Pharmaceutical Biology

ISSN: 1388-0209 (Print) 1744-5116 (Online) Journal homepage: informahealthcare.com/journals/iphb20

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To cite this article: S.K. Senapati, S. Dey & S.K. Dwivedi (2005) Effect of Arjuna (*Terminalia arjuna*.) Extract on Tissue Lead Levels in Rats, Pharmaceutical Biology, 43:8, 696-700, DOI: 10.1080/13880200500384957

To link to this article: https://doi.org/10.1080/13880200500384957

Published online: 07 Oct 2008.

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Pharmaceutical Biology
2005, Vol. 43, No. 8, pp. 696–700

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Effect of Arjuna (*Terminalia arjuna*) Extract on Tissue Lead Levels in Rats

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Abstract

The prophylactic efficacy of arjuna (*Terminalia arjuna* L.) extract to reduce tissue lead (-Pb) concentration was evaluated experimentally in rats. Thirty female rats were divided into five groups, with group A as the control. Rats of groups B, C, D, and E received lead acetate orally at the rate of 5 mg per kg body weight daily for 6 weeks. The arjuna extract was tried in three doses, viz., 50 mg (low), 100 mg (medium), and 200 mg (high) per kg body weight orally and given simultaneously with Pb salt to the rats of groups C, D, and E, respectively. Mean blood Pb concentration in Pb-exposed rats ranged between 0.13 ± 0.02 and 0.96 ± 0.06 μg/ml, whereas in arjuna-treated rats, the range was between 0.15 ± 0.02 and 0.94 ± 0.06, 0.17 ± 0.01 and 0.77 ± 0.06, and 0.13 ± 0.1 and 0.51 ± 0.07 μg/ml in low-, medium-, and high-dose groups, respectively. The mean Pb concentration in liver, kidneys, brain, and bone of Pb-exposed rats was 2.289 ± 0.206, 4.748 ± 0.609, 1.019 ± 0.10, and 44.075 ± 2.60 μg/g, respectively. Concomitant use of arjuna bark extract at three different doses was found to reduce Pb concentration considerably in these vital organs indicating the potential therapeutic activity of arjuna against lead toxicity.

Keywords: Ameliorative potential, blood lead (Pb), *Terminalia arjuna* extract, tissue Pb accumulation.

Introduction

Lead (Pb), a nonbiodegradable heavy metal, continues to pose health hazards to man and animals in India. Industrial, urban, and agricultural activities have resulted in release of this toxic metal in the environment, and increased concentration of this toxic metal has been reported in air, water, and vegetation in certain localities in India (Anon, 1998). Despite recent advances in understanding of toxicity and distribution of this metal, Pb exposure remains unavoidable for man and animals. It affects each and every organ and system in the body (Goyer & Clarkson, 2001). Recent findings with respect to understanding the toxicity of Pb indicate that Pb is capable of inducing toxic effects at exposures far lower than those producing clinical symptoms (Lippman, 1990; Eckerman et al., 1999). Pb is a cumulative poison and accumulates in various organs, viz., liver, kidneys, brain, bone, and hemopoietic system of the body (Klaassen, 2001). Several metal chelators have been used to manage Pb toxicity in the event of acute Pb exposure, but none is suitable in reducing Pb burden in chronic Pb exposure (Smith et al., 1998; Osweiler, 1999). Moreover, the chelating agents (calcium salt of ethylene diamine tetraacetic acid, 2,3-dimercaptopropanol, 2,3-dimercapto-1 propanesulfonic acid, meso-2,3-dimercaptosuccinic acid, etc.) commonly used in acute Pb poisoning have toxic potential in themselves (Mahaffey et al., 2000) and often fail to remove Pb from all body tissues (Brattan et al., 1981; Cory-Slechta et al., 1987; Cory-Slechta, 1988). These chelating agents are never indicated for a prolonged period. There is an urgent need of a safe, nontoxic agent for chronic Pb intoxication that can remove body Pb accumulation without any side effects (Jones & Cherion, 1990).

In the ancient Indian system of medicine (Ayurveda), a number of plants and herbs have been indicated for amelioration of metal poisoning (Dwivedi, 1995). However, these have not been evaluated scientifically to date, and their use is mainly restricted to observable recovery.

Accepted: September 14, 2005

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DOI: 10.1080/13880200500384957 © 2005 Taylor & Francis Ltd.

Arjuna (*Terminalia arjuna* L.) is found all over India and has been used in the treatment of various disease conditions in man and animals. The traditional ethnoveterinary practitioners considered arjuna as an excellent natural product that has immense therapeutic potential in many pathological conditions. The stem bark of arjuna is used as treatment in diseases of the heart, fractures, asthma, ulcers, and leukoderma. Arjuna is used in Ayurveda for amelioration of metal poisoning. The powdered arjuna bark is recommended to be taken orally in Ayurveda (Kirtikar & Basu, 2001). In this study, arjuna bark extract was tested for its potential to reduce body Pb residues in experimentally Pb-exposed rats.

Materials and Methods

Source of plant material

Fresh arjuna stem bark was collected from the natural habitat around Bareilly by the senior author during February–March 1997. It was identified and authenticated from Botanical Survey of India, Central National Herbarium, Howrah (voucher no. CNH/1-1/97 Tech 11), where voucher reference specimens were deposited.

Extract preparation

The plant material (bark of arjuna) was washed with distilled water to remove the dirt and air-dried. The dried plant material was powdered in an electric grinder and stored in an air-tight glass container. Ten grams of the powdered plant material was mixed with 150 ml of distilled water and kept at room temperature overnight. After 24 h, it was stirred in a magnetic stirrer for 1 h and filtered. The extract was dried *in vacuo* and stored refrigerated until used.

Calculation of recovery percentage

For calculation of recovery percentage of *Terminalia arjuna* bark, 2 g of bark powder was dissolved in 100 ml triple-distilled water and was kept overnight. After 24 h, it was filtered and the filtrate was dried at 100°C in an oven to remove the water, and dry matter percentage was calculated as per the following formula

$$\text{Recovery percentage} = X/Y \times 100$$

where X = weight of the dried extract and Y = weight of the plant material taken for extraction.

The recovery percentage was 55.7% in the current experiment.

Animals and treatment

The experiment was conducted on 30 female IVRI 2CQ rats, bred in the Laboratory Animal Research Division of this institute. They were housed in plastic cages with proper bedding and maintained on standard ration and *ad libitum* water. They were acclimatized for 30 days before starting the experiment.

Table 1. Details of the experimental protocol.

Treatment	Group A	Group B	Group C	Group D	Group E
Pb acetate (mg/kg b.wt.)	Nil	5.0	5.0	5.0	5.0
Arjuna extracts (mg/kg b.wt.)	Nil	Nil	50	100	200
Control	Distilled water	Nil	Nil	Nil	Nil

Experimental protocol

The rats were divided into five groups of six animals each. The details of the experimental protocol are given in Table 1.

The Pb was administered orally as 1.0% aqueous solution once daily at the morning hours before feeding. Arjuna extract was administered at two divided doses: half of the required dose at morning and the other half in the evening. All treatments were given daily for 6 weeks.

Sampling methods

Blood

The samples of blood were collected from individual rats on day 0 of the experiment and thereafter at weekly intervals for 6 weeks from the orbital plexus using heparinized microhematocrit capillaries piercing through the outer canthus of the eye. The sample was pooled into nitric acid–washed heparinized vials for estimation of Pb.

Tissue samples

Portions of liver, kidneys, brain, and long bone were collected in polyethylene bags without any preservative. The animals were sacrificed 24 h after the last dose of Pb administration. The samples were subsequently subjected to acid digestion for Pb estimation.

Estimation of Pb

All samples were wet digested (AOAC, 1984) using a 5:1 mixture of concentrated nitric acid and 70% perchloric acid in a heating block under a low heat. The concentration of Pb in the acid digest was estimated by atomic absorption spectrophotometry (Perkin Elmer, Analyst 200, Switzerland) at 217 nm wavelength following the instrument instruction manual. An air-acetylene mixture

was used as oxidant gas. The analytical quality was maintained by repeated analysis of the reference standards (Sigma, St. Louis, MO, USA). The results were expressed as μg/g of sample. The ameliorative potential of the test extract was assessed on the basis of its effect on Pb concentration in blood and different tissues of the body.

Statistical analysis

The data were analyzed statistically to determine whether there was any significant difference among different treatment groups using Fisher's *t*-test and two-way analysis of variance (ANOVA) following standard protocols (Snedecor & Cochran, 1975).

Results

The blood Pb concentrations in rats of different treatment groups are presented in Table 2. Oral Pb exposure increased blood lead concentration in rats of group B compared with the values recorded in healthy rats (group A). Concomitant use of arjuna extract significantly reduced blood Pb concentration in a dose-dependent manner.

Table 3 shows the tissue Pb accumulation pattern in the experimental rats. In healthy rats (group A), the mean Pb concentration was estimated as 0.915 ± 0.038, 1.224 ± 0.180, 0.578 ± 0.068, and 8.542 ± 0.834 μg/g in liver, kidneys, brain, and femur, respectively. Exposure of Pb acetate for 6 weeks led to an increased tissue Pb concentration to 2.943 ± 0.214, 4.780 ± 0.609, 1.019 ± 0.100, and 44.075 ± 2.600 μg/g in liver, kidneys, brain, and femur, respectively. Administration of arjuna extract significantly ($p \leq 0.01$) reduced tissue Pb accumulation. The mean Pb concentrations in liver were estimated as 1.734 ± 0.169, 1.15 ± 0.134 and 1.33 ± 0.433 μg/g in rats of groups C, D, and E, respectively. The mean Pb concentrations in kidneys were recorded as 3.035 ± 0.379, 2.401 ± 0.496, and 2.194 ± 0.360 μg/g in rats of groups C, D, and E, respectively. The Pb concentration in brain decreased nonsignificantly in group C (0.899 ± 0.189 μg/g) and group D (0.97 ± 0.124 μg/g). The reduction was highly significant ($p \leq 0.01$) in group E (0.827 ± 0.137 μg/g). The bone (femur) Pb concentration in rats of groups C, D, and E were 36.693 ± 8.91, 34.810 ± 1.84, and 23.075 ± 0.137 μg/g, respectively. Significant ($p \leq 0.05$) reduction in Pb concentration was recorded in group D (34.81 ± 1.84 μg/g), and the reduction was significant ($p \leq 0.01$) in group E (23.075 ± 0.137 μg/g).

Discussion

The Pb level was increased significantly in liver, kidneys, brain, femur, and blood of rats receiving Pb acetate alone. However, the concomitant use of arjuna bark extract prevented the accumulation of Pb in these organs. The weekly blood Pb profile revealed a significant and consistent dose-dependent decrease in Pb levels in the rats receiving arjuna extract. The highest dose (200 mg per kg body weight) decreased blood Pb concentration most efficiently, almost to the values recorded in healthy rats. The lowest dose (50 mg/kg body weight) reduced Pb level, but it was not sufficient enough to protect from the subtle toxic effects of Pb.

The extract of arjuna stem bark is reported to contain chemical constituents like arjunolone, terminoic acid, and arjunogluside (Chopra et al., 1996). These chemical constituents contain free OH, C=O, and COOH groups in their benzene ring structure. It is reported that compounds containing these radicals in their side chain can chelate Pb and heavy metals (Tandon et al., 1986). The aqueous extract of *T. arjuna* stem bark contains tannin having active principles like catechol, gallocatechol, epicatechol, and epigallocatechol (Chopra et al., 1996). These chemical principles have the potential to bind with bivalent cations (Pandey et al., 1996). The exact mechanism by which arjuna extract interferes with Pb

Table 2. Blood lead concentration in (μg/g) in arjuna-treated rats.

	Weeks of exposure						
Group	0	1	2	3	4	5	6
A	0.14 ± 0.01	0.23 ± 0.01	0.27 ± 0.01	0.28 ± 0.02	0.28 ± 0.02	0.29 ± 0.01	0.31 ± 0.02
B	0.13 ± 0.01	$0.53 \pm 0.01^{**}$	$0.80 \pm 0.04^{**}$	$0.80 \pm 0.02^{**}$	$0.91 \pm 0.03^{**}$	$0.94 \pm 0.04^{**}$	$0.96 \pm 0.06^{**}$
C	0.15 ± 0.02	$0.36 \pm 0.02^{**b}$	$0.48 \pm 0.06^{**a}$	$0.61 \pm 0.07^{**}$	$0.80 \pm 0.04^{**}$	$0.89 \pm 0.04^{**}$	$0.94 \pm 0.06^{**}$
D	0.17 ± 0.01	$0.30 \pm 0.02^{**b}$	$0.36 \pm 0.04^{**b}$	$0.47 \pm 0.05^{**b}$	$0.59 \pm 0.04^{**a}$	$0.67 \pm 0.03^{**a}$	$0.77 \pm 0.06^{**b}$
E	0.13 ± 0.01	$0.17 \pm 0.02^{**b}$	$0.23 \pm 0.03^{**b}$	$0.26 \pm 0.04^{**b}$	$0.40 \pm 0.06^{**b}$	$0.43 \pm 0.04^{**b}$	$0.51 \pm 0.07^{**b}$

*Differ significantly ($p \leq 0.05$) compared with day "0" value of the same group.
**Differ significantly ($p \leq 0.01$) compared with day "0" value of the same group.
[a]Differ significantly ($p \leq 0.05$) compared with group B.R
[b]Differ significantly ($p \leq 0.01$) compared with group B.

Table 3. Lead concentration (μg/g) in different organs of lead and arjuna-treated rats.

Group	Organ			
	Liver	Kidney	Brain	Long bone
A	0.915 ± 0.038	1.244 ± 0.18	0.578 ± 0.068	8.542 ± 0.834
B	2.943 ± 0.214	4.780 ± 0.609	1.019 ± 0.100	44.075 ± 2.60
C	$1.734 \pm 0.169^{**}$	$3.035 \pm 0.379^{*}$	$0.899 \pm 0.189^{*}$	$36.693 \pm 8.91^{*}$
D	$1.15 \pm 0.134^{**}$	$2.401 \pm 0.496^{*}$	$0.970 \pm 0.124^{*}$	$34.81 \pm 1.84^{*}$
E	$1.33 \pm 0.433^{**}$	$2.194 \pm 0.360^{**}$	$0.827 \pm 0.137^{**}$	$23.075 \pm 2.95^{**}$

*Differ significantly ($p \leq 0.05$) compared with group B.
**Differ significantly ($p \leq 0.01$) compared with group B.

deposition in tissues is not clear, and this is the first report that the extract of arjuna bark has the potency to reduce body Pb burden. It is required to study whether it interferes with absorption or facilitates excretion of the metal from the body. However, based on ethnoveterinary practices in Asia, Dwivedi (1995) has hypothesized that arjuna extract could help in excretion of heavy metals from the body. Besides chelating, it is possible that other components of arjuna extract, especially tannins and components like catechol, gallocatechol, epicatechol, and epigallocatechol, might have prevented absorption of Pb from the gastrointestinal tract. Therefore, it can be suggested that the ameliorative potential of arjuna extract was perhaps due to combined effects both on metal absorption and on excretion from the body. It is interesting to note that arjuna extract reduced Pb levels both in blood, soft tissues, as well as hard tissue, which is not found with conventional metal chelators.

Our findings have also revealed that arjuna bark extract had the ability to reduce residues of Pb in soft tissues (liver, kidneys, and brain) as well as in the bone sink in the body. The findings of the current study documented that aqueous extract of bark of arjuna can be used for amelioration of chronic Pb toxicity. However, further studies are required to establish the dose and the molecular basis of the mechanism and the compounds of arjuna involved in it.

Acknowledgments

The financial assistance given to the senior author in the form of an ICAR Jr. Research fellowship by the Indian Council of Agricultural Research is thankfully acknowledged. We acknowledge the technical assistance of Mr. Brijesh Tyagi in estimation of Pb in biosamples.

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