Modulatory effects of *Ginkgo biloba* in Lead induced Genetic Damage in Somatic Cells of Mice.

Karuna Kumari. Jaddu[1] and K Rudrama Devi[2*],
Department of Zoology, Osmania University, Hyderabad
1. karunajaddu@gmail.com
2. rudramadevi_k@yahoo.com*

Abstract

In the present study, modulating effects of *Ginkgo biloba* against lead nitrate induced clastogenicity in bone marrow cells of mice was carried out. Two experiments were conducted, in the first experiment animals were administered with various doses of *Ginkgo biloba* extract 200, 400, 600mg/kg body weight, the percentage of chromosomal aberrations were observed and the statistical analysis showed to be insignificant. The results showed antimutagenic nature of plant extract. In the second experiment animals were primed with *Ginkgo biloba* extract for seven days and lead nitrate was given one day before scarifying the animals. Lead nitrate showed significant increase in the percentage of chromosomal aberrations in 40mg/kg body weight lead nitrate treated animals. However when animals were co-administered for seven days prior to the priming experiment the frequency of chromosomal aberrations showed a decrease after the treatment of *Ginkgo biloba* extract . Thus the results clearly indicate protective nature of *Ginkgo biloba* extract against lead nitrate induced cytogenetic damage in somatic cells of mice.

Key words: Lead nitrate, chromosomal aberrations, *Ginkgo biloba.*

1.Introduction:

Lead (Pb) and one of the earliest metals discovered by the human race. Unique properties of lead, like softness, high malleability, ductility, low melting point and resistance to corrosion, have resulted in its widespread usage in different industries like automobiles, paint, ceramics, plastics, etc. This in turn has led to a manifold rise in the occurrence of free lead in biological systems and the inert environment.

Plants have widely been used in foods to engender good odor , flavor , color and preservative, it is clear fact that they possess antioxidant activity and is the reason for decreasing oxidation potential of lipids in food stuffs , ten of them have been used as a natural defense against diseases and infections .Human exposure to lead occurs through various sources like leaded gasoline, industrial processes such as lead smelting and coal combustion, lead-based paints, lead containing pipes or lead-based solder in water supply systems, battery recycling, grids and bearings, etc. Although lead toxicity is a highly explored and comprehensively published topic, complete control and prevention over lead exposure is still far from being achieved. There is no such level of lead that appears to be necessary or beneficial to the body and no “safe” level of exposure to lead has been found. Lead toxicity is a particularly insidious hazard with the potential of causing irreversible health effects. It is known to interfere with a

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number of body functions and it is primarily affecting the central nervous system, hematopoietic, hepatic and renal system producing serious disorders.[1-4].

. *Ginkgo biloba* is a tree belongs to family Ginkgoaceae. It is thought to have been preserved by priests in China and Japan who cultivated it on temple grounds.[5] The extensive studies of the main bioactive constituents of the *Ginkgo biloba* extract showed important pharmacological effects and was early reported that the *Ginkgo biloba* extract exerts an antioxidant effect by scavenging reactive oxygen species [6], reduces platelet aggregation and showed neuroprotective properties.[7] Previous study demonstrated the potential benefits of *Ginkgo biloba* extract treatment of Alzheimer's disease, learning and memory deficits, cerebrovascular disease, cardiovascular diseases, climacteric vasomotor symptoms and postmenopausal syndrome.[8-12]

So far there are no studies on protective effects of plant extracts on lead nitrate induced genotoxicity in somatic cells of mice. Hence in the present investigations we have made an attempt to evaluate the protective effects of *Ginkgo biloba* extract in lead nitrate induced genotoxicity in bone marrow cells of mice using analysis of chromosomal aberrations in somatic cells of mice.

2. Materials and Method:

2.1. Chemicals :

Lead nitrate from Sigma Aldrich the chemicals used in all the experiments were purchased from Hi-media analytical grade .

2.2.: Animal treatment:

The study was conducted after taking the approval of Institutional Ethical Committee on twenty adult male Swiss albino mice 30 to 50 days old and weighing around to 30 to 40 g were maintained in plastic cages under controlled lighting conditions (12:12 light and dark cycle) relative humidity (50±5%) and temperature (37±2oC) fed with mice feed and were given ad libitum access to water.

2.4. Experimental design Dosage schedule:

In the present study two experiments were conducted. The animals were fed orally with Lead nitrate and GBE extract and categorized in to following groups Group I: controls, Group II: GBE extract 200 mg/kg bwt, Group III: GBE extract 400mg/kg bwt, Group IV: GBE extract 600 mg/kg bwt. In the second experiment modulation studies were carried out as follows: Group I: controls, Group II: lead nitrate 40 mg/kg bwt, Group III: GBE extract 200 mg/kg bwt + lead nitrate 40mg/kg bwt, Group IV: GBE extract 400 mg/kg bwt + lead nitrate 40mg/kg bwt, Group V: GBE extract 600 mg/kg bwt + lead nitrate 40mg/kg bwt.

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2.5. Analysis of chromosomal aberrations in bone marrow cells:

All the control and treated animals groups were killed after 24hr of administration of the test chemical. The control and treated group of animals were scarified after 6 hr of the last treatment by cervical dislocation. The bone marrow was flushed out into clean glass petri dishes with hypertonic solution (0.56% KCl) to get a homogeneous cell suspension. It was then collected in clean centrifuge tubes and incubated at 37ºC for 45 minutes. Four slides were prepared from control and three groups of experimental animals. The staining was done within 24 hr of preparation according to the method of Preston *et al.,* 13] The slides were screened for 100 well spread metaphases per animal to examine the presence of various types of chromosomal aberrations like gaps breaks, fragment, Chromatid separations and polyploids in control and treated group of animals. The data was analyzed using Chi-Square test.

3. Results:

The data on various doses 200, 400, 60 0mg/kg of GBE extract in somatic chromosomes of Swiss albino mice (24hrs) are presented in Table 1. The data clearly indicate that there was a gradual increase n frequency of various types of chromosomal aberrations with duration of exposure as compared with the control group. At 24hr exposure the frequency (%) of breaks also showed an increase with three different concentration at all time intervals. At 24hr exposure the percentage of fragments recorded was 0.20, 0.20 and 0.20 respectively with 200 , 400 and 600mg/kg bwt GBE treated animals respectively against 0.00 in control I (Table 1). Similarly there was a gradual increase in frequency of early chromatid separation. At 24hr exposure, the frequency (%) of early chromatid separation was 0.40 in control 0.60, 0.60, and 0.60 respectively after administration of 200, 400 and 60 0 mg/kg b.wt *Ginkgo biloba* extract treated animals. At 24hr exposure the percentage of total chromosomal aberrations was 2.00, 2.40 and 2.60 after administration of 200, 400 and 600mg/kg bwt GBE respectively as against 1.40 in control II (Table 1).

Table 1: Frequency of chromosomal aberrations in somatic cells of mice treated with various doses of *Ginkgo biloba*. Extract (GBE).

Dose (ml/kg bwt) and duration of treatment (hrs)	**Normal metaphases scored (%)**	**Structural aberrations**			**Numerical aberrations**	**Total No. of aberrations (%)**
		Breaks	**Fragments**	**Exchanges**	**Chromatid separations**	
24hrs						
Control I	493 (98.60)	5(1.00)	0(0.00)	0(0.00)	2(0.40)	7(1.40)
200mgkg GBE	490(98.00)	6(1.20)	1(0.20)	0(0.00)	3(0.60)	10(2.00)
400 mg/kg GBE	488(97.60)	7(1.40)	1(0.20)	1(0.20)	3(0.60)	12(2.40)
60 0mg/kg GBE	487(97.40)	8(1.60)	1(0.20)	1(0.20)	3(0.60)	13(2.60)

The values in parenthesis are percentages, p>0.05(Insignificant).

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The X^2 values for the differences in the incidences of the chromosomal aberrations between control and treated groups at 24hr time interval was subjected to statistical analysis and found to be insignificant at all dose groups (P>0.05, Table 1).

Thus as a result of various types of chromosomal aberrations the percentages of total chromosomal aberrations at 24h exposure to Lead nitrate were 14.60 in 40 mg/kg body weight lead nitrate treated animals to 12.20, 10.20 and 8.40 in 40+200 40 mg/kg bwt, 40+400 mg/kg bwt, 40+600 mg/kg bwt lead nitrate + GBE primed animals respectively against 2.00 in control group (Table 2). The differences in the frequencies of chromosomal aberrations between control and treated groups at 24hr time interval was subjected to statistical analysis and found to be significant (p*<0.01)(Table 2).

Table 2: Classification of different types of chromosomal aberrations in somatic cells of mice analyzed after 24hrs of lead nitrate treated animals primed with various doses of *Ginkgo biloba* extract(GBE).

Dose(mg/kgbwt) and duration of treatment (hr)	**Normal metaphases**	**Various types of chromosomal aberrations**				**Total No. of aberrations (%)**
		Breaks	**Fragments**	**Exchanges**	**Chromatid separations**	
24hrs						
Control I	490(98.00)	5(1.00)	2(0.40)	1(0.20)	2(0.40)	10(2.00)
40mg/kg bwt Lead nitrate	427(85.40)	35(7.00)	17 (3.40)	11(2.20)	13(2.60)	73(14..60)*
200mg/kg bwt GBE + 40mg/kg Lead nitrate	439(87.80)	27(5..40	15(3.00)	9(1.80)	10(2.00)	61(12.20)
400mg/kg bwt GBE+ 40mg/kg Lead nitrate	449(89.80)	23(4.60	12(2.40)	8(1.60)	8 (1.60)	51 (10.20)*
600mg/kg bwt GBE+ 40mg/kg Lead nitrate	458 (91.60)	22(4.40)	8(1.60)	6 (1.20)	6 (1.20)	42 (8.40)*

The values in parenthesis are percentages.
The p*<0.01 level , hence the difference is considered to be statistically significant

4. Discussion:

The actively proliferating cells from bone marrow provide maximum information on the effect of any test compound. [13] Chromosome aberrations observed in the present analysis were classified into structural numerical and other abnormalities these end points serve as indicator for evaluating the mutagenic potentials of test substances. Since these are considered as stable anomalies which continue to next generation. Further such variations in somatic tissues lead to malignancy.

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Supplementation with micronutrients such as
trace elements and vitamins has been reported to provide protection against Pb toxicity [9].

Metals such as calcium (Ca) and zinc(Zn)have similar chemical properties to Pb and can compete for the binding sites of metal absorptive and enzymatic proteins in the gut, blood, and tissues of humans [14]. Zn supplementation also induces the biosynthesis of metallothionein, proteins involved in the detoxification of heavy metals. [15]. Recent studies showed that the intake of Ca and Zn can reduce the level of Pb metal in the blood and bone of pregnant women. [16] and children [17]. Vitamin C is a non-enzymatic antioxidant and a potential chelator of Pb[18][19]. An increasing number of studies have shown that plant extracts with antioxidant properties can effectively alleviate Pb toxicity [20].

Grape seed extract, which is rich in procyanidins, could recover lesions in the cardiovascular system induced by Pb exposure and inhibit alterations in the levels of adrenaline, 5-hydroxytryptamine and glutamic pyruvic transaminase (ALT) in the brain and liver of rats [21]. Another common plant extract, tea polyphenols, was reported to prevent Pb-induced dysfunctions in the brain, blood and liver of humans and animals [22, 23]. Catechins, one of the main constituents of tea polyphenols, were able to ameliorate cytotoxicity in renal and nerve cells under Pb exposure [24].

The results showed that both dietary supplements could effectively decrease Pb levels, protect amino levulinic acid dehydratase, superoxide dismutase and catalase activities and recover glutathione zinc protoporphyrin and malondialdehyde levels in tissues and blood of mice. A step through passive avoidance task confirmed that the dietary supplements could recover the learning and memory capacities of Pb-exposed mice. The protective effects of both dietary supplements to alleviate oxidative stress and cognitive impairments were superior to the chelator treatment. Administration of the dietary supplements during Pb exposure offered more significant protection than administration after Pb exposure. Animal safety evaluation also indicated that these dietary supplements barely induced side effects in the mice. [4]

The in vitro and in vivo studies showed cancer chemopreventive properties by clinical study on 75 years age 3069 participants for a period of 6 years and observed reduced risk of prostate cancer when compared with cancers. Further when GBE extracts were tested in attention deficit disorder (ADD) patients and found to be beneficial and useful for treatment of ADD patients with minimal side effects. In another study GBE extracts is an evidence based option for tinnitus treatment. [25-28]

Several reports have shown the protective nature of garlic its active principle reducing the lead burden in soft tissues [29]. In another study the ability, the protective effects of curcumin in lead induced neurotoxicity has been studied by [30]. Further *Centella asiatica* along with DMSA chelating agent reduced the oxidative stress caused by lead in rats [The present results are comparable with earlier studies which reduced the oxidative stress caused by lead in rats[31] [32].
In the present study showed that Standard *Ginkgo biloba* extract, GBE 761, contains 22-27% flavonoids (ginkgo flavone glycosides) and 5-7% terpenoids (ginkgolides and bilobalides) . The antioxidant effect of GBE has been linked to its main constituents, flavonoids and terpenoids, which

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can scavenge free radicals and reduce levels of reactive oxygen species. [33]

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The present results are comparable with earlier studies in our lab the *Aegle* fruit extract showed reduction in lead induced genotoxicity in mice [34] and protective effects of *Solanum lycopersicum* in lead induced micronuclei in bone marrow cells has been reported [35].

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5. Conclusion:

The overall results of the present study suggest the geno protective activity of *Ginkgo biloba* extract in lead nitrate induced genotoxicity in bone marrow cells of mice.

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