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Research Article

MITOCLASTIC EFFECTS OF ISODON NIGRESCENS (BENTH.) H. HARA EXTRACT USING CHROMOSOME ABERRATION ASSAY

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ABSTRACT

Isodon nigrescens is an enigmatic genus used in Chinese medicines for treatment of tumor, hepatitis, malaria, bacterial infection and inflammation with major bioactive constituent being diterpenoids. In spite of efficacy of this medicinal herb in treatment of various kinds of ailment, mutagenic or genotoxic hazards resulting from high dosage and long term use of the plant have not been well studied. Thus, cytotoxic and genotoxic screening of aqueous extracts of *I. nigrescens* was done using *Allium cepa* assay. Mitotic cell division inhibition and chromosome aberration induction have been widely used as indicators of cytotoxicity and genotoxicity. Studies showed decrease in mitotic index but abnormality percentage was on a high with increasing concentrations and time durations which revealed its toxic effect. Major aberrations observed were mitoclastic which included shift in microtubule organizing centre, misorientations, pole to pole arrangement, scattering and early movement of chromosomes. All values were statistically significant at $p<0.05\%$. Studies conducted revealed that aqueous extracts of *I. nigrescens* possessed genotoxicity especially mitoclastic and mild cytotoxic effect. The cytotoxic and genotoxic activity of *I. nigrescens* extract when specifically targeted can be used to destroy cancer cells. Moreover, it emphasized judicious use of the plant in folk medicines for proper healthcare.

INTRODUCTION

Human beings have always been the victim of diseases not only from infectious organisms but also from the drugs they use to control diseases. Synthetic drugs have affected the mankind with its dangerous side effects and are prompting to return to natural and traditional medicines. Despite the profound therapeutic advantages possessed by some of the medicinal plants, some constituents of it have been found to be potentially toxic, mutagenic, carcinogenic and teratogenic. However, the potential toxicity of herbs has not been recognized by the general public or by professional groups of traditional medicine (Soetan and Aiyelaagbe, 2009).

Plants of the genus *Isodon* (Schrad. ex Benth.) Spach belonging to Lamiaceae have been used in traditional Chinese folk medicine for the treatment of respiratory, gastrointestinal bacterial infections, and as anti-inflammatory and anti-tumor agents. They have become an abundant resource of naturally occurring bioactive *ent*-kauranoid (Fujita and Node, 1984; Sun *et al*., 2001; 2006). *Isodon nigrescens* (Benth.) H. Hara is a perennial unexplored herb mainly growing in marshy areas. Due to the traditional medicinal property of the genus, the plant was chosen for present study.

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Cytotoxic and genotoxic screening of crude aqueous extracts of *I. nigrescens* using *Allium cepa* assay is being attempted in the present study. Inhibition of mitotic cell division and induction of chromosome aberration have been widely used as indicators of cytotoxicity and genotoxicity. *A. cepa* assay which was established by the international program on chemical safety and the World Health Organization (WHO), is one of the most effective and sensitive methods for mutagen testing (Rank and Nielsen, 1994). Fiskesjo and Levan (1993) reported that *Allium* test has been found to have a high correlation with other test systems (MIT-217 cell test with mice, rats or humans *in vivo*) and could be used as an alternative to laboratory animal in toxicological research. Apart from this, the plant tests in some ways are more sensitive than both the microscreen assay and the Ames test. It can even detect some carcinogenic substances that are negative to the Ames test (Rank and Nielsen, 1994). Toxicity screening using crude extracts is being conducted since traditional medicines employs crude forms rather than refined products.

MATERIALS AND METHODS

Plant material and control

Isodon nigrescens (Benth.) H. Hara was collected from Munnar in Idukki district of Kerala (10°6'0"N; 77°4'0"E; 1602 m).

Table 1 Mitotic index and abnormality percentage of *Allium cepa* root meristems treated with *Isodon nigrescens* extract

Extract	Time duration (h)	Total cells ± SE	Dividing cells ± SE	Chromosomal aberrations ± SE	Mitotic index ± SE	Abnormality % ± SE
NC	½	699±4.16^{a}	553±3.84^{b}	0±0.00^{a}	79.11±0.47^{d}	0±0.00^{a}
	1	720±2.89^{a}	567±2.65^{b}	0±0.00^{a}	78.75±0.25^{d}	0±0.00^{a}
	2	682±6.96^{a}	536±4.41^{b}	0±0.00^{a}	78.59±0.52^{e}	0±0.00^{a}
	3	713±3.84^{a}	567±3.05^{b}	0±0.00^{a}	79.52±0.15^{e}	0±0.00^{a}
PC	½	844±13.09^{c}	259±2.96^{a}	362±5.92^{e}	30.69±0.73^{a}	42.89±0.24^{f}
	1	939±8.50^{c}	232±2.73^{a}	450±5.13^{d}	24.71±0.28^{a}	47.92±0.44^{e}
	2	815±13.24^{b}	182±3.71^{a}	416±6.69^{e}	22.33±0.28^{a}	51.04±0.06^{f}
	3	939±34.08^{b}	200±7.31^{a}	577±22.24^{c}	21.30±0.31^{a}	61.45±0.45^{f}
E1	½	1057±2.33^{e}	784±1.76^{e}	325±1.20^{d}	74.17±0.02^{b}	30.75±0.14^{e}
	1	1211±3.48^{d}	890±3.18^{d}	379±0.88^{c}	73.49±0.18^{b}	31.30±0.06^{d}
	2	1007±4.41^{c}	729±3.06^{d}	330±1.15^{d}	72.39±0.14^{b}	32.77±0.11^{e}
	3	1095±3.79^{b}	767±3.18^{c}	371±1.20^{b}	70.04±0.04^{b}	33.88±0.05^{e}
E2	½	815±1.45^{b,c}	625±0.88^{c}	242±0.88^{c}	76.69±0.14^{c}	29.69±0.17^{d}
	1	1283±2.03^{e}	966±2.08^{e}	392±0.33^{c}	75.29±0.19^{c}	30.55±0.17^{d}
	2	1327±2.40^{d}	986±1.20^{e}	418±1.86^{e}	74.30±0.12^{c}	31.50±0.25^{d}
	3	1020±3.61^{b}	751±2.96^{c}	330±1.53^{b}	73.63±0.10^{c}	32.35±0.10^{d}
E3	½	931±1.20^{d}	738±1.00^{d}	264±0.58^{c}	79.27±0.12^{d}	28.35±0.13^{c}
	1	761±3.18^{a,b}	596±2.60^{b}	226±1.20^{b}	78.32±0.05^{d}	29.70±0.14^{c}
	2	953±2.96^{c}	741±2.31^{d}	295±0.67^{c}	77.75±0.16^{d}	30.95±0.10^{c}
	3	1014±1.73^{b}	769±1.76^{c}	316±0.88^{b}	75.84±0.16^{d}	31.16±0.11^{c}
E4	½	761±2.03^{a,b}	613±1.76^{c}	204±1.53^{b}	80.55±0.09^{d}	26.81±0.40^{b}
	1	815±4.48^{b}	650±3.93^{c}	224±0.88^{b}	79.75±0.14^{e}	27.48±0.13^{b}
	2	758±2.60^{a,b}	593±1.86^{c}	217±0.88^{b}	78.23±0.24^{d,e}	28.63±0.09^{b}
	3	947±2.73^{b}	723±2.08^{c}	277±0.88^{b}	76.35±0.04^{d}	29.25±0.04^{b}

NC - Negative control (Distilled water); PC - Positive control (0.01% Methyl parathion); Extracts of *I. nigrescens*: E1- 0.1%, E2- 0.05%, E3- 0.01%, E4- 0.005%; SE - standard error. Means within a column followed by the same letters are not significantly different ($p < 0.05$) as determined by tukey's-b test

Fresh aqueous leaf extracts were prepared with the help of mortar and pestle. Lowest concentrations of the extract *viz.,* 0.005%, 0.01%, 0.05%, 0.1% (E1, E2, E3, E4) were prepared for toxicity analysis. For comparison, distilled water and 0.01% methyl parathion were taken as the negative (NC) and positive control (PC) respectively.

Allium cepa assay

Uniform sized bulbs of *Allium cepa* were sorted and planted in sterilized sandy soil without manure. Germinated bulbs with healthy roots (1-2 cm) were collected at peak mitotic period (9 am-10 am) and washed in distilled water. Bases of onion bulbs bearing roots were suspended in extract solutions. Root tips cut from the samples at different time intervals of ½ h, 1 h, 2 h and 3 h were washed in distilled water and immediately fixed in modified Carnoy's fluid for 1 hour. Mitotic squash preparation was done with the help of improved techniques (Sharma and Sharma, 1990). Hydrolysis was done with 1N HCl and washed thoroughly. Root tips were then stained with 2% acetocarmine. The parameters studied included mitotic index and percentage of abnormality.

All the slides were scanned, tabulated and photomicrographs were taken with Leica ICC 50 digital camera attached to LEICA DM 500 research microscope. Chromosomal aberrations were determined by scoring cells with abnormality in randomly selected six fields from slides prepared with treated onion bulbs for each concentration and time duration.

Statistical analysis

Data obtained on mitotic index and chromosomal aberrations were subjected to statistical analysis. One way ANOVA and tukey's-b test was performed to determine mean separation and significance of treatments using SPSS version 20, SPSS Inc., Chicago, USA. In all cases, $p < 0.05$ was statistically significant.

RESULTS

Cytotoxic and genotoxic effects of aqueous extracts of *Isodon nigrescens* on *Allium cepa* root meristematic cells is shown in Table 1. Studies showed that the mitotic index was decreasing with increasing concentrations and time durations. Mitotic index was found to be much higher than the positive control but less than the negative control. This shows that the aqueous extract of *I. nigrescens* is cytotoxic at increasing concentrations and time durations but not harmfully cytotoxic as in the case of positive control, methyl parathion. All values were statistically significant at $p < 0.05$.

Genotoxic effect of the aqueous extract was revealed through the chromosome aberrations induced. Abnormality percentage was found to increase with increasing concentrations and time durations but was found to be less than the positive control. The mitoclastic aberrations observed includes diagonal meta/anaphases, disturbed meta/anaphases, early movement of chromosomes, equatorial stathmo-anaphase, pole to pole arrangement of chromosomes, scattered meta/anaphases and shift in microtubule organizing centre. Apart from this, bridges, nuclear lesions, nuclear budding and stickiness could also be observed.

DISCUSSION

Herbal drugs have been widely used for thousands of years for the treatment of human diseases. It is well known that the use of plants as a therapeutic material is due to their chemical components of medicinal value (Prabhu *et al*., 2011). The genus *Isodon* is a prolific source of diterpenoids with diverse structures and biological properties. Thus the toxic effect of *Isodon nigrescens* was studied to verify the safety of the species in the form of crude drug. *Allium cepa* assay has been widely used for detection of cytostatic, cytotoxic and mutagenic properties of different compounds, including anticancer drugs of plant origin (Kuraś *et al*., 2006).

Mitotic index and abnormality percentage observed in treated root meristematic cells of *A. cepa* were found to be dose and time dependent. But a lethal effect of the extract was not observed. The major aberrations observed reveal the mitoclastic effects of the extracts of *I. nigrescens*. Mitoclastic agents are found to inhibit the spindle formation and orientation of chromosomes (Ray and Barman, 1987). It is observed that diagonal meta/anaphases, disturbed meta/anaphases, early movement of chromosomes, pole to pole arrangement of chromosomes, scattered meta/anaphases and shift in microtubule organizing centre have resulted from the abnormal orientation of spindle induced by the components in the leaf extracts while equatorial stathmo-anaphase resulted from inhibiton of spindle formation. Depolymerization of spindle fibres cause shifting of poles during both metaphase and anaphase (Mederios and Takahashi, 1987). Scattering of chromosomes occurs due to the interference of the leaf extracts with tubulin or polymerization of the microtubular subunits forming the spindle apparatus (Mathur and Chua, 2000). Thus it is found that *I. nigrescens* extract contain good mitoclastic agents which can be employed in disturbing the uncontrolled division of cancerous cells when specifically targeted.

Studies concerning the toxicity and mutagenicity of medicinal plants are needed to verify the efficacy and safety of their use in the treatment of some diseases (Macêdo *et al*., 2008; Ferreira *et al*., 2009). *Isodon* is a genus extensively used in traditional Chinese medicines for various ailments and the presence of diterpenoids with many biological properties makes it a future prospect in the field of drug research. The herb *I. nigrescens* being a member of this genus thus needs to be tested for its toxicity before being utilized. Investigation of traditional medicinal plants is valuable on two levels: firstly, as a source of potential chemotherapeutic drugs and secondly, as a measure of safety for the continued use of medicinal plants (Vershaeve *et al*., 2004). In the present investigation, *I. nigrescens* is found to be cytotoxic at higher concentrations which emphasize its judicious use in the form of drugs while the genotoxic and mitoclastic effect shown by the extract can be targeted to destroy cancer cells. Thus, it may be concluded that aqueous extracts of *I. nigrescens* are cytotoxic and genotoxic at higher concentrations and it needs to be carefully screened for harmful effects before using in traditional medicines. The mitoclastic property along with genotoxicity reveals a new path in the field of anticancer research.

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