European Journal of Medicinal Plants

19(3): 1-12, 2017; Article no.EJMP.34373
ISSN: 2231-0894, NLM ID: 101583475

Evaluation of Commonly Used Surinamese Medicinal Plants for Their Potential Cytotoxic and Genotoxic Effects Using Cultured Chinese Hamster Ovary Cells

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Authors' contributions

This work was carried out in collaboration among all authors. Author DRAM designed and supervised the study, wrote the protocol, performed the statistical analysis and wrote the first draft of the manuscript. Authors IM and JP performed the plant collections and extractions, the cytotoxicity studies and the micronucleus assays. Authors LJDT and RB performed the cytotoxicity studies and the comet assays. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/EJMP/2017/34373
Editor(s):
(1) Paola Angelini, Department of Applied Biology, University of Perugia, Italy.
(2) Marcello Iriti, Professor of Plant Biology and Pathology, Department of Agricultural and Environmental Sciences, Milan State University, Italy.
Reviewers:
(1) Marjan Vracko, National Institute of Chemistry, Slovenia.
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(3) Eman Mohamed Faruk, Benha University, Egypt.
Complete Peer review History: http://www.sciencedomain.org/review-history/19485

Original Research Article

Received 25th May 2017
Accepted 7th June 2017
Published 12th June 2017

ABSTRACT

Aim: There are often no records about the potential toxicities of medicinal plants including their possible adverse maternal and perinatal effects. In this study, a number of commonly used plant-derived traditional preparations was assessed for their potential cytotoxic and genotoxic effects in a cell culture model.
Place and Duration of Study: The study was carried out for fifteen months at the Departments of Pharmacology and Physiology of the Faculty of Medical Sciences, Anton de Kom University,

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Methodology: Parts from *Aloe vera*, *Apium graveolens*, *Azaradichta indica*, *Carica papaya*, *Cocos nucifera*, *Dioscorea villosa*, *Eryngium foetidum*, *Gossypium barbadense*, *Momordica charantia*, *Musa x paradisiaca*, *Senna reticulata*, and *Spondias mombin* were extracted with distilled water, freeze-dried, and stored at -20℃. The samples were evaluated in cultured Chinese hamster ovary cells for their cytotoxicity using the sulforhodamine B assay, and for their capacity to cause DNA damage using the comet assay and the micronucleus test. The latter studies were validated by establishing the DNA damage caused by etoposide and mitomycin C, respectively. Results were related to data with untreated cell samples.
Results: The extracts from *A. vera*, *G. barbadense*, *M. charantia*, *M. paradisiaca*, and *S. mombin* inhibited cell growth at IC_{50} values of roughly 100 to 400 µg/mL, whereas the remaining samples were barely cytotoxic (IC_{50} values > 1,000 µg/mL). However, only the extracts from *G. barbadense* and *M. paradisiaca* caused appreciable DNA damage in the comet assay (40 and 30%, respectively), and only the former preparation caused the formation of micronuclei (12 ± 5 per 1,000 cells).
Conclusion: The *G. barbadense* extract had caused both repairable and unrepaired, more permanent DNA damage and that from *M. paradisiaca* early, still repairable, more moderate DNA damage. Nevertheless, both preparations may cause genetic toxicity and should be used with caution, particularly by pregnant women.

Keywords: Suriname; medicinal plants; Chinese hamster ovary cells; cytotoxicity; genotoxicity; comet assay; micronucleus assay.

1. INTRODUCTION

Man has probably appreciated the importance of naturally-derived substances for his well-being, health, and survival since his early days [1]. This has resulted in the use of numerous plant-derived substances as, among others, dietary constituents, stimulants, hallucinogens, and medicines [1,2]. The latter practices have led to the development of many life-saving drugs such as the heart glycoside digoxin from the foxglove *Digitalis purpurea* L. (Scrophulariaceae) [3], the hypoglycemic medication metformin from the French lilac *Galega officinalis* L. (Fabaceae) [4], the antineoplastic agent vincristine from the Madagascan periwinkle *Catharanthus roseus* L. (Apocynaceae) [5], and the antimalarial artemisinin from the annual wormwood *Artemisia annua* L. (Asteraceae) [6].

Today, approximately 80% of individuals throughout the world - particularly those living in Third World countries - still relies almost exclusively on herbal substances for their primary health care [7]. Unfortunately, in numerous cases, the scientific evidence to support the therapeutic efficacy of these traditional medicines is insufficient. In many cases there are also no records on the safety of these preparations. However, the bioactive constituents of plants are in general defensive secondary metabolites which act through numerous sophisticated mechanisms and can cause serious harm and even death [8,9].

For instance, the extremely toxic tropane alkaloids atropine, scopolamine, and hyoscyamine in the deadly nightshade *Atropa belladonna* L. (Solanaceae) cause intense delirium and hallucinations [10]. The alkaloid pseudaconitine in wolf's bane species of the genus *Aconitum* (Ranunculaceae) causes potentially fatal ventricular arrhythmias and asystole, and/or paralysis of the heart and the respiratory center [11]. The severely toxic lectin viscumin in mistletoe species of the genus *Viscum* (Santalaceae) inhibits protein synthesis by catalytically inactivating ribosomes [12]. And the calcium oxalate crystals in the dumb cane *Dieffenbachia seguine* (Jacq.) Schott (Araceae) cause severe injury to the cornea if getting into the eyes, and blistering and swelling in the mouth that prevents normal speaking and swallowing as well as gastric and kidney irritation when ingested [13].

Other plant chemicals are even potentially carcinogenic and teratogenic. For instance, the alkaloid arecoline in betel nuts from the areca palm *Areca catechu* L. (Arecaceae) which are habitually chewed in many Asian countries may cause oral cancer in adults [14] and preterm birth as well as low birth weight and height in babies [15]. The mitogenic phorbol esters in Euphorbiaceae family members which are used as folk medicines and herbal teas have been held responsible for the high incidence of nasopharyngeal cancer in China and esophageal

cancer in Curaçao [16,17]. And the pyrrolizidine alkaloids in tea and herbal medicines prepared from comfrey - plants in the genus *Symphytum* (Boraginaceae) - have been associated with liver cancer [18].

The multi-ethnic and multicultural Republic of Suriname is situated on the Guiana Shield, an ecosystem in South America that is renowned for its biodiversity [19]. Suriname also has a rich tradition of medicinal plant use that has its roots not only in South America but also in Africa, India, China, Indonesia, as well as various European and Middle Eastern societies [19]. The traditional wisdom of all these cultures is employed for a myriad of medicinal purposes [20] but often without sufficient scientific evidence of therapeutic efficacy. Furthermore, as in most other countries [21], herbal medicines are introduced into the Surinamese market without any regulations on manufacturing practices, quality standards, and toxicological evaluations. However, these assessments should be mandatory when considering the potential toxicities of these preparations including their possible adverse maternal and perinatal effects [21].

For these reasons, twelve commonly used Surinamese plants (Table 1) have been evaluated for their potential cytotoxic and genotoxic effects in a cell culture model. Parts of these plants are popularly used for treating various microbial infections and parasitic diseases; for alleviating the symptoms of hypertension, diabetes mellitus, and cancer; and/or for certain gynecological conditions, contraception, and/or abortion [20,22-27].

2. MATERIALS AND METHODS

2.1 Plant Collection and Preparation of Plant Extracts

The plants investigated in this study are mentioned in Table 1, which also gives their most common vernacular names, the parts used, the methods of extraction, and the most cited traditional medicinal uses. The plants were collected in rural areas outside Suriname's capital city Paramaribo which had been free from herbicidal or pesticidal use for at least the preceding six months. The collections were done under the auspices of the National Herbarium of Suriname that is in the possession of a collection permit from the Surinamese Ministry of Physical Planning, Land- and Forestry Management. None of the plants are on the International Union for Conservation of Nature's Red List of endangered or threatened species [28].

The collected samples were thoroughly washed with distilled water, dried in open air, washed again, processed as indicated in Table 1, filtered, freeze-dried so as to obtain a stable powder, and divided in aliquots of 2 g which were stored at -20℃. For experiments, the samples were suspended in distilled water and centrifuged for 5 min at 3,000 rpm, and the supernatants were collected and filtered through 0.45-µm pore size Millipore membranes (Sigma-Aldrich, St. Louis, MO, USA).

2.2 Drugs and Chemicals

RPMI-1640 medium and cell culture-grade Tris Base buffer were from Mediatech, Inc., Manassas (VA), USA. *L*-glutamine and cell culture-grade acetic acid were from Amresco LLC, Solon (OH), USA, and fetal calf serum, Hank's Balanced Salt Solution (HBSS), and ethylenediaminetetraacetic acid - trypsin (EDTA-trypsin) from ATCC, Manassas (VA), USA. Penicillin, streptomycin, and amphotericin B were from Corning, Manassas (VA), USA, sulforhodamine B from Biotium, Inc., Hayward (CA), USA, and cell culture-grade trichloroacetic acid from VWR International LLC, West Chester (PA), USA. Low-gelling-temperature agarose and sodium lauryl sarcosinate were from IBI Scientific, Peosta (IA), USA, and Na_2EDTA from Pfaltz & Bauer Inc., Waterbury (CT), USA. Etoposide was from Pharmachemie BV, Haarlem, The Netherlands, and ethidium bromide, mitomycin C, cytochalasine B, and acridine orange from Sigma-Aldrich, St. Louis (MO), USA. All other chemicals used were from our laboratory stock and were of the highest grade available.

2.3 CHO Cells and Maintenance

The cytotoxicity and genotoxicity studies mentioned below have been carried out with the genetically stable Chinese hamster ovary (CHO) cell line that is frequently used for these surveys [29]. The cells had kindly been provided by dr. J.K. Wickleffe (Global Environmental Health Sciences, Tulane University School of Public Health and Tropical Medicine, New Orleans, LA, USA) and were originally from the American Type Culture Collection (Rockville, MD, USA). The cells were maintained in complete medium consisting of RPMI-1640 medium containing

L-glutamine 2% (*w/v*) and supplemented with FCS 10% (*v/v*) in 25-cm^2 culture flasks, at a temperature of 37℃, a minimum relative humidity of 95%, and an atmosphere of 5% CO_2 in air. For experiments, exponentially growing cells were detached from the culture flasks using EDTA-trypsin.

2.4 Assessment of Cytotoxicity of Plant Extracts

Triplicate cultures of CHO cells were inoculated in 96-well microplates at densities of 3 x 10^3 cells per 100 µL complete medium per well and allowed to stabilize for 24 h. The next day, the cell cultures were exposed to the plant extracts at final concentrations of 0 to 1,000 µg/mL and in the presence of penicillin 100 IU/mL, streptomycin 100 µg/mL, and amphotericin B 5 µg/mL. Incubations were carried out for a total of three days and in final volumes of 200 µL per well.

Cellular responses were assessed with the SRB colorimetric assay [30]. Briefly, the cell cultures were *in situ* fixed with trichloroacetic acid 10% (*w/v*) and stained with SRB 0.4% (*w/v*) in acetic acid 1% (*v/v*). Unbound SRB was removed with acetic acid 1% (*v/v*), and cell-bound SRB was solubilized with Tris Base buffer 10 mM, pH 10.5. Absorbance values at a wavelength of 515 nm were measured with an ELx800 TM Absorbance Microplate Reader (BioTek, Winooski (VT), USA), corrected for background absorption, and plotted against extract concentrations. Background absorption was determined from control wells which had received either medium alone or plant extract-containing medium, but no cells. Dose-response profiles were constructed from which IC_{50} values were derived, *i.e.*, concentrations of plant extracts resulting in 50% inhibition of cell growth when compared to untreated controls. The IC_{50} values were taken as a measure of the cytotoxicity of the plant extracts.

2.5 Assessment of DNA Damage by Plant Extracts Using the Comet Assay

The degree of DNA damage caused by the plant extracts in the CHO cells was qualitatively assessed by the comet assay or single-cell gel electrophoresis assay [31]. Thus, triplicate cultures of CHO cells were inoculated in 24-well microplates at densities of 3 x 10^4 cells per mL complete medium per well and allowed to stabilize for 24 h. The next day, the cultures were exposed to a plant extract at its IC_{50} concentration or at 1,000 µg/mL in the case of samples with an IC_{50} > 1,000 µg/mL. Incubations were in final volumes of 1 mL complete medium and in the presence of penicillin 100 IU/mL, streptomycin 100 µg/mL, and amphotericin B 5 µg/mL. Cell samples treated with etoposide (3 µg/mL) - that causes DNA strand breakage [32] - were used as positive controls.

Three days later, the cells were harvested by scraping with a rubber policeman, washed with ice-cold phosphate-buffered saline (PBS), and resuspended in ice-cold PBS at densities of 10^5/mL. These cell samples were mixed with molten low-melting point agarose at a ratio of 1:10 (*v/v*) and at 37℃, and 50 µL of each mixture was rapidly transferred onto a two-well Comet Slide TM (Trevigin Inc., Gaithersburg, MD) which was placed in the dark for 30 min at 4℃ to allow gelling of the agarose. Subsequently, membranes and soluble cell constituents were lyzed and the DNA was liberated at 4℃ in alkaline lysis solution consisting of NaCl 1.2 M, Na_2EDTA 0.1 M, sodium lauryl sarcosinate 0.1% (*w/v*), and NaOH 0.26 M, final pH > 13. One hour later, the DNA was unwinded and salt and detergent were removed with NaOH 0.2 M and Na_2EDTA 0.001 M, final pH > 13. The cell lysates were then electrophoresed for 25 min at 0.6 V/cm in NaOH 0.3 M and Na_2 EDTA 0.001 M, final pH > 13. This resulted in migration of the relaxed and broken DNA fragments away from the nucleus towards the anode and the formation of distinct comet images.

The slides were rinsed and neutralized three times for 5 min in distilled water and stained with 80 µL ethidium bromide 2.5 µg/mL. After removing excess stain with 200 mL distilled water, the comet images were visualized under a fluorescence microscope at 200 x magnification and photographed. The comet tails represent fragmented chromosomal DNA that has migrated away from the nucleus, whereby the comet tail length increases with the degree of DNA strand-breakage, and the extent of DNA liberated from the head of the comet is directly proportional to the amount of DNA damage. Thus, the DNA damage caused by the test samples was estimated from the length of the comet tail with respect to that of the entire comet. This value was expressed as percentage and related to the results obtained with untreated cell samples.

Table 1. Relevant information about the plants evaluated in the current study. All reference vouchers are stored at the National Herbarium of Suriname (BBS) at the Anton de Kom University of Suriname, Paramaribo, Suriname

Plant species (vernacular name; Herbarium voucher)	Plant family	Plant part used (method of processing)	Most cited traditional medicinal uses (literature references)
Apium graveolens L. (Celery; none[a])	Apiaceae	Whole plant (macerated and boiled in distilled water for 15 min)	Urinary conditions including cystitis, and used as a diuretic [22]
Azadirachta indica A. Juss. (Nimtree; UVS-17486)	Meliaceae	Leaves (macerated and boiled in distilled water for 60 min)	Antimicrobial infections, intestinal worms, measles, and diabetes mellitus [23,24]
Aloe vera L. (Aloe; UVS-17469)	Aloaceae	Leaves (macerated at room temperature and juice collected)	Asthma, colds, skin and hair conditions, rheumatism, bleeding [20,22,23]
Carica papaya L. (Papaya; vA 817)	Caricaceae	Immature fruits (macerated at room temperature and juice collected)	Warts, worm infections, hypertension, diabetes mellitus, abortion [20,22,23,25]
Cocos nucifera L. (Coconut; UVS-17801)	Arecaceae	Immature fruits (macerated at room temperature and juice collected)	Bladder problems, furuncles, gout, rheumatism, and abortion [20,22,23,26]
Dioscorea villosa L. (Wild yam; none[b])	Dioscoreaceae	Tubers (macerated and soaked in distilled water for 60 min at 45 ℃)	Menopausal problems, contraception [22]
Eryngium foetidum L. (Culantro; Hei 1056)	Apiaceae	Roots (macerated and soaked in distilled water for 60 min at 45 ℃)	Fevers and stomach pain; abortion [20,22,23,25]
Gossypium barbadense L. (Red cotton; UVS-17433)	Malvaceae	Leaves (macerated and boiled for 60 min in distilled water)	Hypertension, abdominal cramps, painful ovaries, uterus ailments [20,23,25]
Momordica charantia L. (Bitter melon; UVS-17455)	Cucurbitaceae	Whole plant (macerated and soaked in distilled water for 60 min at 45 ºC)	Diabetes mellitus, hypertension, malaria, cancer [23,24,25,27]
Musa x paradisiaca L. (Banana; None[b])	Musaceae	Peels (macerated and soaked in distilled water for 60 min at 45 ℃)	Dysentery, heart problems, skin eruptions, abortion [20,22,23]
Senna reticulata (Willd.) H.S. Irwin and Barneby (Maria mole; LBB 12630)	Caesalpiniaceae	Flowers (macerated and boiled in distilled water for 30 min)	Eczema, ringworm infections, smallpox, purgative [20]
Spondias mombin L. (Hog plum; LBB-12479)	Anacardiaceae	Leaves (macerated and boiled in distilled water for 60 min)	Diarrhea, dysentery, hemorrhage and sores; used as an eyewash [20,23,24]

(UVS: University of Suriname; vA: Tinde van Andel; Hei: D. Heinsdijk; LBB: 's Lands Bosbeheer)

[a] *The current study used commercially available cultivars of these species*

[b] *No voucher number available because the exact species could not be determined*

2.6 Assessment of DNA Damage by Plant Extracts Using the Micronucleus Test

The extent of DNA damage caused by the plant extracts was also assessed by the micronucleus or cytokinesis-block micronucleus test [33]. The results from this assay are positive when a test agent introduces DNA strand breaks or perturbs the integrity of the mitotic spindle during cell division, resulting in the segregation of individual chromosomes or (microscopically visible) parts of chromosomes from the nucleus to become micronuclei [33]. Thus, triplicate cultures of CHO cells were inoculated in 24-well microplates at densities of 3 x 10^4 cells per 1 mL complete medium per well and allowed to stabilize for 24 h. The next day, the cell cultures were exposed to a plant extract at its IC_{50} concentration or at 1,000 µg/mL in the case of samples with an IC_{50} > 1,000 µg/mL. Incubations were in final volumes of 1 mL per well and in the presence of penicillin 100 IU/mL, streptomycin 100 µg/mL, and amphotericin B 5 µg/mL. Cell samples treated with the mutagenic compound mitomycin C at 2 µg/mL [34] were used as positive controls.

Twenty-four hours later, cytokinesis was blocked by the addition of 500 µL cytochalasine B 3 µg/mL to each cell culture. The cultures were grown for another 24 h in the presence of cytochalasine B to allow chromosomal damage to lead to the formation of micronuclei in interphase cells. After removing the cytochalasine B solution, the cell cultures were rinsed with HBSS, harvested by trypsinization, and pelleted by centrifugation for 8 min at 200 rpm. The pelleted cells were collected and lyzed for 20 min in a hypotonic solution of KCl 0.075 M, re-centrifuged, and fixed for 30 min in cold methanol:acetic acid = 3:1 (*v/v*). These preparations were placed on a microscopic slide, air-dried, immediately stained with acridine orange 125 µg/mL, and examined under a fluorescence microscope at 200 x magnification for the presence of binucleated and multinucleated cells. For each treated cell culture, the number of micronuclei per 1,000 cells was scored and related to the number of micronuclei per 1,000 cells in untreated cultures.

2.7 Statistics

All experiments have been carried out at least three times in triplicate. Data are means ± SDs and have been compared using Student's *t* test and taking *P* values < 0.05 to indicate statistically significant differences.

3. RESULTS

3.1 Cytotoxicity of Plant Extracts

As shown in Table 2, the extracts from *A. vera*, *G. barbadense*, *M. charantia*, *M. paradisiaca*, and *S. mombin* inhibited the growth of the CHO cells at IC_{50} concentrations between roughly 100 and 400 µg/mL. The remaining plant extracts produced IC_{50} values that were (well) over 1,000 µg/mL (Table 2). Thus, the plant extracts evaluated in the current study were either lowly or not cytotoxic to the CHO cells.

Table 2. IC_{50} values (± SDs) of the plant extracts in CHO cells after three days exposure

Plant species	IC_{50} (µg/mL)
A. graveolens	> 1000
A. indica	> 1000
A. vera	181 ± 27
C. nucifera	> 1000
C papaya	> 1000
D. villosa	> 1000
E. foetidum	> 1000
G. barbadense	114 ± 36
M. charantia	402 ± 79
M. paradisiaca	259 ± 83
S. reticulata	> 1000
S. mombin	184 ± 38

3.2 Formation of Comet Images by Plant Extracts

The comet images resulting from the DNA of untreated CHO cells did not show signs of a tail (Fig. 1; Table 3). On the other hand, the tails of the comets resulting from the treatment with etoposide represented 73 ± 7% of the total length of the comet images, indicating that this compound had damaged approximately three-quarters of the nuclear DNA (Fig. 1; Table 3). These observations were in agreement with expectations [32] and validated the use of the comet assay to assess the plant extracts for their potential DNA-damaging effects.

The tails of the comets representing the DNA from cells treated with the extract from *G. barbadense* or *M. paradisiaca* represented about 40 and 30%, respectively, of the total fluorescence of the comet images. This suggests that these plant extracts had damaged roughly one-third of the nuclear DNA (Table 1). The remaining samples had hardly any effect on the integrity of the nuclear DNA (Table 3).

3.3 Formation of Micronuclei by Plant Extracts

There were no micronuclei in untreated cell cultures (Fig. 2; Table 3), while those treated with mytomycin C harbored 39 ± 15 micronuclei per 1,000 cells (Fig. 2; Table 3). These observations were again in agreement with expectations [34] and justified the use of the micronucleus test to assess the plant extracts for their potential genotoxicity.

As shown in Table 3, only the use of the *G. barbadense* extract led to the formation of statistically significantly more micronuclei when compared to untreated controls, *i.e.*, 12 ± 5 per 1,000 cells (Table 3). The remaining plant extracts did not produce statistically significantly more micronuclei when compared to the blanc (Table 3).

4. DISCUSSION

Plant-based preparations are widely used in Suriname for health promotion, disease prevention, and managing various conditions [20,22-27]. However, in most cases there are no comprehensive scientific reports about their safety. Using an *in vitro* assay for cytotoxicity as well as two *in vitro* tests for genetic toxicity, the results from the current study suggest that aqueous extracts from *G. barbadense* leaves and *M. paradisiaca* peels were lowly cytotoxic to cultured CHO cells but caused damage to the nuclear DNA of the cells. Thus, these preparations may be associated with the formation of potentially carcinogenic DNA lesions. The remaining plant extracts did not cause apparent DNA damage and were either only slightly cytotoxic (*A. vera* leaves, *M. charantia* whole plant, and *S. mombin* leaves), or did not affect the growth of the CHO cells at all (*A. graveolens* whole plant, *A. indica* leaves, *C. papaya* immature fruits, *C. nucifera* immature fruits, *D. villosa* tubers, *E. foetidum* roots, and *S. reticulata* flowers).

The relatively modest *in vitro* cytotoxic but clear genotoxic effects of the *G. barbadense* and *M. paradisiaca* extracts are in line with data from several previous studies. Exposure to either crude preparations from parts of *G. barbadense* or gossypol [35] – a defensive chemical in several parts of the plant [36] - led to moderate to substantial inhibition of the growth of various cultured tumor cell lines [37-39] as well as inhibition of mitosis in *Allium cepa* L. root tips [40].

Furthermore, gossypol produced DNA strand breaks in cultured human skin fibroblasts and sister chromatid exchanges in human lymphocytes [41], while an intraperitoneally administered aqueous extract from *G. barbadense* seeds led to damage to, among others, testicular tissue in rats [42]. Furthermore, gossypol inhibited ovum implantation and early pregnancy in female rats, presumably by interfering with luteinizing hormone [43], and influenced reproduction, pregnancy, and early embryonic development in rats [44]. This compound also caused infertility in men [45], which was the reason for discontinuing its development as a male contraceptive [45].

As far as the *M. paradisiaca* extract concerns, the relatively low cytotoxicity towards the CHO cells was in the range of that elicited by triterpenes and sterols from an ethanolic *M. paradisiaca* peel extract against cultured RAW 264.7 murine macrophages and LLC-MK2 rhesus monkey kidney epithelial cells [46].

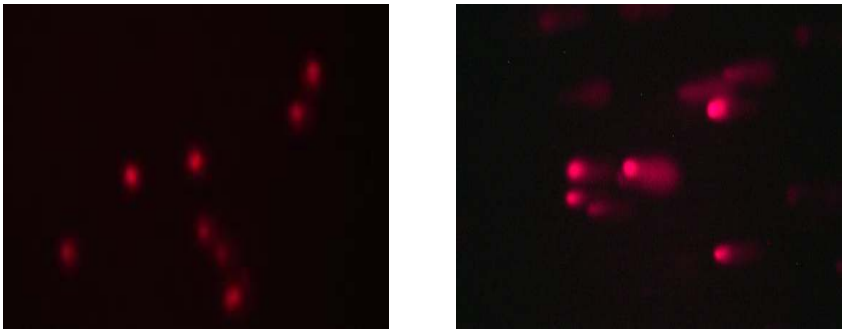

Fig. 1. Comet images representing fragmented nuclear DNA from untreated CHO cells (left) and cells treated with etoposide (right)

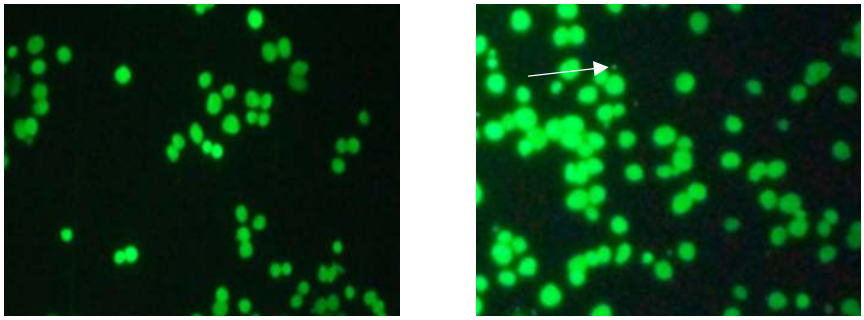

Fig. 2. Untreated cultures of CHO cells without micronuclei (left) and cultures treated with mitomycin C with micronuclei (right). Arrow indicates a micronucleus

Table 3. Degree of DNA strand-breakage and number of micronuclei per 1,000 cells caused by the plant extracts in CHO cells after three days exposure. Data are means ± SDs of at least three independent experiments performed in triplicate

Plant species (popular name; Plant family)	% of DNA in comet tail	Number of micronuclei per 1,000 cells
Blanc	0 ± 0	0 ± 0
Etoposide	73 ± 7[1]	-
Mitomycin C	-	39 ± 15[1]
A. graveolens	3 ± 0	4 ± 4
A. indica	12 ± 3	6 ± 5
A. vera	7 ± 1	1 ± 1
C. nucifera	7 ± 2	0 ± 1
C. papaya	10 ± 3	3 ± 3
D. villosa	8 ± 2	2 ± 2
E. foetidum	5 ± 2	2 ± 2
G. barbadense	41 ± 13[1]	12 ± 5[1]
M. charantia	8 ± 4	4 ± 2
M. paradisiaca	28 ± 9[1]	7 ± 6
S. reticulata	7 ± 2	3 ± 2
S. mombin	6 ± 3	3 ± 4

[1]*Statistically significantly different from blanc (P < 0.05)*

And comparably to the current results, an *M. paradisiaca* peel extract produced significantly more comet images but not more micronuclei in the peripheral blood cells of Swiss mice treated orally with this preparation when compared to untreated animals [47].

Comet images probably represent still repairable injuries whereas micronuclei most likely represent injuries that have survived at least one mitotic cycle and reflect unrepaired, fixed DNA damage [48]. This would imply that the *G. barbadense* preparation - causing both comet images and micronuclei - had produced both repairable and unrepaired, permanent DNA damage, whereas the *M. paradisiaca* extract - only causing comet images – had produced early, still repairable, more moderate DNA damage. Obviously, these assumptions should be verified in more comprehensive studies.

The relatively modest or nonexistent cytotoxicity and the absence of DNA damage by the remaining plant extracts noted in the current study are not in accordance with literature data. Extracts from *A. vera* leaves reportedly elicited appreciable cytotoxicity in the *A. cepa* root tips test [49], towards isolated murine bone marrow cells [50], and against cultured human carcinoma cells [51], and caused the formation of binucleate cells in *A. cepa* root tips [49] and chromosomal aberrations in murine bone marrow cells [50]. Parts of *M. charantia* displayed antitumor activity in laboratory animals [52] and were genotoxic in the *Drosophila melanogaster* wing spot test [53] albeit not in a mouse bone marrow micronucleus

test and a chromosome aberration test [54]. And an ethanolic extract from *S. mombin* leaves displayed an antifertility effect in laboratory rats [55] while a hydromethanol extract increased the formation of micronucleated polychromatic erythrocytes in Swiss albino mice [56].

Furthermore, a methanolic extract from *A. graveolens* seeds, the tetranortriterpenoid nimbolide in *A. indica* leaves and flowers, the hexane fraction and a methanol extract from *C. nucifera* peels and endocarps, the steroidal saponin diosgenin in *D. villosa* roots, methanolic extracts from *E. foetidum* leaves, and certain anthraquinones in the plant genus *Senna* exhibited cytotoxicity towards various preclinical models [57-63]. However, although nimbolide reportedly introduced DNA lesions in a comet assay [60], none of the other preparations or compounds have been associated with DNA abnormalities or genetic toxicity [64-69].

The reasons for the discrepancies among these and our data are not clear, but may be related to differences in plant parts investigated, extraction procedures applied, and laboratory models used. For instance, an ethanol extract of *A. vera* whole leaf produced reproductive toxicity and increased mortality in laboratory mice [70], but a preparation from the inner leaf did not produce toxicity in rats [71]. Furthermore, an aqueous extract from the aerial parts of this plant was clearly genotoxic while an aqueous extract from the fruits displayed antigenotoxic effects [53]. And azadichtin was much more potent against insect models than against mammalian cell lines [72]. Whether and to which extent these considerations hold true must be determined in future studies.

5. CONCLUSIONS

Preparations from particularly *G. barbadense* but also from *M. paradisiaca* may possess relatively low cytotoxic properties but definite genotoxic properties, warranting caution when used, especially by pregnant women. Those from *A. vera*, *A. indica*, *A. graveolens*, *C. papaya*, *C. nucifera*, *D. villosa*, *E. foetidum*, *M. charantia*, *S. reticulata*, and *S. mombin* did not seem to be associated with these effects. However, in all cases, the scientific evidence for lack of genetic toxicity is insufficient and sometimes conflicting, indicating that they also should be used with care until more definitive data on their safety are available.

DISCLAIMER

This manuscript's title and abstract presented in a conference: "Caribbean Public Health Agency. Caribbean Public Health Agency: 60th Annual Scientific Meeting. Kingston, the University of the West Indies".
Conference link:http://bases.bireme.br/cgi-bin/wxislind.exe/iah/online/?IsisScript=iah/iah.xis&src=google&base=MedCarib&lang=p&nextAction=lnk&exprSearch=18074&indexSearch=ID

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

ACKNOWLEDGEMENTS

The VLIR/ADEKUS-partner program and the Suriname Conservation Foundation are kindly acknowledged for financially supporting for part of this work.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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