

# Aqueous Extract of *Juglans Nigra* Prevents Lead Induced Testicular Toxicity in Rats

Abdul Alhassan<sup>1</sup>, Mohamed Mabrouk<sup>1</sup>, Ramatu Idris<sup>1</sup>, Emmanuel Salawu<sup>2</sup>, Abiodun Oyerinde<sup>2</sup>, Zainab Bauchi<sup>1</sup>

<sup>1</sup>Ahmadu Bello University, Department of Human Physiology, Zaria, Kaduna State, Nigeria; <sup>2</sup>Ladoke Akintola University of Technology, Department of Physiology, Ogbomoso, Oyo State, 234038, Nigeria

## Abstract

**Citation:** Alhassan A, Mabrouk M, Idris R, Salawu E, Oyerinde A, Bauchi Z. Aqueous Extract of *Juglans Nigra* Prevents Lead Induced Testicular Toxicity in Rats. *Maced J Med Sci.* 2010;3(3):289-294. doi:10.3889/MJMS.1957-5773.2010.0109.

**Key words:** *Juglans nigra*; lead; testis; testicular; toxicity; fertility; sperm; testosterone.

**Correspondence:** Emmanuel O Salawu. Ladoke Akintola University of Technology, Department of Physiology, Ogbomoso, Oyo State, 234038, Nigeria. Phone: 2348056916409. E-Mail: seocatholic@gmail.com

Received: 01-Apr-2010; Revised: 01-May-2010; Accepted: 04-May-2010; Online first: 02-Jun-2010

**Copyright:** © 2010 Alhassan A. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

**Competing Interests:** The author have declared that no competing interests exist.

**Background.** Lead is a heavy metal that has been known for its adverse effects on many body organs and systems and thus their functions.

**Aim.** In this study, the toxic effect of lead on the testicular system was investigated, and Aqueous Extract of *Juglans Nigra* (JnE) (a well known antioxidant) was administered orally to prevent this effect.

**Materials and Methods.** Twenty-four adult male Wister rats, randomly divided into four groups (n = 6), were used for this study. Group B and Group D were given 3g of JnE/Kg Body Weight/Day (orally) and 1% sodium acetate and Lead acetate respectively, while group A (control) and group C were given sodium acetate and lead acetate respectively. All treatments were for eight weeks. The animals were sacrificed twenty-four hours after the last treatment. Sperm count, motility, morphology, and testosterone level were determined. The control and "test" groups were compared using independent-sample t-test.

**Results.** The results obtained showed that lead caused a significant decrease in epididymal weight, sperm count, sperm mortality and testosterone level; and significant increase in abnormal structure of spermatozoa. These (abnormalities) were however, prevented in the JnE treated groups.

**Conclusion.** It is therefore concluded that oral administration of JnE promote fertility and annuls lead induced testicular toxicity.

## Introduction

Lead is one of the first metals to be smelted and used [1]. It probably was first mined in Turkey around 3500 BC [2]. Its density, workability and corrosion resistance were among the metals attractions [3].

Lead poisoning (plumbism or painter's Colic [4]) is one of the oldest known environmental hazards [3]. But the level of Lead found today in most people is in order of magnitude greater than those of pre-industrial times

[5, 6]. Humans in one way or the other get exposed to lead through air, water, soil, food and consumer products [7]. Still, the modern understanding of their full extent and the small amount of Lead necessary to produce the hazards is relatively new [5]. In fact, blood lead levels once considered safe are now considered hazardous. Also, no level of lead in the body below which no harm can occur has been discovered [5].

Lead interferes with a variety of body processes and is toxic to the body systems including the cardiovas-

cular, reproductive, haematopoietic, gastrointestinal, renal and nervous systems [4], renal functions [8], release of glutamate learning [9].

In the humans, lead inhibits porphobilinogen synthase and ferrochelatase preventing both porphobilinogen formation and the incorporation of iron into protoporphyrin XI, the final stage in heme synthesis. This causes ineffective heme synthesis and subsequent microcytic anemia [10]. Also, research has shown that lead affects both male and female reproductive system mainly because of its ability to cause lipid peroxidation [8]. And a growing body of evidence suggest that metal cation of Lead as well as Iron, Aluminum stimulate free radical formation. Also, Quinlan *et al.*, [10] observed that even when lead ions alone do not induce peroxidation, they do accelerate the rate of peroxidation caused by iron ions.

These oxidation properties of lead suggest that a source of antioxidant such as Walnut (*Juglans nigra*) should have the tendency to prevent the adverse effect of Lead on the body. *Juglans nigra* contains a high amount of copper which is believed to take part in the removal of free radicals from the body, hence preventing cell structure damage [11]. Studies funded by the California Walnut Commission [12] show the effectiveness of walnut against condition ranging from heart diseases to diabetes, osteoporosis, lung cancer and other diseases [13].

Since humans are inseparable from their environment and almost entirely not free from exposure to Lead, it is very necessary to figure out ways by which our body can be made to still maintain homeostasis (basis of good health) even on exposure to relatively high level of lead exposure. This research work, therefore, aims at knowing whether or not oral administration of *Juglans nigra* will prevent Lead induced testicular toxicity base on the knowledge that *Juglans nigra* has very strong antioxidant components. This research could, therefore, propose another way by which lead toxicity could be tackled.

## Materials and Methods

Twenty four adult male Wister rats [average Body Weight (BW)  $140.75 \pm 4.41$  g] obtained from the animal house section of Faculty of Pharmaceutical Science, Ahmadu Bello University, Zaria, Nigeria, were used for this study. The animals were kept in the animal house section of Human Physiology Department, Ahmadu Bello University, Zaria, Nigeria, and allowed to acclima-

tize over a period of ten days.

### Plant Materials

The nuts of *Juglans nigra* were bought from Zaria, Kaduna state, Nigeria and authenticated at the Department of Biological Science, Ahmadu Bello University, Zaria, Nigeria.

### Preparation of Aqueous Extract of *Juglans nigra*

The preparation of aqueous extract of *Juglans nigra* was done in Department of Pharmacognosy and Drug Development, Ahmadu Bello University, Zaria following the method described by Henry *et al.*, [14]. The shells were removed and the *Juglans nigra* were grounded to a fine powder. The fine powder was left to dry under shade, and after then soaked in distilled water (1:1, by volume) in a separating funnel plugged with cotton wool. It was drained after twenty-four hours and rewashed with more of the solvent. The filtrate was poured in an evaporating dish and placed on a water bath at 80 °C, and reduced pressure (70% atmospheric pressure) to remove the solvent. After all the solvent had evaporated, the extract was scrapped and stored in a dry glass container at a temperature of 0 – 4 °C. Yield was 5.67% w/w. Fresh solution of the extract was prepared in distilled water just before use to maintain its potency.

### Animal Treatment

The twenty four rats were randomly grouped into four (Group A, B, C and D, n = 6). Rats in group A served as the control and were neither exposed to lead nor treated with *Juglans nigra* extract (JNE), but they were allowed to drink 1% sodium acetate *ad libitum*. Group B rats were allowed to drink 1% sodium acetate *ad libitum* and also administered 200 mg of JNE/Kg BW/Day. Group C and D were allowed to drink 1 % lead acetate *ad libitum*, while Group D rats were in addition administered 200 mg of JNE/Kg BW/Day. Groups A and B were allowed to drink 1% sodium acetate *ad libitum* so as to cancel out the potential effects of acetate, and to ensure that only the effects of lead were measured in the test "groups" (C and D). All treatments were for eight weeks.

### Animal Sacrifice and Collection of Samples

Twenty-four hours after the last treatment, the rats were weighed and then sacrificed by cervical dislocation and blood samples (4 ml/rat) were collected by cardiac puncture into a standard test tubes and allowed to clot, serum was collected by centrifugation at 5.1 x g

for 15 minutes ( $g = 9.821 \text{ m/s/s}$ ). Epididymes and testes were excised.

### Collection of Data and Statistical Analysis

The epididymes were dissected free of the surrounding tissues and were weighed. Testosterone level was assayed using radioimmunoassay as described by Odell *et al.* [15]. Strictly following the methods used by Salah *et al.* [16], sperm count and sperm motility were determined. Sperm morphology was accessed using the method described by Saalu *et al.* [17].

The control and "test groups" were compared using independent-sample t-test. The significant level was set to  $P < 0.05$ .

## Results

The following results were obtained and are presented as mean  $\pm$  SEM and level of significance is taken at " $P < 0.05$ " (\*), " $P < 0.001$ " (\*\*) and/or " $P < 0.0001$ " (\*\*\*).

### Weight Gain and Epididymal Weight (g)

Gain in body weight of group C and group D were not significantly ( $P > 0.05$ ) difference from that of the

**Table 1: Gain in body weight and epididymal weight.**

Groups	Final Body Weight	Initial Body Weight	Gain in Body Weight	Epididymal Weight
A (Control)	183.17 $\pm$ 12.9	141.67 $\pm$ 7.4	41.50 $\pm$ 9.5	0.195 $\pm$ 0.009
B (JnE)	232.83 $\pm$ 2.8	168.83 $\pm$ 4.9	64.00 $\pm$ 3.4*	0.228 $\pm$ 0.007**
C (Pb acetate )	179.83 $\pm$ 5.0	146.67 $\pm$ 4.4	33.17 $\pm$ 6.6	0.162 $\pm$ 0.004**
D (Pb acetate + JnE)	169.67 $\pm$ 7.6	126.67 $\pm$ 4.4	43.00 $\pm$ 9.0	0.178 $\pm$ 0.011

\* = " $P < 0.05$ " \*\* = " $P < 0.001$ "

control, while that of group B was significantly ( $P < 0.05$ ) higher (Table 1).

Epididymal weight of group B was significantly ( $P < 0.001$ ) higher than that of the control, while that of group C was significantly ( $P < 0.001$ ) lower. There was,

**Table 2: Sperm count ( $10^{12}/L$ ) and serum testosterone level ( $\mu\text{mol}/L$ ).**

Groups	Sperm count	Serum Testosterone Level
A (Control)	1.717 $\pm$ 0.03	4.967 $\pm$ 0.32
B (JnE)	1.800 $\pm$ 0.04	5.433 $\pm$ 0.14
C (Pb acetate )	1.350 $\pm$ 0.04***	4.217 $\pm$ 0.25*
D (Pb acetate + JnE)	1.567 $\pm$ 0.06 *	5.333 $\pm$ 0.34

\* = " $P < 0.05$ ", \*\*\* = " $P < 0.0001$ "

however, no significant difference in the epididymal weight of group D and the control (Table 1).

### Sperm Count and Serum Testosterone Level

Sperm count in group C was noted to be significantly ( $P < 0.001$ ) lower, while those of group B and D were not significantly different from that of the control. A similar trend was found for serum testosterone level (Table 2).

### Sperm Motility

Sperm motility was found to be significantly ( $P <$

**Table 3: Sperm motility.**

Groups	Fast movement	Slow movement	Non motile
A (Control)	69.17 $\pm$ 3.7	20.83 $\pm$ 2.4	10.00 $\pm$ 2.2
B (JnE)	74.17 $\pm$ 3.0	17.50 $\pm$ 3.8	8.33 $\pm$ 1.7
C (Pb acetate )	50.83 $\pm$ 1.5 **	32.50 $\pm$ 2.1 **	16.67 $\pm$ 1.7 *
D (Pb acetate + JnE)	67.50 $\pm$ 4.0	19.67 $\pm$ 4.0	13.33 $\pm$ 1.7

\* = " $P < 0.05$ " \*\* = " $P < 0.001$ "

0.01) lower in group C compared to the control, while the sperm motility for group B and that of group D were not significantly different from that of the control (Table 3).

### Sperm Morphology

Spermatozoa of group B were found to be of significant ( $P < 0.05$ ) better morphology, those of group C were of significant ( $P < 0.05$ ) poorer morphology, while those of group D were not significantly ( $P > 0.05$ ) difference from those of the control (Table 4).

**Table 4: Sperm morphology.**

Groups	Normal	Abnormal
A (Control)	69.17 $\pm$ 3.7	30.83 $\pm$ 3.7
B (JnE)	77.50 $\pm$ 2.1 *	22.50 $\pm$ 2.1 *
C (Pb acetate )	60.00 $\pm$ 3.2 *	40.00 $\pm$ 3.2 *
D (Pb acetate + JnE)	70.83 $\pm$ 2.9	29.00 $\pm$ 2.0

\* = " $P < 0.05$ "

## Discussion

The significantly higher weight gain in group B compared to the control, and the non significant difference in weight gain of group C and group D rats with respect to the control prove that oral administration of aqueous extract of *Juglans nigra* for 8 weeks is able to

increase body weight gain while exposure to Lead acetate for 8 weeks did not significantly affect gain in body weight. This can be accounted for by the high fat component of *Juglans nigra* [18] and the anti-metabolic effect of Lead [19]. This is in support of the findings of Ronis *et al.* [20] that rats exposed to lead for 8 weeks showed significant impairment in neither growth nor weight gain. Longer period of exposure (for example 12 weeks and above) have been shown to significantly decrease gain in body weight [21].

The significant ( $P < 0.001$ ) increase and decrease in epididymal weight of group B and group C respectively, and the non significant difference in epididymal weight of group D show that while *Juglans nigra* extract protects and promotes the cells and tissues of the epididymis, lead exposure does the opposite. These further show that the extract is able to effectively reduce or even annul the cyto-toxic effect of lead (in the case of group D). These results coincide with the findings of Marchlewicz *et al.* [22] that lead exposure (and toxicity) causes epididymal damage and degeneration of epithelial and interstitial tissues, and those of Batra *et al.* [23] that lead has spermicidal effect on high exposure. These suggest that oral administration of extracts of *Juglans nigra* promotes maintenance of normal epididymal and interstitial metabolic activities. Similar effects of *Juglans nigra* noted by Wang *et al.* [24] were linked to its high copper content which also forms its antioxidant basis.

The highly significant ( $P < 0.0001$ ) decrease in sperm count in group C supports the finding of Xu *et al.* [9], that the testicular sperm count are important indicators of adverse effect of Lead on spermatogenesis, and this can be accounted for by direct influence of Lead on testicular tissues [23], these observations go in line with those of Salawu *et al.* [25] that exposure to Lead is capable of inducing infertility in male wister rats by significantly reducing sperm count. Meanwhile group B showed no significant difference in sperm count compared to the control. However, group D compared to the control also showed significant ( $P < 0.05$ ) decrease in sperm count, but group D had significantly ( $P < 0.05$ ) higher sperm count compared to group C. Thus, *Juglans nigra* although did not totally prevent the adverse effect of Lead on sperm count, it significantly reduce the Lead's adverse effect on the sperm count and significantly ( $P < 0.05$ ) increased Sperm count of Group D compared to Group C. This is a confirmation of the importance of *Juglans nigra* as a potent antioxidant and free radical scavenger [26, 27], which ameliorated the potential increase in free radicals generation from lead toxicity.

The non significant difference in testosterone level of group B and group D, and the significant decrease in testosterone level of group C compared to the control support the findings of Martin *et al.* [28] and Salawu *et al.* [25] that the impairment of spermatogenesis in lead toxicity is mainly a consequence of the decline in serum testosterone level. This further establishes that the normalcy found in group D despite their lead exposure must have resulted from the administered *Juglans nigra*. In this case, it could be proposed that *Juglans nigra* somehow affects the hypothalamo-pituitary-testicular axis, at least at a point in the axis.

The fact that groups B and D showed no significant difference in sperm motility from the control, Group C showed highly significant decrease in fast and slow motility relative to the control and a significant increase in non motile cells shows that Lead highly affect Sperm motility, a sign of infertility. This is in support of the findings of Jensen *et al.* [29], and Berry *et al.* [30] that administration of Lead acetate resulted in decline in sperm density, motility and viability. This could be due to the influence of lead acetate on sperm quality [23]. This result also gives evidence that *Juglans nigra* supports fertility in male rats. Based on its ability to improve motility, it is reasonable to predict that *Juglans nigra* has an effect on the mitochondria in the body of the tail of the spermatozoon, more so that the synthesis of energy (in the form of adenosine triphosphate) for sperm motility takes place in these mitochondria [31].

The significant decrease in normal and a significant increase in abnormal sperm morphology in group C rats compared to the control further confirms the anti-fertility effects of lead. These support the findings of Jensen *et al.* [29] and Berry *et al.* [30] that the administration of Lead acetate resulted in significant increase sperm abnormalities. This could be accounted for by the Lead's ability to cross the cell membrane in various ways that causes structural abnormality [32]. The significant increase in normal sperm morphology and a significant decrease in abnormal sperm morphology in group B, and the non significant difference of group D from the control confirms that *Juglans nigra* is effective in retaining cell structural components. This observed cyto-protective effect of *Juglans nigra* is in support of the findings of Batra *et al.* [22], but could still be linked to its nutritional components as well as the electrolyte components which actively competes with lead and thus prevents lead's adverse effects [23].

It can be concluded that exposure to Lead causes testicular toxicity by increasing abnormalities in sperm

characteristics (sperm count, sperm morphology, sperm motility), decrease in epididymal weight and testosterone level. *Juglans nigra*, however, prevented (significantly reduced) Lead induced testicular toxicity, which is linked to its antioxidant activity.

## References

- Aicheson L. A History of Metals. Interscience: New York, 1960:54-61.
- Alan WC. A Short History of Metals. <http://neon.mems.cmu.edu/cramb/Processing/history.html> Accessed April 1, 2010.
- Flora SJ, Mittal M, Mehta A. Heavy metal induced oxidative stress & its possible reversal by chelation therapy. *Indian J Med Res.* 2008;128(4):501-23. [PMID:19106443](#).
- Meyer PA, Brown MJ, Falk H. Global approach to reducing lead exposure and poisoning. *Mutat Res.* 2008;659(1-2):166-75. [doi:10.1016/j.mrrev.2008.03.003](#) [PMID:18436472](#).
- Vaziri ND. Mechanisms of lead-induced hypertension and cardiovascular disease. *Am J Physiol Heart Circ Physiol.* 2008;295(2):H454-65. [doi:10.1152/ajpheart.00158.2008](#) [PMID:18567711](#).
- Patrick L. Lead toxicity, a review of the literature. Part 1: Exposure, evaluation, and treatment. *Altern Med Rev.* 2006;11(1):2-22. [PMID:16597190](#).
- Hammond PB. Exposure of humans to lead. *Annu Rev Pharmacol Toxicol.* 1977;17:197-214. [doi:10.1146/annurev.pa.17.040177.001213](#) [PMID:326164](#).
- Emmerson BT. Chronic lead nephropathy. *Kidney Int.* 1973;4(1):1-5. [doi:10.1038/ki.1973.73](#) [PMID:4353288](#).
- Xu HH, Chen ZP, Shen Y, Wu X, He F. [Meta analysis for effect of lead on male productive function]. *Zhonghua Lao Dong Wei Sheng Zhi Ye Bing Za Zhi.* 2006;24(10):634-6. Chinese. [PMID:17096959](#).
- Quinlan GJ, Halliwell B, Moorhouse CP, Gutteridge JM. Action of lead(II) and aluminium (III) ions on iron-stimulated lipid peroxidation in liposomes, erythrocytes and rat liver microsomal fractions. *Biochim Biophys Acta.* 1988;962(2):196-200. [PMID:3167077](#).
- Neathery MW, Miller WJ. Metabolism and toxicity of cadmium, mercury, and lead in animals: a review. *J Dairy Sci.* 1975;58(12):1767-81. [doi:10.3168/jds.S0022-0302\(75\)84785-0](#) [PMID:1107364](#).
- California Walnut Commission Website (2005). Scientific research update for health professionals. Available online: [http://www.walnuts.org/health/han\\_science\\_news.asp](http://www.walnuts.org/health/han_science_news.asp) Science news. Accessed April 1, 2010.
- Green RE, Hunt WG, Parish CN, Newton I. Effectiveness of action to reduce exposure of free-ranging California condors in Arizona and Utah to lead from spent ammunition. *PLoS One.* 2008;3(12):e4022. [doi:10.1371/journal.pone.0004022](#) [PMID:19107211](#).
- Hurt GS, Howards SS, Turner TT. Repair of experimental varicoceles in the rat. Long-term effects on testicular blood flow and temperature and cauda epididymal sperm concentration and motility. *J Androl.* 1986;7(5):271-6. [PMID:3771366](#).
- Hurt GS, Howards SS, Turner TT. Repair of experimental varicoceles in the rat. Long-term effects on testicular blood flow and temperature and cauda epididymal sperm concentration and motility. *J Androl.* 1986;7(5):271-6. [PMID:3771366](#).
- Salah N, Miller NJ, Paganga G, Tijburg L, Bolwell GP, Rice-Evans C. Polyphenolic flavanols as scavengers of aqueous phase radicals and as chain-breaking antioxidants. *Arch Biochem Biophys.* 1995;322(2):339-46. [PMID:7574706](#).
- Saalu LC, Oluyemi KA, and Omotuyi IO. Alpha-tocopherol (vitamin E) attenuates the testicular toxicity associated with experimental cryptorchidism in rats. *African Journal of Biotechnology.* 2007;6(12):1373-1377.
- Foster S and Duke JA. Black walnut in Medicinal Plants. Hughton Mifflin Company, New York; 1990:276.
- Biswas NM, Ghosh P. Effect of lead on male gonadal activity in albino rats. *Kathmandu Univ Med J (KUMJ).* 2004;2(1):43-6. [PMID:19780287](#).
- Ronis MJ, Badger TM, Shema SJ, Roberson PK, Shaikh F. Reproductive toxicity and growth effects in rats exposed to lead at different periods during development. *Toxicol Appl Pharmacol.* 1996;136(2):361-71. [doi:10.1006/taap.1996.0044](#) [PMID:8619245](#).
- Wadi SA, Ahmad G. Effects of lead on the male reproductive system in mice. *J Toxicol Environ Health A.* 1999;56(7):513-21. [doi:10.1080/009841099157953](#) [PMID:10201637](#).
- Marchlewicz M, Michalska T, Wiszniewska B. Detection of lead-induced oxidative stress in the rat epididymis by chemiluminescence. *Chemosphere.* 2004;57(10):1553-62. [doi:10.1016/j.chemosphere.2004.08.102](#) [PMID:15519400](#).
- Batra N, Nehru B, Bansal MP. Influence of lead and zinc on rat male reproduction at 'biochemical and histopathological levels'. *J Appl Toxicol.* 2001;21(6):507-12. [doi:10.1002/jat.796](#) [PMID:11746199](#).
- Wang L, Xun P, Zhao Y et al. Effect of lead exposure on sperm concentration and Testes weight in male rats. *J Toxicol Environ Health.* 2008;71(7):454-463. [doi:10.1080/15287390701839331](#) [PMID:18306093](#).

25. Salawu EO, Adeeyo OA, Falokun OP, Yusuf UA, Oyerinde A, Adeleke AA. Tomato (*Lycopersicon esculentum*) prevents lead-induced testicular toxicity. *J Hum Reprod Sci.* 2009;2(1):30-4. doi:[10.4103/0974-1208.51346](https://doi.org/10.4103/0974-1208.51346) PMID:[19562072](https://pubmed.ncbi.nlm.nih.gov/19562072/).
26. Ngadjui BT, Abegaz BM, Keumedjio F, Folefoc GN, Kapche GW. Diterpenoids from the stem bark of *Croton zambesicus*. *Phytochemistry.* 2002;60(4):345-9. doi:[10.1016/S0031-9422\(02\)00034-1](https://doi.org/10.1016/S0031-9422(02)00034-1) PMID:[12031423](https://pubmed.ncbi.nlm.nih.gov/12031423/).
27. Okokon JE, Ofodum KC, Ajibesin, KK, Danladi B and Gamaniel KS. Pharmacological screening and evaluation of antiplasmodial activity of *Croton zambesicus* against *Plasmodium berghei berghei* infection in mice. *Indian J Pharmacol.* 2005;37:243-246. doi:[10.4103/0253-7613.16571](https://doi.org/10.4103/0253-7613.16571).
28. Ronis MJ, Badger TM, Shema SJ, Roberson PK, Shaikh F. Reproductive toxicity and growth effects in rats exposed to lead at different periods during development. *Toxicol Appl Pharmacol.* 1996;136(2):361-71. doi:[10.1006/taap.1996.0044](https://doi.org/10.1006/taap.1996.0044) PMID:[8619245](https://pubmed.ncbi.nlm.nih.gov/8619245/).
29. Jensen TK, Bonde JP, Joffe M. The influence of occupational exposure on male reproductive function. *Occup Med (Lond).* 2006;56(8):544-53. doi:[10.1093/occmed/kql116](https://doi.org/10.1093/occmed/kql116) PMID:[17151390](https://pubmed.ncbi.nlm.nih.gov/17151390/).
30. Berry WD Jr, Moriarty CM, Lau YS. Lead attenuation of episodic growth hormone secretion in male rats. *Int J Toxicol.* 2002;21(2):93-8. doi:[10.1080/10915810252866060](https://doi.org/10.1080/10915810252866060) PMID:[12022635](https://pubmed.ncbi.nlm.nih.gov/12022635/).
31. Duke J. The ultimate compendium of Natural remedies from the worlds foremost Authority on healing and herbs. The Green Pharmacy Rodale press 1997;248; 334; 463; 481.
32. Thoreux-Manlay A, Vélez de la Calle JF, Olivier MF, Soufir JC, Masse R, Pinon-Lataillade G. Impairment of testicular endocrine function after lead intoxication in the adult rat. *Toxicology.* 1995;100(1-3):101-9. doi:[10.1016/0300-483X\(95\)03066-O](https://doi.org/10.1016/0300-483X(95)03066-O) PMID:[7624866](https://pubmed.ncbi.nlm.nih.gov/7624866/).