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"MODULATING EFFECTS OF GINKO BILOBA IN CYCLOPHAMIDE INDUCED GENETIC DAMAGE IN SWISS ALBINO MICE"

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ABSTRACT

The present study the experiments were carried out to investigate the possible protective effect of *Ginkgo biloba* extract (GBE) on germ cells damage induced by the anticancer drug cyclophosphamide (CP) in male albino mice. The animals treated with various doses of *Ginkgo biloba* 200, 400 & 600 mg/kg individually and primed after cyclophosphmaide treatment. The animals were sacrificed on 35th day by cervical dislocation and slides were prepared according to the standard method. The prepared slides were screened for the presence of different type of sperms head abnormalities in control and treated mice. The results showed when animals treated with CP caused significant increase in the percentage of aberrant sperms in germ cells of mice. However after co administration of three doses of GBE extract there was a dose dependent decrease in % of aberrant sperms. Thus the results clearly indicate the protective role of GBE on Cyclophosphamide induced genotoxic damage in germ cells of mice. The results of this study indicated that GBE protected mice against CP induced germ cell damage. Such studies are useful chemo therapeutic strategy.

KEYWORDS: Cyclophosphamide, Ginkgo biloba, Aberrant Sperms Protection.

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. The tree has a long medicinal history being recorded as reported that repeated intake of GBE enhanced cell proliferation and neuroblasts differentiation in the mouse hippocampal dentate gyrus and consumption of GBE may be helpful to increase endogenous neurogenesis in adults. GBE administration to cisplatin-treated rats effectively alleviated all of the cisplatin induced reproductive toxicity that accompanied with increased germ-cell degeneration and increased germ-cell apoptosis.[8] The effect of GBE on CP-induced reproductive toxicity in male albino mice and morphological deterioration in cisplatin-induced peripheral neuropathy has been reported.[9] Yoo et al.[10] reported that repeated intake of GBE enhanced cell proliferation and neuroblasts differentiation in the mouse hippocampas dentate gyrus and consumption of GBE may be helpful to increase endogenous neurogenesis in adults. Not much work has been reported against drug induced toxicity hence the present investigation studies were carried out in germ cells of mice using the plant extract *Ginkgo biloba* against cyclophosphamide induced cytogenetic damage by sperms morphology assay.

MATERIALS AND METHODS

Animals

The animals were procured from National Institute of Nutrition, Hyderabad and maintained in the departmental animal house under an absolute hygienic condition as per the recommended procedures by fulfilling all the necessary ethical standards. Five animals were housed in separate cages with sterile bedding, (rice husk procured locally and autoclaved to free from micro-organisms) and kept in air-conditioned room, at 25°C *(± 2*°C) and RH 65 ± *5%* and a photo-cycle of 12:12 h light and dark periods, were fed with pelleted diet (purchased from National Institute of Nutrition, Hyderabad) and water *ad libitum.* Animal care and handling were performed according to guidelines issued by the World Health

Organization (Geneva, Switzerland) and the Indian National Science Academy (New Delhi, India). Precautions were taken to avoid infections by periodical changing of rice husk(paddy) beds, cleaning of cages with dettol, cleaning of water bottles since the pathological changes will affect the toxicity studies and thereby cause changes in the final results and their interpretation during experimental period.

Dosage schedule

The control animals were given 0.5 ml d.dil H_2O and the experimental animals were administered with plant extract of *Ginkgo biloba* (GBE) obtained in the form of EGb761 purchased from standard pharma company and prepared according to the required dosage based on literature.

First experiment

First group: Control I (non treated)

Second Group: GBE 200 mg/kg

Third Group: GBE 400 mg/kg

Fourth Group: GBE600 mg/kg.

Second experiment

First group: Control II (non treated)

Second Group: 16mg/kg CP+GBE 200 mg/kg

Third Group: 16mg/kg CP+GBE 400 mg/kg

Fourth Group: 16mg/kg CP+GBE600 mg/kg.

Sperm Morphology Assay

All the control and treated animals were sacrificed on 35th day. This is because spermatogenesis takes about 34.5 days to complete in mice. Sperms were sampled from the caudal epididymis after the animals had been sacrificed by cervical dislocation. Sperm suspension was prepared from the caudal of each testis by mincing the caudal in physiological saline. To the suspension 2- 3 drops of 1% aqueous eosin was added and kept for about 20 min undisturbed. Smears were made on clean slides and allowed to dry in air. 1000 sperm cells/mouse were assessed for morphological abnormalities according to the criteria of Wyrobek and Bruce.[11] The various types were observed as banana, Amorphous, hammer head shaped were observed in control and treated group of animals and data was analysed using Chi-Square test.

RESULTS

The results on the frequency of aberrant sperms are presented in table 1-4. Various abnormalities in sperm morphology were observed in the treated animals. The determinant abnormal characteristics of the spermatozoa head abnormalities included amorphous and head lack the unusual hook. The frequency was 3.20% in controls and after treating with three doses of GBE the frequencies were 3.40, 3.73 and 4.20(Table 1 P>0.05) the percentages of aberrant sperms were also showed no significant difference between control and GBE treated group. However, CP treated group (19.40%) showed significant increase in sperm head and tail abnormalities (P<0.05) over the control group(3.20%). Animals treated with CP and GBE showed significant reduction in sperm abnormalities when compared to CP the percentages were decreased from 19.40 in CP treated mice and in primed treated groups the percentage was decreased to 14.80, 13.66 and 11.98 in GBE treated group(P<0.05).

Table 1: Frequency of sperm head abnormalities in mice treated with *Ginkgo biloba* extract (GBE).

Treatment	Normal sperms %	Abnormal shape of sperms %
Control	2904 (96.80)	96 (3.20)
200 GBE	2868 (96.60)	102 (3.40)
400 GBE	2888 (96.27)	112 (3.73)
600 GBE	2874 (95.80)	126 (4.20)

The values in parentheses are percentage

*P>0.05.

Table 2: Classification of various types of sperm head abnormalities in *Ginkgo biloba* fruit extract treated animals.

Dose	Different shape of abnormities				Total
	Amorphous	Banana shaped	Hammer head	Pin shaped	
Control	40 (1.33)	20 (0.66)	21 (0.70)	16 (0.53)	96 (3.20)
200 GBE	42 (1.40)	21 (0.70)	21 (0.70)	18 (0.60)	102 (3.40)
400 GBE	44 (1.46)	24 (0.80)	23 (0.76)	21 (0.70)	112 (3.73)
600 GBE	49 (1.63)	28 (0.93)	27 (0.90)	22 (0.73)	126 (4.20)

The values in parenthesis are percentages

Table 3: Frequency of aberrant sperms in cyclophosphamide treated animals primed with *Ginkgo biloba* extract.

Grouping	Treatment & Dose	Normal sperms %	Abnormal shape of sperms %	% of inhibition
Group I	**Control**	2904 (96.80)	96 (3.20)	
Group II	**16 mg/kg CP**	2418 (80.60)	582 (19.40)*	
Group III	**200 GBE + 16mg/kg CP**	2556 (85.20)	444 (14.80)	28.39
Group IV	**400 GBE + 16mg/kg CP**	2590 (86.34)	410 (13.66)	35.26*
Group V	**600 GBE + 16mg/kg CP**	2643 (88.10)	357 (11.98)	46.30*

The values in parentheses are percentage *p<0.05.

Table 4: Classification of various types of sperm head abnormalities in cyclophosphamide treated animals primed with *Ginkgo biloba* extract.

Treatment	Various types of abnormities				% of abnormal cells
	Amorphous	Banana Shaped	Hammer head	Pin shaped	
Control	40 (1.33)	24 (0.8)	22 (0.73)	10 (0.33)	96 (3.20)
16 mg/kg Cp	194 (6.46)	163(5.43)	150 (5.00)	75 (2.50)	582 (19.40)
200 GBE + 16mg/kg CP	170 (5.70)	132(4.40)	102 (3.40)	40 (1.33)	444 (14.80)
400 GBE + 16mg/kg CP	156 (3.20)	128 (2.54)	96 (3.13)	38 (1.26)	410 (13.66)
600 GBE + 16mg/kg CP	156 (5.20)	96(3.20)	72 (2.40)	31 (1.33)	357 (11.98)

The values is parenthesis are percentage.

DISCUSSION

The mouse sperm morphology test is commonly used for measurement of spermatogenic damage induced by test agents. Studies have shown that induced changes in sperm morphology reflect the genetic damage in male germ cells. Sperm assays are commonly used to detect the causes of infertility. Here, mainly sperm counts, motility of sperms and sperm morphology are being used as test parameters.[12] There are several reports on chemically induced abnormal sperms.[13] reported the sperm abnormalities in mouse germ cells after short term exposure to pesticides acetamiprid, propineb and their mixture. From various studies it has been concluded that chemicals yielding positive response in mouse sperm morphology test should be regarded as suspected germ cell mutagens in mammals and agent's positive responses in these sperm tests should be considered with high priority against human applications.[14-15] Sperm abnormality assay is extensively being used for the evaluation of genotoxicity of chemicals and also for the study of antigenotoxic protective effects of natural compounds.[16-20]

Cyclophosphamide is widely used drug among alkylating agents in cancer chemotherapy. Acrolein and phosphoramide are active compounds of CP and further these compounds reduce the growth of cancerous cells by acting at DNA level.[21] There are many studies showing chemotherapeutic agents and CP cause gene mutations, CA and aneuploidy and rearrangements in somatic and germ cells of mice in vivo and in vitro test systems[22-23] and an elevated frequency of secondary treatment related tumors in human cancer survivors.[24] Earlier studies have shown that post meiotic germ cells are specifically sensitive to cyclophosphamide treatment The administration of low doses of cyclophosphamide to male rats for 6 week produced greater than 95% post implantation loss among their progeny.[24] This loss caused to male rat with cyclophosphamide was characterized by early pre implantation embryonic death.[25] Some abnormalities in progeny outcome caused by cyclophosphamide treatment persisted to a subsequent generation. Thus the effects of cyclophosphamide exposure were both specific and heritable. Further chronic low dose exposure to cyclophosphamide produced adverse effects on progeny by altering sperm nuclear components.[26] The results of present study showed that there is a significant increase in the frequency of chromosomal aberrations when compared with control values ($p<0.05$). The mutagenicity of CP is clearly related to the formation of ultimate cytotoxic metabolite phosphoramide mustard through intermediate agent is hydroxyl cyclophosphamide and deschloroethyl cyclophosphamide which is responsible DNA cross links and strand lesions.

Further when rats were treated with CP caused significant decreases in epididymal sperm concentration and motility and significant increases in MDA levelsy.[27] This plant extract has been identified as novel pharmacologically active early as 2800 BC in the Chinese literature. Besides, Ginkgo bilobaleaves extract (EGb) has been used in the treatment of cerebrovascular and peripheral vascular disorders. GBE administration to cisplatin-treated rats effectively alleviated all of the cisplatin induced reproductive toxicity that accompanied with increased germ-cell degeneration and increased germ-cell apoptosis.[28] Further, Ilbey et al.[29] observed that CP caused excessive production of oxygen-derived free radicals in testes. Agarwal et al.[30] denoted that reproductive cells and tissues remain stable when the balance between free radical production and scavenging anti-oxidants is maintained. Ceribasi et al.[31] suggested that the damage observed after CP treatment in the histological structure of testes of rats may be elucidated with the direct or indirect effect of CP, which later induces lipid peroxidation that is a chemical mechanism capable of disrupting the structure and function of testis. CP induced degeneration of spermatogenic cells, significant increase in sperm head abnormality

and decline in sperm count in mice. The authors attributed these changes to direct cytotoxic effects of the drug on the highly proliferating germ cellstail were observed after CP treatment.[32] Similarly, Elangovan et al.[33] recorded a reduction in sperm count and motility after CP treatment in mice and considered it as indication of drug toxicity. Many investigators agreed that damage of sperms might be attributed to the presence of large quantities of polyunsaturated fatty acids in the sperm plasma membrane and low concentrations of scavenging enzymes in sperm cytoplasm, which make spermatozoa particularly susceptible to the damage induced by excessive reactive oxygen species.[30]

Protected from cadmium chloride induced histological alterations.[33] Furthermore, GBE administration to cisplatintreated rats effectively alleviated all of the cisplatin induced reproductive toxicity that accompanied with increased germ-cell degeneration and increased germ-cell apoptosis. This confirms the essential antioxidant potential of GBE.[28] Elevation in sperm count and reduction in sperm abnormalities was observed in the current study after GBE treatment. Due to the essential antioxidant potential of EGb, it was able to elevate epididymal sperm count and motility induced in rats by different anticancer drugs.[34] antioxidant status, protects against naphthalene-induced oxidative organ injury. In this respect. Standard *Ginkgo biloba* extract, GBE 761, contains 22-27% flavonoids (ginkgo flavone glycosides) and 5-7% terpenoids (ginkgolides and bilobalides).[35] 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.[36] It was reported that due to the antioxidant potential of EGb, it was able to prevent oxidative stress- related diseases including cancers, cardiovascular diseases, degenerative diseases and central neural system disorders.[37] In addition, ginkgolides A, B, C and M have been shown to check the platelet activating factor thereby preventing the bronchoconstriction, hypotension, cutaneous vasodilatation and finally the release of inflammatory compounds. Extract of *Ginkgo biloba* has antioxidant and hepatoprotective effects and can inhibit liver fibrosis in rat of and morphological deterioration in cisplatin-induced peripheral neuropathy.[38]

In the present study the protective nature of GBE is because of presences of antioxidants activity such as ginkgolides A, B, C, M and flavonaids. Earlier in our lab, the protective of plant extracts such as *Phyllanthus emblica, Garlic extract, Aegelos marmelos, Solanum lycopersicum, Curcumin longa* against antneoplastic drugs induced cytogentic damage in germs cells of mice has been reported.[39-42]

CONCLUSION

The results of the present study indicate that cyclophosmide is found to be mutagenic however when animals were primed with *Ginko biloba* extract the frequency of sperm head abnormalities showed a significant decrease in germ cells of mice. Such studies are necessary for use of GBE Extract along with chemotherapy treatment for medical.

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REFERENCES

1. Smorenburg CH, Sparreboom A, Bontenbal M and Verweij J. Eur J Cancer, 2001; 37: 2310-23.
2. Akram H, Ghaderi Pakdel F, Ahmadi A, Zare S. Cell J. Summer, 2012; 14(2): 116-21.
3. Deshpande SS, Kewatkar SM, Paithankar V V. Indian J Pharmacol, 2013; 45(2): 184-6.
4. Fleming RE., Pharmacotherapy, 1997; 17: 1465–1545.
5. Perini P, Calabrese M, Rinaldi L, Gallo P. Expert Opin Drug Saf, 2007; 6: 183–190.
6. Uber WE, Self SE, Van Bakel AB, Pereira NL. Am J Transplant, 2007; 7: 2064–2074.
7. Sweetman, S., Martindale C. The Complete Drug Reference. 36th Ed., Pharmaceutical Press, London; 2007; 702-705.
8. Raskin, I.et al. Plants and human health in the twenty-first century. Trends Biotechnol, 2002; 20(12): 522-531.
9. Kar, A. Pharmacognosy and Pharmacobiotechnology. 2nd Ed., New Age International Limited Publishers, New Delhi, 2007; 234-235.
10. Yoo, D. Y. et al. Effects of *Ginkgo biloba* extract on promotion of neurogenesis in the hippocampal dentate gyrus in C57BL/6 mice. J Vet Med Sci., 2011; 73(1): 71-76.
11. Wyrobe K and Bruce.
12. Sinha N., R. Narayan, R. Shanker, D.K. Vet. Hum. Toxicol., 1995; 37(6): 547–549.
13. Rasgele P.G. Arh. Hig. Rada Toksikol., 2014; 65(1): 47–56.
14. A.J. Wyrobek, L.A. Gordon, J.G. Burkhart, M.W. Francis, J.R.W. Kapp, G. Letz, H.V. Malling, J.C. Topham, M.D. Whorton Mutat. Res., 1983; 15(1): 73–148.
15. Wyrobek A.J., L.A. Gordon, J.G. Burkhart, M.W. Francis, R.W. Kapp Jr., Letz, H.V. Malling, J.C. Topham, M.D. Whorton Mutat. Res., 1983; 115: 1–72.

16. Mohammadi F., H. Nikzad, M. Taghizadeh, A. Taherian, A. Azami-Tameh, S. M. Hosseini, A. Moravveji. Andrologia, August 2014; 46(6): 680–686.
17. Zarei L1, Sadrkhanlou R1, Shahrooz R1, Malekinejad H1, Eilkhanizadeh B2, Ahmadi A1.Vet Res Forum., 2014 Winter; 5(1): 21-7.
18. Sushma Ch and K. Rudrama Devi*. Int. J. Pure App. Biosci., 2015; 3(5): 178-183.
19. K. Rudrama devi, s srivani, P.P Reddy and Minny Jael. P. Indian journal of applied research. 2015 Volume: 5 | Issue: 10 | October | ISSN - 2249-555X.
20. Yalçin E1, Oruç E, Cavuşoğlu K, Yapar K. J Med Food., 2010 Aug; 13(4): 917-25.
21. Wyrobek AJ, Bruce WR. Proc Natl Acad Sci, 1975; 72: 4425-29.
22. Wyrobek AJ. In: JA Heddle (Ed). New York: Academic Press Inc., 1982; 337-49.
23. Haubitz, M., Tx Med., 2007; 19: 26-31.
24. Anuradha, S. and Rudrama Devi, K., The Bioscan., 2010; 4(4): 641-644.
25. Lakshmi Sowjanya, B., Rudrama Devi, K. and Madhavi, D., Journal of Environmental biology, 2011; 30(5): 663-666.
26. Kruawanand, K. and Kangsadalampai, K., Thai J. Pharm. Sci., 2006; 30: 28-35.
27. Turk, G. et al., Reprod Fertil Dev., 2010; 22(4): 587-596.
28. Amin, A. et al., J Biomed Biotechnol, 2012; doi: 10.115 5/362049.
29. Ilbey, Y.O.et al., Fertil Steril, 2009; 92(3): 1124-1132.
30. Agarwal, A. et al., Am J Reprod Immunol, 2008; 59(1): 2-11.
31. Ceribasi, A.O. et al., Basic Clin Pharmacol Toxicol, 2010; 107(3): 730-736.
32. Khan, S., Jena, G. B. Toxicol Int, 2013; 20(1): 68-76.
33. Elangovan, N. et al., Toxicology, 2006; 222(1-2): 60-70.
34. Yeh, Y. C. et al., Br J Pharmacol, 2009; 156(1): 48-61.
35. Sharafzadeh, S., Int Res J Appl Basic, 2011; Sci, 2(9): 334-338.
36. De Feudis, F. V. et al, Fundam Clin Pharmacol, 2003; 17(4): 405-417.
37. Liu, S. Q. et al., Am J Chin Med, 2006; 34(1): 99-114.
38. Ozturk, G. et al., Toxicol Appl Pharmacol, 2004; 196(1): 169-175.
39. Rudrama Devi and Keshav Rao. Inter. J. pure and applied bio sciences, 2016; 4(5): 90-97.
40. K. Rudrama devi*, Sri Vani, Minny Jael .P Innovative Journal of Medical and Health Science, 2014; 4: 2 March – April, 67 – 70.
41. K. Rudrama Devi, Ch. Prabhakar Reddy and J.Karuna Kumari. World Journal of Pharmaceutical Research. Volume 3, Issue 6, 1724-1729. SJIF Impact Factor 5.045.
42. Karuna Kumara and Rudrama Devi W. J. Pharma. Research, 7[9]: 1839-1850.