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Evaluation of antimutagenic activity of methanolic extract of leaves of *Momordica charantia* Linn

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Abstract
Cancer is not a single disease, but rather a group of diseases characterized by abnormal cell growth. Present study was taken up to evaluate antimutagenic activity of hydroalcoholic extract of leaves of *Momordica charantia* (MC) by bone marrow micronucleus assay (MNT) and chromosomal aberration Assay (CAA) in mice. Cyclophosphamide (50 mg/kg,) was used as a genotoxic challenge and bone marrow of control and MC treated mice was collected after. In MNT, the bone marrow smears were stained with May-Grunwald's followed by Giemsa stain. Polychromatic and Normochromatic erythrocytes were counted and P/N ratio was calculated. In CAA, Colchicine (0.250gm /100 ml DDW), i.p, was administered 90 min before sacrifice, bone marrow smears were prepared, stained with Giemsa stain and observed under 100X for different types of chromosomal aberrations. Mitotic index was calculated. The MC has significantly decreased the formation of micronuclei, increased the P/N ratio, inhibited the formation of chromosomal aberrations and increased the mitotic index. The micronucleus study showed that. *Momordica charantia* has significant antimutagenic activity.

Keywords: Cyclophosphamide, micronucleus assay, momordica charantia, antimutagenic activity

1. Introduction
Cancer cells develop because of damage to DNA. This material is in every cell and directs all activities. Most of the time when DNA becomes injured the body is able to repair it. In cancer cells, the damaged DNA is not repaired. People can inherit damaged DNA, which accounts for inherited cancers. More often, though, a person's DNA becomes damaged by exposure to something in the environment, like smoking. Cancer usually forms as a tumor. Some cancers, like leukemia, do not form tumors. Instead, these cancer cells involve the blood and blood-forming organs and circulate through other tissues where they grow [1, 2]. Micronuclei (MN) are extra nuclear bodies that contain damaged chromosome fragments and/or whole chromosomes that were not incorporated into the nucleus after cell division. MN can be induced by defects in the cell repair machinery and accumulation of DNA damages and chromosomal aberrations. A variety of genotoxic agents may induce MN formation leading to cell death, genomic instability, or cancer development [3].
Cancer begins with damage (mutations) in your DNA. Your DNA is like a set of instructions for your cells, telling them how to grow and divide. Normal cells often develop mutations in their DNA, but they have the ability to repair most of these mutations. Or, if they can't make the repairs, the cells often die. However, certain mutations aren't repaired, causing the cells to grow and become cancerous. Mutations also cause cancer cells to live beyond a normal cell life span. This causes the cancerous cells to accumulate [1, 2].
M. charantia is also used in folklore medicine worldwide [4, 5]. *M. charantia* a member of the Cucurbitaceae family is known as bitter melon, bitter gourd, balsam pear, karela, and pares. The seeds, fruit, leaves, and root of the plant have been used in traditional medicine for microbial infections, intestinal gas, menstrual stimulation, wound healing, inflammation, hypertension, and as a laxative, antipyretic & emetic. Medical situation for which *M. charantia* extracts are currently being used include diabetes, dyslipidemia, microbial infections, & potentially as a cytotoxic agent for certain types of cancer [6, 7]. MAP30, a 30-kDa protein isolated from seeds of *M. charantia*, has shown promising effects for treating tumors and HIV infection [8, 9], While momordin, a protein found in *M.charantia*, has anticancer activity in animal experiment [10]. Triterpenoids isolated from the fruit of *M. charantia* on tumor cells has been demonstrated by numerous *in vitro* and *in vivo* studies [11, 12, 13]. And the present study was designed to investigate the mutagenic effects of *karela's leaves* extracts in comparison with the mutagenic effects of cycloplasmide.

2 Materials and Methods

2.1 Preparation of extract

The collected, cleaned and powdered leaves of *M. charantia* were used for the extraction purpose. 500g of powder of leaves of *M. charantia* was evenly packed in a soxhlet apparatus. It was extracted with 50% methanol. The solvent used were purified before use. The extracts were concentrated by vacuum distillation to reduce the volume 1/10.The concentrated extract was transferred to 100 ml beaker and the left over solvent was evaporated on the water bath, then collected and placed in a desiccators to remove excessive moisture. The dried extract was packed in air tight container and use for further studies such as phytochemical screening and pharmacological activity.

2.2 Procurement of experimental animals

Swiss albino Mice (18-30 g) were obtained from Dept. of Research, JN Cancer Hospital & Research Centre, Bhopal (M.P.). They were housed in good condition in the departmental animal house and given standard mouse pellet and water *ad Libitum.* All the mice were kept at controlled light & humidity condition (light: dark, 12:12 hr), and the temperature 22 ± 1º C. (According to CPCSEA norms). Organization for Economic co-operation and Development (OECD) regulates guideline for oral acute toxicity study.

2.3 Experimental design

The animals were divided into five groups consisting of six animals each. Group one served as normal control, group two was treated with clastogen, Cyclophosphamide 50 mg/kg, i.p. Group third was treated with *M. charantia* (250 mg/kg body weight in DMSO) for seven days. Groups four was treated with *M. charantia* (500 mg/kg body weight in DMSO) for seven days and group five was treated with *M. charantia* (750 mg/kg body weight in DMSO) for seven days followed by Cyclophosphamide as a challenge. The crude extracts were prepared in normal saline and were applied orally.

2.4 Bone marrow micronucleus assay

On seventh day, the animals were killed by cervical dislocation. Bone marrow cells were harvested. From freshly killed animal bone marrow were removed from muscle by use of gauze and fingers. Bone marrow cell was aspirated by flushing with HBSS solution with help of a syringe. The femur and tibia were excised. Bone marrow MN slides were prepared by using the modified method of Schmid. Marrow suspension from femur and tibia bones ready in 5% bovine serum albumin (BSA), was centrifuged at 1000 rpm and the pellet was resuspended in BSA solution. A drop of this suspension was placed on a clean glass slides and smears were prepared and the slides were air-dried. The slides were fixed in methanol, stained with May-Grunwald-Giemsa and MN was identified in two forms of RBCs (i.e. polychromatic erythrocytes as PCEs and norm chromatic erythrocytes as NCEs). About 2000 PCEs and corresponding NCEs per animals were scanned for the presence of MN [14, 15, 6].

2.5 Statistical analysis

All quantitative values are presented as mean ± standard deviation. Each experiment in triplicate was performed three to five times, and the figures show results from one representative experiment. Statistical differences were analyzed using the Student's t-test, and a value of $p < 0.05$ was considered significant.

3 Result & Discussion

The bone marrow MN assay indicated that *M. charantia* had inhibited the MN percentage induced by the clastogens. When *M. Charantia* (50 mg/kg) alone was tested, a non-significant increase in the frequency of micronucleated erythrocytes was observed in bone marrow MN tests. The micronucleus study showed that the single application of the *M. charantia* extract at the dose of 250,500,700mg/kg body wt. prior to the administration of cyclophosphamide have significantly prevented the micronucleus formation in dose dependent manner. The PCE/NCE ratio of *M. charantia* also not suppressed as compared to control group.

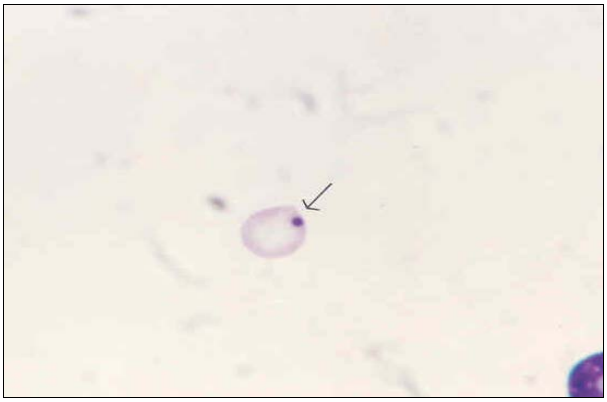

Fig 1: Photograph Showing Micronucleus Formation in PCE Cells

Table 1: Effect of *M. charantia* leaves extract on MN formation in mouse bone marrow cell

Group	MNPCE+SE	PCE/NCE Ratio
Cyclophasphomide (CP)	3. 26±0.7	2.78±0.46
M. charantia + CP (250mg/kg)	1.16±0.6*	1.06±0.02
M. charantia +CP (500mg/kg)	1.25±0.48*	1.09±0.06
M. charantia +CP (750mg/kg)	1.5±0.7*	1.1±0.07
Normal	0.6±0.5	1.06±0.03

Values are expressed as Mean ± SEM of 3 mice in each group
*P<0.001 comparison to CP group

Abbreviation: MN = Micronuclei, PCEs = Polychromatic erythrocytes, NCEs = Normochromatic erythrocytes, CP = Cyclophosphamide,

Numbers of micronuclei prevention are found increase with the increase in the concentration of *M. charantia* extract (250mg/kg- 1.16±0.6, 500mg/kg- 1.25±0.48, 750mg/kg- 1.5±0.7). On the other hand, there was a very much decrease of micronuclei in case of the group with *M. charantia* extract alone (0-75±0.4) as compared to control group (Cyclophosphamide only).

4 Conclusions

Cyclophosphamide is a alkylating anti-tumor agents. These agents after biochemical activation react with electron rich areas of susceptible molecules such as nucleic acid and proteins. The nuclear damage is re-sponsible for the mutagenicity while the effect on proteins will further aggravate the malfunctioning of the host cell.In the present investigation, *M. charantia* had produced anti mutagenic effect evident from the decreased micronuclei frequency observed in polychromatic and normochromatic serythrocytes. *M. Charantia* prevented the nuclear damage induced by cyclophosphamide, in bone blood micronucleus tests.

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