

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

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Abstract

The effects of Ginkgo biloba on lead nitrateinduced clastogenicity in mouse bone marrow cells were investigated in this work. In the first experiment, mice were given different doses of Ginkgo biloba extract (200, 400, 600 mg/kg body weight), the proportion of chromosomal abnormalities was measured, and the statistical revealed that the results were analysis inconsequential. The results revealed that the plant extract is antimutagenic. In the second experiment, animals were administered Ginkgo biloba extract for seven days and then given lead nitrate one day before being scarified. In 40mg/kg body weight lead nitrate treated animals, the percentage of chromosomal abnormalities increased significantly. However, when mice were co-administered with Ginkgo biloba extract for seven days prior to the priming experiment, the frequency of chromosomal abnormalities decreased. Thus, the findings clearly show that Ginkgo biloba extract protects mice somatic cells against lead nitrateinduced cytogenetic damage.

Key words: Ginkgo biloba, lead nitrate, chromosomal abnormalities

Introduction:

Humans have discovered lead (Pb) since the dawn of time. As a result of lead's unique features such as its softness and malleability as well as its ductility, ductility, low melting point and corrosion resistance, it has been widely used in a variety of industries. Because of this, the amount of free lead found in biological systems and the inert environment has risen dramatically. In addition to providing flavour, colour, and preservation benefits, plants have long been employed for their antioxidant properties, which have been shown to lower the oxidation potential of lipids in food. Ten of these plants have even been used to fight sickness and infection naturally. Toxins from leaded gasoline and industrial operations like lead smelting and coal combustion can be found in paints, leadbased pipes, lead-based solder used in water supply systems, grids and bearings, as well as lead-based batteries. There has been a lot of research done on lead poisoning, but there is still a long way to go before we can say we've completely eliminated our exposure to lead. There is no "safe" level of lead exposure, and no level of lead that appears to be essential or helpful to the body has been found. Toxic exposure to lead has the potential to have



long-term and often permanent impacts on one's health. It has been reported to have an effect on

These include the central nervous system, hematopoietic, hepatic, and renal systems, all of which are impacted by the disease. Numbered from

identified in thorough studies of the Ginkgo biloba extract's major bioactive components [6]. [7] Alzheimer's illness, learning and memory deficits, cerebrovascular disease, cardiovascular disease, climacteric vasomotor symptoms and postmenopausal syndrome have previously been found to benefit from Ginkgo biloba extract. [8-12]

Mice exposed to lead nitrate-induced genotoxicity in somatic cells have not yet been protected against the genotoxic effects of plant extracts. Somatic cell chromosomal aberrations in bone marrow cells from mice exposed to lead nitrate were examined in the current investigations to study the protective effects of Ginkgo biloba extract on bone marrow cells.

What I Used and How I Did It

In this case, we're talking about chemicals.

For all experiments, we used lead nitrate analytical grade from Sigma Aldrich.

: Treatment of animals:

Following the approval of the Institutional Ethical Committee, the study was carried out using 20 adult male Swiss albino mice, 30 to 50 days old and weighing between 30 and 40 grammes, in plastic cages under controlled lighting conditions (12:12)

[1-4]. \s. Ginkgo biloba is a member of the Ginkgoaceae family. According to legend, priests in China and Japan planted it on temple grounds, where it was protected from the weather. [5] A reduction in platelet aggregation and the demonstration of neuroprotective properties were

light and dark cycle), relative humidity (50–5 percent) and temperature (37–2oC), fed with mice feed, and given ad-libitum access to water in a controlled environment.

Dosage schedule for the experiment

Two experiments were carried out in this investigation. Lead nitrate and GBE extract were administered orally to the animals, and they were then divided into the following groups: There were four groups: the control, 200 mg/kg bwt GBE extract, 400 mg/kg bwt and 600 mg/kg bwt GBE extract. The following modulation experiments were conducted out in the second experiment: A total of five groups were studied: control, lead nitrate 40mg/kg bwt, GBE extract 200 mg/kg bwt, GBE extract 400 mg/kg bwt, GBE extract 600 mg/kg bwt, and GBE extract 600 mg/kg.

Analysis of chromosomal aberrations in bone marrow cells:

1. Toxicology tests were conducted on both the control and treated groups of animals following 24 hours of exposure to the test substance. Animals in both the control and treated groups were scarified six hours following their final cervical dislocation treatment. To obtain a uniform cell suspension, the



bone marrow was drained into clean glass petri dishes with hypertonic solution (0.56 percent KCl). After that, it was centrifuged and incubated for 45 minutes at 37oC in clean tubes. Four slides were made, one for each of the three experimental groups and the two controls. According to Preston et al., 13] the staining was completed within 24 hours of preparation. For each animal, 100 well-spread metaphases were examined for the presence of various chromosomal abnormalities, such as gaps breaks, fragments of chromosomes, Chromatid separations, and polyploidy. The Chi-Square test was used to examine the data.

2. **Results:**

Table 1 shows the effects of GBE extract at doses of 200, 400, and 600 mg/kg on Swiss albino mice's somatic chromosomes over the course of a day. Compared to the control group, the findings clearly

show that the frequency of various types of chromosomal abnormalities increased over time. The frequency (percentage) of breaks increased with three different concentrations during the course of a 24-hour exposure. With 200, 400, and 600mg/kg bwt GBE-treated animals, the proportion of fragments reported was 0.20, 0.20, and 0.20, respectively, at 24 hours of exposure, as opposed to 0.00 in the control I (Table 1). Similarly, the frequency of early chromatid separation has increased steadily over the years. The frequency (percentage) of early chromatid separation was 0.40 in the 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 mice at 24 hours of exposure. After injection of 200, 400, and 600mg/kg bwt GBE, the percentage of total chromosomal abnormalities was 2.00, 2.40, and 2.60, respectively, compared to 1.40 in control II at 24hr exposure (Table 1).

Chromosome aberration frequency in somatic cells of mice treated with varied dosages of (see Table 1)

Ginkgo biloba. Extracted from the plant (GBE).

Dose (ml/kg bwt) and duration of treatment (hrs)	Normal metaphases scored (%)	Structura	al 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).



Chromosome aberrations were found to be minor at all dose levels (P>0.05, Table 1) when X2 values were compared between control and treatment groups at 24hr intervals.

This is because chromosome aberrations of various types were found in the animals exposed to Lead Nitrate for 24 hours, ranging from 14.60 in animals exposed to 40 mg/kg bwt Lead Nitrate to 12.20, 10.20, and 8.40 in animals exposed to 40+200 mg/kg bwt Lead Nitrate to 40+400 mg/kg bwt, 40+600 mg/kg bwt Lead Nitrate + GBE primed animals to 2.00 in the control group (Table

2). To determine if the differences between control and treatment groups in the frequency of chromosomal abnormalities were statistically significant (p*0.01), statistical analysis was conducted (Table 2).

Somatic cell chromosomal abnormalities in mice treated with lead nitrate and primed with various dosages of Ginkgo biloba extract are shown in Table 2. (GBE).

Dose(mg/kgbwt) and duration of treatment (hr)	Normal metaphase s	Various t	Total No.			
		Breaks	Fragments	Exchanges	Chromatid separation s	aberration s (%)
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	427(85.40)	35(7.00)	17 (3.40)	11(2.20)	13(2.60)	73(1460)*
nitrate						
200mg/kg bwt GBE +	439(87.80)	27(540	15(3.00)	9(1.80)	10(2.00)	61(12.20)
40mg/kg Lead nitrate						
400mg/kg bwt GBE+	449(89.80)	23(4.60	12(2.40)	8(1.60)	8 (1.60)	51 (10.20)*
40mg/kg Lead nitrate						
600mg/kg bwt GBE+	458 (91.60)	22(4.40)	8(1.60)	6 (1.20)	6 (1.20)	42 (8.40)*
40mg/kg Lead nitrate						

The values in parenthesis are percentages.

The p*<0.01 level, hence the difference is considered to be statistically significant





3. Discussion:

Bone marrow cells, which are constantly dividing, provide the most accurate information about the effects of any test chemical. [13] To determine the carcinogenic potential of test chemicals, the chromosome aberrations detected in this study were divided into structural numerical and other abnormalities. Due to the fact that these are considered to be stable anomalies, they will be passed down to the next generation without change. Malignancy is the result of further somatic tissue changes.

Micronutrients such as vitamins and trace minerals have been shown to protect against Pb poisoning [9].

Chemically, metals like calcium (Ca) and zinc (Zn) have characteristics similar to those of lead (Pb) and can compete for binding sites in human tissues gut, blood, and with Pb Metallothionein, a protein involved in heavy metal detoxification, also induced is by supplementation. [15]. Pregnant women's blood and bone levels of Pb metal can be reduced by supplementing with Ca and Zn, according to a new study. [16] and children [17]. [16] and Antioxidant: Vitamin C is a non-enzymatic antioxidant and a possible chelator of Pb[18][19]. Plant extracts with antioxidant capabilities have been found in a growing number of studies to effectively relieve Pb poisoning [20]. Preservative-free grape seed extract, which contains procyanidins, was able to restore cardiovascular system lesions generated by Pb exposure and prevent changes in the levels of adrenaline and 5-hydroxytryptamine (ALT) in the brain and liver of rats [21]. [20,21]. Tea

polyphenols, a common plant extract, have been shown to protect the brain, blood, and liver of people and animals from Pb-induced dysfunctions [22, 23]. Pb-induced cytotoxicity in renal and nerve cells was reduced by tea polyphenols, including catechins [24].

Pb levels were reduced, amino levulinic acid dehydrase activity was protected and catalase and glutathione zinc protoporphyrin levels were restored by both dietary supplements in tissues and blood of mice. Pb-exposed mice were able to regain their learning and memory abilities through a passive avoidance test. In comparison to the chelator treatment, the protective benefits of both dietary supplements to reduce oxidative stress and cognitive deficits were superior. supplementation during exposure to Pb was more effective than supplementation after exposure to Pb. These nutritional supplements have little negative effects in mice, according to a review of their safety conducted on animals. [4] In a sixyear clinical trial including 75-year-old men, researchers found that the drug had cancerfighting qualities in both in vitro and in vivo investigations. They also found a lower incidence of prostate cancer than in the control group. Additional research has shown the positive effects of the GBE extracts on attention deficit disorder (ADD) patients, as well as the low risk of side effects when used for therapy purposes. GBE extracts have been found to be an effective treatment for tinnitus in another investigation. [25-28]

Numerous studies demonstrate the preventive properties of garlic, demonstrating that its active principle lowers soft tissue concentrations of lead.



Curcumin's capacity to protect against leadinduced neurotoxicity has been examined in another study [30]. The oxidative stress in rats generated by lead was also reduced by Centella asiatica in conjunction with the DMSA chelating agent [The present results are comparable with past research that reduced the oxidative stress in rats caused by lead(31)]. [32].

There were 22-27 percent flavonoids (ginkgo flavone glycosides) and 7 percent terpenoids (ginkgolides and bilobalides) in the standard Ginkgo biloba extract, GBE761. Flavonoids and terpenoids, two of GBE's primary ingredients, have been shown to have an antioxidant effect.

free radicals and reactive oxygen species can be scavenged and reduced. [33].

Solanum lycopersicum has been shown to protect against the formation of lead-induced micronuclei in bone marrow cells in previous investigations in our lab [34, 35]. The current results are comparable to those of those earlier studies..

4. **Conclusion:**

Ginkgo biloba extract showed strong protective action against lead nitrate-induced genotoxicity in mouse bone marrow cells, according to the findings of this investigation.

5. **References:**

[1]. Kalia K and Flora SJ are the authors (2005). Therapeutic options for chronic arsenic and lead poisoning that are safe and effective. Occupational Health.;47:1–21.;

- [2]. Drs. D.Madhavi and K.Rudrama Devi (2007). Procedings of the AP Science Academy, Vol. 11(1): 56-60, "Lead nitrate induced chromosomal abnormalities in in vitro human cells,"
- [3]. Lead-based paints can cause chromosomal abnormalities in industrial painters who have been exposed, according to a study published in the journal Environmental Toxicology, Oncology, and Pathology.
- [4]. Qixiao Zhai, Liu Yang, Jianxin Zhao, Hao Zhang, Fengwei Tian, and Wei Chen are the seven members of this group (2018). Nutritional Supplements Rich in Plant Extract and Probiotic Enrichment Protect Mice against Lead Toxicity. The journal Front Microbiol. 2018; 9: 2134
- [5] Clinical and preclinical studies of Ginkgo biloba's efficacy, safety and use. [5] McKenna DJ, Jones K, Hughes K. 2001;7:70–86. 88–90. Altern Ther Health Med
- [6] The following six researchers have contributed to this work: L. Brunetti, G Orlando, L. Menghini, and M. Vacca all of Ferrante and C. Chiavaroli and M. In vitro, Ginkgo biloba leaf extract prevents the synthesis of isoprostanes caused by amyloid betapeptide in the rat brain. In 2006, Planta Med. 72:1296–1299.

In a study published in Prog Neurobiol in 2002, Maclennan KM, Darlington CL, and Smith PF looked at the effects of ginkgo biloba extracts and ginkgolide B on the central nervous system.

[8]. Ginkgo biloba extract (Egb 761) reduces - amyloid formation by reducing free cholesterol levels, according to ZX, Han Z, Drieu K, and Papadopoulos V. 2004;15:749–756.



- [9] Extraction of Ginkgo biloba leaf extract from the leaves of model rats has been shown to protect against aluminum-induced learning and memory deficits. 2006;12:37–41 in the Chinese Journal of Integrative Medicine (CJIM).
- 10. Hrehorovska M, Burda J, Domorakova I, and Mechirova E. Effect of Tanakan on postischemic activity of the rat brain's protein synthesis system. Biophys Genomics. 2004;23:457–465.
- 11 Koch E, Furst R, Vollmar AM and Zahler S. Increased endothelial nitric oxide generation by Ginkgo biloba extract EGb 761 in vitro and in vivo. Biochem Biophys Res Commun 2007;64:1715–1721. [PubMed]
- [12]. It's SM Chung KH (2004). In vitro studies on the estrogenic properties of Ginkgo biloba extracts
- [13]. Brewen JG Carrano AV Heddle JA Mc Fee AF Wolfe Sand Preston RJ Au N Bondar NH Brewen JG Carrano A V Wasson J.S. (1981). U.S. Epa's Gene-Tox programme report on the mammalian in vitro and in vivo cytogenetie assay, Mutat. Res., 87:143-783.
- [14]. Basha, D. C., Rani, M. U., Devi, C. B., Kumar, M. R., and Reddy, G. R. (2012). (2012). To reverse the effects of perinatal lead exposure, calcium co-administration was administered to the rat brain. Int.J.Dev.Neurosci.30, 343–350.doi:10.1016/j.ijdevneu.2012.01.004.
- [15]. A.K. Baltaci, K. Yuce, and R. Mogulkoc (2018). Metallothioneins and Zinc Metabolism. Doi: 10.1007/s12011-0171119-7 in Biol. Trace Elem. Res., 183, 22–31.

- [16]. A review of the literature by Gulson, Mizon, Palmer, Korsch, Taylor, and Mahaffey (2000). Review of prenatal and postpartum estimations of skeletal blood supply. 249–251 J Lab Clin Med 136
- [17]. Allan T. Cantoral, Maria M. Téllez-Rojo; Thomas SS, Levy; Hernández-vila; L. Schnaas; H. Hu; and others (2015). In two-year-old Mexican youngsters, zinc status has a distinct effect on lead exposure and length.

Environment and Health. 2015; 14: 95. -

- [18]. R.A. Goyer and M.G. Cherian (1979). Treatment of lead toxicity in rats using ascorbic acid and EDTA. LifeSci.24,433–438.doi:10.1016/0024-3205(79)90215-7.
- [19]. These researchers studied the effects of the Rendon-Ramirez sulfonamide on the brains of mice and found that the effects of the sulfonamide on the brains of mice were similar to those found in the brains of healthy mice (2014). Lead-exposed workers' oxidative damage and total antioxidant capacity can be reduced by treatment with vitamin E and C. Applied Toxicology and Pharmacology 37: 45–54, 2013.
- [20]. Liu, Xue, Zhu and Zhang Y's research team included Jiao, Gü, Liu, Zhu, Zhu, H., Zhu H., Jiao, J., and Lü, G. (2011). Dietary supplements and naturally occurring antioxidants reduce the blood levels of lead in mice that have been exposed to lead. J.Sci.FoodAgric.91,485–49.



- [21]. Waggas, A.M. (2012). Male albino rats treated with lead acetate were given grape seed extract (Vitisvinifera), which reduced the rats' neurotoxicity and hepatotoxicity. Neurosciences of Behaviour, 2, 176–18
- [22]. E. A. Hamed, A. R. Meki, and N. A. A. El-Mottaleb are the authors of this paper (2010). Green tea protects rat blood and brain tissue homogenates against lead-induced oxidative damage. Physical and Biochemical Reviews, 1966, 64–151.
- [23]. EE Mehana, AR Meki, and K. M. Fazili are the authors (2012). Green tea extract has been shown to reduce the harmful effects of lead on rats' livers. Exposure Toxicology Pathol. 64: 291–295
- [24]. Chen,L.,Yang,X.,Jiao,H.,andZhao,B.(2002). It is thought that catechins in tea can reduce the harmful effects of lead on cells, lipids, and the membrane of these tissues 32,787–794. doi:10.1016/j.nutres.2012.09.004..
- [25] Chen XH, Miao YX, Wang XJ, and others Human colon cancer cells treated with Ginkgo biloba extract EGb761. Biology of the Cell 2011;27:227–232.
- [26]. Ginkgo biloba extract's effects on human hepatocellular carcinoma cell proliferation and cytotoxicity were studied by Chao JC and Chu CC. WJG 10:37–41, World J Gastroenterol
- [27]. Chinese researchers found that the Ginkgo biloba extract kaempferrol was effective at slowing the growth of pancreatic cancer cells and inducing their death through the process of

- programmed cell death (apoptosis). Journal of Surgery, 148:17–23, 2008.
- . [28] Xu, AH; Chen, HS; Sun, BC; and colleagues. Ginkgo biloba exocarp polysaccharides may have a role in the treatment of stomach cancer. World J Gastroenterol. 2003;9:2424-2427.
- 29]. Sharma V, Sharma A, and Kansal L are the three members of the research team (2010). The effects of Allium sativum preparations taken orally
- [30]. A study by Shukla, Khanna, Khan, and Srimal (2003). In rats, curcumin protects against lead neurotoxicity. Human Exposure to Toxicology;22:653–658. [31]. G. Saxena and S.J. Flora (2006). Succimers and Centella asiatica have been shown to alter brain biogenic amines and haem production in lead-poisoned rats. 547–559. J Pharm Pharmacol.
- [32]. SB Sainath; R Meena; C Supriya; K P R Reddy; and PS Sainath; (2011). Antioxidant and reproductive health benefits of Centella asiatica in male rats exposed to lead. Environmental Toxicology and Pharmacology, 32, 146–154.
- [33]. Zhang, Ying, and Zhou, et al (2010). Efficacy of Ginkgo biloba extract in rats with non-alcoholic steatohepatitis. The Saudi Medical Journal, 31(10), 1114-1118
- [35]. Dilip reddy and Rudramadev (2014). Lead-induced micronucleus in bone marrow cells can be prevented by Solanum lycopersicum fruit extract, according to the World Journal of Pharmaceutical Research.