RESEARCH PAPER:

Protective effect of vitamins on lead induced reproductive toxicity in female swiss mice

NAZERA QURESHI, RAGINI SHARMA AND SHEETAL MOGRA

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See end of the article for authors' affiliations

Correspondence to :
NAZERA QURESHI
Environmental Toxicology Research Laboratory, Department of Zoology, University College of Science, M.L. Sukhadia University, UDAIPUR (RAJASTHAN) INDIA

SUMMARY

In the present study, effects of lead toxicity on fertility of female Swiss mice has been investigated. Lead toxicity was induced by lead acetate given orally as a daily dose 160mg/kg and 320mg/kg for 3 months. The treatment of lead poisoning through chelating agents can remove essential elements, resulting in kidney damage. Oxidative damage associated with presence of lead has been illustrated as one possible mechanism involved in lead toxicity which suggests that antioxidant (vitamin C and E) might play a role in the treatment of lead induced fertility. The role of vitamins in treating/preventing chronic lead toxicity in animals is receiving wide attention. Therefore, along with above study, therapeutic effects of ascorbic acid and alpha tocopherol on lead induced toxicity have also been investigated. In low as well as high lead treated groups, there was decline in fertility. Supplementation of ascorbic acid along with lead did not provide successful beneficial results, but treatment of alpha tocopherol resulted in improved fertility outcomes.

Key words :
Lead, Ovary, Alpha tocopherol, Ascorbic acid, Fertility

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A number of heavy metals are still widely used in industry and lead, in particular, is generally considered as one of the most toxic metals to humans as well as animals (Yu, 2001). Lead has a long environmental persistence and never losses its toxic potential. Today, lead is still used in batteries and some insecticides and is found in cigarette smoke, varying with range of 0.017 and 0.98 μg per cigarette (Timbrell, 1995; Williams *et al.*, 1999). Lead is highly toxic to humans, with the deleterious effects on the hemopoietic, nervous, reproductive systems and the urinary tract. Lead has been shown to cross the placenta during pregnancy and has been associated with intrauterine death, prematurity and low birth weight (Papanikolaou *et al.*, 2005). Reproductive toxicity, which can be defined as the adverse effect of chemicals, lead being one that can affect the gonadal structure and functions, can cause alterations in fertility and impaired gamete function (British National Formulary, 1999; Hu, 1998; Timbrell, 1995). Lead poisoning causes reduced fertility, miscarriages and stillbirths since antiquity (Bell and Thomas, 1980).Gestational lead exposure has an adverse effect on development, with an effects that may be most peonounced during the first trimester(Mogra *et al.*, 2009).

The treatment of lead poisoning, especially at subclinical level, is equally important. The prospects for successful chemical treatment of long term, low dose lead toxicity, are not promising. Chelating agents can remove essential elements, resulting in kidney damage (Mahaffey *et al.*, 2000). Hence, metal chelation therapy has not been more successful to treat lead poisoning (Bondy, 1988).

Oxidative damage associated with the presence of lead has been illustrated as one possible mechanism involved in lead toxicity (Adonoylo and Oteiza, 1999), which suggests that antioxidant (vitamin C and E) might play a role in the treatment of lead induced fertility (Gurer *et al.*, 2001). Naturally occurring antioxidants have been extensively studied for their capacity to protect organisms and cells from damage induced by oxygen reactive species (Cozzi *et al.*, 1997). The role of vitamins in treating/preventing chronic lead toxicity in animals is receiving wide attention (Anitra and Frei, 1999).

Ascorbic acid has long been associated with fertility (Luck *et al.*, 1995), is a definite antioxidant and plays protective role against metal toxicity (Rao *et al.*, 2001; Houston and Johnson, 2000). Vitamin E is known as the antisterility vitamin because it is found to be

necessary for normal reproduction in animals.

MATERIALS AND METHODS

Inbred, healthy, female Swiss mice in the age group of 5-6 weeks, with 22-28 gm body weight were used for the experiment and divided into ten groups containing ten animals in each. These groups were treated orally with a low (160 mg/ kg / day) and high (320 mg / kg / day) dose of lead acetate, vitamin C (200 mg / kg/ day) and vitamin E (160 mg / kg / day) for 3 months according to following schedule:-

Group I (Control group) – This group served as control group, and was given distilled water through Canula.

Group II (LL) – Treated with lower dose of lead acetate (160 mg / kg/ day).

Group III (LH) – Treated with higher dose of lead acetate (320 mg / kg / day).

Group IV (LLC) – Treated with lead acetate + vitamin C (160 + 200 mg / kg / day, respectively).

Group V (LLE) – Given lead acetate + vitamin E (160 + 160 mg / kg / day, respectively).

Group VI (LLCE) – Treated with lead acetate + vitamin C + vitamin E (160 + 200 + 160 mg / kg / day, respectively).

Group VII (LHCE) – Treated with lead acetate + vitamin C + vitamin E (320 + 200 + 160 mg / kg / day, respectively).

Group VIII (Only vitamin C) – Given only vitamin C (200 mg / kg/ day).

Group IX (Only vitamin E) – Given only vitamin E (160 mg / kg / day).

Group X (Only peanut oil) – Animals of this group treated with 0.05 ml of peanut oil only for a period of 3 months.

In each group there were 12 animals (10 females, 2 males). Total X group were selected for this study.

In all the groups after two months, male was introduced in the breeding cages. Each animal of the group was checked in the morning for vaginal plug. The animals showing vaginal plugs were separated marked and put for further observations.

RESULTS AND DISCUSSION

The results obtained from the present investigation are summarized below :

Group I (Control group):

After 19-20 days females delivered pups and they were healthy.

Group II (Only lead treated group):

After 19-20 days females delivered pups, but the young ones were eaten by the females.

Group III (Lead + Vitamin C treated group):

The pregnancies were continued for approximately thirteen days but may be due to resorption, the gestation period was not completed and no litters were born.

Group IV (Lead + Vitamin E treated group):

After 19-20 days young ones were born and they were healthy.

Group V (Lead + Vitamin C + Vitamin E treated group):

In these, females pregnancy continued till mid gestation period but no parturation occurred.

Group VI, VII and VIII (Only vitamin C, Vitamin E and peanut oil):

After 19-20 days females delivered the litters and they were healthy.

Group IX (High dose of lead treated group (320 mg/ kg/day/animal]):

The treatment for this group continued only for one month because animals could not survive. After one month of the treatment, male was introduced in this group. Each animal of the group was checked in the morning for vaginal plug. The animals showed vaginal plugs, but no pregnancy was observed in this group.

Group X (High dose of lead + Vitamins treated group [320 mg lead/kg/day/animal, 200 mg vitamin C/kg/day/ animal and 160 mg vitamin E/kg/day/animal])

The treatment for this group was also given for one-month period. After one month of the treatment, male was introduced in this group. Each animal of the group was checked in the morning for vaginal plug. The animals showed vaginal plugs, but no pregnancy was observed in this group.

In the present investigation, lead treated females showed reduced number of pregnancies, small litter size and decreased body weight. Lead affects female fertility, the classical signs of lead poisoning in pregnant women risk spontaneous abortion and increased blood pressure. Wide (1985) studied the exposure to lead at a time of early organogenesis, caused fertility decrease by interfering with the development of the female germ cells.

The effects of 200 and 400 ppm lead acetate in drinking water on reproduction and development as well as on renal and hepatic parameters of rats at different

Table 1 : Effects of lead and vitamins on fertility

Sr. No.	Groups	No. of Animals	Average weight (g)	Mortility	Treatments	No. of pregnant females after 2 months	Average weight after treatment (g)	Litter size (Number of pups)	Average weight of single pup
1.	Group I	12 10 (Female) + 2 (Male)	27	Nil	Control	6 + 1	33	8 - 10	1.78 g/each
2.	Group II	12	26	4	Only lead (160mg)	3 Preg. 3 Px	23	4 - 6	Abnormal
3.	Group III	12	26	5	Lead + Vitamin C	2 Preg. 2 Px	20	—	—
4.	Group IV	12	28	2	Lead + Vitamin E	4 Preg. →, 2 Px	30	6 - 7	1.22 g/each
5.	Group V	12	27	4	Lead + Vitamin C + Vitamin E	3 Preg. →, 2 Px	22	—	—
6.	Group VI	12	28	1	Only Vitamin C	6 Preg. →	28	8 - 9	1.25 g/each
7.	Group VII	12	27	Nil	Only Vitamin E	6 Preg. →	29	9 - 10	1.27 g/each
8.	Group VIII	12	27	Nil	Peanut oil	7 Preg. →	30	3 - 8	1.60 g/each
9.	Group IX	12	28	7	Only lead (320mg) (only one month)	Nil	23	—	–
10.	Group X	12	27	4	Lead + Vitamin C + Vitamin E (320+200+160 mg)	Nil	24	—	—

Px : No pregnancy
Preg:Pregnancy persist

life stages, from gestation to 3 months post weaning were studied by Teijon *et al.* (2006). They concluded a dose dependent effect on reproduction with variations in the number of births and in pups' weight.

Previously, Tang and Zhu (2003) also noted that occupational lead exposure of female workers could lead to the impairment of the functions of reproductive system.

In the present investigation, animals treated with high dose showed vaginal plug but no pregnancies were observed in these females and body weights of females were also decreased. Animals treated with low dose showed vaginal plug but only few females showed pregnancy and litter size was also small.

In the present study, animals treated with low dose of lead revealed reduce fertility as compared to control group animals; the present studies are in conformity with the findings of above investigators.

Agrawal *et al.* (2005) reported that ROS (reactive oxygen species) affect multiple physiological processes from oocyte maturation to fertilization, embryo development and pregnancy.

OS (oxidative stress) influences the entire reproductive span of women's life and even thereafter (*i.e.* menopause) {de Bruin *et al.* (2002), Myatt and Cui (2004), Fainaru *et al.* (2002), Mocatta *et al.* (2004), Wall *et al.* (2002) and Pressman *et al.* (2003). It has been suggested that OS modulates the age related decline in fertility.

During the last 90, years since the discovery of vitamin E, research has focused on different properties of this molecule, the focus often depending on the specific techniques and scientific knowledge present at each time.

Originally discovered as a dietary factor essential for reproduction in rats, vitamin E has revealed in the meantime many more important molecular properties, such as the scavenging of reactive oxygen and nitrogen species with consequent prevention of oxidative damage associated with many diseases (Zingg, 2007).

In the present study, order of effectiveness of vitamins were found to be as follows: vitamin C plus vitamin E > vitamin E > vitamin C.

In the present investigation lead and vitamins (vitamin C and E) revealed protective role on the reproduction of female Swiss mice. In lead + vitamin C treated group the animals showed vaginal plugs and the pregnancy were continued for approximately thirteen days but may be due to resorptions, the gestation period not completed and no litters were born.

In lead + vitamin E treated group, the animals showing vaginal plugs were separated, and young ones were born and they were healthy. In lead + vitamin C + vitamin E treated group, the animals exhibited vaginal plugs, in these females pregnancy continued till mid gestation period but partutation did not occur.

Luck *et al*. (1995) noted that ascorbic acid has long been associated with fertility, but no consistent study of its mechanisms of action in reproductive tissues has been made. Ascorbic acid's principal functions, namely its promotion of collagen synthesis, its role in hormone production, and its ability to protect cells from free radicals may explain its reproductive actions. Ascorbic acid may also prevent gametes from damage by free radicals during reproduction and fertilization.

Authors' affiliations
RAGINI SHARMA AND SHEETAL MOGRA
Environmental Toxicology Research Laboratory, Department of Zoology, University College of Science, M.L. Sukhadia University, UDAIPUR (RAJASTHAN) INDIA

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