International Journal for Pharmaceutical Research Scholars (IJPRS)

V-5, I-1, 2016 ISSN No: 2277 - 7873

RESEARCH ARTICLE

Genotoxicity Study of *Vinca differmis* using Rat Bone Marrow

Mathew George*, Lincy Joseph, Balawant Singh, Naina Sara Sabu

Pushpagiri College of Pharmacy, Tiruvalla, Kerala, India.

Manuscript No: IJPRS/V5/I1/00029, Received On: 16/02/2016, Accepted On: 24/02/2016

ABSTRACT

The study aimed to investigate the phytochemical and therapeutic effects of aerial parts of Vinca differmis for genotoxicity activity in normal and cyclophosphamide treated group. Aerial parts of Vinca differmis was extracted with ethanol: acetone mixture. Presences of alkaloid, glycosides, steroids, phenolic compounds and volatile oils were identified in the extract. Genotoxicity studies showed that P>0.05 when compared to drug treated group with control and P<0.05 when compared to cyclophosphamide group and P<0.05 for drug with cyclophopshamide group in respect to control. Thus the results suggests that the aerial parts of Vinca differmis extract contains some active principles which may possess significant non-genotoxic activity.

KEYWORDS

Genotoxicity, cyclophosphamide, chromosomal aberration, *Vinca differmis*

INTRODUCTION

Chemicals that exert their adverse effect through interaction with the genetic material (DNA) of cells are called genotoxic. Most human carcinogens are genotoxic in nature. The science of genotoxicity mainly concerns that chemicals which induce mutations in various experimental models, may conceivably after the incidence of heritable mutations in man. Genotoxicity tests can be defined as in vitro or in vivo tests designed to detect drugs, which can induce genetic damage directly or indirectly by various mechanism of action. Genotoxicity tests enable hazard identification with respect to DNA damage and its fixation in the form of gene mutations, large-scale chromosomal damage, recombination and numerical chromosome changes. Drugs that are positive in these tests that detect such kind of damage have the potential to be human carcinogens and /or mutagens[1].

***Address for Correspondence:**
Mathew George,
Pushpagiri College of Pharmacy,
Tiruvalla, Kerala, India.
E-Mail Id: mathewlincg@yahoo.com

In Vitro Genotoxicity Assays[2]

In vitro Chromosomal Aberration Assays

Chemicals causing chromosomal aberrations may be identified with an in vitro cytogenetics assay. Cell cultures are treated with the test chemical and then mitosis is arrested in metaphase with an inhibitor, such as colchicines. The metaphase spreads are examined by light microscopy to detect chromosome or chromatid aberrations, or polyploidy cells. A biologically significant increase in the requency of cells with structural or numerical aberrations compared with that of the concurrent control group indicates the chemical is clastogenic or aneugenic.

In vitro Gene Mutation Assays

Mammalian forward mutation assays, such as thymidine kinase(*Tk*) assay or the hypoxanthine-guanine phosphoribosyl transferase(*Hprt*) assay, detect mutations at the heterozygous *Tk* or hemizygous *Hprt* gene. Cells having forward mutations at the TK or Hprt genes survive in the presence of the selective agent, while wild-type

cells accumulate a toxic metabolite and do not proliferate. Comparison of the mutant frequency of the treatment groups with the concurrent negative group allows the identification of a mutagenic chemical.

Rodent Erythrocyte Micronucleus Assay

Because of its relative simplicity and sensitivity to clastogens, the rodent erythrocyte micronucleus assay has now become the most commonly conducted in in vivo assay. It has achieved widespread acceptances and recommended test methods have been published as OECD Test Guideline 474. The micronucleus assay detects chromosome damage and whole chromosome loss in polychromatic erythrocytes and eventually in nonchromatic erythrocytes in peripheral blood as the red cells mature. Structural aberrations are believed to result from direct or indirect interaction of the test chemical with DNA, while numerical aberrations are often a result of interferences with the mitotic apparatus preventing normal nuclear division. Bone marrow is the major hematopoietic tissue in the rodent.

Mammalian Bone Marrow Chromosomal Aberration Assay

Mammalian bone marrow chromosomal aberration assay can detect clastogenic effects of a test agent. However, in the chromosomal aberration assay, these effects are observed directly by examination of metaphase chromosome spreads. The recommended methodology has been published in OECD Test Guideline 475. The assay is based on the ability of a test agent to induce chromosome structural or numerical alterations that can be visualized microscopically. The target tissue for the chromosomal aberration assay is the bone marrow because it is a rapidly dividing, well vascularized tissue.

Herbal drugs have been used since ancient times as medicines for the treatment of a range of diseases. Medicinal plants have played a key role in world health. In spite of the great advances observed in modern medicine in recent decades, plant still makes an important contribution to health care[2]. The genus Vinca (Apocyanaceae) is an evergreen shrubs or herbaceaous perennials, native to western Europe. Vinca differmis known commonly as intermediate periwinkle. There are at least 86 alkaloids extracted from plants in the *Vinca* genus. The chemotherapy agent vincris tine is extracted from *Vinca rosea* (current name *Catharanthus roseus*), and is used to treat some leukemias, lymphomas, and childhood cancers, as well as several other types of cancer and some non-cancerous conditions. Vinblastine is a chemical analogue of vincristine and is also used to treat various forms of cancer. Dimeric alkaloids such as vincristine and vinblastine are produced by the coupling of smaller indole alkaloids such as vindoline and catharanthine. In addition, the nootropic agent Vincamine is derived from Vinca minor[7].

To the best of our knowledge, there are no genotoxicity data available for this plant, and, therefore, we have investigated the mutagenic activity, anti-mutagenicity and chromosomal aberration of the extract of *Vinca differmis*, by chromosomal aberration test based on Organization for Economic Co-operation and Development (OECD) guidelines.

MATERIAL AND METHODS

Plant Material

Collection of Plant Material

The aerial part of *Vinca differmis* was collected from garden of Cagliari, Italy and powdered using grinder mill. The powdered drug packed in a paper bags and stored in air tight container until use.

Authentification

The botanical identity was confirmed by Universita degli studi di Cagliari, Dipartimento di scienze botaniche, Italy, the voucher specimen number is 925/B.

Preparation of Extracts

The powdered drug was extracted with ethanol : acetone 1:1 by maceration. After completion of extraction solvent was recovered and the saturated solvent was dried over water bath at 40-50°C. The semisolid paste formed is transferred

to petri plates and kept in hot air oven at 60°C for further drying and stored in air tight container and kept at 2-8°C for further use.

Phytochemical Screening[3]

The freshly prepared crude extracts of the aerial parts of *Vinca differmis* were qualitatively tested for the presences of alkaloids, Tannins and Phenolic compounds, Flavanoids, Steroids, Glycosides, Saponins, Proteins and Amino acids.

Experimental Animals

Male Wistar rats 150-200 gm of avg.wt. have been used. The animals maintained under standard environmental conditions had free access to standard diet and water ad libitum. Rats were housed in groups of six per cage. All the animals were maintained under standard conditions; that is room temperature 26±1°C, relative humidity 45-55% and 12:12 hrs light-dark cycle. The cages were maintained clean, and all experiments were conducted between 9 am and 4 pm.

Acute Toxicity Study[4]

Swiss Albino mice (25 - 30 gm weight) were used for acute oral toxicity study. The study was carried out as per the guidelines set by OECD 425 and animals were observed for mortality and behavioral changes.

Ethical Approval

The experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC).All the experiments were conducted according to the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Pharmacological Screening

Micronucleus Assay[5]

Procedure

Extraction of Bone Marrow: Test substances applied intraperitoneally and animals were sacrificed by cervical dislocation and 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 5% BSA solution with help of a syringe.

Preparation of the Smear: Tube was centrifuged at 1000rpm for 5min. the supernatant is removed the cells in the sediment are carefully mixed by aspiration and a small drop of the viscous suspension was put on the end of a slide and spread by pulling the material behind a polished cover glass held at an angle of 45°. The preparation was then dried and fixed for 2-5min.

Staining: Staining was carried out in ordinary vertical staining jars. Stain for 10 min in Giemsa then slides rinsed in distilled water, blotted, clean back side of slides with filter paper then dry on the slide warmer.

Analysis of Slides: PCEs were scored for micronuclei under the microscope at least 1000PCEs per animals were scored for the incidence of micronuclei. The ratio of PCEs to NCEs was determined for each animal by counting a total 2000 erythrocytes.

Experimental Design: Animals were divided into four groups. Total number of animals for each group is five rats. Group 1 receives vehicle alone, Group 2 receives standard drug cyclophosphamide 50mg/kg, Group 3 receives V.D.E 400mg/kg alone and Group 4 receives 400mg/kg V.D.E and Cyclophosphamide 50mg/kg.

Chromosomal Aberration Assay[6]

Procedure:

Administered the drug *Vinca differmis* 400mg/kg extract orally and after 24hr Cyclophosphamide 50mg/kg was administered i.p. Colchicine I.P (3-5mg/kg) was injected after 24hrs of Cyclophosphamide. Animals were sacrificed by cervical dislocation after 90 min of colchicines injection.

Animal dissected and femur bone was excised. Bone marrow was aspirated by flushing with normal saline in the centrifuge tube. Flush the suspension in the tube properly to get good cell suspension. Centrifuged for 15 min at 1000rpm. Supernatant discarded and pellet was treated with pre-warmed (37°C) 0.56% KCl on cyclomixer.

Left the suspension in a water bath (37°C) for 30 min. Centrifuge and supernatant discarded.

Pellet was treated with freshly prepared cornoy's fixative on cyclomixer. Centrifuged and supernatant discarded. Above step of treatment with cornpy's fixative was repeated three times to get debris free white pellet. To the pellet add cornoy's fixative to get a good cell suspension and made slide with air drop method. Stained and observed under microscope in 40x and then in 100x magnifications. Number of cells having aberration and the particular aberrations were scored (total 50 metaphase plates were counted) and data was analysed by t-test.

The animals were divided into four different groups each consists of five animals. Group1 received control with colchicines (3-5mg/kg) i.p, Group 2 received standard cyclophosphamide (50mg/kg) and colchicines (3-5mg/kg) i.p. group 3 receives V.D.E (400mg/kg) and colchicines (3-5mg/kg) i.p and Group 4 received (400mg/kg) V.D.E, cyclophosphamide (50mg/kg) and colchicines (3-5mg/kg) i.p, respectively.

Statistical Analysis

Statistical analysis was carried out using primer of Bio-statistical software. All results were expressed as mean ±standard error mean (SEM). Data were analyzed using one-way ANOVA followed by Bonferroni t-Test. In the entire tests the criterion for statistical significance was P‹ 0.05.

RESULTS

Phytochemical Analysis

The results of the chemical tests performed in the screening, revealed the presences of flavonoids, alkaloids, tannins, glycosides, saponins, terpenoid in the extract of aerial parts of *Vinca differmis*.

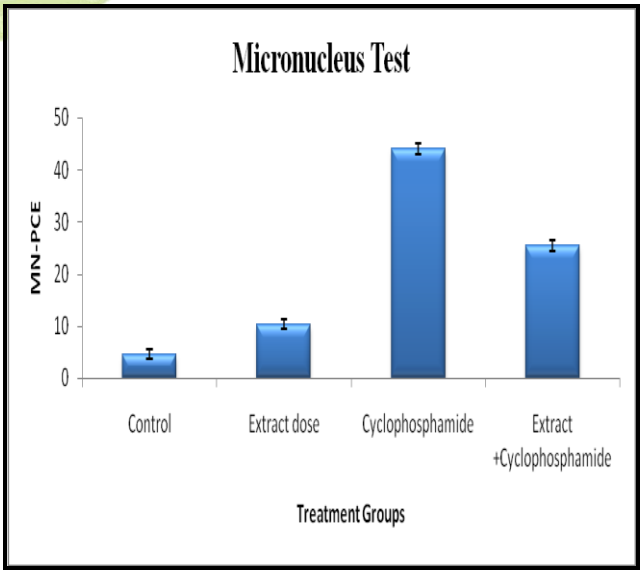
Table 1: Phytochemical Analysis of Vinca differmis extract

Sl.No.	Tests	Inferences
1.	Carbohydrates	+
2.	Alkaloids	+
3.	Glycosides	+
4.	Flavanoids	+
5.	Tannins and Phenolic compounds	+
6.	Steroid	+

Acute Oral Toxicity

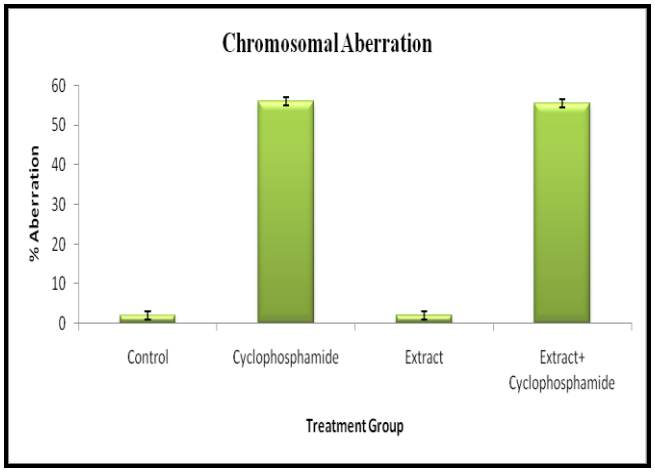
Acute oral toxicity studies revealed the non-toxic nature of the *Vinca differmis*. The extract of *Vinca differmis* did not show any sign and symptoms of toxicity and mortality up to 2000 mg/kg dose after fourteen days of study. This indicates that the extracts were found to be safe up to the dose levels studied. Since, all the animals survived at a dose of 2000 mg/kg body weight, the LD_{50} of the extract will be >2000 mg/kg body weight. No major behavioral changes were observed during the period of study. Therefore $1/10^{th}$ and $1/5^{th}$ of the maximum tolerated safe dose was selected for further pharmacological activity.

Micronucleus Test for Genotoxicity



Graph 1: Effect of Genotoxicity study of arial parts of *Vinca differmis* compared with treated groups

Chromosomal Aberration Method for Genotoxicity Study



Graph 2: Percentage Chromosomal for Genotoxicity Study

Chromosomal Aberrations Photographs

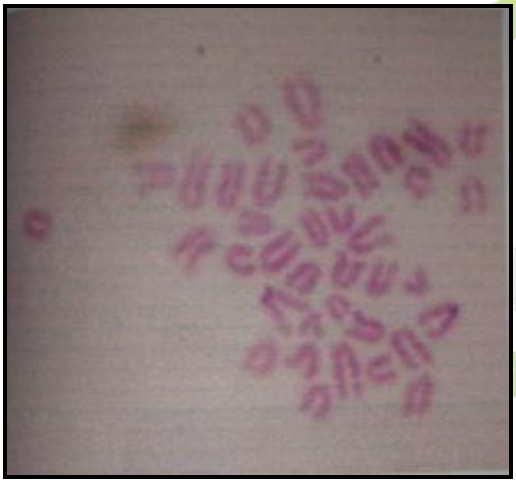


Figure 1: Control only treated group

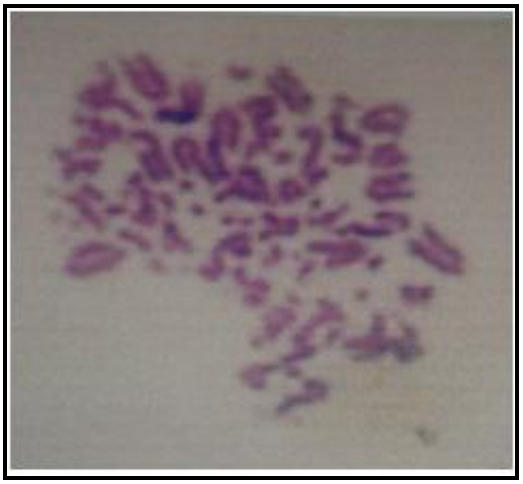


Figure 2: Cyclophosphamide treated Group

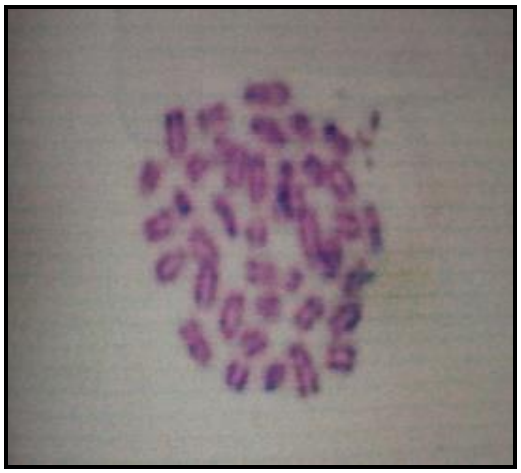


Figure 3: Drug (V.D.E 400mg/kg) treated Group

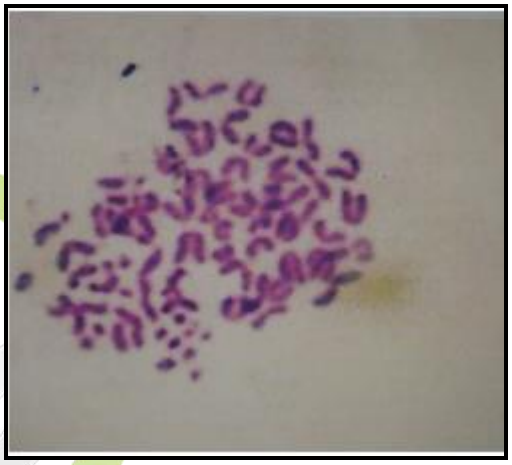


Figure 4: Drug+Cyclophosphamide Group

DISCUSSION

Decrease in the percentage of PCEs and increase percentage of MNPCEs and MNPCEs are the indicators of the chromosomal aberrations and these damages implicate as the major cause for the appearances of nuclei at the last stage of mitosis and as an indicator of genotoxic insult to the nuclei. It was shown that the alkylating agents exert their action through DNA cross-linking or by inducing oxidative stress. It was pointed out that the chemotherapeutic agents used in the treatment of neoplasia are either clastogenic, these agents mainly induces damage to the DNA in the form of primary DNA or secondary lesions that may lead to chromosomal aberration and point mutations. Micronucleus test was carried out by counting of MN-PCE cells per 2000 PCE cells. From the graph cyclophosphamide group showed positive control group and bone depression is major side effect of

cyclophosphamide and it also affect the normal physiology of erythrocyte.

The drug treated group had no significant difference in relation to control group thus the extract was not having any toxicity as genetic level. Significant was present between extract dose treated group and drug + cyclophosphamide group. Drug + Cyclophopshamide group had less count of MN-PCEs in compare to extract group that is drug itself does not cause sign of genotoxicity but drug did not protect from cyclophosphamide toxicity. The extract was nongenotoxic but the extract was not having any protective potential against cyclophosphamide induced genotoxicity. The extract was unable to protect chromosomes from different type of damages. The extract was also not effective against micronucleus formation by cyclophosphamide.

CONCLUSION

On molecular level *Vinca differmis* did not possess any sign of toxicity in respect to chromosomal aberration. The frequency of for only extract treated group and control treated group was significantly similar. It was observed that there was less frequency of fragment, less occurrence of ring formation and less presences of breakage. Percentage chromosomal aberration was not significantly high, thus drug did not cause genotoxicity, same results were observed for micronucleus assay. Thus *Vinca differmis* is a non-genotoxic plant.

REFERENCES

1. Mavournin, K. H., Blakey, D. H., Cimino, M. C., & Heddle, J. A. (1990). The in vivo micronucleus assay in mammalian bone marrow and peripheral blood. A report of the US Environmental Protection Agency Gene-Tox Program. *Mutation Research/Reviews in Genetic Toxicology*, *239*(1), 29-80.
2. OECD. (1980). *OECD Guidelines for the Testing of Chemicals*. Organization for Economic. http://www.oecd.org/dataoecd/39/12/34446120.pdf.
3. Khaldelwal, K. R. (2005). *Practical pharmacognosy*. Nirali Prakashan, Pune, 149-153.
4. Gosh, M. N. (1984). Toxicity Studies in Fundamentals of Experimental Pharmacology. Scientific Book Agency, Calcutta, 153-158.
5. Schmid, W. (1975). The micronucleus test. *Mutation Research/Environmental Muta genesis and Related Subjects*, *31*(1), 9-15.
6. Wongpa, S., Himakoun, L., Soontornchai, S., & Temcharoen, P. (2007). Antimutagenic effects of piperine on cyclophosphamide-induced chromosome aberrations in rat bone marrow cells. *Asian Pac J Cancer Prev*, *8*(4), 623-627.
7. Vinca differmis, (2006). https://en.wikipedia.org/wiki/Vinca_differmis.

