Available online at www.ijpab.com

DOI: http://dx.doi.org/10.18782/2582-2845.8029

ISSN: 2582 – 2845

Ind. J. Pure App. Biosci. (2020) *8*(2), 238-247

Research Article

Indian Journal of Pure & Applied Biosciences

Peer-Reviewed, Refereed, Open Access Journal

Modulatory Effects of *Ginkgo biloba* in Cyclophosphamide Induced Genotoxicity in Germ Cells of Mice

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Received: 13.03.2020 | Revised: 16.04.2020 | Accepted: 20.04.2020

ABSTRACT

Cyclophosphamide has been used for treatment of various cancers, hence there are side toxic effects has been noted hence studies were carried to evaluate the protective effects of Ginkgo biloba in germ cells of mice two experiments were carried out. In first experiment the animals were treated with various doses of GBE 200, 400 and 600 mg/kg were given orally. All the animals were killed on 60th day if treatment for meiotic prepares were made according to the standardized protocol. In second experiment animals were given Cyclophosphamide co administered with GBE for seven days prior to the cp . the prepared slides were screened for the presence of various types of aberrations such as autosomal univalent and sex chromosomal univalent and translocations in control and treated animals . the data was analyzed using standard statistical analysis the results showed increased incidence of chromosomal aberration in cp induced animals however in primed with GBE there is a significant reduction in the frequency of aberrations thus the data clearly indicate protective nature of Ginkgo biloba in Swiss albino mice the present observations are useful in ca cancer chemotherapy regimen.

Keywords: *Cyclophosphamide, Ginkgo Biloba Extract, Chromosomal Aberrations, Germ Cells*

INTRODUCTION

A wide 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, and mammals and in exposed population (Smorenburg et al., 2001; Akram et al., 2012; Deshpande et al., 2013). Cyclophosphamide (CP) is a nitrogen mustard alkylating agent from the oxazophorines group.

Cite this article: Ravi Prasad, A., & Rudrama Devi, K. (2020). Modulatory Effects of *Ginkgo biloba* in Cyclophosphamide Induced Genotoxicity in Germ Cells of Mice, *Ind. J. Pure App. Biosci. 8*(2), 238-247. doi: http://dx.doi.org/10.18782/2582-2845.8029

It is used to treat Hodgkin's disease, lymphomas, leukemia, Wegener's granulomatosis, severe rheumatoid arthritis, and lupus erythematosus (Fleming, 1997; Perini et al., 2007; Uber et al., 2007). 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. Sweet man (Sweetman & Martindale, 2007) 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 (McKenna et al., 2001). The extensive studies of the main bioactive constituents of the *Ginkgo biloba* extract showed important pharmacological effects was early reported that the *Ginkgo biloba* extract exerts an antioxidant effect by scavenging reactive oxygen species (Brunetti et al., 2006), reduces platelet aggregation and showed neuroportective properties (Maclennan et al., 2002), previous study demonstrated the potential benefits *Ginkgo biloba* extract treatment of Alzheimer's disease, learning and memory deficits, cerebrovascular disease, cardiovascular diseases, climacteric vasomotor symptoms and postmenopausal syndrome (Han et al., 2004; Gong et al., 2006; Hrehorovska et al., 2004; Koltermann et al., 2004; Oh & Chung, 2004), Further the *Ginkgo biloba* extract possesses anti-tumor properties (Oh & Chung, 2006), may induced cancer cells apoptosis and differentiation and inhibit the progression of human colon cancer, hepatocellular carcinoma, pancreatic and gastric cancer (Feng et al., 2009; Chen et al., 2011; Chao, J.C. & Chu, 2004; Zhang et al., 2008; Xu et al., 2003). The present work was aimed to study the effect of GBE on CP-induced germ cell damage in male albino mice.

MATERIALS 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 libitum 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 was 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 m 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 of chromosomal aberrations in germ cells of mice:

Both control and treated groups of animals were sacrificed after 28days of exposure to test compounds with a view to cover spermatogenetic cycle of mouse. In the present study the air drying technique of Evans et al.

(1964) was employed with slight modifications to study the effect of test compounds on meiotic cells of mice

All the animals were sacrificed by cervical dislocation on 28th day. Animals were dissected out for testis and kept in 0.9% physiological saline. Tunica albugenia, the membrane covering the testes was removed carefully and the tubules were transferred to another Petri dish containing 5ml of 1.2% trisodium citrate. The tubules of the testes were teased in hypotonic solution. The cell suspension was collected in clean centrifuge tubes and incubated at 37°C for 45minutes. After incubation the tubes were centrifuged for 10 minutes at 1000 rpm. The supernatant was discarded and to the pellet 5ml of freshly prepared pre-chilled fixative (3:1 methanol and acetic acid) was added drop wise from the sides of the centrifuge tubes and immediately dispersed the cell suspension by aspirating several times with a Pasteur pipette. The tubes were left undisturbed for 10 minutes at room temperature. This step was repeated 4 to 5 times. In the final change the cells were suspended in 0.5ml of fresh fixative3 to 4 drops of cell suspension dropped from a height of 30-35cms on clean grease free pre chilled slides with a pipette. The slides were stained with 2% giemsa for 8-10 min and they were rinsed in double distilled water and allowed to dry for screening various types of chromosomal aberrations.

The slides were screened and a total of 500 well spread spermatocytes at the diakinesis of metaphase-1 of meiosis were observed per animal for the presence of various types of chromosomal aberrations like structural and numerical aberrations in control and treated groups.

RESULTS

The genotoxic effects of *Ginkgo biloba* extract and their modulatory effects against cyclophosphamide inducted genotoxicity in germ cells of mice are presented in tables 1-4 and illustrated graphically in graphs 1-3.

The mutagenic effects of various doses of the GBE were conducted to study the effects on the germ cells of mice and the results were tabulated in Tables-1&2 and depicted graphically in Graph-1. The frequencies of abnormal metaphases in the controls recorded were 3.66%, when compared to 200, 400, 600 mg/kg bw of GBE treated mice were 3.33%, 3.66% & 4.00% respectively (Table-1 and graph-1).

Changes in the chromosomal number were recorded as autosomal univalents in controls were 1.33% when compared to GBE treated mice were 1.66%, 1.66% and 1.66%. Sex chromosomal univalents in controls were 1.66% with that of 200, 400 & 600 mg/kg GBE treated mice were 1.33%, 1.33% and 1.66% respectively. Aneuploidy results in controls were 0.66% when compared to that of 200, 400 & 600 mg/kg GBE treated mice was 0.33%, 0.66% and 0.66% respectively. Polyploidy were not observed in control and the treated groups. Structural changes included on translocations were noted and observed to be 0.00% in control and 200, 400 and 600 mg/kg GBE treated groups (Table-2).

The modulatory effects of increasing the concentrations of GBE (200, 400 and 600 mg/kg) was primed to mice and the animals were administered with 16 mg/kg of CP to evaluate the antimutagenic effects of GBE and the results were tabulated in tables 3-4 illustrated in graph-2.

Among the groups the controls have shown only 3.33% of abnormal metaphases when compared to 18.00% in CP alone administered mice. There was a significant mitigation in the frequencies of abnormal metaphases in mice primed with GBE 200, 400 and 600 mg/kg bw as 13.33%, 11.66% and 11.00% respectively (table 3 and graph 2). The inhibitory effects of GBE against CP inducted chromosomal aberrations in germ cells of male mice were 31.81%, 43.181% and 47.72% in III, IV & V grouped animals, table-3 and graph-3.

Table 1: Frequency of Chromosomal Aberrations recorded in germ cells of mice with various doses of *Ginkgo biloba* extract

Treatment	Normal metaphases scores	Abnormal metaphases scores
Control	289 (96.33)	11 (3.66) ± 3.16
200 mg/kg GBE	290 (96.66)	10 (3.33) ± 3.80
400 mg/kg GBE	289 (96.33)	11 (3.66) ± 2.54
600 mg/kg GBE	288 (96.00)	12 (4.00) ± 3.53

The values in the parenthesis are percentages

The *p>0.05 level, hence the difference is considered to be statistically insignificant

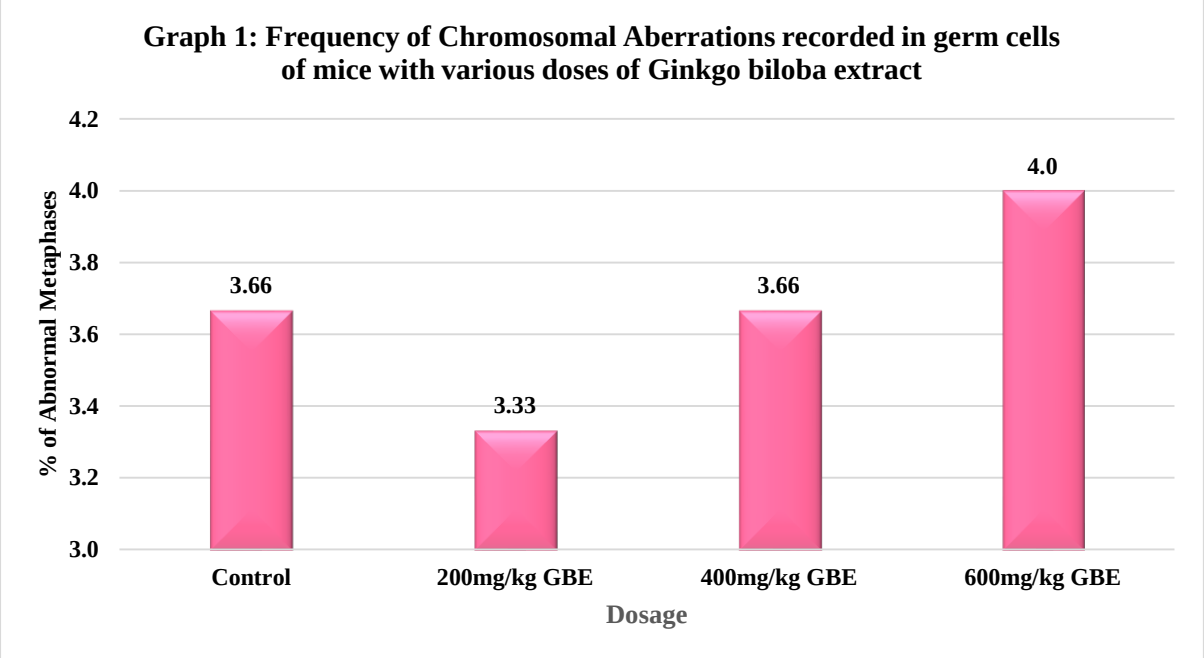


Table 2: Classification of Chromosomal aberrations in germ cells of mice treated with *Ginkgo biloba* extract

Group	Treatment dose (mg/kg)	Changes in chromosomal number				Structural changes
		Autosomal univalents	Sexchromosomal univalents	Aneuploids	Polyploids	Translocations
I	**Control**	4 (1.33)	5 (1.66)	2 (0.66)	0 (0.00)	0 (0.00)
II	**200 mg/kg GBE**	5 (1.66)	4 (1.33)	1 (0.33)	0 (0.00)	0 (0.00)
III	**400 mg/kg GBE**	5 (1.66)	4 (1.33)	2 (0.66)	0 (0.00)	0 (0.00)
IV	**600 mg/kg GBE**	5 (1.66)	5 (1.66)	2 (0.66)	0 (0.00)	0 (0.00)

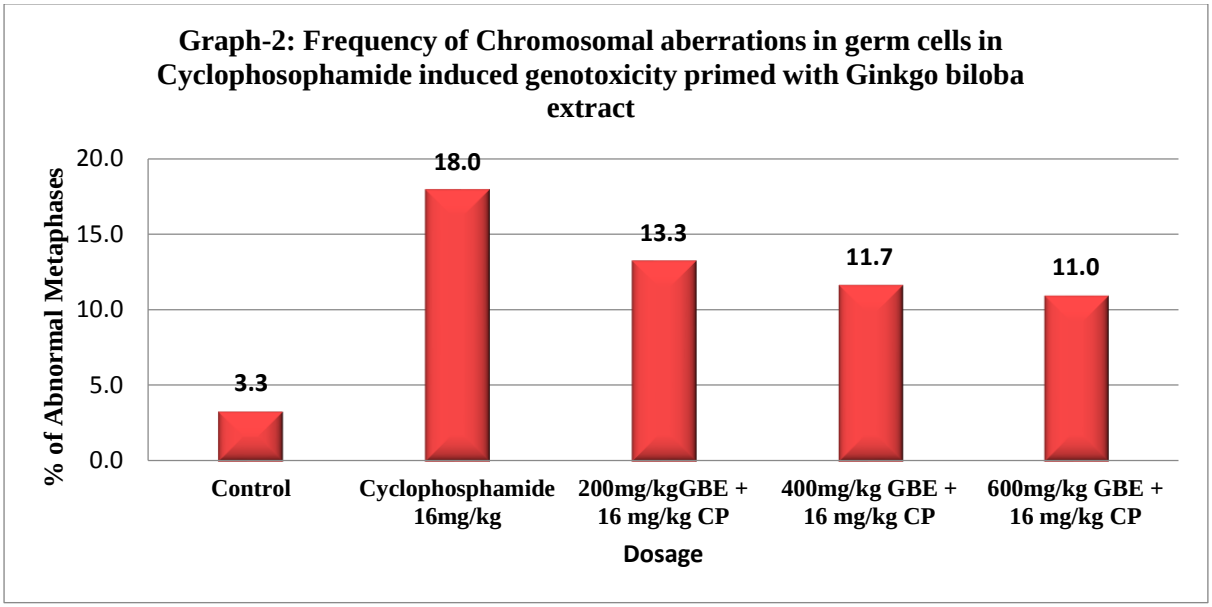
The values in the parenthesis are percentages

The *p>0.05 level, hence the difference is considered to be statistically insignifican

Classification of the chromosomal aberrations in germ cells was tabulated in Table-4. The Changes in chromosomal number were recorded as autosomal univalents in controls were 1.00%, in CP alone treated were 6.60% when compared to GBE co administered mice were 4.66%, 4.33% and 4.33 %. Sex chromosomal univalents in controls were 1.66%, in CP alone treated were 4.66% with that of 200, 400 & 600 mg/kg GBE co administered mice were 3.33%, 3.00% and 3.00% respectively. Aneuploidy results in controls were 0.33%, in CP alone treated were 2.00% when compared to that of 200, 400 & 600 mg/kg GBE co administered mice were 1.66%, 1.33% and 1.33% respectively. Polyploids observed in control were 0.33%, in CP alone treated were 3.33% compared to that of 200, 400 & 600 mg/kg bw GBE co administered were 3.00%, 2.66% and 2.00% respectively. Structural changes included on translocations were noted and observed to be 0.00% in control, 1.33% in CP alone treated and 0.66%. 0.33% and 0.33% in 200, 400 and 600 mg/kg GBE co administered groups (Table- 4

Table 3: Frequency of Chromosomal aberrations in germ cells of mice recorded in C cyclophosophamide induced genotoxicity primed with *Ginkgo biloba* extract

Group	Dose	Normal metaphases	Abnormal metaphases	Inhibition %
Group I	**Control**	290 (96.66)	10 (3.33) ± 2.54	
Group II	**16 mg/kg CP**	246 (82.00)	54 (18.00) ± 3.16	
Group III	**200 GBE + 16mg/kg CP**	260 (86.66)	40 (13.33) ± 3.53	31.81
Group IV	**400 GBE + 16mg/kg CP**	265 (88.33)	35 (11.66) ± 3.80	43.181
Group V	**600 GBE + 16mg/kg CP**	267(89.00)	33 (11.00) ± 2.	47.72



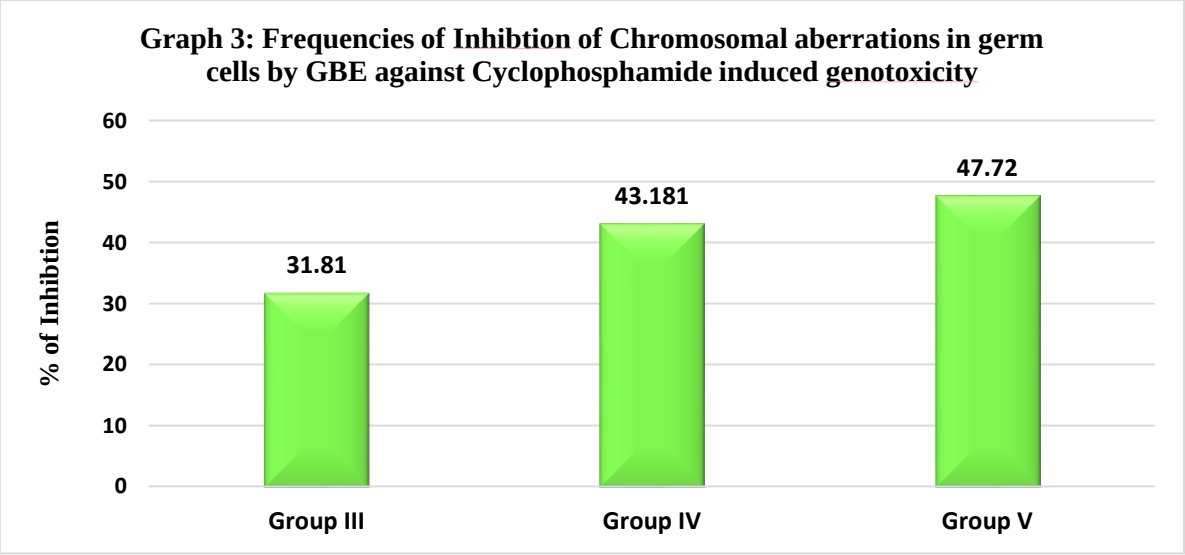


Table 4: Classification of chromosomal aberrations in germ cells of mice treated with Cyclophosphamide and primed with *Ginkgo biloba* extract

Group	Treatment dose (mg/kg)	Changes in chromosomal number				Structural changes	Abnormal Chromosomes Total
		Autosomal univalents	Allosomal univalents	Polyploids	Aneuploids	Translocations	
I	Control	3 (1.00)	5 (1.66)	1 (0.33)	1 (0.33)	0 (0.00)	10(3.33)
II	16 mg/kg CP	20 (6.60)	14 (4.66)	10 (3.3)	6 (2.00)	4 (1.33)	54(18.00)
III	200 GBE + 16mg/kg CP	14 (4.66)	10 (3.33)	9 (3.00)	5 (1.66)	2 (0.66)	40(13.33)
IV	400 GBE + 16mg/kg CP	13 (4.33)	9 (3.00)	8 (2.66)	4 (1.33)	1 (0.33)	35(11.66)
V	600 GBE + 16mg/kg CP	13 (4.33)	9 (3.00)	6(2.00)	4 (1.33)	1 (0.33)	33(11.00)

The values in the parenthesis are percentages

The *p<0.05 level, hence the difference is considered to be statistically significant.

DISCUSSION

The toxicity of Cyclophosphamide (CP) in germ cells has been reviewed and germ cells are more delicate to CP at post mitotic stages (Anderson et al., 1995) cyclophosphamide influenced dominant lethal in F1 generation heritable translocations, specific locus mutations in F1 embryos further generated sister chromatid exchanges in germ cells. Many studies showed positive outcome in rats and rabbits in testes) and in hamsters (Waters & Nolan, 1995). Schimenti et al. (1997) showed the CP treatment induced intra chromosomal gene conversion and mutations in meiotic stages, generation of dominant lethal mutations (Dobrzyńska et al., 1998). In another study Cusido et al. (1995) reveals the effect of CP in female rats, after 16 days during gestation period, increased nucleolar and synaptinimal complex fragmentation was noticed. Further Barton et al. (2003), reported harmful effects on spermatongesis after chronic exposure to CP. (Sharma & Agrawal, 2015; Ghaly et al., 2015; Singh et al., 2015).

Jytothirmaye and Lingupelly (2015) studied the anti claslogenic effects of *Ricinus communis* extract against CP induced classogenicity in mice. In another studythe Euphorbia triaculeata extract is not genotoxic and it hits the clastogenicity induced by Cyclophosphamide, Hanaa Mahmoud et al. (2018).

The present results are comparable with that Amar Amin et al. (2012) who described the protective nature of *Ginkgo biloba* extract against cisplatin induced reproductive toxicity. Even the studies on these lines are, however the beneficial effects of *Ginkgo biloba* extract has been investigated extensively as it is used in Chinese medicines 3000 years ago, it is one of the herbal drugs used to modify the vasomotor function, stimulates neurotransmitter (Wyrobek et al. 1983b).

The present outcomes are comparable with that of Sakr et al. (2012) who manifested CP induced many histological changes such as appearance of irregular seminiferous tubules, reduction in spermatogenic cells, declining of spermatogenic cells, degeneration of spermatogenic cells and leydig cells, but when Fenugreek seeds extract was given there is significant mitigation in reproductive toxicity. In another study Saberet al. (2017) announced that protective effect of Fennel oil on cyclophosphamide induced spermatogenesis. Similar results were observed in rat liver Akram et al. (2018) studied the chronic exposure of cyclophosphamide induced remarkable oxidative damage, but stimulated structure and function of testis when co-administration of American Ginseng has showed in recovery from gonadal dysfunction

Since many decades plants derivatives has been considered as important source of new discovery of novel pharmacologically active compounds. *Ginkgo biloba* has been preserved in china and Japan by prients and has been cultivated (McKenna et al., 2001). It has been known that it leaf extracts has been used for the treatment of peripheral vascular disorders, cerebral disorders Alzheimer diseases dementia (Brunetti et al., 2006; Maclennan et al., 2002; Han et al., 2004; Gong et al., 2006; Hrehorovska et al., 2004; Koltermann et al., 2004; Oh & Chung, 2004; Oh & Chung, 2006). 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 (Feng et al., 2009; Chen et al., 2011; Chao & Chu, 2004; Zhang et al., 2008; Xu et al., 2003).

The extracts have GBE has antioxidant and ehpatoprotective impact and reduces the liver fibrosis in rat of nonalcoholic steato hepatitis (Zhou et al., 2010). Further the extract protects liver damage induced by ccl4 in male rats (Chavez-Morales, 2011) Yoo et al. (2011) 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. (2004) found the beneficial effects on nervous system and prevented morphological deterioration and functional in cisplatin induced neuropathy. The present study showed that Standard *Ginkgo biloba* extract, EGb 761, contains 22-27% flavonoids (ginkgo flavone glycosides) and 5-7% terpenoids (ginkgolides and bilobalides) (Chavez-Morales, 2011). 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 (Yoo, 2011).

CONCLUSION

From the above studies, it is concluded that *Ginkgo biloba* was a potential candidate as protective agent to cyclophosphamide induced genotoxic effect in germ cells of mice. The combined treatment of cyclophosphamide and

GBE holds a promise as a safe and effective chemotherapeutic strategy.

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