2012 International Conference on Nutrition and Food Sciences
IPCBEE vol. 39 (2012) © (2012) IACSIT Press, Singapore

Genotoxicity of *Euphorbia Hirta* on *Allium Cepa* Assay

Kwan Yuet Ping[1], Ibrahim Darah[2], Umi Kalsom Yusuf[3], Sreenivasan Sasidharan[1+]

[1]Institute for Research in Molecular Medicine (INFORMM), Universiti Sains Malaysia, 11800, Pulau Pinang, Malaysia

[2]School of Biological Sciences, Universiti Sains Malaysia, 11800, Pulau Pinang, Malaysia

[3]Department of Biology, Faculty of Science, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

Abstract. The genotoxic effects of crude extract of *Euphorbia hirta* on was investigated using *Allium cepa* assay. Different concentrations of extract were tested on root meristems of *A. cepa*. Ethylmethanesulfonate was used as positive control and distilled water as negative control. The result showed that mitotic index decreased as the concentrations of crude extracts increased. The increase of the genotoxic effect corresponds to a decrease of mitotic activity. A dose-dependent increase of chromosome aberrations was observed. Abnormalities scored were stickiness, c-mitosis, bridges and vagrant chromosomes. Result of this study suggested that the methanol crude extracts of *E. hirta* exerted significant genotoxic and mitodepressive effects at 1000µg/ml.

Keywords: Genotoxicity; *Allium cepa*; Mitotic index; Chromosome aberrations

1. Introduction

The use of medicinal plants in remedial pursuits is gaining attention worldwide. Despite the profound therapeutic advantages possessed by the medicinal plants, some constituents of medicinal plants 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 [1]. Hence, evaluating the toxicological effects of any herbal extract intended to be used in humans is of utmost importance. *Euphorbia hirta* L., commonly known as asthma weed is widely used in traditional medicine to treat a variety of diseased conditions including asthma, coughs, diarrhea and dysentery [2]. The sedative, anxiolytic, analgesic, antipyretic and anti-inflammatory properties of *E. hirta* have been reported in previous study [3]. Recent studies have shown that long-term exposures to traditional medicinal herbs might be associated with increases in the rates of morbidity and mortality. In addition to systemic toxicity, the possible genotoxicity of herbal plants has been investigated in recent years. The aim of this study was to contribute to a better understanding of the genotoxic effect of crude extract of *E. hirta* using the in vitro mutagenicity bioassay on mitotic cells in *A. cepa* root tips.

2. Materials and Methods

2.1. Plant Extraction

Fresh *E. hirta* were collected and authenticated at the Herbarium of the School of Biological Sciences, Universiti Sains Malaysia, Pulau Pinang, Malaysia. The plant material was washed and dried in an oven at 50°C. Powdered dried plant material was extracted with methanol for 7 days. The whole extract was filtered and the solvent was evaporated to dryness using rotary evaporator at 40-50ºC to afford a paste mass.

2.2. *Allium Cepa* Assay

The *A. cepa* bulbs were grown in tap water for 2-3 days. When the roots were 2-4 cm in length, the bulbs were treated with different concentrations of the crude extracts (125, 250, 500, 1000µg/ml). Another set of plants was placed in ethylmethanesulfonate (125, 250, 500, 1000µg/ml) as positive controls while for the negative control, a set of *A. cepa* was growing in water. The solutions were changed daily and after 48 hours, root tips from each bulb were harvested, fixed in Carnoy's fixative (1:3 acetic acid: alcohol) for 24 hours. It was then proceed to slides preparation. After pre-treatment, the root tips were washed a few times with distilled water. They were hydrolyzed with 1N HCl at 60-70°C for 5 minutes. After hydrolysis, the roots were washed. Then, about 1-2 mm of the root tips were cut and placed on the slide. A small drop of aceto-orcein was dropped on the root tip and wait for 2 minutes. The root tip was then squashed with metal rod and another small drop of aceto-orcein was added and waited for another 2 minutes. The cover slip was carefully placed and the sides of the slides were sealed. The experiment was replicated 3 times. The microscopic observations and the photomicrographs were obtained using Olympus light microscope with digital camera. The mitotic index was determined by the examination and counting of cells in mitotic phases from among 100 cells per slide [9].The mitotic index was obtained as follows:

Mitotic index=Number of cells in mitosis/Total number of cells

3. Results

Table 1 shows the cytological effects of *E. hirta* extract on root tip cells of *A.cepa*. Exposure of *E. hirta* inhibited the mitotic index in a concentration-dependent manner when compared to the mitotic index of 0.509 in the control. The lowest MI value of 0.299 was recorded for 1000µg/ml treated with *E. hirta* extract. The mitotic index for *E. hirta* extract decreased significantly at 500µg/ml and 1000µg/ml. The mitotic indexes were 0.379 and 0.299 respectively as compared to mitotic index at 125µg/ml and 250µg/ml which were 0.403 and 0.406 respectively. This may indicate that *E. hirta* methanol crude extract exerted a genotoxic effect at 1000µg/ml. The mitotic indexes in treated cells were lower compared to the distilled water (negative control) which was 0.509. Ethylmethanesulfonate was used as positive control. As shown in Table 1, the mitotic index decreased at the same rate as the concentration increased from 125µg/ml to 1000µg/ml.

Chromosome aberrations were observed in all stages of mitosis. Table 1 showed the types and frequencies of chromosome aberrations induced by treatments. *E. hirta* extract showed concentration-dependent increase in the frequency of chromosome aberrations. At high concentration (1000µg/ml), sticky chromosomes and chromosome bridges were the most common chromosome aberrations observed. Other chromosomal abnormalities observed were and c-mitosis and vagrant chromosomes. *E. hirta* at 1000µg/ml showed half as much % aberrations as compared to positive control. For ethylmethanesulfonate, stickiness and c-mitosis were found to be the frequent aberrations observed.

4. Discussion

Findings of the present study reflected the utility of root tips of cells of *A. cepa* for monitoring the genotoxic effects of crude extracts. *A. cepa* assay enabled the assessment of different genetic endpoints, which are mitotic index and chromosome aberration. Mitotic index is used as an indicator of cell proliferation biomarkers which measures the proportion of cells in the M-phase of the cell cycle. Hence, the decrease in the mitotic index of *A. cepa* meristematic cells could be interpreted as cellular death. The cells of *A. cepa* root tips after treatment with extracts of *E. hirta* showed decreased mitotic index with increasing concentration. The mitotic activity of *E. hirta* methanol extract was significantly decreased at concentration of 1000µg/ml. Ethylmethanesulfonate was used as positive control in this study. A dose dependent decrease of mitotic index was observed in the ethylmethanesulfonate. Ethylmethanesulfonate is a genotoxic chemical, where positive results have been consistently reported in numerous in vitro mutagenicity and genotoxicity assays. The mitodepressive effect suggests that *E. hirta* extract had some effects on cell division of *A. cepa*. This may be due to abnormal conditions of the cells after induced by the treatments. The abnormalities of chromosomes could be due to the blockage of DNA synthesis or inhibition of spindle formation. They may not even allow the initiation of their biosynthesis [4]. The reduction of the mitotic

index might be explained as being due to the obstruction of the onset of prophase, the arrest of one or more mitotic phases, or the slowing of the rate of cell progression through mitosis [5].

Table 1: Cytological effects of *E. hirta* extract on cells of *A. cepa*

Treatments Concentration (µg/ml)	No. of cells	Mitotic Index	Chromosome aberrations			
			Stickiness	Bridges	C-mitosis	Vagrant
E. hirta						
125	1059	0.403 ± 0.042	3	2	3	4
250	1124	0.406 ± 0.063	4	5	2	4
500	1045	0.379 ± 0.040	2	3	2	14
1000	1070	0.299 ± 0.035	12	18	7	2
Ethylmethanesulfonate						
125	1225	0.410 ± 0.035	4	2	5	5
250	868	0.339 ± 0.035	8	3	12	3
500	1065	0.294 ± 0.049	20	5	2	6
1000	1105	0.184 ± 0.021	26	14	28	6
Distilled water	1183	0.509 ± 0.034	0	1	0	1

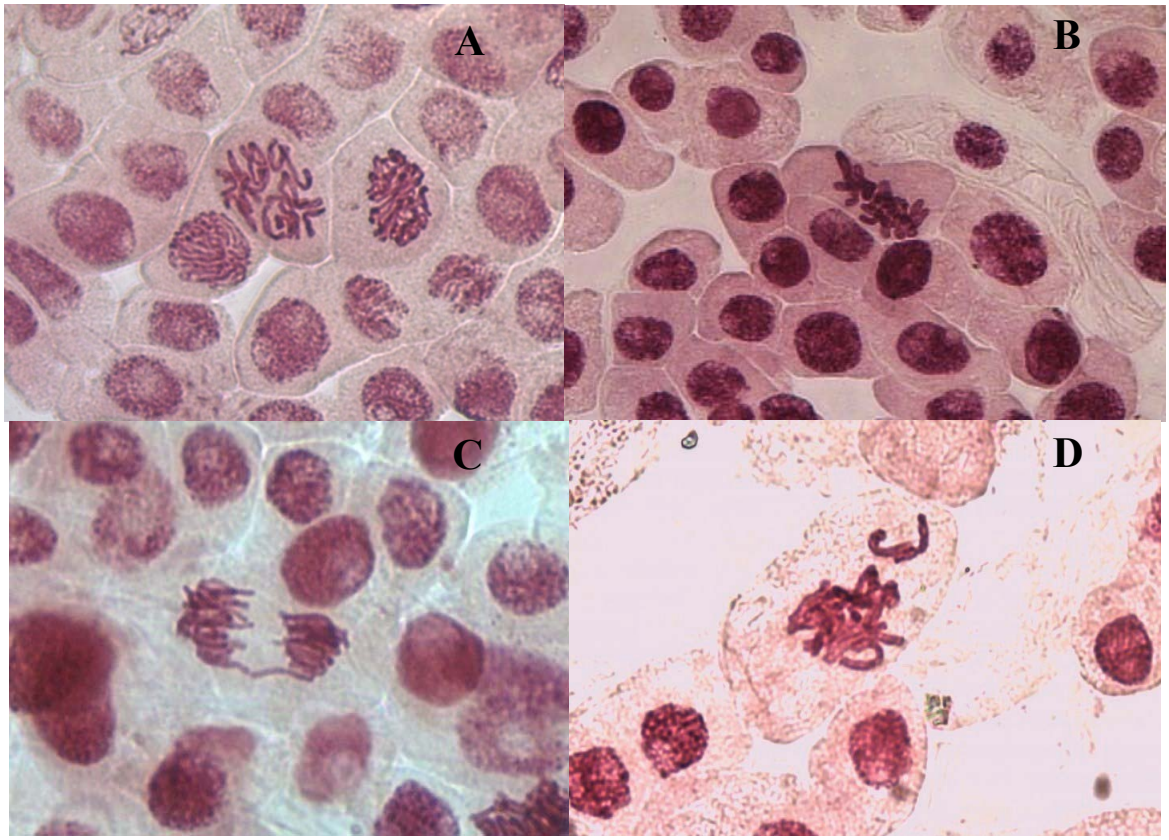

Fig. 1: Chromosome aberrations observed in cells of *A. cepa* treated with methanol extracts of *E. hirta*. (A) C-mitosis (B) Sticky chromosomes (C) Chromosome bridge (D) Vagrant chromosomes. Magnification: 400X

The chromosome aberrations observed at all concentrations of the treatment were chromosome stickiness, bridges, c-mitosis and vagrant chromosomes. These aberrations were due to the effect of the extract on the spindle formation and thus resulted in cell division disturbances. Chromosome bridges demonstrated signs of clastogenic effects caused by chromosome breaks, and vagrant chromosomes and c-metaphases, which increased the risk for aneuploidy [6]. Some of the physiological aberrations that were commonly observed in

this study were stickiness (Figure 1B). A remarkable correlation between the frequencies of stickiness in prophase and metaphase cells and the bridges in anaphase and telophase cells was observed. This supports the hypothesis that stickiness may result from improper folding of chromosome fibers which makes the chromatids connected by means of subchromatid bridges [7, 8]. According to Fiskesjo [9], sticky chromosomes indicated a highly toxic, irreversible effect, probably leading to cell death. Chromosome bridges were commonly observed during anaphase (Figure 1C). The bridges noticed in the cells were probably formed by breakage and fusion of chromatids or subchromatids [10]. A low frequency of c-mitosis (Figure 1A) and vagrant chromosomes was also observed. Their presence may be attributed to the failure of the spindle apparatus to organize and function in a normal way. Similar observations have been made by other workers where c-mitosis was regarded as indicative of a weak toxic effect which may be reversible [9]. Vagrant chromosomes that were not organized to a specific stage of the mitotic division were also observed (Figure 1D). This abnormality may be caused by unequal distribution of chromosomes with paired chromatids in which resulted from nondisjunction of chromatids in anaphase. Vagrant chromosomes were weak c-mitotic effect indicating risk of aneuploidy [9].

5. Conclusion

The results obtained from this study led to the conclusion that methanol extracts of *E. hirta* at 1000µg/ml exerted significant genotoxic and mitodepressive effects on *A. cepa* cells. Further studies to determine carcinogenicity potential of *E. hirta* are necessary to obtain a more comprehensive genotoxic assessment in animal test systems for human welfare.

6. Acknowledgements

Kwan Yuet Ping was supported by MyPhD fellowship from Ministry of Higher Education, Government of Malaysia, Malaysia.

7. References

[1] Soetan, K. O., and Aiyelaagbe, O. O. (2009). The need for bioactivity-safety evaluation and conservation of medicinal plants: A review. *J. Med. Plants Res.* **3**(5), 324-328.

[2] Ogbolie, J.N., Ogeke, C.C., Okoli, I.C. and Anyanwu, B.N. (2007). Antibacterial activities and toxological potentials of crude ethanolic extracts of *Euphorbia hirta. Afr. J. Biotechnol*. 6(13): 1544-1548.

[3] Lanhers, M.C., Fleurentin, J., Dorfman, P., Mortier, F. and Pelt, J.M. (1991). Analgesic, antipyretic and anti-inflammatory properties of Euphorbia hirta. *Planta Med*. 57: 225-231.

[4] Akinboro, A., and Bakare, A. A. (2007). Cytotoxic and genotoxic effects of aqueous extracts of five medicinal plants on *Allium cepa* Linn. *J. Ethnopharmacol*. **112,** 470– 475.

[5] Christopher, H. B., and Kapoor, M. B. (1988). The cytogenetic effects of sodium salicylate on the root meristem cells of *Allium sativa* L. *Cytologia.* **54,** 203-209.

[6] Leme, D. M., and Marin-Morales, M. A. (2009). *Allium cepa* test in environmental monitoring: A review on its application. *Mutation Res.* **682**(1), 71-81.

[7] McGill, M., Pathak, S., and Hsu, T. C. (1974). Effects of ethidium bromide on mitosis and chromosomes: A possible material basis for chromosome stickiness. *Chromosoma*. **47,** 157 -167.

[8] Klasterska, I., Natarajan, A. T., and Ramel, C. (1976). An interpretation of the origin of subchromatid aberrations and chromosome stickiness as a category of chromatid aberrations. Hereditas. 83, 153-169.

[9] Fiskesjo, G. (1985). The *Allium* test as a standard in environmental monitoring. *Hereditas.* **102,** 99-112.

[10] Shehab, A. S., and Adam, Z. M. (1983). Cytological effects of medicinal plants in Qatar III. Mitotic effect of water extract of *Anastatica hierochuntico* L. on *Allium cepa. Cytologia.* **48,** 343-348.