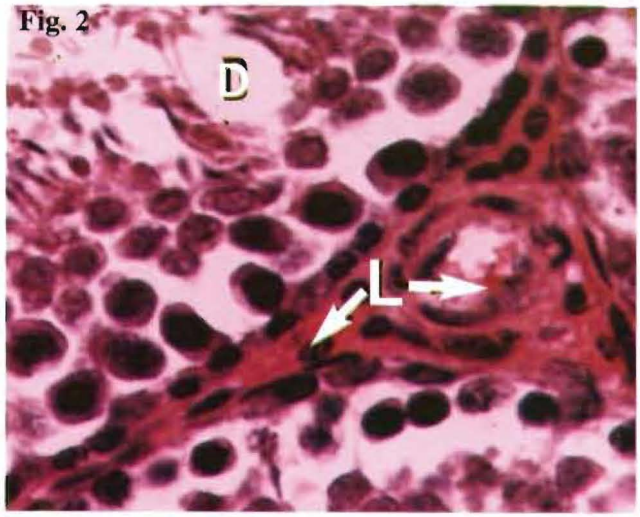
The sperm motility (Table 4) decreased significantly (p<0.01) in the rats administered lead or lead with vitamin E, by 21% and 10% respectively. Similarly, sperm count per epididymis significantly decreased in the animals that ingested lead (p<0.01) and lead with vitamin E by 34% and 18%, respectively. Sperm abnormalities were significantly elevated in all treated groups as compared to the control. The sperm abnormality values of animals treated with lead and vitamin E in combination were significantly less than those of animals which ingested lead only. Moreover, sperm motility and sperm count were significantly higher in animals given lead with vitamin E as compared to rats administered lead only.

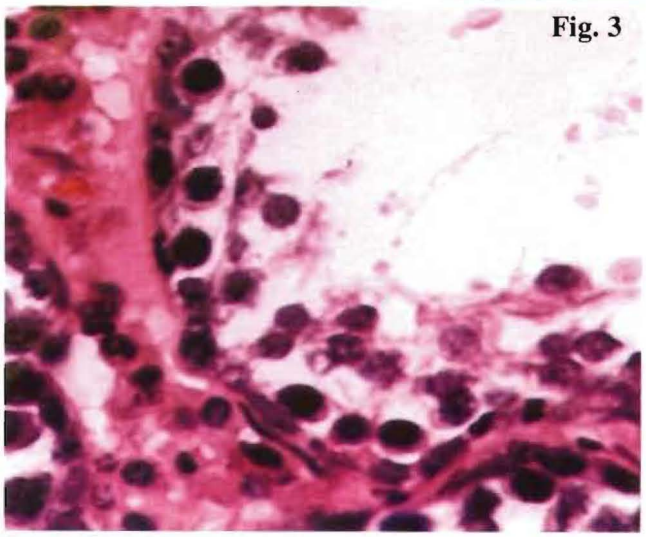
Table 4: Serum viability of male rats treated with lead singly and in combination with vitamin E for 6 weeks.

Treatment	Parameter				
	Sperm motility (%)	Sperm count per epididymis (%)	Sperm abnormalities (%)		
			Head	Tail	Total
Control	84.50 ±0.56	24.57 ±0.50	2.48 ±0.13	3.06 ±0.08	5.53 ±0.20
Lead	66.60** ±1.84	16.23** ±0.66	15.36** ±1.12	9.92** ±0.82	21.39** ±1.64
Lead and vitamin E	76.03**•• ±1.01	20.05**•• ±0.50	5.46**•• ±0.63	4.15**•• ±0.39	9.25**•• ±0.83

Each value is mean of 10 replicates + S.E.
*,**: Significant difference from control at $p<0.05$ and $p<0.01$ respectively.
•,•• : Significant difference from lead group at $p<0.05$ and $p<0.01$ respectively.







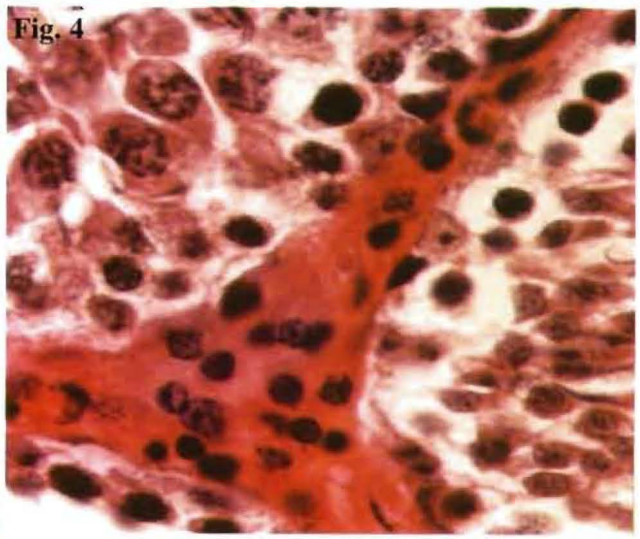


Figure 1 - 4

Fig. 1: TS of testis of control rat illustrates normal structure (Hx & E x400)

Fig. 2: TS of testis of rat treated with lead for 6 weeks. Note deformation of leydig cells (L) and degeneration (D) of some spermatogenic cells (Hx & E x400)

Fig. 3: T S of testis of rat treated with lead for 6 weeks. Note arrest of spermatogenesis in a few seminiferous tubles (Hx & E x400)

Fig. 4: T S of testis of rat treated with lead and vitamin E in combination for 6 weeks to show normal structure (Hx & E x400)

A few seminiferous tubules of the rats exposed to lead (Fig. 2) appeared devoid of spermatozoa. Moreover, Leydig cells were deformed with interstitial hemorrhage and degenerative areas in spermatogenic cells (Fig. 3). No histological changes in the testes of animals treated with vitamin E and lead in combination were observed (Fig. 4).

Discussion

Lead is an environmental and industrial pollutant that has been detected in all biological systems. In the present study, the body gain of the rats treated with lead alone was less than that of control rats. It has been suggested that the growth depression induced by lead involved a depression of appetite rather than reduced release of thyroxin (Hammond *et al.* 1989) or growth hormone secretion (Comoratto *et al.* 1993). The growth of the accessory sex organs, i.e. the seminal vesicles and prostate, is highly dependent on the level of testosterone. The decrease in the weight of these accessory sex organs in the rats treated with lead might be due to a decreased level of testosterone observed in the present work. The significant decreases in seminal vesicle and prostate weights suggest that lead could affect the male reproductive function via its direct or indirect action on male accessory organs (Pinon-Lataillade *et al.* 1995).

Lead compounds are common pollutants in many areas and can impair the reproductive function of males by various mechanisms. Prenatal and neonatal exposures to lead reduce the binding of FSH and LH to their receptors in male rats and significantly suppress testicular synthesis of testosterone in rats (Wiebe *et al.* 1983). Reduced concentrations of testosterone in the blood were found in human populations occupationally exposed to lead (Rodamilans *et al.* 1988). Our data demonstrate a decrease in LH and testosterone levels of rats given lead only and deformation of Leydig cells. The possible effect of lead on the endocrine testicular function may be due to a direct inhibitory action of lead on androgen biosynthesis in Leydig cells (Wiebe *et al.* 1983) or to a defect in LH regulation at the hypothalamic or the pituitary level (Braunstein *et al.* 1978).

The basal testosterone production by Leydig cells is markedly reduced in lead exposed rats and the ability of Leydig cells from lead treated animals to respond to hCG diminishes drastically. These findings provide a demonstration that Leydig cells are a direct target for the harmful action of lead (Thoreux-Manlay *et al.* 1995).

A number of enzymes are altered by lead exposure. Such exposure decreases the biosynthesis of heme and reduces the hepatic content of heme-containing cytochromes, which are involved in steroid hormone metabolism (Alvares, 1979). The data published by Wiebe and coworkers (1983) demonstrated that in vitro and in vivo neonatal exposure to lead act on testicular cells by inhibiting the activity of steriodogenic enzymes and the synthesis of testosterone receptor proteins.

Reduced spermatogenesis in rats after exposure to lead compounds has been reported by Sokol *et al.* (1985). Evidence for reduced spermatogenesis in men exposed to toxic levels of organic lead has also been reported (Eyden *et al.* 1981). Reduced fertility with increased frequency of asthospermia, hypospermia and teratospermia was found in lead exposed workers (Lacranjan *et al.* 1975). In the present study, the sperm motility and count decreased significantly in the rats subjected to lead. Moreover, sperm abnormalities were elevated in the lead-treated rats. Alterations in sperm count, morphology and motility are useful indices of chemically induced effects on spermatogenic function (Wyrobek *et al.* 1983). The decrease in sperm count observed in lead exposed animals could be secondary to the decreased testosterone concentration or it may represent direct lead toxicity on spermatogenesis.

Lead was detected in blood, seminal plasma and spermatozoa of all bulls treated with lead (Alexaki *et al.* 1990). One of the major concepts concerning the mechanism of heavy metal toxicity is attributed to its ability to generate reactive oxygen species which cause peroxidation of membrane lipids (Hermes-Lima *et al.* 1991). Lead is known to promote oxidative damage by enhancing peroxidation of lipids (Stohs and Bagchi, 1995). It seems that the increase in sperm abnormalities and the observed histological changes in the testes of treated rats with lead resulted from lipid peroxidation. Another mechanism for lead-induced oxidative stress is on the antioxidant defense systems of cells. Lead has been shown to alter antioxidant activities by inhibiting functional SH groups in several enzymes such as superoxide dismutase (Ito *et al.* 1985) and catalase (McGowan and Donaldson, 1986), which in turn causes the cells to be more susceptible to oxidative attacks.

Our data have demonstrated a potential effect of vitamin E in the protection against lead toxicity. Vitamin E is an integral part of cellular membranes and functions as a free radical scavenger preventing

the propagation of lipid peroxidation (Boreck, 1988 and Huang *et al.* 2003). Vitamin E also stabilizes membranes by forming complexes with unsaturated fatty acids. The membrane stabilization is regarded as a molecular mechanism against the damaging action of free fatty acids (Erin *et al.* 1984). Vitamin E supplementation produced increased levels of alpha-tocopherol in semen and testes. The increased vitamin E concentration in the spermatozoa has been associated with a reduction in their susceptibility to lipid peroxidation (Surai *et al.* 1998). One of the protective roles of vitamin E on lead- induced damage was preventing lead's effects on increased lipid peroxidation and inhibition of superoxide dismutase and catalase activities (Chaurasia and Kar, 1997). It has been observed that animals with lead poisoning who were simultaneously fed with vitamin E show lower lead levels in the blood. The protective effect of vitamin E is also due to its capacity to inhibit lead absorption (Pace and Iannucci, 1994).

Consequently, in male rats exposure to lead might affect reproductive function by acting on accessory sex organs and pituitary-gonadal axis. Vitamin E can play a protective role in the prevention of lead intoxication.

References

Alexaki, E., Samara, C., Alexopoules, F., Tsafaris, F. and Smokovitis, A. (1990) Detection of lead in blood, seminal plasma and spermatozoa of bulls. Effect in vitro of lead acetate on sperm motility. Bull. Environ. Contam. Toxicol. **45**: 824-828.

Alvares, A. P. (1979) Lead and polychlorinal euphenyls: effects on heme and drug metabolism. Drug Meta. Rev. **10**: 91-98

Baloch, K. and Cohen, R. B. (1964) A cytochemical technic for studying oxidative enzyme system of mammalian spermatozoa in semen smears. Fertil. Steril. **15** (1): 35-39

Bekyarova, G. and Yankova, Y. (1998) Alpha-tocopherol and reduced glutathione deficiency and decreased deformability of erythrocytes after thermal skin injury. Acta Physiol. Pharmacol. Bulg. **23** (2): 55-59.

Bjorklund, H., Lind, B., Piscator, M., Hoffer, B. and Olson, L. (1981) Lead, zinc and copper levels in intraocular brain tissue grafts, brain and blood of lead exposed rats. Toxicol. Appl. Phamaco. **60** 424-430

Boreck, C. (1988) Oncogenes, hormones and free radical processes in malignant transformation in vitro. Ann. N. Y. Avad. Sci. **551**: 95-101

Braunstein, G. D., Dahlgren, J. and Loriaux, D. L. (1978) Hypogonadism in chronically lead poisoned men. Infertility **1**: 33-51.

Chaurasia, S. S. and Kar, A. (1997) Protective effects of vitamin E against lead-induced deterioration of membrane associated type-I iodothyronine 5-monodeiodinase (5'. D-I) activity in male mice. Toxicology **124**: 203-209.

Comoratto, A. M., White, L. M., Lau, Y. S., Ware, G. O., Berry, W. D. and Moriarty, C. M. (1993) Effect of exposure to low level lead on growth and growth hormone release in rats. Toxicology **83**: 101-114.

Cullen, M. R., Kayne, R. D. and Robins, J. M. (1984) Endocrine and reproductive dysfunction in men associated with occupational inorganic lead intoxication. Arch. Environ. Health **39**: 431-440.

Erin, A. N., Spirin, M. M., Tabidze, L. V. and Kagan, V. E. (1984) Formation of alpha-tocopherol complexs with fatty acids. A hypothetical mechanism of stabilization of biomembranes by vitamin E. Biochem Biophys. Acta. **774** (1): 96-102.

Eyden, B. P., Maisin, T. R. and Mattelin, G. (1981) Long-term dietary effects of dietary lead acetate on survival, body weight and seminal cytology in mice. Bull. Environ. Contam. Toxicol. **20** (3): 226-231.

Hammond, P. B., Chernausek, S. D., Succop, P. A., Shukla, R. and Bornschein, R. L. (1989) Mechanisms by which lead depresses linear and ponderal growth in wealin rats. Toxicol. Appl. Pharmacol. **109**: 80-87

Hermes-Lima, M., Pereira, B. and Bechara, E. J. H. (1991) Are free radicals involved in lead poisoning? Xenobiotica **21**: 1085-1090.

Hildebrand, D. C., Der, R., Griffin, W. T. and Fahim, M. S. (1973) Effects of lead acetate on reproduction. Am. J. Obstet. Gynecol. **115**: 1058-1065.

Horwitt, M. K. (1991) Data supporting supplementation of humans with vitamin E. J. Nutr. **121**: 424-429.

Huang, C. H., Chang, R. J., Huang, S. L. and Chen, W. (2003). Dietary vitamin E supplementation affects tissue lipid peroxidation of hybrid tilapia, Oreochromis niloticus. Comp. Biochem. Physiol. B; Comp. Biochem. Mol. Biol. **134** (2): 265-270.

Ito, Y., Niiya, Y., Kurita, H., Shima, S. and Sarai, S. (1985) Serum lipid peroxide level and blood superoxide dismutase activity in workers with occupational exposure to lead. Int. Arch. Occup. Environ. Health **56**: 119-127.

Johansson, L. and Wide, M. (1986) Long-term exposure of the male mouse to lead: effects on fertility. Environ. Res. **41**: 481-487

Lacranjan, I., Popescu, H. I., Gavanescu, O., Kelepsch, J. and Serbanescy, M. (1975) Reproductive ability of warkmen occupationally exposed to lead. Arch Environ Health **30** 396-401.

Machlin, L. J. and Bendich, A. (1987) Free radical tissue damage: protective role of antioxidant nutients. FASEB J. **1**: 441-445

McGowan, C. and Donaldson, W. E. (1986) Changes in organ nonprotein sulfhydryl and glutathione concentrations during acute and chronic administration of inorganic lead to chicks. Biol. Trace Elem. Res. **10**: 37-46.

McGregor, A. J. and Mason, H. J. (1990) Chronic occupational lead exposure and testicular endocrine function. Human Exp. Toxicol. **9**: 371-376.

McVean, M. and Liebler, D. C. (1999) Prevention of DNA photodamage by vitamin E compounds and sunscreens: roles of ultraviolet absorbance and cellular uptake. Mol. Carcinog. **24** (3): 169-176.

Omara, F. O. and Blakley, B. R. (1993) Vitamin E is protective against iron toxicity and iron-induced hepatic vitamin E depletion in mice. J. Nutr. **123**: 1649-1655.

Pace, V. and Iannucci, E. (1994) The importance of vitamins in relation to the presence of heavy metals in food. Panminerva Med. **36**: 80-82.

Paget, G. E. and Barnes, J. M. (1964) Evaluation of drug activities. Pharmacometrics, **Vol. 1**. Academic Press, London and New York.

Pauling, L (1970) Ascorbic acid and other diseases. In: Vitamin C, the common cold and the flu. W. H. Freeman, New York, pp. 187-196.

Pinon-Lataillade, G., Thoreux-Manlay, A., Goffigny, H., Masse, R. and Soufir, J. C. (1995) Reproductive toxicity of chronic lead exposure in male and female mice. Hum. Exp. Toxicol. **14**: 872-878.

Rodamilans, M., Mtz-Osaba, M. J., To-Figueras, J., Rivero-Fillat, F., Torra, M., Perez, P. and Corbella, J. (1988) Inhibition of intratesticular testosterone synthesis by inorganic lead. Toxicol. Lett. **42**: 285-290.

Sokol, R. Z., Madding, C. E. and Swerdloff, R. S. (1985) Lead toxicity and the hypothalamic pituitary-testicular axis. Biol. Reprod. **33**: 722-728.

Stohs, S. J. and Bagchi, D. (1995) Oxidative mechanisms in the toxicity of metal ions. Free Radical Biol. Med. **18**: 321-336.

Surai, P., Kostjuk, I., Wishart, G., Macpherson, A., Speake, B., Noble, R., Ionov, I. and Kutz, E. (1998) Effect of vitamin E and selenium supplementation of cockerel diets on glutathione peroxidase activity and lipid peroxidation susceptibility in sperm testes and liver. Biol. Trace Elem. Res. **64** (1-3): 119-132.

Thoreux-Manlay, A., LeGoascogne, C., Segretain, D., Jegou, B. and Pinon-Lataillade, G. (1995) Lead affects steroidogensis in rat Leydig cells in vivo and in vitro. Toxicology **103**: 53-62.

Wiebe, J. P., Salhanik, A. I., Myers, K. I. (1983) The mechanism of action of lead in the testis: in vitro suppression of FSH receptors, cyclic AMP and steroidogenesis. Life Sci. **32**: 1997-2005.

Wyrobek, A. J., Gordon, L. A., Bukhart, J. G., Francis, M. W., Kapp, R. W. (1983) An evaluation of human sperms as indicators of chemically induced alterations of spermatogenic function. Mut. Res. **115**: 73-148.

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