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Research Article

Chemoprotective Effect of Carwin Capsules against Cyclophosphamide Induced Genotoxicity by *In Vivo* Micronucleus Assay

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Abstract

The protective effects of carwin capsules (CAR) against toxicity induced by cyclophosphamide (CYC) in mice were investigated using the micronucleus assay for anticlastogenic activity in mouse bone marrow cells. 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). The standard drug (cyclophosphamide, 50 mg/kg bw) was dissolved in saline and injected intraperitoneally. The results demonstrate that carwin was found to be significant ($P<0.05$) as compared to cyclophosphamide treated as the no. of MNPCEs 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 very effective antigenotoxic ($P<0.01$) as compared to cyclophosphamide in scoring of PCE: NCE ratio. All the groups were compared with cyclophosphamide (-ve control). So, it was concluded that the used polyherbal formulation (carwin) was found to be very effective drug in cancer therapy as it has very less genotoxic effect as compared to cyclophosphamide and also has a protective effect with cyclophosphamide.

Keywords: Carwin capsules, Cyclophosphamide, Micronucleus assay, Antigenotoxic, Polyherbal formulation.

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1. INTRODUCTION

Genotoxicity assessments are performed to detect whether a compound can induce direct or indirect DNA damage that may result in sequence alterations, loss of heterozygosity and chromosome disorders [1, 2]. These events play an important role in many malignancies and induce heritable effects leading to congenital disorders[3, 4]. The evaluation of potential genotoxic effects is essential in the risk/benefit analysis of new drugs as well as for preparations already used in traditional medicine[5, 6]. International regulatory agencies have validated and recommend various genetic tests for genotoxic assessment, such as comet and micronucleus assays[7]. The micronucleus test is based on the detection of DNA breaks that result in chromosome fragments which are not included in the nucleus during mitosis, forming an additional nucleus. The comet assay is a technique used for detecting the presence of single strand breaks of DNA at alkali-sensitive sites or sites of incomplete excision repair in mammalian cells[8]. These tests have been used to analyze the toxicity[9] as well as anti-genotoxic, anti-mutagenic and anti-carcinogenic effects[10, 11] of extracts and isolated compounds from plants. The genotoxicity of anticancer drugs can result in secondary tumors in non tumor cells. For example, cyclophosphamide (CP) has been in clinical use for the treatment of malignant and nonmalignant disorders for over 40 years. However, despite its wide spectrum of clinical uses, CP also possesses wide spectrum cytotoxicity to normal cells[12, 13]. The cellular toxicity of CP, an alkylating drug, is primarily connected to the damage it causes to DNA[14]. Carwin is the time tested marvelous combination of precious antineoplastic and tissue promoting herbo-minerals. Carwin is very much useful in treating various types of benign, malignant and degenerative conditions[15]. In this study we investigated the ability of Carwin capsule to modulate the genetic damage induced by CP in the mouse erythropoietic system by determining the frequency of micronuclei in immature erythrocytes of bone marrow.

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. Hank's balanced salt solution (HBSS) was purchased from Hi Media Laboratories Pvt. Ltd. (Mumbai, India). Giemsa stain and May Gruen wald stain, bovine albumin and EDTA were purchased from SD Fine-Chem. Ltd. (Mumbai, India). Vehicle (Acacia suspension) 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.

2.2.2. Chemical solutions

Hank's Balanced Salt Solution (HBSS): 4.75 gm of powder product (Hank's salt) was suspended in 400 ml of 0.9% saline solution. 1% albumin solution and 0.15% EDTA solution was prepared separately in saline solution and added to the Hank's salt solution and final volume was made upto 500 ml. (Schmid W, 1975)

2.2.3. Staining Solutions

Giemsa stain solution: The solution was prepared in 1:6 ratios in phosphate buffer.

May Gruenwald stain solution*:* The solution was prepared in 1:1 ratio in phosphate buffer[16].

2.3. Animals

In the present investigation the 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.

2.3.1. Grouping and treatments of experimental animals

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

2.3.2. *In-vivo* micronucleus test

The micronucleus test is a mammalian *in vivo* test which detects damage to the chromosome or the mitotic apparatus induced by chemicals. Polychromatic erythrocytes in the bone marrow of rodents are used in the assay. When an erythroblast develops into an erythrocyte, the main nucleus is extruded and may leave micronuclei in the cytoplasm. Visualization of micronuclei is facilitated in these cells because they lack a nucleus. Micronuclei form under normal conditions. The assay is based on an increase in the frequency of micro nucleated polychromatic erythrocytes in the bone marrow of treated animals. Micronuclei are small particles consisting of acentric fragments of chromosomes or entire chromosomes which lag behind at anaphase stage of cell division. After telophase, these fragments may not be included in the nuclei of daughter cells and form single or multiple micronuclei in the cytoplasm. Animals are exposed to the test substance by an appropriate route. They are sacrificed, the bone marrow extracted and smear preparations are made and stained. Polychromatic erythrocytes are scored for micronuclei under the microscope [17].

2.3.2.1. Procedure

The preparation and staining of bone marrow cells were carried out according to Schmid (1975) [17]. Each group consisted of four mice. 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. The animals were sacrificed by cervical dislocation 24 h after injecting the standard drug. The bone marrow cells from both femurs were flushed using syringe in the form of a fine suspension with the help of HBSS into a centrifuge tube. This cell suspension was centrifuged at 1000 rpm for 5 min, and the supernatant was discarded, leaving a small drop of HBSS. Smears were prepared by putting a small drop of vicsous suspension on the end of a slide and spreaded by pulling the material on a glass slides and air dried. Dried slides were stained with May-Gruenwald stain solution for 2 min. and with Giemsa stain solution for 5 min., rinsed with distilled water and dried. All the slides were coded prior to microscopic analysis. At least 1000 polychromatic erythrocytes per animal were scored for the incidence of micronuclei. The ratio of polychromatic to normochromatic erythrocytes is determined for each animal by counting a total of 1000 erythrocytes. All the data were analyzed by statistical method for comparing the effects of treatments on genotoxicity.

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 examples 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 (-ve control) was treated i.p. with cyclophosphamide (50 mg/kg, bw.), Group II (control) was treated orally with vehicle (acacia suspension- 1ml/100gm), 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 parameters are frequencies of MNPCEs/1000 PCE and ratio of PCE: NCE. The results demonstrate that carwin was found to be significant ($P<0.05$) as compared to cyclophosphamide treated as the no. of MNPCEs 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 very effective antigenotoxic ($P<0.01$) as compared to cyclophosphamide in scoring of PCE: NCE ratio. All the groups were compared with cyclophosphamide (-ve control) (Table 1 & Figure 1). 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.

Table 1: Frequencies of micro nucleated polychromatic erythrocytes (MNPCEs) in mice bone marrow cells after 7-day oral administration of 1.5 mg/animal of carwin capsule suspension

Group No.	Drug	Dose	MNPCEs/1000 PCEs (Mean±SEM)	PCE:NCE Ratio (Mean±SEM)
I	Cyclophosphamide treated	50 mg/kg	6.2±0.93	4.28±0.33
II	Vehicle treated	1 ml/100gm	1.9±0.84**	1.16±0.17**
III	Carwin treated	1.5 mg/animal	2.6±0.53*	1.38±0.23**
IV	Cyclophosphamide + Carwin treated	1.5 mg/animal + 50 mg/kg	4.3±0.73	2.22±0.14**

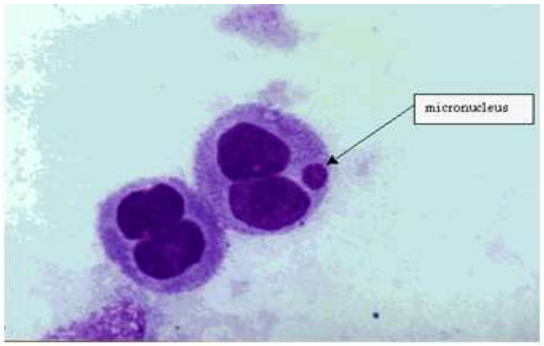
No. of animals in each group = 4, Data expressed as mean ± S.E.M, One way ANOVA followed by Dunnett's t-test Group I- negative control, Group II-control, Group III- test, Group IV test + cyclophosphamide. All test and standard groups were compared with group I (negative control) MNPCE - Micronucleated Polychromatic Erythrocytes, PCE - Polychromatic Erythrocytes, NCE - Normochromatic Erythrocytes ** P<0.01, * P<0.05

(A)

(B)

(C)

(D)



(E)

Figure 1: Dissection of animal (A), Extracted femur (B), Femur before flushing (C), Femur after flushing (D) Picture showing micronucleus in PCE cell (E)

4. CONCLUSION

The present study was the first *in vivo* evaluation of the genotoxicity of an carwin capsule. From our results, it was concluded that the used polyherbal formulation (carwin) was found to be very effective drug in cancer therapy as it has very less genotoxic effect as compared to cyclophosphamide and has also has a protective effect with cyclophosphamide.

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