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Original Research Article

Evaluation of genotoxicity potential of plants traditionally used for mosquito control in Kenya's South coast

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Abstract

Plants are widely used for control of mosquitoes in Kenya but there is little documentation on effects of their long term use. Six plants used traditionally for mosquito control in Kenya's south coast were evaluated for their genotoxicity potential using the *Allium cepa* test. The plants were *Tagetus minuta* L., *Adansonia digitata* Linn., *Ocimum suave*, *Plectranthus barbatus* A., *Azadirachta indica* A. Juss., *Lantana camara* L. The plant extracts were assessed for mitotic index, early anaphases, chromosomal bridges/fragments, stickiness and c-mitosis. Leaf extracts of *Azadirachta indica* induced mitotic inhibition, produced binucleate and ghost cells in the *allium cepa* root meristems. Among the normal cells in the extracts of *P.berbatus*, there were cells which had a high cytoplasm: nucleus ratio including a bulging cytoplasm. The plants in this study may be used for topical application but *Azadirachta indica* should not be used systemically and continuously for long periods.

Key words: *Allium cepa* test, genotoxicity, Mitotic inhibition, Msambweni district

INTRODUCTION

Genotoxicity is the ability of a substance to interact with DNA and/or the cellular apparatus that regulates the fidelity of the genome (Maurici *et al*., 2005). Genotoxicity tests have been used for prediction of carcinogenicity and their outcomes are valuable for interpreting carcinogenicity studies (OECD, 1997a). Ames *et al*., 1975 developed bacterial reverse mutation test using *Salmonella typhimurium*. The test detects mutations which revert mutations present in the test strains and restore the functional capability of the bacteria to synthesize an essential amino acid. It is rapid, inexpensive and easy to perform. The limitation is that the test utilizes prokaryotic cells which differ from mammalian cells in such factors as uptake, metabolism, chromosome structure and DNA repair processes. It requires the use of an exogenous source of metabolic activation which cannot mimic entirely the mammalian *in vivo* conditions and therefore does not provide direct information on the genotoxicity potency of a substance in mammals (Maron and Ames, 1983; OECD, 1997b). Another test is *in vitro* mammalian cell micronucleus test (MNvit) is a genotoxicity test for the detection of micronuclei (MN) in the cytoplasm of interphase cells. It is a basis for investigating chromosome damaging potential *in vitro* because both aneugens and clastogens can be detected in cells that have undergone cell division during or after exposure to the test chemical. Micronuclei represent damage that has been transmitted to daughter cells and their formation prevents cell survival. The results obtained cannot mimic *in vivo* mammalian conditions (ESAC, 2006; OECD, 2014a). *In vitro* mammalian chromosomal aberration test (OECD TG 473) identifies substances that cause structural chromosomal aberrations in cultured human or other mammalian cells. The cell cultures are exposed to the test chemical both with and without an exogenous source of metabolic activation and then treated with a metaphase-arresting substance (e.g. Colcemid® or colc-

hicine), harvested, stained and metaphase cells are analyzed microscopically for the presence of chromatid-type and chromosome-type aberrations. With this test, there is a likelihood of artifactual positive results such as chromosome damage due to other factors other than direct interaction between the test chemicals and chromosomes (Honma, 2011; OECD, 2014b). Mammalian erythrocyte micronucleus test (OECD TG 474) is useful in assessing genotoxicity since it caters for factors such as *in vivo* metabolism, pharmacokinetics and DNA repair processes. The test is useful in identifying substances that cause cytogenetic damage which results in the formation of micronuclei containing lagging chromosome fragments or whole chromosomes in erythrocytes sampled in the bone marrow or peripheral blood cells of animals, mostly rodents (Parton *et al*., 1996; OECD, 2014 c).

The *Allium cepa* test uses the *Allium* root system. It has been in use since 1938 (Levan, 1938) for investigating environmental pollution factors, genotoxic and anticancer potential of medicinal plants (Majewska *et al*., 2003; Babatunde and Bakare, 2006 Camparoto *et al*., 2003; Akaneme and Amaefule, 2012; Vicentini, *et al*., 2001). It lasts 4 days, is easy, of low cost and provides good chromosomal conditions for study of chromosome and cell damage which can be extrapolated on animal cells. (Fiskesjö, 1985). Rank and Nielsen (1994) showed a correlation of 82% between the *A. cepa* test and the carcinogenicity test in rodents and concluded that it is more sensitive than the Ames test.

METHODOLOGY

Study area

The plants for this study were collected in Msambweni district which is hot and humid all year round. Annual mean temperatures are 23 ^{0}C – 34 ^{0}C and humidity is 60% - 80%. Climate is monsoon being hot and dry from January to April and cool in June to August. Short rains are in October – December and long rains from March/April to July (Muthaura et al., 2007).

Selection and collection of plant material

This study was initiated to establish whether the six plants that have been reportedly used traditionally as anti mosquitoes have genotoxic effect on long term use. Selection of plants for this study was based on the ethnobotanical and ethnopharmacological surveys carried on the area (Nguta et al., 2010) coupled with review of relevant literature on ethnomedicinal plants used in the Kenya's south coast that have been reported to have activity against mosquitoes. Field collection and initial identification of the plants was done with the assistance of traditional herbal practitioners from the area and further identified by a plant taxonomist at the department of Land Resource Management and Agricultural Technology (LARMAT), University of Nairobi where voucher specimens were deposited. The plants' parts were harvested during the optimal season of the months of September and November 2012 when plants in the area have adequate foliage due to rains and material of best quality is ensured (Muthaura et al., 2007). Each plant part was harvested alone, devoid of weeds, cleaned of foreign matter and soil using water, allowed to dry off the water and then stored in a dry, clean bag then placed on a dry area to prevent decomposition. The collected plant material was then transported to the Department of Public Health, Pharmacology and Toxicology, University of Nairobi.

Preparation of plant material

The plants' parts were scrutinized for any foreign matter or moulds and cleaned with distilled water and dried off the water. They were air dried under shade ground using a laboratory mill to fine powders in a fume chamber. The powdered plant material obtained was packed in 500gram portions and stored in clean air tight paper bags (Wagate et al., 2010).

Extraction

One thousand grams (1000 grams) of each plant powder was extracted separately using acetone by placing the powder in conical flasks and analytical grade acetone added until the powder was fully submerged. Stirring was done to ensure proper mixing and shaking was done regularly to allow percolation. On the fifth day, the extracts were filtered using Whatman No.1 filter paper into another conical flask. Acetone was removed in a rotary evaporator at 60^{0}C. The resultant viscous substance was dried and stored in amber coloured bottles and in a refrigerator at $^+$4^{0}C pending *Allium cepa* test.

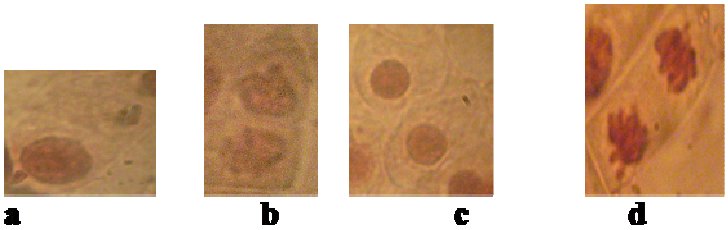
Allium cepa test for genotoxicity

Sixteen (16) *Allium cepa* bulbs (2.5–2.8 cm diameter) grown in tap water at room temperature for 3 days and were used per concentration per test (Çelik, and Aslantürk, 2010; Olusegun *et al*., 2010). The roots of the bulbs were measured daily and when they reached 2–4 cm in length, the bulbs were removed from the tap water and treated with different concentrations of the test samples of the plant extracts in the ranges of 125, 250, 500, 1,000 µg/mL. Positive control was Vincristine in concentrations of 125, 250, 500, 1,000 µg/mL and for the

Table 1. Mitotic inhibition of acetone extracts of six plants used for mosquito control in Kenya's south coast

Treatment	Concentration			
	0.125mg/ml	0.25mg/ml	0.5mg/ml	1mg/ml
Lantana camara	23.55±0.79^{Ad}	40.76±0.93^{Be}	50.36±0.66^{Cd}	62.45±1.10^{De}
Tagetes minuta	-	27.49±0.68^{Ad}	33.80±0.63^{B}	44.93±0.95^{Cd}
Azadirachta indica	28.91±0.98^{Ae}	41.06±0.92^{Be}	54.57±0.67^{Ce}	70.36±0.68^{Df}
Ocimum suave	13.76±0.51^{Ab}	23.40±1.17^{Bc}	36.01±2.21^{Cc}	37.83±0.70^{Cc}
Adansonia digitata	1.02±0.46^{Aa}	16.27±1.02^{Bb}	23.62±1.02^{Cb}	29.53±0.61^{Db}
Plectranthus barbatus	18.61±2.33^{Ac}	23.59±1.24^{Bc}	33.55±0.73^{Cc}	45.16±1.14^{Dd}
Tap water	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
Vincristine sulphate	94.99±0.38^{f}	94.99±0.38^{f}	94.99±0.38^{f}	94.99±0.38^{f}
P-value	<0.001	<0.001	<0.001	<0.001

Mean values followed by the Same small letter(s) within the same column do not differ significantly from one another and those followed by the same capital letter(s) within the same row do not differ significantly from one another (One –way ANOVA,SNK-test, α=0.05)



a) Ghost cell (*A.indica*) b**)** Binucleate cells (*A.indica*) **c)** High cytoplasm to nucleus ratio (*p. barbatus*) **d)** Normal metaphase (tap water)

Figure 1. Some of the observed effects of plant extracts and controls on *Allium cepa* meristem cells

negative control *A. cepa* were grown in water (Ping *et al*., 2012). The solutions were changed daily. After 48 hours 2 mm of the root tips were cut and fixed in ethanol: glacial acetic acid (3:1 v/v) hydrolyzed in 1N HCL at 60 °C for 5 minutes and washed in distilled water. They were squashed on a microscope slide and stained with aceto-orcein for 10 minutes. Excess stain was removed and cover slips placed on the smear then sealed on the slides with clear fingernail polish as suggested by Grant (1982). The slides were observed under light microscope at ×40 magnification. Photomicrographs were made and analyzed for early anaphases, chromosomal bridges/fragments, stickiness and c-mitosis. Mitotic index was calculated as = Number of cells in mitosis/Total number of cells (Fiskesjo, 1985; Ping *et al*., 2012)

Statistical analysis

Data was stored in excel spread sheet then analyzed using the SPSS V22 (Statistical Package of Social Sciences) software for means, standard deviations of means followed by One way ANOVA and Student Newman Keul (SNK) test. Results were considered statistically significant if $P< 0.05$.

RESULTS

Chromosomal analysis was assessed through mitotic inhibition and presence of abnormal chromosomes. There was significant difference among the extracts and mitotic inhibition was dose dependent increasing with dose. *A. Indica* at concentration of 1mg/ml produced most mitotic inhibition at 70.36±0.68 which was not statically different from the positive control. Table 1 show mitotic inhibition values of different plants' extracts and Figure 1 shows some chromosomal aberrations produced by some of the plants' extracts.

DISCUSSION

The *Allium cepa* test is useful for detecting pollution, toxicity, and evaluating potential anticancer properties of substances (Majewska *et al*., 2003; Babatunde and

Bakare, 2006). It has been validated by researchers (Vicentini, *et al*., 2001) and has been used to evaluate the genotoxic potential of medicinal plants (Camparoto *et al*., 2003; Akaneme and Amaefule, 2012). From the results, there was significant difference among the extracts and mitotic inhibition increased with dose. *Azadirachta indica* extracts caused greater mitotic inhibition than the others and produced binucleate cells and ghost cells. Binucleate cells result from interference of cell wall formation (Baeshin et al., 1999). Soliman, (2001) observed chromosomal aberrations such as micronuclei, bridges, stickiness, laggards and polyploidy in *Azadirachta indica* extracts. *Adansonia digitata* induced least mitotic inhibition. *P.berbatus* extracts induced high cytoplasm: nucleus ratio including a bulging cytoplasm in some cells.

CONCLUSION AND RECOMMENDATIONS

This study identified that leaf extracts of *Azadirachta indica* induced mitotic inhibition, produced binucleate and ghost cells in the *allium cepa* root meristems which is an indication of mutagenic potential. The plants in the study may be used for topical application. However, *Azadirachta indica* should not be used systemically and continuously for long periods. There is need for further research on animal systems to investigate other possible genotoxicological effects these plants may possess.

Conflict of Interests

The authors declare no conflict of interest

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