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Evaluation of the therapeutic efficiency of raw garlic on reproduction of domestic rabbits under lead induced toxicity

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ABSTRACT

The aim of this study is to evaluate the therapeutic use of raw garlic against Pb toxicity. Male rabbits were exposed, either to a diet containing 400 Pb acetate/Kg diet, or combined with garlic (5g/100g diet) for a period of 2 weeks. Semen analysis was performed from the epididymis, and the levels of testosterone, cholesterol, haemoglobin, hematocrit and blood cells were evaluated. The obtained results have indicated that testosterone concentration was not affected significantly by both treatments compared to the control. Sperm concentration, motility and viability were reduced in the Pb group and also in the Pb-garlic group when compared to the control. Contrary, sperm speed was significantly reduced in the Pb group only. There was a significant decrease of red blood cells accompanied by a significant rise of white blood cells compared with the control. On the other hand, hematocrit, hemoglobin and cholesterol of the Pb group were almost similar as that of the control. Concerning the Pb-garlic group, red blood counts and cholesterol level were not significantly different when compared to the control, but that of white blood counts was still elevated. To conclude, in Pb intoxicated rabbits, garlic may exert a protective role by maintaining cholesterol, red blood cells and sperm speed to almost normal levels, but it failed to preserve those of white blood cells and most sperm parameters.

Key words: Garlic, Pb, spermogram, testosterone, testis

INTRODUCTION

Many xenobiotics including trace metals are suspected of being responsible for the decline of male and female fertility observed over the last century. Lead alters cell functions by disrupting many metabolic pathways and various physiological processes [1]. It also alters calcium homeostasis and interferes with cellular processes and molecular mediated by calcium levels at the cytoplasmic membranes [2]. These changes are probably the cause of the effects of lead on the central nervous system [3]. Lead is considered to be one of the oxidative stress inducer in

different cells and organs [4, 5]. It has been reported that chronic exposure to lead contributes to blood, reproductive, renal and hepatic disorders disturbances [5, 6, 1]. Workers exposed to Pb have suffered from reproductive disorders [7, 8]. Lead has a direct toxic effect on the testis and epididymis responsible for oligoasthenospermy, causing infertility [2]. A decrease in libido with diminished plasma testosterone may increase LH and / or FSH, was also reported. Sperm abnormalities are usually reversible with cessation of exposure and/or after chelation therapy [2]. Many factors contribute to the absorption of lead as deficiencies of iron, calcium, phosphorus, zinc, vitamin B1, vitamin D, magnesium and fiber plants [9, 10].

In this study, therefore, garlic *Allium sativum* L has been chosen as a therapeutic nutrient because it contains many important nutritive and antioxidant constituents as selenium, sulfur compounds, vitamins A, B, C and E [11]. It was reported that the consumption of garlic extract as a dietary supplement improves blood lipid profile, strengthen the anti-oxidants capacity and also causes a decrease in blood levels of oxidants. Garlic can reduce lipid peroxidation and increase antioxidant defense mechanism in animals [13].

The present work aims to evaluate the therapeutic efficiency of local raw garlic against lead toxicity on some reproductive and haematological parameters of domestic rabbits *Oryctolagus cuniculus*.

MATERIALS AND METHODS

Domestic rabbit *Oryctolagus cuniculus* of 6 months of age and an average body weight of 1400g were chosen. The experiment was made in the breeding house of the University under controlled conditions of temperature, humidity and photoperiod. They were placed in specific cages (50 x 60 x 53cm) and fed on a standards diet. Twenty four males were divided equally into 03 groups; control, treated with Pb (400 mg lead acetate / kg diet) and treated with Pb-garlic (400 mg lead acetate/kg diet and 50g raw garlic) for a period of two weeks.

After sacrifice by decapitation, blood was collected either in EDTA tubes for blood counts (Automatic Cell Counter) or in dry tubes to obtain the serum. Cholesterol was measured by the enzymatic colorimetric method [14] and testosterone was evaluated by Enzyme-Linked Immunosorbent Assay (ELISA) method using commercial kits, Spain.

Concerning semen analysis, a small opening at the epididymis was performed to obtain a drop of sperm of about 1 μ l, then 49 μ l of 0.9% NaCl was added to evaluate the concentration, speed, motility and vitality) [15]. Results were evaluated statistically using the Minitab 13. However, the Student *t*- test was used by comparing each of the treated group with the control. The significance level of $p < 0.05$ was considered.

RESULTS

Results have indicated a non significant variation in testosterone concentration in both treated groups compared with the control.

Sperm concentration was diminished significantly in the Pb and in the Pb-garlic group alike. Moreover, sperm speed and motility in the Pb group have reached zero, but these two parameters have been increased in the Pb-garlic compared to control. However, sperm viability was reduced in the Pb group and also in the Pb-garlic group when compared to the control.

Results of white blood counts showed a significant rise in the Pb group when compared to the control and also in the Pb-garlic group as well. There was a significant decline in red blood cell counts of the Pb treated group compared to the control. Contrary, this difference is not significant in the group of Pb-garlic compared to the control. Concerning hematocrit, hemoglobin and cholesterol, no significant differences were recorded in both treated groups compared to the control.

Table 1. The level of blood biomarkers (X±SD) of rabbits *Oryctolagus Cuniculus* exposed to lead and raw garlic during 15 consecutive days.

	Control	Pb	Pb-garlic
Testosterone (ng/d)	1.36±0.31	1.22±0.07	1.25±0.04
Sperm concentration (ml/10 ⁶)	402±10.12	44±27.57*	175±15.45*
Sperm speed (µm/s)	46.85±4.2	00*	32±2.69
Sperm motility (%)	72±8.36	00*	28±12.58*
Sperm viability (%)	66±4.18	26.60±5.27*	30.60±7.23*
Cholesterol (mg/l)	160±17.23	118.98±29.72	125±67.95
WBC (µl/10 ³)	9.80±0.94	13.83±1.24*	7.98±0.39
RBC (µl/10 ⁶)	5.32±0.97	5.15±1.0*	4.09±0.42
Hb (g/dl)	9.04±1.86	9.66±0.85	9.23±0.81
Ht (%)	31±5.54	31.50±2.98	29±2.55

*: Significantly different than that of the control at $p < 0.05$. (Student ^t-test).

DISCUSSION

Results show a non variation of the serum testosterone levels in the group exposed to Pb alone, and also in the group treated with garlic for a period of two weeks. Contrary, Pb was reported to have a cytotoxic effect on the interstitial leydig cells which secrete testosterone [16]. Indeed, serum testosterone in rats showed a significant drop (60% - 80%) during the first 45 days of treatment with 6 mg/ml of Pb acetate[16]. Accordingly, [17] have mentioned that Pb inhibits the expression of certain enzymes involved in the biosynthesis of steroid hormones. On the other hand, raw garlic was confirmed to provide reparations to the male reproductive function [18,19]. Thus, garlic is considered as a protective regime for different tissues against arsenic induced toxicity [20]. Garlic has also proved to play a protective role against the damage induced to testes and spermatogenesis in cadmium intoxicated rats [13]. Such protection may reduce lipid peroxidation and increase antioxidant defense mechanism. In addition, [21] have explained the garlic mechanism of action in several target tissues, due to the presence of organo-sulfur compounds, which alters the activity of several enzymes controlling the metabolism of cytochrome P450 or the detoxification of glutathione S-transferase.

In our study, results indicate a significant decrease in the concentration, motility and vitality of sperm either in the group treated with Pb alone or combined with garlic. It can be assumed that Pb has a direct effect on spermatogenesis, causing decline in sperm concentration and their full growth and maturation. Concerning the fall of motility, it is suggested that this metal may act on the mitochondria function of the intermediate piece, which inhibits the energy needed for sperm movement. Though, a disruption of concentration, motility and morphology of sperm were observed in rodents, monkeys and humans exposed to lead[8, 22]. Also, the injected mature rabbits by a dose of 3.85 mg Pb acetate/kg for 15 weeks revealed a very marked change in ejaculate volume, sperm count, motility, speed and morphology [23], accompanied with an inhibition of spermatogenesis in the histopathological study. Furthermore, among workers

exposed to Pb during working hours as those of police and taxi drivers, a decline in male fertility associated with a decrease in the number and motility of sperm and reduced seminal fluid volume were observed [7, 8]. Thus, Pb chronic poisoning was known to induce degeneration of the testicles, reduce the size of seminiferous tubules and destruct the spermatocytes, spermatids and interstitial tissue, which may eventually provoke a blockage of spermatogenesis and cause azoospermia [23, 24, 25]. Other research has indicated that testes are most sensitive to oxidative stress because of their high content of polyunsaturated fatty acids [26]. However, oral supplementation of garlic juice added to lead nitrate significantly reduced the metal concentration in blood and tissues, indicating that garlic may be a protecting food and has therapeutic potential against Pb intoxication [27, 28].

The treatment of rabbits with Pb polluted diet for 2 weeks caused a remarkable rise in the number of white and red blood cells, but the supplementation of garlic with Pb have protected only red blood cells from any change. Some studies suggested that lead might induce oxidative damage to red blood cell membranes [29], resulting in an inhibition of the haem and haemoglobin synthesis [30, 31]. Moreover, [32] have shown the existence of a high level of Pb in white and red blood cells. In the current study, the levels of hemoglobin and hematocrit have not been affected even by Pb alone. In contrast, [33] reported a remarkable decrease in both parameters and also in the number of erythrocytes of female mice exposed Pb acetate in drinking water for 5 weeks. It has been suggested that the hematological changes may start at a dose of 420-1600 mg Pb/kg [34, 35]. But the influence of garlic was evident on rats treated with a dose of 100 mg/day of garlic extract in the diet for 30 days where there has been an increase in the number of red blood cells, neutrophils and lymphocytes and in the hematocrit [36].

Concerning cholesterol concentration, no noticeable variation in rabbits exposed to Pb alone or combined with garlic has been registered. Recently, the treatment of rats with 400 ppm in drinking water for two weeks also indicated no evident effect of Pb on plasma cholesterol (HDL) and triglycerides, whereas red blood cell cholesterol have marked a profound increase in different doses of 200, 300 and 400 ppm [37]. Moreover, serum cholesterol level was raised after exposure to Pb nitrate [27].

CONCLUSION

Modern medicine has recognized that garlic is a natural food containing important nutrients and antioxidants that can play roles as preventive and therapeutic agent. Its supplementation with Pb has relatively reduced its toxicity.

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