"Protective Effects of Ginko Biloba Againgnest Cyclophosphamide Induced Genotoxicityin Swiss Albino Mice"

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***Abstract*:** The present investigation the antimutagenic effects of ginko biloba extract (GBE) has been evaluated against cyclophosphamide induced genotoxicity in bone marrow cells of mice. The administration ofGBE extract at various doses i.e. 200, 400and 600 mg /kg. When treated individually did not induce chromosomal aberrations in somatic cells of mice in 24hrs. A single intraperitoneal administration of 50mg/kg of cyclophosphamide induced significant increase in the percentage of CAS in bone marrow cells of mice. However after co administration of three doses of GBE extract there was a dose dependent decrease in the % of CAS was observed. When animals were administeredwith GBE 200, 400 & 600 mg/kg/bw orally for two weeks and on sixteenth day CP (50 mg/kg/bw) was given intraperitonially as a single dose. For each experimental group control, animals were maintained. Two days after the administration of the last dose, the animals were sacrificed and air dried metaphase preparations were made and processed for identification of chromosomal aberrations in somatic cells of mice. In animals treated with single dose of CP, an increase was observed when compared with the values of control group. But when animals primed with GBE, there was a decrease in the frequency of chromosomal aberrations. Thus the results clearly indicated the protective role of GBE on cyclophosphamide induced genotoxic damage in somatic cells of mice.

Key Words: *Cyclophosphamide protection, ginko biloba and somatic cells.*

1. Introduction

A number of antineoplastic drugs are in common use to combat various types of cancers. These are shown to be mutagenic in different test systems and these antineoplastic drugs such as Cyclophosphamide, Cisplatin, Tamoxifen, Gemcitabine and Paclitxel etc., have shown clastogenic effects in various test systems. Potential genetic damage due to drugs and other chemicals is well recognized. Extensive studies have been carried out on mutagenicity of various drugs in microorganisms, insects, mammals and in exposed population. [1-3] Cyclophosphamide (CP) is a nitrogen mustard alkylating agent from the oxazophorines group. It is used to treat Hodgkin's disease, lymphomas, leukemia, Wegener's granulomatosis, severe rheumatoid arthritis, and lupus erythematosus.[4-6] It is also used in combination with other drugs to treat breast cancer, leukemia, and ovarian cancer. The drug also has immunosuppressant action when it has used in smaller doses. In spite of CP therapeutic importance, a wide range of adverse effects were recorded. Sweetman [7] reported many side effects; including hemorrhagic cystitis, alopecia and hyperpigmentation of skin may develop after high or prolonged dosages and can be life-threatening..

Ginkgo biloba Linné 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 [8]. The extensive studies of the main bioactive constituents of the *Ginkgo biloba* extract showed important pharmacological effects.

It was early reported that the *Ginkgo biloba* extract exerts an antioxidant effect by scavenging reactive oxygen species[9], reduces platelet aggregation and showed neuroportective properties [10], previous study demonstrated the potential benefits G*inkgo biloba* extract treatment of Alzheimer's disease, learning and memory deficits, cerebrovascular disease, cardiovascular diseases, climacteric vasomotor symptoms and postmenopausal syndrome[11-15], Further the ginko biloba extract possesses antimuour properties [16], may induced cancer cells apoptosis and differentiation and inhibit the progression of human colon cancer , heptocellular carcinoma , parncretic and gastric cancer [17-21]. The present work was aimed to study the effect of GBE on CP-induced germ cell damage in male albino mice.

MATIALS AND METHODS

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 libitium access to water. A group of 5 mice per experiment were taken and treated with CP and GBF. The doses were prepared daily in distilled water and were administered by gastric gavage method for GBF and 26G needle intraperitoneal injection for CP treatment dose protocols were as follows Group I control group were treated with 5 ml of physiological saline. Group II the animals were treated with CP 5 mg/animal/day intraperitoneally. Group III control were treated with GBE 200, 400 & 600 mg/animal/day for two weeks daily. Group IV Experimental batch were pretreated with 200, 400 & 600 mg/kg BW GBE for 15 days on the 16th day single intraperitoneal dose of cyclophosphamide 5 mg/kg/bw, were administered.

Dosage schedule: In the present study two experiments were conducted. The animals were fed orally with cyclophosphamide and PFE extract and categorized in to following groups Group I: controls Group II: PFE extract 200 mg/kg Group III: GBE extract 400mg/kg Group IV: GBE extract 600 mg

In the second experiment for modulation studies all the three groups as follows:
Group I: controls
Group II: Cyclophosphamide 50 mg/kg
Group III: GBE extract 2000 mg/kg + Cyclophosphamide 16 mg/kg
Group IV: GBE extract 400 mg/kg + Cyclophosphamide 16 mg/kg
Group V: PFE extract 600 mg/kg + Cyclophosphamide 16 mg/k

Analysis chromosomal aberrations in somatic cells of mice: The animals were killed two days after administration of the last dose. The bone marrow was flushed into clean glass Petri dishes with hypertonic solution (0.56% KCl) were used to get a homogeneous cell suspension. It was then collected in clean centrifuge tubes and incubated at 37oC for 45 minutes. Four slides for each were prepared from control and experimental animals. The staining was done within 24 h of preparation according to the method Preston et al.[22] The slides were screened for 50 well spread metaphases per animal for the presence of various types of chromosomal aberrations like gaps breaks, fragment, chromatid separations and polyploids in control and treated group of animals. The differences in the frequencies of chromosomal aberrations between control and treated groups were analyzed using ChiSquare test. For calculating mitotic index (MI) a minimum of 1000 cells were counted for each animal results selected for GINKO BILOBA extract were 200, 400 and 600 mg/kg body weight at various time intervals. The mutagenic effects of the extract were studied on somatic cells of mice for different time intervals. The results were recorded (Table 1).

Results:

At 24 hrs the percentage of chromosomal aberrations for 200, 400and 600 mg/kg body weights of fruit extract in the treated groups recorded were 2.00, 2.80 and 2.4% respectively when compared with that of controls 1.6% (Table- 1). The differences in the frequencies of chromosomal aberrations between controls and PFE treated mice for 24 were analyzed by X2 test and the results were found to be insignificant (P>0.05, Table- 1).

In the present study various doses of the cyclophosphamide of 50 mg/kg were primed with different doses of Ginkobilobat extract of 200, 400 and 600 mg/kg body weight and the results were presented in Table 2

At 24 hrs of the study the controls have shown 2.4% of the chromosomal abnormalities when 16 mg/kg body weight of cyclophosphamide were recorded (Table 2). The uprimed mice with cyclophosphamide have shown the chromosomal aberrations were 19.20% respectively. The highest dose has shown maximum abnormal metaphases. Priming with 200 mg/kg body weight of GB E, the effect has decreased. There was decrease observed and the aberrations were 18.80% respectively. Similarly with 400mg/kg body weight the recorded values were 12.6% and with 600 mg/kg body weight there was a decrease for 16 mg/kg body weight of cyclophosphamide with 11.20% respectively at 24 hrs (Table-3). The differences in the frequencies of the chromosomal aberrations were analyzed by X2 test and the results observed were found to be significant (P<0.01, Table 2

Frequency of CA recorded in somatic cells of mice analyzed after 24, 48, 72h treatment with various doses of *Ginkgo biloba*extract

Dose(mg/kg) and Duration of treatment	**24hrs**	
	Normal metaphases %	**Abnormal metaphases %**
Control	488(97.20)	14(2.80)
200 mg/kg GbE	485(97.00)	15(3.00)
400 mg/kg GbE	484(96.70)	16(3.30)
600 mg/kg GbE	483(96.50)	17(3.50)

The values in parentheses are percentages
*P>0.05

Classification of various types of chromosomal aberrations in somatic cells of mice analyzed after 24, 48, 72hrs of *Ginkgo biloba* extract

Dose (mg/kg) and duration	Structural aberrations				Numerical aberrations		Total of no. of aberrations (%)
	Gaps	Breaks	fragments	exchanges	polyploidy	Chromatid separations	
24hrs Control I -	4(0.80)	6(1.20)	0(0.00)	0(0.00)	0(0.00)	4(0.80)	14(2.80)
200 mg/kg GbE	5(1.00)	6(1.20)	0(0.00)	0(0.00)	1(0.20)	4(0.80)	15(3.00)
400 mg/kg GbE	5(1.00)	6(1.20)	1(0.20)	0(0.00)	1(0.20)	3(0.60)	16 (3.30)
600 mg/kg GbE	5(1.00)	6(1.20)	2(0.40)	0(0.00)	1(0.20)	3 (0.10)	17 (3.50)

Table3 Frequency of CA recorded in somatic cells of mice treated with Dox^{+} and primed with *Ginkgo biloba* extract**24hrs**

Grouping	Dose(mg/kg)		
		Normal metaphases %	Abnormal metaphases %
Group I	**Control**	498(97.70)	11(2.20)
Group II	**Cp16mg/kg**	404(80.8)	90(19.20)
Group III	**200 GbE + 16mg/kg Cp**	427(85.4)	73(14.60)
Group IV	**400 GbE + 16mg/kg Cp**	437(87.4)	63(12.60)
Group V	**600 GbE + 16mg/kg Cp**	444(88.80)	56 (11.20)

The values is parentheses are percentages ** $p<0.05$

Classification of CA recorded in somatic cells of mice treated with Dox and primed with *Ginkgo biloba* extract

Dose (mg/kg) and duration of treatment	Structural aberrations				Numerical aberrations		Total of no. of aberrations (%)
	Gaps	Breaks	fragments	exchanges	Polyploidy	Chromatid separations	
24hrs							
Control	3(0.60)	4(0.80)	2(0.40)	0(0.00)	0(0.00)	2(0.40)	11(2.20)
Cp 16 mg/kg	24(5.20)	20(4.00)	26(4.60)	4(0.40)	6(0.20)	10(2.00)	90(19.20)
200 GbE + 16 mg/kg Cp	20(4.00)	16(3.20)	24(4.00)	3(0.60)	4(0.80)	10(2.00)	73(14.60)
400 GbE + 16 mg/kg Cp	18(3.40)	15(3.00)	16(3.20)	2(0.40)	4(0.80)	8(1.60)	63(12.60)*
600 GbE + 16 mg/kg Cp	15(3.00)	11(2.20)	15(3.00)	2(0.40)	3(0.60)	8(1.60)	56(11.20)*

The values in parenthesis are percentages $p>0.05$

DISCUSSION:

The actively proliferating cells from bone marrow provide maximum information on the effect of any test compound. The transition from proerythroblast to erythrocytes takes about seven cell division cycles. Each cell cycle takes 10-11 hrs and the terminal mitosis icompleted in about 10hrs before the transition of orthochromatid erythroblast to polychromatic erythrocytes. In view of the above to see the long and short term effect of test compound on cells, the sampling time ranged was from 6-72 hrs has taken in present observation There are different type of chromosomal aberrations observed in present analysis These aberration are classified into structural, numerical and other abnormalities. Structural aberration includes gaps, breaks, fragments, terminal deletion and centric fusion these end points serve as indicators for assessing the mutagenic effect of test substance.

The present results are comparable with that of Asita el al,[23] who investigated the intraperitoneal injection of mice with a single dose of 40 mg/kg body weight of Cyclophosphamide induced a significant increase in the frequency of MNPCE, 24 h after injection, when compared with animals that received water treatment. The present results are comparable to Santos Renato et al.,[24] who reported that Cyclophosphamide at 135mg/kg dose induced a significant increase in the frequency of micronuclei in polychromatic erythrocytes of male mice

Since many decades plants derivatives has been considered as important source of new discovery of novel pharmacologically active compounds. *Ginko biloba* has been preserved in china and japanby prients and has been cultivated [8]. It has been known that it leaf extracts has been used for the treatment of peripheral vasucular disorders, cerebral disorders alzemier diseases dementia [9-16]. The in vitro and invivo 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 [17-21].

Zhou et al [25]. The extracts have GBE has antioxidant and ehpatoprotective impact and reduces the liver fibrosis in rat of non alcoholic steato hepatitis. Further the extract protects liver damage induced by ccl4 in male rats [26] Yoo et al [27] reported that when repeated intake of Egb increases cell proliferation and neuroblast differentiation in mice hippocampal dentate gyrus and enhances neurogenesis in adults. Ozuturk et al [28] found the beneficial effects on nervous system and prevented morphological deteriotion and functional in cisplatin induced neuropathy.

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) [26]. The antioxidant effect of GBE has been linked to its main constituents, flavonoids and terpenoids, which can scavenge free radicals and reduce levels of reactive oxygen species[27]. The results of present study it indicate protective nature of GBE in cyclophosphamide induced chromosomal aberration in animals. Such protective nature of other plant extracts such as *Phylthanthus emblica, Garlic extract, Solanum lycopersiun, Curcumin extract* against anti-cancer drug has been reported[29-32].

LIST OF CONCLUSION:

From the above studies, it is concluded that *Ginkgo biloba* was a potential candidate as protective agent to cyclophosphamide induced genotoxic effect in somatic cells of mice. The combined treatment of cyclophosphamide and GBE holds a promise as a safe and effective chemotherapeutic strategy.

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