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Antigenotoxic effects of a polyherbal drug septilin against the genotoxicity of cyclophosphamide in mice

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ABSTRACT

Septilin (Spt) is a polyherbal drug formulation from Himalaya Drug Company, consisting of extracts from different medicinal plants and minerals. In the traditional system of medicine, septilin is being used as immunomodulatory, antioxidant and anti-inflammatory agent. In the present study, the protective effects of septilin against the genotoxicity of cyclophosphamide (CP) a widely used alkylating anticancer drug was evaluated by using *in vivo* micronucleus (MN) and sperm shape abnormality assays in Swiss albino mice. CP administered intraperitoneally at a dose of 50 mg/kg b.w. was used as positive mutagen. Different doses of septilin viz., 125, 250 and 500 mg/kg b.w. was orally administered for 5 consecutive days. CP was administered intraperitoneally on 5th day. MN and sperm preparations were made after 24 h and 35 days respectively. CP induced significant MN in both bone marrow and peripheral blood cells and also a high frequency of abnormal sperms. In septilin supplemented animals, no significant induction of MN and abnormal sperms was recorded. In septilin supplemented groups, a dose dependent significant decrease in CP induced clastogenicity was observed. Thus the current *in vivo* study revealed the antigenotoxic effects of septilin against CP induced damage, in both somatic and germ cells of Swiss albino mice.

1. Introduction

Ayurveda, the science of life, derived from spiritual visions were practiced by Indian Rishis since time immemorial. It still continues as an important system of medicine and drug therapy, in India. Throughout the history of civilization, plants have played major role in medication for the treatment of various kinds of human diseases. Plants and plant based medicine are the backbone of modern pharmaceutical preparations and they are the major contributors to the pharmaceutical industry both in India and other countries [102,14]. According to world health organisation more than 80% of the world population depends on the traditional medicines for their primary healthcare units. Plant secondary metabolites are the primary active ingredients of ayurvedic drugs. Plants harbour large number of active ingredients in valuable in controlling the diverse group of diseases. Several prescription drugs in the developed countries contain plant components and more than 100 important prescription drugs are derived from plants [49]. The medicinal values of plants are due to some specific chemical substances which produce definite physiological action on the human body [14]. A single plant may contain number of bioactive compounds which may act singly or synergistically to impart beneficial health effects. At global level, there is an increased demand for the pharmaceutical products of plant origin or other natural sources, because of the fact that the allopathic drugs have unwanted side effects which may be hindrance for their therapeutic use. Further, plant based therapy are marked due to its low cost, easy availability [58].

In recent years much attention is being given for the discovery of cell/genoprotective agents from the natural sources against the damaging effects of chemicals and radiations. Numerous studies have been carried out in the last four decades to identify the compounds that might protect the humans against the DNA damage and its consequences. In this line, more emphasis has been given to the medicinal plants and their isolated bioactive components. Septilin, is one of the ayurvedic herbo mineral [46] preparation of the Himalayan Drug Company containing extracts of six different plants and powders of *Blasmodendron mukul* and shankha bhasma. There are reports on the antibacterial, antiinflammatory,

Abbreviations: A, amorphous; B, banana shaped; BSA, bovine serum albumin; CMC, carboxymethyl cellulose; CP, cyclophosphamide; DH, double headed; DT, double tailed; F, folded; H, hookless; MN, micronucleus; MNNCE, micronucleus in normochromatic erythrocytes; MNPCE, micronucleus in polychromatic erythrocytes; NCE, normochromatic erythrocytes; PCE, polychromatic erythrocytes; Spt, septilin.

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immunomodulatory and immunopotentiating effects of septilin and it is extensively used for the treatment of several acute and chronic infections [62,45].

Among the currently available test systems to evaluate the genotoxicity/clastogenicity of the various test agents, micronucleus assay is the most widely applied methods due to its simplicity, reliability, sensitivity and proven suitability for genotoxicity evaluation [27,15].

Micronuclei (MN) are chromatin containing bodies, appearing as small satellite nucleus in the cell arising from acentric chromosomal fragments or from entire chromosome that is lagging at anaphase [56,89,81]. Presence of micronuclei in cells is an indicator of damage to the DNA. The *in vivo* micronucleus tests using haematopoietic bone marrow and peripheral blood cells are widely used methods for the assessment of genotoxicity of chemicals in exposed organisms. The rodent bone marrow micronucleus assay is the measure of both clastogenicity/aneugenicity of target chemical [59]. It has helped a lot to understand the dose response relationship for aneugens and clastogens [23]. Micronucleus is a biomarker, which is used widely in biomonitoring studies to determine the genetic risk due to the exposure to environmental chemicals [10]. Presence of micronuclei in the peripheral blood is found to be as an important biomarker, which shows the risk of cancer development [11,25]. Hernandez et al. [36] investigated the applicability of MN data to derive cancer potency information. They explored relationship between dose response data from genotoxicity tests and carcinogenicity studies and they observed a positive correlation between these two.

The number of mature (normochromatic) erythrocytes in the peripheral blood that contain micronuclei among a given number of mature erythrocytes can also be used as the end point of the assay when animals are continuously treated with the test agents [65]. Micronucleus assay is also being used as biological dosimeter of *in vivo* ionising radiation exposure [107]. There are many reports of clastogenecity and anticlastogenecity studies where bone marrow micronucleus assay was used as one of the parameters [57,95,67,73,74,63]. Several workers used peripheral blood micronucleus assay method to evaluate the clastogenic and anticlastogenic potency of different agents [21,50,64].

In animals, exposure of the males to toxic chemicals and radiation can result in wide variety and combination of reproductive dysfunction such as changes in the sexual behaviour, spermatogenic killing, diminished sperm quantity and quality, chromosomal defects in germ cells, reduced fertility *etc*. [108]. Epidemiological studies have shown cytotoxic and genotoxic effects of chemotherapeutic drugs and impaired fertility in males [7]. 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 [93]. There are several reports on chemically induced abnormal sperms. [75] 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 [110,111]. 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 [40,9,26,73,74,4,12,24,91]. In addition, sperm abnormality assay is also being used to study the endocrine mediated effects to assess the potency of endocrine disrupting chemicals on hormone homeostasis and also studies indicated the risk of lowered fertility due to decreased spermatogenesis in such exposed animals [114].

Cyclophosphamide (CP) is widely used anticancer and chemotherapeutic drug [90]. However, despite its wide spectrum of clinical benefits it can also induce cytotoxic effects on the normal cells in humans and experimental animals [44]. CP is an alkylating agent capable of inducing gene mutations, chromosomal aberrations, micronucleus (MN), sister chromatid exchanges, as well as other genotoxic effects [51,1]. Since CP is a well-known mutagen/genotoxin, in the present study it was used as a positive mutagen. There are several reports on the use of CP to evaluate the anticlastogenic/antigenotoxic effects of various natural compounds and other chemicals [87,61,28,92,32,33,18,70]. [42] reported the anticlastogenic effect of *Ricinus communis* extract against CP induced clastogenicity in mice bone marrow cells.

Since there are very few reports on the chemoprotective effects of septilin, the present study was undertaken to investigate its *in vivo* anticlastogenic effects against CP induced clastogenicity by using micronucleus assays in bone marrow and peripheral blood cells and sperm abnormality assay in germinal cells. Both test systems used in present study have the same sensitivity, specificity and accuracy and are being used as short term tests for the evaluation of carcinogenecity. Whereas, sperm morphology assay is useful in screening test for compounds that constitutes a potential genetic hazard for mammals.

2. Materials and methods

2.1. Chemicals

Septilin (The Himalayan drug company, Peenya Industrial Area, Bangalore, Batch No.-37300170B), containing the extracts of *Maharasanadi qoath* (130 mg), *Tinospora cordifolia* (98 mg), *Rubia cordifolia* (64 mg), *Emblica officinalis* (32 mg), *Moringa pterigosperma* (32 mg), *Glycyrriza glabra* (12 mg) and powders of *Balsamodendron mukul* (324 mg), *Shankha bhasma* (64 mg) was used.

Cyclophosphamide (CP-CAS No.-6055-19-2), Endoxan- N Baxter Oncology, Germany, Batch No.-JN1045 was used as the positive control. All other chemicals were obtained either from Merck, SRL and Hi-media, India.

2.2. Animals

Swiss albino mice belonging to *Mus musculus* species, bred and maintained in the institutional animal house were used for the experiment. They were housed in polypropylene shoe box type cages, bedded with rice husk and kept in air-conditioned room, at 23° C (±2° C) and RH 50 ± 5%, were fed with a pelleted diet (Amruth Feeds, India) and water *ad libitum*. 8–10 weeks old animals with average body weight of 25 ± 2 gms were used for the experiments. Five animals (3 females + 2 males) were used for each treatment and control group in micronucleus assays. In sperm abnormality assay, 8 week old male animals were used. All groups of animals were kept under an absolute hygienic condition as per the recommended procedures by fulfilling the necessary ethical standards. Care and experimental procedures were conducted as per the guidelines of CPCSEA, India. *In vivo* animal studies were conducted after obtaining the prior approval from Institutional Animal Ethics Committee (IAEC) of Mangalore University (MU/AZ/99/2013-14/IAEC dt: 2.04.2013).

2.3. Dose and treatment schedule

Scheme of an appropriate dosing schedule and regimen should be based on clinical use, exposure pattern, pharmacokinetics and practical consideration. If the substance is genotoxic highest dose