Journal of Medicinal Plants Research Vol. 4(12), pp. 1102-1107, 18 June, 2010
Available online at http://www.academicjournals.org/JMPR
DOI: 10.5897/JMPR09.128
ISSN 1996-0875 ©2010 Academic Journals

Full Length Research Paper

Interactive roles of terpenoid extract from the leaves of neem plant (*Azadirachta indica*, A. Juss) on lead induced toxicity in pregnant rabbits

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Accepted 11 May, 2010

This study was designed to investigate the interactive roles of terpenoid extract from the leaves of Neem plant (*Azadirachta indica*, A. Juss) on lead-induced toxicity in pregnant rabbits and the resulting litters. Terpenoid was extracted from the air dried leaves of *A. indica* by previously reported procedure. Pregnant rabbits (does) weighing between 1.8 - 2.0 kg was randomly divided into 4 treatment groups: Group I (Control Group) received olive oil (2 ml/kg body weight per day). Group II (Lead Group) received Lead acetate solution (50 mg/kg body weight per day). Group III (Positive Control Group) received ascorbic acid (400 mg/kg body weight per day) and Group IV (Terpenoid Group) received with terpenoid extract (300 mg/kg body weight per day). Lead acetate solution (50 mg/kg body weight per day) was later administered to animals in groups III and IV. The animals were treated for 11 days starting from day 14 of gestation period of the animals. The does and the litters (young rabbits) were sacrificed 4 weeks after parturition. Blood plasma, whole blood was obtained for biochemical and lead analysis respectively. The liver, kidney, heart and lungs were removed for lead analysis. Lead concentration in the blood and the tissues was determined by atomic absorption spectroscopy (AAS). The total protein concentration in the plasma and liver homogenates were determined using biuret reaction method. The plasma albumin concentration, hepatic marker enzymes, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were also determined by standard spectrophotometric methods. The results revealed that, the terpenoid extract was able to reduce lead concentration in the blood, liver and kidney of pregnant rabbits. The levels of reduction was found to be statistically significant ($p < 0.05$) but the extract had no significant effect on its concentration of lead in the tissues of the litters, suggesting that the extract could not prevent inter placenta transfer of lead. Liver protein, plasma protein and albumin concentration, ALT and AST activities were not significantly different in all the groups. Conclusively, the extract was also able to reduce the lead burden in pregnant rabbits but could not produce the same effects on the litters.

Key words: Pregnant rabbit, lead, toxicity, terpenoid, blood.

INTRODUCTION

Virtually all metals are toxic to humans if they are ingested in large sufficient quantities. Exposure to these metals occurs in many diverse circumstances. Inadvertent environmental exposure is perhaps the major concern of toxicologist. Cadmium, lead, mercury, arsenic and nickel are examples of these toxic metals. Out of these, lead is the most common metal in the environment because of its wide industrial uses such as an additive in petrol, paint, hair dye, solder and battery manufacturing etc (Rusell - Jones, 1982; Timbrel, 1995; Babalola et al., 2005). In recent years, there has been concern among the environmental toxicologists that low-level chronic exposure to lead may be responsible for the increase cardiovascular disease, central nervous system disorders

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especially in children and infertility problems. Earlier researches had also shown that lead is a multi-targeted toxicant causing effects in many systems and organs. (Babalola et al., 2005). Further studied has however revealed that body lead burden can be reduced by the use of synthetic chemical agents called chelators such as meso -2, 3- dimercaptosuccinic acid (DMSA) and calcium disodium ethylene diaminetetra acetic acid ($CaNa_2$-EDTA). (Flora et al., 1995; Yokoyama et al., 1998) Ascorbic acid has also been shown to offer protection against lead toxicity in laboratory animals (Gajowat et al., 2006). Current effort was undertaken to investigate and examine the possibility of terpenoid extract from the leaf of *Azardirachta indica* to ameliorate the toxic effect of lead in pregnant rabbits. The findings could be useful to understand lead toxicity in pregnant animals and shed more light on the possibility of using natural products to combat lead toxicity as against synthetic chemicals with various side effects.

MATERIALS AND METHODS

Plant

Fresh leaves of *A. indica* were collected in March, 2007 from Obafemi Awolowo University Campus Ile-Ife, Nigeria. The plant was identified and authenticated by Dr. F. A. Oloyede of Botany department, Obafemi Awolowo University, Ile-Ife. The leaves were air dried in the laboratory for 21days. Tetracyclic triterpenoids was extracted from dried leaves of *A. indica* by the procedure described by Siddique et al. (2003).

Experimental animals

Eight healthy matured virgin female rabbits (Does) with a weight range of 1.8 - 2.0 kg were purchased from the rabbitry unit of Training and Research farm, Obafemi Awolowo University, Ile-Ife. The animals were acclimatized for 28 days in the animal house where they had free access to standard pellets (Guinea Feeds, Benin City, Nigeria) and clean water. They were caged individually. At the expiration of isolation period, each female rabbit (Doe) was mated with matured male rabbits (Buck) for 2 consecutive days. The first and second mating days were designated day 0 and day 1 of pregnancy respectively. Pregnancy test called palpation was performed on day 12 of gestation period to ascertain the pregnancy. The rabbits were randomly divided into 4 treatment groups, each of these were treated between Day 14 - 24 of the gestation period as follows:

Group 1 (normal control) - Animals received 2 ml/kg body weight (b.wt) per day of olive oil (the vehicle for administration of extract).
Group 2 (test 1) - Animals received lead acetate solution only (50 mg/kg b.wt per day).
Group 3 (test 2) - Animals were pretreated with terpenoid extract (300 mg/kg b.wt per day) and one hour later, lead acetate (50 mg/kg b.twt per day) was given to each of the animals.
Group 4 (positive control) - Animal were pretreated with ascorbic acid (400 mg/kg b.wt per day) one hour before the administration of lead acetate (50 mg/kg b.wt per day).

When the treatment was completed on day 24, a nest box was placed in each of the cages for the doe to give birth in. The Does were allowed to nurse the litters for 4 weeks. At the end of the 4th week, the Does and the litters (minimum of six in each group) were then sacrificed by stunning method. Blood samples were collected from each of the animals by cardiac puncture using disposable pyrogen free needle and stunning method. Blood samples were collected from each of the animals by cardiac puncture using disposable pyrogen free needle and syringe. The blood samples were carefully transferred into labeled heparinized tubes containing lithium heparin. Livers, lungs, kidneys and the heart were also removed and processed for further biochemical studies.

Preparation of blood plasma

A portion of the blood samples collected was centrifuged at 3000 rpm for 10 min in Gallenhamp Junior Table centrifuge at room temperature. The supernatants were collected into sterile sample bottles, labeled and kept in the deep freezer for further analyses.

Preparation of liver homogenates

One gram me (1 g) of Liver was homogenized separately in 10 ml of 100 mM phosphate buffer, pH 7.4 and centrifuged at 12000 rpm for 30 min. The supernatants were collected into clean sterile bottles, labeled and kept in the deep freezer for further analyses.

Digestion of plant material

Dried leaf of *A. indica* (1 g) was suspended in 100 ml of distilled water. 10 ml of Aqua Regia (1:4 v/v Conc. $HN0_3$: HCl) was added, mixed in a conical flask and put in water bath at 100°C. It was heated for two hours, allowed to cool down, filtered and the filtrate made up to 50 ml with distilled water. The filtrate was kept in polyethylene bottle for AAS analysis.

Digestion of whole blood

Whole blood sample was used for this analysis. 1 ml of the blood was measured into a clean test tube; 2 ml of concentrated HNO_3 containing 0.1% Triton x-100 was added and mixed carefully to prevent spillage. The tube was covered with cotton wool and left on the bench overnight to solubilize the sample. The content was made up to 25 ml with distilled water. The digested sample was stored in 30 ml polyethylene bottle for later analysis by Atomic Absorption Spectrophotometer (AAS) at a wavelength of 293.3 nm.

Digestion of organs

One gramme of each organ was homogenized in 5 ml of concentrated $HN0_3$, the homogenate was poured into a test tube, covered with cotton wool and left on the bench overnight to solubilize the tissue. The sample was heated at 100°C in water bath for 20 min. It was allowed to cool before the addition of 1.0 ml of hydrogen peroxide (H_2O_2). The sample was then diluted to a final volume of 25 ml with distilled water and stored in a 30 ml polyethylene bottle for later analysis by AAS.

Estimation of total protein concentration in plasma and liver homogenate

The total protein concentration, in the plasma and liver homogenates were estimated using a modified Biuret reaction method as described earlier by Gornall et al. (1949).

Table 1. Lead concentration in blood and various organs of the four treatment groups.

Blood/organs	Does	Litters
Blood		
Group 1		2.00 + 1.08
Group 2		10.00 + 1.00
Group 3		1.00 + 0.10
Group 4		12.00 + 0.41
Liver		
Group 1		0.50 + 0.05
Group 2		11.00 + 1.41
Group 3		0.25 + 0.25
Group 4		10.00 + 2.35
Kidney		
Group 1	2.50 + 0.50	0.25 + 0.02
Group 2	21.50 + 0.50	13.50 + 1.50
Group 3	1.50 + 0.50	0.00+ 0.00
Group 4	7.00 + 1.70	12.75 + 0.70
Heart		
Group 1	0.00 + 0.00	0.00 + 0.00
Group 2	3.50 + 0.50	1.00 + 0.00
Group 3	0.00 + 0.00	0.00 + 0.00
Group 4	0.00 + 0.00	0.00 + 0.00
Lungs		
Group 1	0.00 + 0.00	4.50 + 0.50
Group 2	0.00 + 0.00	22.00 + 1.00
Group 3	0.00 + 0.00	8.00 + 1.00
Group 4	0.00 + 0.00	17.00 + 2.00

Values are in µg/dl and expressed as means + SEM (standard error of mean).
Group1 = Control group.
Group 2 = Lead only treated group.
Group 3 = Ascorbic acid pretreated group (POSITIVE control).
Group 4 = Terpenoid extract pretreated group.

Estimation of plasma albumin

The plasma albumin was estimated using an Albumin kit and the procedure as described by Pinnel and Northam, 1978.

Plasma alanine aminotransferase (ALT)

The plasma alanine aminotransferase activity was estimated as described by Reitman and Frankel, 1957.

Plasma aspartate aminostransfeaze (AST)

The plasma aspartate aminotranferase was assayed as described by Reitman and Frankel, 1957. One unit of AST activity was defined as the amount of protein that liberated 1.0 µmole of oxaloacetate per min at 37°C under experimental condition.

Statistical analysis

Data were analyzed by one way analysis of variance (ANOVA) follow by Duncan's multiple range test (DMRT) using statistical software package (SPSS for Windows). The results were presented as mean ± SD. $P < 0.05$ were regarded as statistically significant.

RESULTS

Table 1 shows the lead concentration in the blood and various organs of experimental animals (the does and the litters) in the four treatment groups. Table 2 shows the comparison of lead concentration in blood, liver and kidney of control group versus lead group and control

Table 2. Comparison of lead concentration in blood, liver and kidney of controls vs. lead treated group and control vs terpenoid pretreated treated groups.

Experimental group	Blood (µg/dL)	Liver (µg/dL)	Kidney (µg/dL)
Group 1 (D)	4.50 ± 0.50[b]	3.00 ± 2.00[a]	2.50 ± 0.05[a]
Group 2 (D)	22.00 ± 100[a]	25.00 ± 2.00[a]	21.50 ± 0.05[a]
Group 1 (L)	2.00 ± 1.08[b]	0.05 ± 0.05[a]	0.025 ± 0.02[a]
Group 2 (L)	10.00 ± 1.00[a]	11.00 ± 1.44[a]	13.5 ± 1.50[a]
Control (D)	4.50 + 0.05b	3.00 + 1.00a	2.50 + 0.50a
Group 4(D)	17.00 + 2.00a	17.50 + 2.50a	7.00 + 1.00b
Group 1 (L)	2.00 + 1.08b	0.50 + 0.05a	0.25 + 0.02a
Group 4 (L)	12.00 + 0.41b	10.00 + 2.35b	2.75 + 1.70a
Group 3 (D)	8.00 + 1.00b	7.50 + 0.50b	1.50 + 1.50a
Group 4 (D)	17.00 + 2.00b	17.50 + 2.50b	7.00 + 1.00a
Group 3(L)	1.00 + 0.10b	0.25 + 0.25a	0.00 + 0.00b
Group 4 (L)	12.00 + 0.41b	10.00 + 2.35a	2.75 + 1.70a

Each value represents Means ± SEM (Standard error of mean)
Values not sharing a common superscript within each row differ significantly at $P < 0.05$
(D) - Does, (L) - Litters
Group1 = Control group
Group 2 = Lead only treated group
Group 3 = Ascorbic acid pretreated group (positive control)
Group 4 = Terpenoid extract pretreated group.

Table 3. Comparison of total protein and albumin concentration in plasma and liver of the control and lead treated groups.

Protein/Albumin	Control	Lead
Plasma albumin (g/dL)-(D)	4.40+0.10[a]	4.28+0.08[a]
Plasma albumin (g/dL)-(L)	2.53+0.13[a]	2.48+0.25[a]
Plasma protein (g/dL)-(D)	6.81+0.11[a]	6.47+0.17 [a]
Plasma protein (g/dL)-(L)	4.07+0.06[a]	3.51+0.22 [a]
Liver albumin (g/dL)-(D)	3.26+0.06[a]	3.47+0.17[a]
Liver albumin (g/dL)-(L)	1.98+0.05[a]	1.82+0.29[a]
Liver protein (g/dL)-(D)	3.57+0.22[a]	3.24+0.08[a]
Liver protein (g/dL)-(L)	2.51+0.14[a]	2.29+0.30[a]

Each value represents mean + SEM (Standard Error of mean).
Values not sharing a common superscript within each row differ significantly at $P < 0.05$.
D – Does, L – Litters.
D – Does, L – Litters.

Table 4. Comparison of total protein and albumin concentration in plasma and liver of the control and terpenoid treated groups.

Protein/Albumin	Control	Terpenoid
Plasma albumin (g/dL)-(D)	4.40+0.10[a]	4.02+0.10[a]
Plasma albumin (g/dL)-(L)	2.53+0.13[a]	2.32+0.16[a]
Plasma protein (g/dL)-(D)	6.81+0.11[a]	6.30+0.05[a]
Plasma protein (g/dL)-(L)	4.07+0.06[a]	3.36+0.28[a]
Liver albumin (g/dL)-(D)	3.26+0.06[a]	3.09+0.07[a]
Liver albumin (g/dL)-(L)	1.98+0.05[a]	1.95+0.18[a]
Liver protein (g/dL)-(D)	3.57+0.22[a]	3.38+0.14[a]
Liver protein (g/dL)-(L)	2.51+0.14[a]	2.48+0.25[a]

Each value represents mean + SEM (Standard Error of Mean)
Values not sharing a common superscript within each row differ significantly at $P < 0.05$
D – Does, L- Litters.

group versus terpenoid treated groups. Tables 3 and 4 show the comparison of total protein and albumin concentration in plasma and liver of the controls vs lead treated Groups and control vs terpenoid treated Groups respectively. While Tables 5 and 6 show the comparison of hepatic maker enzyme activity in control vs. lead only treated group, and control vs terpenoid treated group respectively.

DISCUSSION

Terpenoid are present in relatively small quantity in the leaves of many plants where they play protective role against the herbivores (Rosenthal, 1979). In this study, terpenoid extract was well tolerated by the pregnant rabbits as no death was recorded among the does exposed to the extract. No abortion was also recorded at the end of the gestation periods, this shows that the extract is not an abortificient, and no still birth, this also indicates that the extract is probably not embryotoxic. The extract is also not teratogenic because the litters did not show any deformity or malformation when they were examined at birth. Potential natural drugs for pregnant animals must possess all these qualities.

Table 1 showed lead concentration in the blood and other tissues namely the kidney, liver, heart and lung of the experimental animals. These data shows that animals

Table 5. Comparison of hepatic maker enzyme activity (Control vs. Lead group).

Enzyme	Control	Lead
Plasma ALT (µmole/ml)-(D)	12.00+0.00[a]	13.25+1.50[b]
Plasma ALT (µmole/ml)-(L)	8.00+0.30[a]	8.38+074[a]
Plasma AST (µmole/ml)-(D)	21.75+1.50[a]	24.00+0.00[b]
Plasma AST (µmole/ml)-(L)	12.88+0.85[a]	11.00+0.50[b]
Liver ALT (µmole/ml)-(D)	20.00+2.50[a]	21.50+1.20[a]
Liver ALT (µmole/ml)-(L)	10.88+0.80[a]	10.00+0.05[a]
Liver AST (µmole/ml)-(D)	24.50+0.50[a]	24.50+1.00[a]
Liver AST (µmole/ml) -(L)	12.13+1.06[a]	14.25+1.05[b]

Each value represents mean + SEM (Standard Error of mean).
Values not sharing a common superscript within each row differ significantly at $P < 0.05$.
D – Does, L- Litters.

Table 6. Comparison of hepatic makers enzyme activity (Control vs terpenoid group).

Enzyme	Control	Terpenoid
Plasma ALT (µmole/ml) - (D)	12.00+0.00[a]	12.75+1.50[a]
Plasma ALT (µmole/ml) - (L)	8.00+0.30[a]	7.38+0.38[b]
Plasma AST (µmole/ml) - (D)	21.75+1.50[a]	23.50+0.75[b]
Plasma AST (µmole/ml) - (L)	12.88+0.85[a]	8.50+0.15[b]
Liver ALT (µmole/ml) - (D)	20.00+2.50[a]	22.00+1.00[b]
Liver ALT (µmole/ml) - (L)	10.88+0.80[a]	9.88+0.20[b]
Liver AST (µmole/ml) - (D)	24.50+0.50[a]	24.75+0.05[a]
Liver AST (µmole/ml - (L)	12.13+1.06[a]	10.63+0.32[b]

Each value represents Mean + SEM (Standard Error of mean).
Values not sharing a common superscript within each row differ significantly at $P < 0.05$.
D – Does, L- Litters.

in control group contained traces of lead in the blood, Liver and kidney. This was not expected because animals in the control group were not exposed to lead. However the level were significantly lower to that obtained in the lead only treated group. The trace amount in the controls could probable be from the commercial feeds given to the animal. Animals in lead treated group showed highest concentration of lead in the blood, liver and kidney compared with the other experimental group. Lead in the blood is transient; it migrates to the liver and kidney after few weeks of exposure. Lead also leaves the soft tissues and bioaccumulate in the bone permanently (Needleman et al., 1972; Shapiro et al., 1975).

No abortion and no still birth were recorded in the animals exposed to lead only. Lead is a known abortifacient at high concentration; it was extensively used in the past to terminate pregnancies, when the toxic effect of lead was not known. This observation has been confirmed by various researchers through the studies carried out on samples of human placenta from spontaneous abortions, still births and miscarriages. An amazingly high lead concentration was found in all the placenta studies (Bryce-Smith, 1974; Hricko, 1978). However, at relatively low lead concentration, toxic effects on pregnant animals enumerated above were absent as observed in this study. Presence of substantial quantity of lead in the blood, liver and kidney of litters produced by does exposed to lead during pregnancy confirmed the findings of reproductive scientists that lead can easily cross the placenta barrier during foetal development in utero (Rajegowda et al., 1972; Carpenter, 1974).

Experimental animals in the ascorbic acid group contained the least quantity of lead in the blood and tissues among the experimental group. The result showed that ascorbic acid was able to reduce the lead burden in the pregnant does. The litters produced also showed very low lead concentration, comparable with what was obtained in control group. This result is in agreement with findings by other researchers that vitamin c has the ability to reduce the lead burden in laboratory animals and in human (Gajawat et al., 2006).

Lead concentration in animals pre-treated with terpenoid extract is not significantly different from the lead only treated group. This observation may suggest that, terpenoid extract from the leaves of *A. indica* probably does not possess therapeutic property to reduce lead burden in pregnant rabbits as was observed in ascorbic acid group. Comparison of metabolites concentration between the control and experimental groups did not show significant differences. Plasma protein and plasma albumin showed no significant difference lead, terpenoid and ascorbic acid treated groups respectively when compared with control group. This appears to be similar to what was obtained for liver proteins dut in the does and the litters.

Concentration of total protein and albumin in the plasma and liver are commonly used to evaluate liver functions. Significant reduction in the concentration of these metabolites is an indication of severe liver injury because liver is the principal organ responsible for the synthesis of these proteins (Herper, 1961). Albumin synthesis is sensitive to amino acid supply and thus nutrition state plays important roles in albumin concentration.

The activities of L-alanine aminotrasferase (ALT) and L-aspartate aminotrsferse (AST) in the control group and experimental group were also compared. ALT and AST is hepatocellular injury marker (Herper, 1961; Higgins, 2000). Results of the comparison show that there were insignificant differences between the control and experimental group. Hepatic enzymes activities were also similar in the litters from control group and experimental group. Normal liver function observed in all the experimental groups probably suggests that the level of lead at this dose is well tolerated.

In conclusion, from the results of this research work it is clear that terpenoid extract from the leaves of *Azadiracta indica* is not embryotoxic, non-teratogenic and does not posses abortifacient property. Also, the extract did not

show ability to interfere with lead from crossing the placenta and to reduce lead burden in the pregnant rabbits under laboratory conditions. Further studies are recommended to determine the effects of this extract on higher doses of lead.

REFERENCES

Babalola OO, Ojo LO, Aderemi MO (2005). Lead levels in some biological samples of aeromechanics in Abeokuta, Nigeria. Indian. J. Biochem. Biophys. 42: 401-403.

Bryce-Smith D, Despande RR, Hughes J, Waldron HA (1977). Lead and Cadmium levels in the still births. Lancetn pp. I: 1159.

Bryce-Smith D (1983). Lead induced disorders and mentation in children. Nutr. Health 1: 179-194.

Carpenter SJ (1974). Placenta permeability of lead. Env. Health Perspect. 7: 129-130Ong, CN, Lee WR (1980). High affinity of lead for fetal hemoglobin Br. J. Int. Med. 37: 292-298.

Flora GJ, Seth PK, prakash AO (1995). Therapentic efficacy of combined meso 2,3-dimeracap to succinic acid and calcium disodium edentate treatment during acute lead intoxication in rats. Hum. Exp. Toxicol. 14: 410-413.

Gajowat S, Sancheti G, Goyal PK (2006). Protection against lead-induced hepatic lesions in swiss albino mice by ascorbic acid. Pharmacology online 1: 140-149.

Gornall AG, Bandawil CJ, David MM (1949). Determination ofserum proteins by Biuret reaction Jibiol Chem.117:.751-756.

Goyer RA (1971). Lead Toxicity a problem in the environment. Am. J. Pathol. 64: 167-169.

Herper HA (1961). The functions and tests of the liver In: " Review of publications Los Attos, California pp. 271-283.

Higginys C (2000). Clinical Biochemistry Tests. In understanding Laboratory Investigations". A test for Nurses and health care professionals Black well science Ltd,,USA pp. 33-70.

Hricko A (1978). A social policy consideration of occupational health standards: The example of lead and reproductive effects. Prev. Med. 7: 394-406.

Needleman HL, Gunnoe G, Leviton A, Reed D (1979). Deficis in Psychologic and classroom performance of Children with elevated dentine lead levels. New Engl. J. Med. 290: 245-250.

Pinnel AE, Northam BE (1978). New automated bye-binding method for serum albumin determination with bromocresol purple. Clin. Chem. pp. 24: 80.

Rajegowda BK, Glass L, Evans HE (1972). Lead concentrations in newborn infants J. Pediates. 80: 116-117.

Reitman S, Frankel S (1957). A Colorimetric Method for the Determination of Serum Glutamic oxaloacetic and Glutamic pyruvic transaminase . Am. J. Clin. Pathol. 28: 58-63.

Rosenthal G (1990). Herbivores: Their interaction with secondary plant metabolites, New York: Acad. Press. pp. 502-507.

Russell-Jones R (1982). Lead in petrol. Brit. med. J. pp. 284:902.

Shapiro IM, Needleman HL, Tuncay OC (1972) The lead content of human deciduous and permanent teeth. Env. Res. 5: 467.

Shapiro IM, Mitchell G, Davidson I, Katz SH (1975). The lead content of teeth. Arch. Environ. Health 30: 483-486.

Siddique JB, Afshan F, Gulzar T, Sultama R (2003) . Tetracyclic triterpenoid from the leaves of Azadirachta indica and their insecticides activities. Chem. Pharm. Bull 51(4): 415 - 417

Timbrel JA (1995). Lead Pollution in introduction to toxicology 2nd edition, London: Taylor and Francis pp. 111-114.

Weiss ST, Munoz A, Stein A, Sparrow D, Speizer FE (1986) The relationship of blood Lead to blood pressure in a longitudinal study of working men. Am. J. Epid. 123: 800-808

Yokoyama K, Araki S, Aono H (1998). Calcium disodium ethylenediamine tetra acetate-chelated lead as a predictor for sub clinical lead neurotoxicity: follow up study on gun-metal fundry workers. Int. Arch. Occup. Environ. Health 71: 459-464.