

ORIGINAL ARTICLE

PROTECTIVE ROLE OF GINGER ON LEAD INDUCED DERANGEMENT IN PLASMA TESTOSTERONE AND LH LEVELS OF MALE SPRAGUE DAWLEY RATS

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Background: Lead is one of the most serious environmental threats to human health especially in developing countries. It damages multiple body systems including the reproductive system. Ginger's antioxidant and androgenic activity is reported in multiple animal studies. The aim of this study was to investigate the ameliorative effect of *Zingiber officinale* (ginger) on lead induced derangement in plasma testosterone and luteinizing hormone (LH) levels of male rats. **Methods:** Sixty adult male Sprague Dawley rats were used in this study in four groups. Group A served as normal control, Group B received 0.3% lead acetate in drinking water, Group C and group D received supplementary 0.5 and 1 gm/Kg bodyweight of ginger respectively along with lead acetate in drinking water. Five rats from each group were sacrificed at the end of 2nd, 4th and 6th weeks. Serum testosterone and LH levels were analysed using ELISA technique. **Results:** After co administration with different doses of ginger, serum testosterone level which was significantly decreased in lead treated group, showed a significant rise as compared to lead treated group. LH levels which had exhibited no significant change by lead treatment, after co administration with different doses of ginger, again showed no significant change. **Conclusion:** Oral administration of ginger ameliorated lead induced testicular toxicity in male rats by increasing serum testosterone level at all durations which might be a product of both its androgenic and antioxidant properties.

Keywords: Lead toxicity, testosterone, luteinizing hormone, *Zingiber officinale*, male rats, antioxidant

INTRODUCTION

Lead (Pb) is one of the oldest and commonest environmental pollutant, which is reported to cause damage in multiple body systems.¹ In a developing country like Pakistan, people are specially exposed to lead pollution through air, water and soil.² Studies which have been conducted in Pakistan, have revealed that major population have blood lead levels above the internationally acceptable limits.³

Like other toxic metals, lead causes oxidative damage and disrupts the pro-oxidant/antioxidant balance.⁴ Lead toxicity imposes adverse effects on male reproduction and fertility both in clinical and animal studies.⁵ It may have direct testicular toxicity or indirect effects through targeting the endocrine control of reproduction or both.⁶ Studies in male rats have shown that lead intoxication disrupts testicular steroidogenesis by inhibiting the activities of testicular steroidogenic enzymes.⁷

Thus, it is very essential to find out ways, by which our body can maintain homeostasis and good health even if exposed to high levels of lead toxicity. The standard management for the treatment of lead toxicity is chelation therapy which has many side effects.⁸ Based on the observation that lead induced pathogenic processes involved generation of free radicals, it was presumed that supplementation of antioxidants could be an alternative method for chelation therapy.⁹

Nowadays, a lot of research has been conducted on the use of herbal products as natural antioxidants because of their fewer side effects, easy and cheap availability. Ginger (*Zingiber officinale*) is commonly used as food spice in this region since ancient times.¹⁰ It has long been used as a remedy for common ailments like digestive problems, cold, fever, morning sickness. Studies have revealed a wide range of biological activities like anticancer, antioxidant, anti-inflammatory and antimicrobial.¹¹ All major active ingredients of ginger such as zingerone, gingerdiol, zingibrene, gingerols and shogaols have antioxidant activity.¹² Ginger's androgenic activity in male rats has also been reported.¹³ Gingers protective role has also been studied in animal models, in various reproductive toxicities like those induced by cyclophosphamide¹⁴, cisplatin¹⁵ and Di-(2-ethylhexym) phthalate¹⁶. All have reported oxidative stress to be a major factor as evidenced by decreased antioxidant enzyme activity and increased lipid peroxidation marker, MDA. These studies confirm ginger's antioxidant and androgenic activity.

However, to our knowledge, its antioxidant and androgenic role on lead induced derangement in plasma testosterone and LH levels have not been studied. The present work was conducted to study the protective role of ginger on lead induced derangement in plasma testosterone and LH levels of male rats.

MATERIAL AND METHODS

Sixty adult male Sprague Dawley rats between the ages of 60–90 days, weighing 130–200 gm were randomly selected. They were divided into four groups with fifteen rats in each group. Group A served as normal control, group B was given 0.3% lead acetate dissolved in drinking water, whereas group C and group D were given 0.5 gm/Kg body weight and 1 gm/Kg body weight of ginger along with lead acetate. Lead and ginger were given in clean, inverted drinking bottles specific for the rat cages. All the groups were fed on standard pellet diet and water *ad libitum* in the animal house of National Institute of Health (NIH), Islamabad and kept in separate standard cages designed accordingly. Drinking water consumption in all the groups was recorded daily and rats were weighed on weekly basis to adjust the dose of ginger. Treatment in all groups continued for 42 days with 5 rats being sacrificed in each group at the end of 2nd, 4th and 6th week.⁷ Three to five ml blood was drawn by intra cardiac catheterisation. Samples were immediately transferred into labelled gold top vacutainers without anticoagulant kept in an ice packed rack. Samples were then shifted in an hour from NIH to Riphah Diagnostic and Research Laboratory at Riphah College, Islamabad. Serum was separated by centrifugation, transferred into labelled 1.5 ml eppendorf tubes, frozen and stored at -80 °C till assayed. Testosterone and LH levels in each group were quantitatively determined using solid phase ELISA.⁸ Testosterone and LH ELISA kit were

procured from DRG International, Inc. (Lot # 16k096). Using semi-algorithmic graph paper a standard curve was constructed by plotting the mean absorbents obtained from each standard against its concentration with absorbance values at Y-axis and concentration on the horizontal X-axis. Thus the corresponding concentration for each sample was determined from the standard curve.

Statistical analysis was done using SPSS-13. Mean±SD of all observations were calculated. For multiple comparison, ANOVA was used. When ANOVA showed significant difference, post hoc analysis was performed; $p < 0.05$ was taken as significant.

RESULTS

There was a significant decrease in serum testosterone level in lead treated group (group B) at all durations as compared to control group (group A). In groups C and D no significant changes were observed when compared to group A (control group) when they were simultaneously treated with 0.5 and 1 gm/Kg body weight of ginger. However, there was a significant increase in both groups C and D when compared to group B (Table-1).

Multiple comparison of LH levels shows that there was no significant change in LH levels of lead treated group (group B) at all durations as compared to control group (group A). Similarly groups C and D exhibited no significant change when co administered with 0.5 and 1 gm/Kg body weight of ginger although it did show a rise (Table-2).

Table-1: Comparison of serum Testosterone levels at day 14, 28 and 42 (Mean±SD)

Groups n=5	Mean±SD		p		Mean±SD		p		Mean±SD		p	
	Day 14				Day 28				Day 42			
Control group	3.0250±0.22174		p*	p#	2.8500±0.61914	p*	p#	3.0500±0.83865	p*	p#		
Lead treated group	1.4500±0.54467	0.001	0.001		1.3250±0.48563	0.032	0.032	1.9500±0.26458	0.028	0.028		
Lead with low dose ginger	3.1000±0.35590	0.993	0.000		2.6750±0.84607	0.982	0.061	3.0250±0.27538	1.000	0.031		
Lead with high dose ginger	3.1250±0.41130	0.984	0.000		2.6000±0.67823	0.951	0.080	3.0000±0.18257	0.999	0.036		

p* represents comparison between control and experimental groups, p# represents comparison between lead treated and other experimental groups

Table-2: Comparison of serum LH levels at day 14, 28 and 42 (Mean±SD)

Groups n=5	Day 14		Day 28		Day 42	
	Mean±SD	p*	Mean±SD	p	Mean±SD	p
Control group	4.8750±1.07199		4.2000±0.78740		4.8250±0.82614	
Lead treated group	5.2500±0.85829	0.888	4.8250±0.51235	0.609	5.2000±0.77460	0.929
Lead with low dose ginger	6.2250±0.41130	0.096	5.5250±0.86554	0.609	6.1250±0.80571	0.209
Lead with high dose ginger	6.0750±0.35000	0.153	5.3750±0.60759	0.141	6.5250±1.07199	0.074

p* represents comparison between control and experimental groups

DISCUSSION

Lead treated group in our study showed significant decrease in serum testosterone levels at all time periods as compared to control group. These results were in accordance with a study conducted by Ait Hamadouche and Slimani M in which dose dependent suppression of serum testosterone level was seen by lead acetate.¹⁹ In another study, lead acetate suppressed serum

testosterone, FSH and LH levels along with testicular spermatogenesis, showing that lead acts at all levels of reproduction.⁶ Administration of 0.1% lead acetate in male wistar rats by Yasser El-Sayed 2010 again suppressed serum testosterone levels and blood lead levels exceeded by 22 µg/dl again confirming our results.⁵ Regarding different durations of treatment, data of another study verified that increased duration of

exposure to lead acetate after 14 days did not further suppress serum testosterone levels or spermatogenesis.²⁰ However, in contrast to our study, serum testosterone level remained unaffected in a study conducted by Foster¹⁷ while some others observed an increase in testosterone level after lead administration.²¹ Their data could be viewed as a result of a mixture of specific lead toxicity (at the enzyme level) with other more general actions (at the level of hypothalamic-pituitary-testicular axis).

In our study serum LH levels in lead treated group, revealed no significant change when compared to the control group showing that the major target of lead intoxication are the Leydig cells with only a modest effect on pituitary axis. The non-significant increase in LH levels in the study after lead intoxication was similar to another study conducted by Lynda Allouche.²² Contrary to our study of LH results, a decrease in LH was observed recently by some studies.¹⁹ This difference in LH levels could be attributed to the longer duration of lead exposure (rats were exposed to lead for 90 days) or difference in the technique of measuring LH (electrochemiluminescence).⁵ Lead along with other commonly found persistent toxic metals like mercury, arsenic and cadmium damages cellular material and produce alteration in genetic material.²³ Lead adversely affects steroidogenesis either directly or through endocrinological system.²⁴ The mechanism underlying all these metals is common involving oxidative damage.²⁵ Hamadouche *et al* confirmed it by showing increased lipid peroxidation products (LPPs) in lead intoxicated rats.¹⁹ Based on these and similar observations, in the present study ginger was co administered with lead acetate, since ginger is well known for its antioxidant activity.

Regarding the effect of ginger on plasma testosterone level, comparable results were obtained with a study conducted by Morakinyo in which ginger was administered to male rats orally in similar doses. Treatment lasted for 14 and 28 days showing a significant increase in serum testosterone level in a dose and duration dependent manner.¹⁰ In another study ginger administration in adult male albino rats of same age and weight as ours raised serum testosterone level with no significant change in LH levels.²⁶ However lead was not administered in these studies.

Ginger's antioxidant role has also been observed in another environmental pollutant, sodium arsenite in the same dose (0.5 mg/Kg body weight) in adult male Sprague Dawley rats for a period of 30 days by oral gavage. Serum testosterone and LH levels showed a significant rise when co administered with ginger. Gingers role was attributed again to its antioxidant and androgenic properties as it successfully lowered MDA marker and raised antioxidant enzyme levels.²⁷ Similarly, Amr Amin successfully evaluated

the protective role of ginger against testicular damage and oxidative stress in a cisplatin induced rodent model.¹⁵

Lead induced testicular toxicity has also been ameliorated by the concurrent administration of other herbal products such as walnut. *Juglans nigra* ameliorated the toxic effects and increased serum testosterone level again due to its antioxidant activity.¹⁸ Comparable results were obtained by a study conducted by Sakr *et al*²⁸ which evaluated gingers role in Mancozeb fungicide induced testicular damage. It was suggested that testicular injury induced by mancozeb is mediated by the same mechanism of depletion of antioxidants and elevation of lipid peroxidation.²⁸ Serum testosterone and LH levels were measured after 2, 4 and 6 weeks as in the present study. Serum testosterone exhibited significant increase when co-administered with ginger, whereas LH level did not show a significant increase.²⁸

CONCLUSION

Ginger effectively ameliorated lead toxicity in adult male Sprague Dawley rats by significantly raising serum testosterone levels at all durations. This might be a product of both its antioxidant and androgenic potential.

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