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Original Research Article

Protective effect of carwin capsules against cyclophosphamide induced chromosomal aberrations in mice

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ABSTRACT

The concept of polyherbalism has been highlighted in *Sharangdhar Samhita*, an Ayurvedic literature dating back to 1300 AD. Polyherbal formulations enhance the therapeutic action and reduce the concentrations of single herbs, thereby reducing adverse events. In the present study, the clastogenic effect of carwin capsules has been evaluated against cyclophosphamide (CP)-induced chromosomal aberrations in the bone marrow cells of the mice. Genotoxicity was carried out in mouse bone marrow cells. Animals were divided into four groups each containing four animals. Group I (control) was treated orally with vehicle (acacia suspension-1ml/100gm), Group II was treated i.p. with cyclophosphamide (50 mg/kg, bw.), Group III was treated orally with carwin alone (1.5 mg/animal, bsa.), and Group IV was treated with cyclophosphamide + carwin. Animals were pretreated for 7 days with test drug (carwin). The evaluating parameter was to count total number of aberrated chromosomes and its various types. The results demonstrate that carwin was found to be significant ($P<0.01$) as compared to cyclophosphamide treated as the no. of total aberrated chromosomes in carwin treated was decreased effectively. And also carwin was able to significantly ($P<0.01$) protect the action caused by cyclophosphamide as well was also found to be effective antigenotoxic ($P<0.01$) as compared to cyclophosphamide, when total no. of aberrated cells and their types were scored. Therefore, the results suggest a genotoxic potential of carwin capsules.

1. Introduction

Medicinal plants have always been on the vanguard whether regarding the treatment of a number of ailments or even cancer. Over decades plants have been prized for their medicinal properties and used pragmatically as drugs, initially as traditional preparations and then as pure active principles, with this knowledge and practice being passed from generation to generation.[1] It has been suggested that the use of antimutagens/anticarcinogens in everyday life can be the most effective way to avert human cancer and genetic diseases.[2] The bioactive compounds in medicinal plants act as a blueprint to block or reverse carcinogenesis at early stages.[3] Moreover, they are considered to be an inexpensive, effective and easily applicable approach to control cancer.[4] Herbal medicines remain an important component of the health care system. Medicinal plants are the food supplements which have not only nutritional value but therapeutic value as well. The medicinal value of plants is due to the presence of secondary metabolites which includes alkaloids, saponins, terpenoids, flavonoids, tannins, sterols and phenolic compounds. Hence the importance of any plant lies in its biologically active principles. Almost four decades ago, the antimutagens were reported. Many reports have shown the rising trends of antimutagenic studies with the plant extracts.[5–7] Medicinal plants and

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their extracts have been used by man from prehistoric times to cure various diseases and this has resulted in the discovery of some very important drugs. It is now been well established that the traditional herbal therapies contain a diverse array of chemopreventive agents as well.[8] Herb-herb combinations also known as polyherbal therapy have been used in Chinese medicine practice for thousands of years, yet scientific evidence of their therapeutic benefits is lacking.[9] Drug combination often produces a promising effect in treatment of diseases over a single drug. The concept of drug combination has been well established in Western medicine and remarkable success has been achieved over the decades. In recent years, drug combination therapies in cancer and infectious diseases have offered new hope to patients.[10] Cyclophosphamide (CP) is a cytotoxic bifunctional alkylating agents belonging to nitrogen mustards class. It is widely used in the treatment of various malignant and non-malignant tumors. It is also used in organ transplant rejection and autoimmune diseases due to its immunosuppressant activity.[11–13] CP has been classified as known human carcinogen by the International Agency for Research on Cancer (IARC).[14] Acrolein, a metabolite of CP, is responsible for its carcinogenic activity. Acrolein causes damage to normal cell DNA and toxicities to various target organs by inducing oxidative stress.[15] Effective cancer chemotherapy as well as immunosuppressive therapy with CP is severely limited due to its unwanted toxicity to normal tissue. Thus, it is necessary to defend normal cell DNA from cyclophosphamide induced damage for improving clinical efficacy of cyclophosphamide. In the present study, we have made an attempt to evaluate the beneficial effects of carwin capsules against CP-induced mutagenesis in the bone marrow cells.

2. Materials and Methods

2.1. Material

Drug sample (Carwin capules) was gifted from Unjha Pharmacy plant- Tonix Health Care, Ahmedabad along with its literature for research work. Standard drug (Cyclophosphamide) was purchased from Sigma Chemical Co, St Louis, MO, USA. Colchine and Giemsa stain was purchased from SD Fine-Chem. Ltd. (Mumbai, India). Vehicle (Acacia suspension), Potassium chloride solution and Conroy's fixative (methanol & acetic acid) was prepared in lab. And saline solution was purchased from local market. All other chemicals used were of the analytical grade.

2.2. Preparation of solutions

2.2.1. Drug sample solution

2% acacia suspension was prepared by suspending 2 gram of accurately weighed acacia powder in 100 ml of 0.9% saline. 10 ml of vehicle was taken separately to which 250 mg of powdered drug (capsule content) was added and sonicated to produce a suspension of 25 mg/ml strength. The dose was calculated based on body surface area of animals.

2.3. Chemical solutions

2.3.1. Mitotic inhibitor

Colchicine (4 mg/kg bw): 0.04% cochicine solution was prepared by dissolving 40 mg colchicine in 100 ml distilled water.

2.3.2. Fixative

Cornoy's fixative: Methanol (3 ml): Acetic acid (1 ml).

Potassium chloride solution: 0.56% KCl solution was prepared by dissolving 560 mg KCl in 100 ml saline solution.

2.4. Staining solution

Giemsa stain solution (5%): The solution was prepared by mixing 5 ml giemsa stain solution in 100 ml saline solution.

2.5. Animals

In the present investigation the healthy adult male Swiss albino mice (10-12 weeks, 20-25 gm) were group housed (n= 4) under a standard 12 h light/dark cycle and controlled conditions of temperature and humidity (25±2 °C, 55–65%). Mices received standard rodent chow and water *ad libitum.* Mices were acclimatized to laboratory conditions for 7 days before carrying out the experiments. All the experiments were carried in a noise-free room between 08.00 to 15.00 h. Separate group (n=4) of mices was used for each set of experiments. The animal studies were approved by the Institutional Animal Ethics Committee (IAEC), constituted for the purpose of control and supervision of experimental animals by Ministry of Environment and Forests, Government of India, New Delhi, India (Reg No. 1196/PO/Re/S/08/CPCSEA). Protocol Approval Reference No. TIP/IAEC/PN- 133.

2.6. Grouping and treatments of experimental animals

Four animals in each group were taken and total four groups were divided. Group I (vehicle treated, 1ml/100gm, oral), Group II (cyclophosphamide treated, 50mg/kg bw. i.p), Group III (carwin treated 1.5mg/animal bsa. oral), Group IV (cyclophosphamide + carwin treated).

2.7. In-vivo chromosomal aberration test

The experimental animals were pre-treated orally with the drug suspension (carwin capsule suspension) for seven consecutive days (drug alone in group III and in combination with standard drug in group IV). Control animals received same volume of vehicle (acacia

suspension) as that of experimental animals. The standard drug (cyclophosphamide, 50 mg/kg bw) was dissolved in saline and injected intraperitoneally, after 24 hrs of the drug treatment, then colchicine (4 mg/kg bw) was injected (i.p.). The animals were sacrificed by cervical dislocation 2 h after injecting the colchicine. The bone marrow cells from both femurs were flushed using syringe in the form of a fine suspension with the help of normal saline solution into a centrifuge tube. This cell suspension was centrifuged at 1000 rpm for 10 min, and the supernatant was discarded. Pellet was treated with pre-warmed (37°C) KCl solution on cyclomixer. Above suspension was left in a water-bath (37°C) for 20 min. Centrifuged and supernatant discarded. Pellet was treated with freshly prepared cornoy's fixative on cyclomixer. Centrifuged and supernatant discarded. Above step of treatment with cornoy's fixative was repeated three times to get debris free white pellet. Then add a small amount of cornoy's fixative to get a good cell suspension. Slides were prepared by Air Drop method, by dropping a small drop of viscous suspension onto the clean chilled slide and air dried. Dried slides were stained with Giemsa stain solution for 3 min., rinsed with distilled water and dried. All the slides were coded prior to microscopic analysis. Number of cells having aberration and the different types of aberrations were scored (total 100 cells were counted). All the datas were analyzed by statistical method for comparing the effects of treatments on genotoxicity.[16]

3. Result and Discussion

Genotoxicity is a property possessed by substances that makes them harmful to the genetic information contained in organisms and the substance that cause genotoxicity are called genotoxins. One of the common example of genotoxin is cyclophosphamide (anticancer drug). Anticancer drugs have the property to decrease the growth rate (cell division) of the cancer cells but along with this they also affect the healthy, normal cells which naturally have a rapid turnover of cells which results in various severe side effects. Till now since after a long time research there is no available anticancer drug without having side effects. There are several ways to reduce or prevent the action of genotoxins. Chemicals which interfere with DNA repair or with genotoxin metabolism can be used as effective antigenotoxin. The present investigation was directed to study the possible protective activity of orally administered carwin suspension against cyclophosphamide induced genotoxicity (in vivo) in mice and to compare its genotoxic potential in contrast to cyclophosphamide. Genotoxicity was carried out in mouse bone marrow cells. Animals were divided into four groups each containing four animals. Group I (control) was treated orally with vehicle (acacia suspension- 1ml/100gm), Group II was treated i.p. with cyclophosphamide (50 mg/kg, bw.), Group III was treated orally with carwin alone (1.5 mg/animal, bsa.), and Group IV was treated with cyclophosphamide + carwin. Animals were pretreated for 7 days with test drug (carwin). The evaluating parameter was to count total number of aberrated chromosomes and its various types. The Table 1 represents total chromosomal aberration count and its types. It is found that the total chromosomal aberration in normal group is 13.5, while cyclophosphamide group leads this to 64.75, which is controlled in the carwin treated group (24.75) and this is found to be more significant than carwin+cyclophosphamide group (35.5). The various types of chromosomal aberrations observe were Chromatid Brake, Chromatid Fragment, Chromatid Gap, Ring Formation and Centromeric Associatiodn. Among these chromatid brake was most common while centromeric association was found negligible in almost all groups. Overall the percentage protection by carwin was 61.78% against genotoxicity induced by cyclophosphamide (Table 2). The results demonstrate that carwin was found to be significant ($P<0.01$) as compared to cyclophosphamide treated as the no. of total aberrated chromosomes in carwin treated was decreased effectively. And also carwin was able to significantly ($P<0.01$) protect the action caused by cyclophosphamide as well was also found to be effective antigenotoxic ($P<0.01$) as compared to cyclophosphamide, when total no. of aberrated cells and their types were scored and Figures 1, 2, 3 and 4- 7.6 shows pictures of various types of chromosomal aberrations observed. Thus we reveal that the possible common mechanism of action of drug may be due to its antioxidant property, free radical scavenging property or even gene regulation could contribute to its direct and indirect antigenotoxic data. It is known that alkylating agent cyclophosphamide is one of a group of anticancer drugs that are administered as inactive prodrugs and that are activated in vivo via one or more metabolic steps. The initial step in the bioactivation of cyclophosphamide involves cytochrome P-450-mediated hydroxylation at C-4.

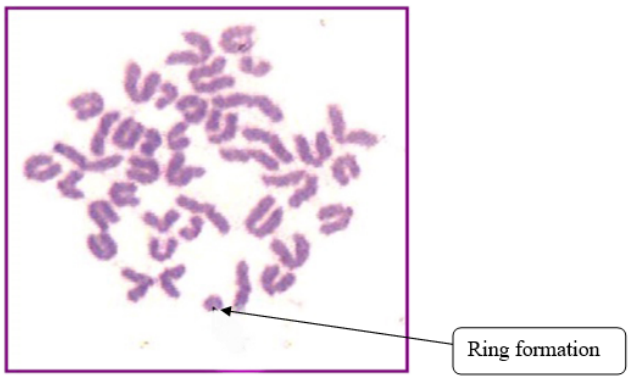

Fig. 1: Picture showing ring formation of chromosome.

Table 1: Effect of carwin capsule on chromosomal aberrations in mice bone marrow cells:

Group No.	Group	Total CA (Mean±SEM)	Various aberrations observed (mean)				
			C.B.	C.F.	C.G.	R.F.	C.A.
I	Vehicle treated (1 ml/100gm)	13.5±3.1	6	3	2	1	-
II	Cyclophosphamidetreated (50 mg/kg)	64.75±4.78 a**	39	14	6	3	1
III	Carwin treated (1.5 mg/animal)	24.75±3.78 a*, b**	12	7	2	2	-
IV	Cyclophosphamide + Carwin treated (50 mg/kg+1.5 mg/animal)	35.5±2.4 a**, b**	18	8	6	3	-

CA –Chromosomal Aberration, C.B. – Chromatid Brake, C.F. – Chromatid Fragment, C.G.– Chromatid Gap, R.F.– Ring Formation, C.A. – Centromeric Association. No. of animals in each group = 4. Data expressed as Mean ± S.E.M, One way ANOVA followed by Dunnett's t-test. a-comparison with group I (control) of all groups. b-comparison with group II (cyclophosphamide) of III & IV group. **P<0.01, * P<0.05

Table 2: Effect of carwin capsule against cyclophosphamide induced chromosomal aberrations and its percentage protection.

Group No.	Treatment	Total CA	Relative % of CA	% Protection
I	Vehicle treated (1 ml/100gm)	13.5±3.1	20.8	79.2
II	Cyclophosphamide treated (50 mg/kg)	64.75±4.78 a**	100	0
III	Carwin treated (1.5 mg/animal)	24.75±3.78 a*, b**	38.22	61.78
IV	Cyclophosphamide + Carwin treated (50 mg/kg+1.5 mg/animal)	35.5±2.4 a**, b**	54.8	45.2

CA – Chromosomal Aberration. No. of animals in each group = 4. Data expressed as Mean ± S.E.M, One way ANOVA followed by Dennett's t-test. a-comparison with group I (control) of all groups. b - comparison with Group II (cyclophosphamide) of III & IV group. **P<0.01, * P<0.05.

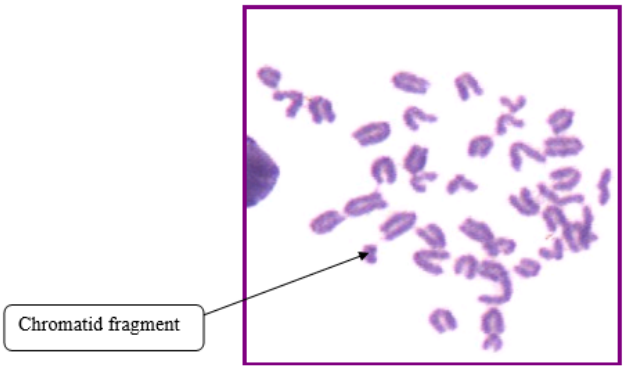

Fig. 2: Picture showing fragment of chromosome.

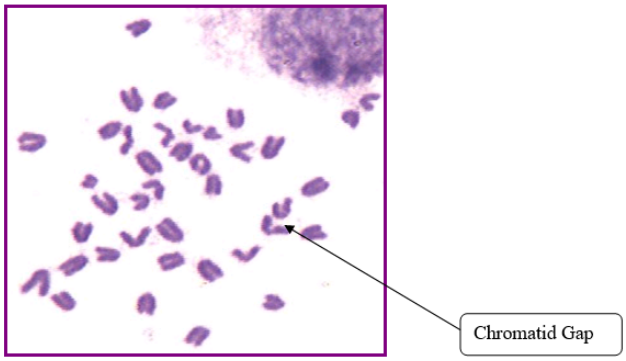

Fig. 3: Picture showing chromatid gap.

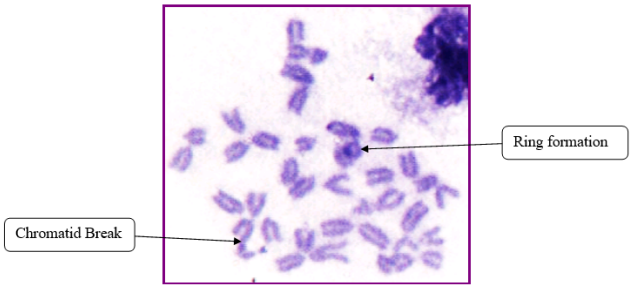

Fig. 4: Picture showing chromatid break and ring formation.

4. Conclusion

The observed results show that carwin is an antigenotoxic agent when compared to cyclophosphamide, and it also acts as a protective agent when taken in conjunction with cyclophosphamide, according to the present study, which offers evidence of this for the first time. We can therefore draw the conclusion that the herbal formulation utilised for this study is a good and useful treatment in cancer therapy as opposed to cyclophosphamide and other carcinogenic drugs, which have serious side effects.

5. Source of Funding

None.

6. Conflict of Interest

None.

References

1. Taylor JSL, Rabe T, Mcgaw LJ, Jager AK, Van Staden J. Towards the scientific validation of traditional medicinal plants. *Plant Growth Regul*. 2001;34:23–37.
2. Ferguson LR. Antimutagens as cancer chemopreventive agents in the diet. *Mutat Res*. 1994;307(1):395–410.
3. Lippman SM, Benner SE, Hong WK. Cancer chemoprevention. *J Clin Oncol*. 1994;12(4):851–73.
4. Wattemberg LW. Chemoprevention of cancer. *Cancer Res*. 1985;54(3):1–8.
5. Khader M, Bresgen N, Eckl PM. Antimutagenic effects of ethanolic extracts from selected Palestinian medicinal plants. *J Ethnopharmacol*. 2010;127:319–324.
6. Chen H, Chiang W, Chang J, Chien Y, Lee C, Liu K, et al. Antimutagenic constituents of adlay (Coixlachryma-jobi L. var. mayuenStapf) with potential cancer chemopreventive activity. *J Agric Food Chem*. 2011;59(12):6444–52.
7. El-Sayed WM, Hussin WA. Antimutagenic and antioxidant activity of novel 4- substituted phenyl-2, 2′-bichalcophenes and aza-analogs. *J Drug Des Dev Ther*. 2013;7:73–81.
8. Aruoma OI. Methodological considerations for characterizing potential antioxidant actions of bioactive components in plant foods. *Mutat Res*. 2003;523-24:9–20.
9. Che CT, Wang ZJ, Chow M, Lam C. Herb-herb combination for therapeutic enhancement and advancement: Theory, practice and future perspectives. *Molecules*. 2013;18(5):5125–66.
10. Risberg K, Andersson YF. Synergistic anticancer effects of the 9.2.27PE immunotoxin and ABT-737 in melanoma. *PLoS One*. 2011;6(9):1–9.
11. Perini P, Calabrese M, Rinaldi L, Gallo P. The safety profile of cyclophosphamide in multiple sclerosis therapy. *Expert Opin Drug Saf*. 2007;6(2):183–90.
12. Starz TE, Putnam CW, Halgrimson CG, Schroter GT, Martineau G, Launois B. Cyclophosphamide and whole organ transplantation in human beings. *Surg Gynecol Obstet*. 1971;133(6):981–91.
13. Uber WE, Self SE, Van Bakel AB, Pereira NL. Acute antibody-mediated rejection following heart transplantation. *Am J Transplant*. 2007;7(9):2064–74.
14. IARC Monograph on the evaluation of carcinogenicity: an update of IARC monographs 1 to 42.; 1987.
15. Korkmaz A, Topal T, Oter S. Pathophysiological aspects of cyclophosphamide and ifosfamide induced hemorrhagic cystitis; implication of reactive oxygen and nitrogen species aswell as PARP activation. *Cell Biol Toxicol*. 2007;23(5):303–12.
16. Preston RJ, Dean BJ, Galloway S, Holden H, Mcfee AF, Shelby M. Mammalian in vivo cytogenetic assays: analysis of chromosome aberrations in bone marrow cells. *Mutation Res*. 1987;189(2):157–65.

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