Evaluation of *Procavia capensis* hyraceum used in traditional medicine for modulation of mutagen-induced genotoxicity

Asita Okorie Asita*, Thabang Rants‘o, Sibusisiwe Magama and Matsepo Taole

Department of Biology, National University of Lesotho, P. O. Roma 180, Maseru 100, Lesotho, Southern Africa.

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Hyraceum (HM) is used in traditional medicine in Southern Africa. Three concentrations of HM (mg/ml in distilled water) (0.0156, 0.03125 and 0.0625) were assessed for cytotoxicity (CT), genotoxicity (GT) and modulation of cyclophosphamide (CP) and ethyl methanesulfonate (EMS)-induced GT using the *Allium cepa* assay following 24 h treatment. CP (1.00 mg/ml) and EMS (0.0375 mg/ml) were not cytotoxic but genotoxic. HM (0.03125, 0.0625 mg/ml) and its mixtures with CP or EMS induced significant reduction (p <0.05) of the mitotic index (MI) and were adjudged cytotoxic. HM alone and its mixtures with CP or EMS induced statistically significant genotoxicity (p < 0.05). Mixture of HM (0.016 mg/ml) with CP was not significantly more genotoxic than CP alone (ME 0.57). Each mixture of HM (0.03125, 0.0625 mg/ml) with CP was insignificantly less genotoxic than CP alone with modulatory effect (ME) of -0.14 and -0.01, respectively, which suggested no interaction between HM and CP. Mixtures of HM with EMS induced positive and significant (>2-fold) MEs and each mixture was significantly (p < 0.05) more genotoxic than HM or EMS alone which indicated a synergistic interaction. Sticky chromosomes, chromosome laggards, chromosome fragments, anaphase and telophase bridges, binucleate interphase cells were observed.

Key words: Cytotoxicity, cyclophosphamide, ethyl methanesulfonate (EMS), genotoxicity; anti-genotoxicity.

INTRODUCTION

Since time immemorial, animals and animal products have been an important component of traditional medicine in practically every human culture in all parts of the world, used to treat and relieve a myriad of illnesses and diseases (Padmanabhan and Sujana, 2008). The importance of traditional medicine based on animals and animal products is well established and, in some cases, approved by pharmaceutical companies as it complements modern medical science (Debas et al., 2006; Jäger et al., 2005). The animal parts and products used in traditional medicine include the fresh manure of the dromedary (*Camelus dromedaries*) to alleviate arthritis (El-Kamali, 2000), and metabolic products such as urine and excreta (dung) separately or in combination with other herbs and minerals (Padmanabhan and Sujana, 2008) are used by tribes in Western Ghats of
India to manage or cure about 21 diseases including tuberculosis, rheumatic and joints pain, asthma, piles, night blindness, paralysis, debility, impotence, rheumatic and muscle pain, paralysis, impotency, skin burns and rickets (Sinha and Sinha, 2001). Notwithstanding the reported value of animals, their products and their body parts in traditional medicine, studies on their therapeutic effects and, also their safety, have not received enough attention, when compared to plants (Solovan et al, 2004). The concoctions used in traditional medicine are usually crude extracts in water, alcohol, distillates or essential oil, which contain many secondary metabolites (SMs) from several structural groups and their activity is often due to synergistic interactions of SMs present (Eid et al., 2012; Mulyaningsih et al., 2010). The apparent broad-spectrum activity of concoctions used in traditional medicine has been ascribed to phenolic compounds and polysaccharides (Wink, 2015). At high concentrations, SMs change membrane fluidity and increase permeability. Therefore, many lipophilic SMs (especially those in essential oils) exhibit antimicrobial and cytotoxic activities (van Wyk and Wink, 2015).

Some SMs such as pyrrolizidine alkaloids and furanocoumarins are both lipophilic, aromatic and planar which allows them to intercalate or alkylate DNA, thereby causing mutations and even cancer and show substantial antibacterial, antifungal, antiviral and cytotoxic properties (van Wyk and Wink, 2015; Wink and Schimmer, 2010; Schmeller et al., 1997).

In Lesotho the fossilized excreta of Procavia capensis (rock hyrax) called hyraceum or “moroto oa pela” in Sesotho is used to treat respiratory infections, urinary tract or bladder infections, measles and non-communicable diseases such as diabetes mellitus. The hyraceum is also used in combination with other medicinal plant species to enhance its efficacy (Seleteng-Kose et al., 2015). The fossil is formed from the faeces and urine of P. capensis as the major components which accrete to form dark brown, resin-like masses to which plant material, pollen grains and other digestive remains are trapped. Fossilization occurs with time in arid regions. P. capensis inhabits shelters in rocky outcrops in a variety of biomes and feeds on a variety of grasses, shrubs, tree leaves, fruits and berries including the bark of trees (Olsen et al., 2007).

The only literature found on the biological activity of hyracei was that on its effect on the GABA-benzodiazepine receptor, which indicated that since the hyracei exhibited a high affinity for GABA-benzodiazepine they could be used to treat epilepsy, a non-communicable disease (Olsen et al., 2007). Other than that, no records were found on the scientific validation of traditional medicinal uses of hyraceum (van Wyk, 2008).

In view of complex nature of hyraceum, composed of animal metabolic waste and plant materials and its use to treat both communicable and non-communicable diseases, the present study was performed and involved the analyses of the methanolic extract of hyraceum for cytotoxic, genotoxic and modulation of mutagen-induced genotoxicity in the Allium cepa root tip. The A. cepa root tip assay was used in this study because plant genotoxicity assays are relatively inexpensive, fast, give reliable results and chemicals which cause chromosomal aberration (CA) in plant cells also produce CA in cultured animal cells that are frequently identical (Grant, 1978; Ma et al., 1994).

**MATERIALS AND METHODS**

**Test organism**

Onion (A. cepa) seeds of the variety, Texas Grano 502 P.R.R., a product of Sakata seeds, Lanseria 1748, Republic of South Africa were purchased from Maseru Garden Centre, Lesotho, Southern Africa.

**Mutagens and chemicals**

Cyclophosphamide (CP) and ethyl methanesulfonate (EMS) are products of Fluka (Biochemika, Germany). Methanol (absolute) is a product of Associated Chemical Enterprises (Pty) Ltd (Johannesburg, South Africa); hydrochloric acid glacial and acetic acid are products of UNILAB (Krugerdorp, South Africa); acetocarmine stain was obtained from Carolina Biological Supply Company, Burlington, North Carolina, USA.

**Procurement and processing of hyraceum**

A sample of hyraceum was purchased at the main Maseru open air market. Maseru is the capital of Lesotho with the geographical coordinates of: latitude: 29° 19’ 0.01” S and longitude: 27° 28’ 59.99” E. The sample was fragmented and dried in a fanned oven (Labcon) at 35°C to a constant weight and brittle. The dried hyraceum was ground to a fine powder using an electric pulverizer (Kenwood), dissolved in water as solvent and assessed for cytotoxicity and genotoxicity in the A. cepa chromosome aberration assays.

**Genotoxicity, cytotoxicity and modulatory effects of hyraceum crude extract using the A. cepa root tip chromosome aberration assay**

The assays were conducted according to the methods of Asita et al. (2017) namely, preliminary dose selection assay to determine the concentrations of mutagens and hyraceum to use in the assays; cytotoxicity that is, mitotic index (MI), genotoxicity (GT) and the modulatory effect (ME) of hyraceum extract on mutagen-induced genotoxicity, including the root harvest, slide preparation and scoring of slides. In each assay, three root tips (triplicate) were assessed at each concentration.

On each of three slides (n = 3) per treatment, a total of 2000 cells, classified into interphase or dividing cell, that is, prophase (normal, N or aberrant, ABN), metaphase (N or ABN), anaphase (N or ABN) or telophase (N or ABN) were scored; that is, a total of 6000 cells each for the control and treatment groups. The aberrations observed were: Binucleate interphase (Bin), nuclear bud (NB); micronuclei (MN), pyknotic interphase nuclei (PN), sticky chromosomes (S), C-metaphase (C-Mit), lagging chromosomes (L),
polyplody (PP), anaphase and telophasic bridges (A.B), chromosome fragment (F), multipolar anaphase and telophasic (MP). For calculating the GT, only aberrant mitotic cells were considered.

**Analysis of slide preparations**

Cytotoxicity, as determined by the mitotic index (MI) was expressed as the number of dividing cells per 100 cells scored according to the formula:

\[ MI = \frac{\text{No. of dividing cells}}{\text{Total No. of cells scored}} \times 100 \quad (1) \]

Genotoxicity (GT) was expressed as the number of aberrant mitotic cells (AMC) per 100 mitotic cells [that is, AMC + normal mitotic cells (NMC)].

Frequency of GT = \( \frac{\text{AMC}}{\text{AMC} + \text{NMC}} \times 100 \) \quad (2)

Modulatory effect (ME) of hyraceum extract on CP- or EMS-induced GT was calculated as:

\[ \text{ME} = \frac{(B - C) \cdot (A - C)}{(A - C)} \quad (3) \]

where ME is the number of times of the mutagen-induced GT by which the GT of the mutagen was reduced (ME is negative) or increased (ME is positive) in mixtures with hyraceum extract; A is the GT induced by CP or EMS alone; B is the GT induced by each mixture of hyraceum extract and CP or EMS and C is the GT induced by tap water alone.

A positive (+) value of ME indicated that the mixture of mutagen and hyraceum extract was more genotoxic than the mutagen alone. If mixture is more genotoxic than both the mutagen alone and the genotoxic hyraceum extract alone then synergism is indicated. However, if the mixture is more genotoxic than the mutagen and also more or less genotoxic than the non-genotoxic hyraceum extract alone then mutagen potentiation is indicated.

A negative (-) value of ME indicated that the mixture of mutagen and hyraceum extract was less genotoxic than the mutagen alone. If mixture is less genotoxic than both the mutagen alone and the genotoxic hyraceum extract alone then antagonism is indicated. However, if the mixture is less genotoxic than the mutagen and also more or less genotoxic than the non-genotoxic hyraceum extract and hyraceum extract alone then antimutagenicity is indicated.

ME values, whether positive (increase) or negative (reduction) were considered to be significant when greater than 2.

**Data analysis**

Data were expressed as mean ± SD of three values. The mean value of each group of three slides per concentration of test agent was compared with that of the negative control group using student's t-test. P-values less than 0.05 (p < 0.05) were considered as indicative of significance.

**RESULTS**

**Cytotoxicity (CT), genotoxicity (GT) and modulatory effects (ME) of hyraceum extract in the A. cepa root tip chromosome aberration assay**

Photographs of the most representative pictures of normal mitotic cells and cells containing the different types of chromosome aberrations that were observed and scored are presented in Figure 1. The results of the CT, GT and modulatory effects (ME) of hyraceum extract in the A. cepa root tip chromosome aberration experiments with the HM extracts and the mutagens are presented in Table 1.

**Cytotoxicity and genotoxicity analysis experiments with the hyraceum extracts, CP and EMS (P + M)/(A + T) ratio**

Examination of the (P + M)/(A + T) ratio in column 8 of Table 1 shows that none of the treatments, that is, CP, EMS, the three concentrations of hyraceum extract alone or mixtures of CP or EMS with each concentration of hyraceum induced a significant change in the (P + M)/(A + T) ratio, when compared with the water-treated negative control group (p > 0.05).

**Cytotoxicity**

Examination of the MI in column 9 of Table 1 shows that the concentration of CP (1.00 mg/ml) or EMS (0.0375 mg/ml) used was not toxic to the root meristem cells of A. cepa when compared to the water-treated negative control (p > 0.05). Of the three concentrations of HM used (0.016, 0.03125 and 0.0625 mg/ml) only the top two concentrations induced significant reduction of the MI compared to the water-treated negative control (p > 0.05) and were considered toxic to the root meristem cells. The mixture of CP (1.00 mg/ml) or EMS (0.0375 mg/ml) with each of the three individual concentrations of extract of HM induced significant reduction of the MI compared to the water-treated negative control (p > 0.05) and were considered toxic to the root meristem cells.

**Genotoxicity**

Examination of induction of GT in column 10 of Table 1 shows that the concentration of CP (100 mg/ml) or EMS (0.0375 mg/ml) used was genotoxic to the root meristem cells of A. cepa when compared to the water-treated negative control (p < 0.05). The three concentrations (0.016, 0.03125 and 0.0625 mg/ml) of HM used and their individual mixtures with CP (1.00 mg/ml) or EMS (0.0375 mg/ml) also induced statistically significant levels of aberrant mitotic cells (p < 0.05) and were therefore adjudged to be genotoxic to the root meristem cells.

**Modulatory effect of HM on CP-induced genotoxicity**

Examination of the ME in column 11 of Table 1 shows that the mixture of the lowest concentration (0.016 mg/ml) of HM with CP induced a positive but none-significant (<2-fold) value of ME of 0.57 which indicated that the mixture was more genotoxic than CP alone, but the
increase was not significant. The mixture of the middle (0.03125 mg/ml) or the highest (0.0625 mg/ml) concentration of HM with CP induced a negative but none-significant (<2-fold) value of ME of -0.14 and -0.01 respectively which indicated that each mixture was less genotoxic than CP alone, but the decrease was not significant in each case. Therefore, neither synergistic (CP plus lowest concentration) nor antagonistic or anti-genotoxic (CP mixture with either the middle or highest concentration) interaction could be inferred.
Table 1. Cytotoxicity and genotoxicity of hyraceum (HM), EMS and CP to meristem cells of onion root tip and the modulatory effects (ME) of HM on EMS- or CP-induced genotoxicity.

<table>
<thead>
<tr>
<th>TC concentration (mg/ml) and treatment</th>
<th>Cells scored</th>
<th>Genotoxicity Modulatory effect on genotoxicity</th>
<th>Statistics</th>
<th>Mean</th>
<th>SD</th>
<th>Mean</th>
<th>SD</th>
<th>Mean</th>
<th>SD</th>
<th>Mean</th>
<th>SD</th>
<th>Mean</th>
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<tbody>
<tr>
<td></td>
<td>Cells in interphase</td>
<td>Cells in mitosis</td>
<td>Total cells in mitosis</td>
<td>Total number of cells scored</td>
<td>(P+M)/(A+T)</td>
<td>MI</td>
<td>Genotoxicity</td>
<td></td>
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<td>AMC</td>
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<tr>
<td>Water</td>
<td>Mean 1807.67</td>
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<td>0.33</td>
<td>192.33</td>
<td>2000.00</td>
<td>2.69</td>
<td>9.62</td>
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<td>CP (1.00)</td>
<td>Mean 1867.00</td>
<td>127.67</td>
<td>5.33</td>
<td>133.00</td>
<td>2000.00</td>
<td>2.34</td>
<td>3.00</td>
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<td>EMS (0.0375)</td>
<td>Mean 1824.00</td>
<td>161.67</td>
<td>14.33</td>
<td>176.00</td>
<td>2000.00</td>
<td>2.69</td>
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<td>HM (0.0156)</td>
<td>Mean 1821.67</td>
<td>167.67</td>
<td>10.67</td>
<td>178.33</td>
<td>2000.00</td>
<td>2.23</td>
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<td>7.09</td>
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<td>HM (0.03125)</td>
<td>Mean 1857.67</td>
<td>132.33</td>
<td>10.00</td>
<td>142.33</td>
<td>2000.00</td>
<td>2.18</td>
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<td>7.02</td>
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<td>CP + HM (0.0156)</td>
<td>Mean 1930.67</td>
<td>64.67</td>
<td>4.67</td>
<td>69.33</td>
<td>2000.00</td>
<td>3.43</td>
<td>3.47</td>
<td>6.29</td>
<td>0.57</td>
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<tr>
<td>CP + HM (0.03125)</td>
<td>Mean 1903.00</td>
<td>93.33</td>
<td>3.67</td>
<td>97.00</td>
<td>2000.00</td>
<td>3.36</td>
<td>4.85</td>
<td>3.52</td>
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<tr>
<td>CP + HM (0.0625)</td>
<td>Mean 1875.67</td>
<td>119.67</td>
<td>4.67</td>
<td>124.33</td>
<td>2000.00</td>
<td>2.71</td>
<td>6.22</td>
<td>4.00</td>
<td>-0.01</td>
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<tr>
<td>EMS + HM (0.0156)</td>
<td>Mean 1986.00</td>
<td>4.00</td>
<td>10.00</td>
<td>14.00</td>
<td>2000.00</td>
<td>6.78</td>
<td>0.70</td>
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<tr>
<td>EMS + HM (0.03125)</td>
<td>Mean 1966.00</td>
<td>24.67</td>
<td>9.33</td>
<td>34.00</td>
<td>2000.00</td>
<td>3.10</td>
<td>1.70</td>
<td>28.42</td>
<td></td>
<td>2.44</td>
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<tr>
<td>EMS + HM (0.0625)</td>
<td>Mean 1990.67</td>
<td>3.00</td>
<td>6.33</td>
<td>9.33</td>
<td>2000.00</td>
<td>4.06</td>
<td>0.47</td>
<td>71.43</td>
<td></td>
<td>7.68</td>
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</table>

TC = Test compound; NMC = Normal mitotic cells (comprising prophase, metaphase, anaphase and telophase); AMC = Aberrant mitotic cells; SD = Standard deviation; CP = Cyclophosphamide; EMS = Ethyl methanesulphonate; HM = Hyraceum; MI = Mitotic index; J = P+M/A+T ratio (significant increase in ratio compared to negative control, P<0.05 in the t-test, n = 3); * = TC is Toxic (MI treatment significantly different from negative control, P<0.05 in the t-test, n = 3); † = TC is genotoxic (significant difference from negative control, P<0.05 in the t-test, n = 3); # = HM + Mutagen mixture more genotoxic than mutagen or HM alone (Synergism); ‡ = HM + Mutagen mixture less genotoxic than mutagen or HM alone (antagonism); †† = PC + Mutagen mixture more genotoxic than mutagen alone but less genotoxic than HM alone; †‡ = HM + Mutagen mixture less genotoxic than mutagen alone (antimutagenicity) but more than HM alone.
Modulatory effect of HM on EMS-induced genotoxicity

Examination of the ME in column 11 of Table 1 showed that the mixture of EMS (0.0375 mg/ml) separately with each of the three concentrations (0.016, 0.03125 and 0.0625 mg/ml) of HM induced a positive and significant (>2-fold) value of ME of 7.60, 2.44 and 7.68 respectively, and from the symbols against the ME values, each mixture was significantly more genotoxic than EMS or HM alone. The results indicated a synergistic interaction between HM extract at all three concentrations and EMS.

DISCUSSION

The results of the cytotoxicity and genotoxicity analysis in Table 1 showed that the concentrations of CP (1.00 mg/ml) and EMS (0.0375 mg/ml) used in the present study reduced the mitotic index (MI) of treated roots but the decreases were not significant (p > 0.05) and were not considered cytotoxic. They however induced GT in the root meristem cells of A. cepa. In one study, EMS at a concentration of 2 × 10^{-2} M (0.2484 mg/ml) was both toxic and mutagenic to root meristem cells of A. cepa (Çelik and Aslanı́́türk, 2010). CP at a concentration of 1% (1 mg/ml) was also both toxic, that is, significantly reduced the mitotic index (MI), and clastogenic, significantly induced chromosome aberrations, in treated onion root meristem cells (Akeem et al., 2011). In the present study, the concentration (0.0375 mg/ml) of EMS induced more types of aberrations than CP (1.00 mg/ml).

Of the three concentrations of HM extracts tested (0.016, 0.03125 and 0.0625 mg/ml) (Table 1), only 0.0625 and 0.03125 mg/ml induced significant reduction of the MI compared to the water-treated negative control (p <0.05) and were considered cytotoxic to the root meristem cells. The mixture of CP (1.00 mg/ml) or EMS (0.0375 mg/ml) with each of the three individual concentrations of extract of HM induced significant reduction of the MI compared to the water-treated negative control (p > 0.05) and were considered toxic to the root meristem cells.

A decrease in the proportion of dividing cells in A + T is an indication of metaphase arrest due to the poisoning of the spindle fibers, akin to the action of the well-documented spindle poison, colcemid (Parry et al., 1999). In the present study, none of the concentrations of the hyraceum extract tested significantly increased the (P + M)/(A + T) ratio. In a study by Ćenanović and Đuraković (2015), treatment of A. cepa root tip cells with 825 μg/ml of ascorbic acid induced a decrease in the frequency of cells in metaphase, anaphase and telophase while the frequency of cells in prophase increased significantly (p < 0.05). Decrease in the number of cells in telophase had no statistical significance.

The three concentrations (0.016, 0.03125 and 0.0625 mg/ml) of HM used and their individual mixtures with CP (1.00 mg/ml) or EMS (0.0375 mg/ml) induced statistically significant levels of aberrant mitotic cells (p < 0.05) and were therefore adjudged to be genotoxic to the root meristem cells.

The change in the GT of the mixtures compared to the extract or mutagen alone was measured by the ME, which was defined as the number of times of the mutagen-induced GT by which the GT of the mutagen alone was reduced (ME is negative) or increased (ME is positive) in mixtures with HM extracts, was 0.57 for the mixture of HM (0.016 mg/ml) with CP (1.0 mg/ml) and ME of -0.14 and -0.01 for mixture of HM (0.03125 or 0.0625 mg/ml) separately with CP. The ME values were less than two-fold increase for mixture of the lowest concentration with CP or two-fold decrease for mixtures of CP with the middle or top dose. No interaction between HM extract and CP could therefore be inferred. However, the mixture of EMS (0.0375 mg/ml) separately with each of the three concentrations (0.016, 0.03125 and 0.0625 mg/ml) of HM induced a positive and significant (>2-fold) value of ME of 7.60, 2.44 and 7.68 respectively, and each mixture was significantly more genotoxic than EMS or HM alone which indicated a synergistic interaction between HM extract and EMS in all concentrations.

There is little information on the toxicity of hyraceum. Hyraceum is reported to have been used in some areas as a poison ingredient (Watt and Breyer-Brandwijk, 1962) but it is not thought to be particularly poisonous and is used in perfumes and traditional medicine (Khoza and Hamer, 2013). As a tea, hyraceum is used to treat women’s ailments but large doses are said to result in abortion (Laidler, 1928). Castoreum, the yellowish exudate from the castor sacs of the mature North American beaver (Castor canadensis) and the European beaver (Castor fiber) is another complex product of animal metabolic waste and plant materials, used also in traditional medicine but as an analgesic, analeptic, and nerve agent to treat conditions such as amenorrhea, dysmenorrhea, hysteria, and restless sleep (Müller-Schwarze, 2003). The activity of castoreum has been credited to the accumulation of salicin from willow trees (Salix spp.) in the beaver’s diet, which is transformed to salicylic acid and has an action very similar to that of aspirin (Pincock, 2005). Camel urine, another animal metabolic waste showed cytotoxic and anti-tumor activity to Ehrlich ascite carcinoma (EAC) cells and a constituent of cow urine, creatinine, was highly cytotoxic to EAC cells (Al-Rejaie et al., 2007).

The organic matter of hyraceum contained traces of urea together with uric, hippuric and benzoic acids since hyraceum is partly derived from urine and faecal matter (Greene and Parker, 1879). In an in vitro study, sodium benzoate produced no significant chromosomal aberrations in human tissue culture cells at any of the
tested doses. The chromosome abnormalities as well as the mitotic indices were within normal values (Nair, 2001). In in vivo cytogenetic assays, the micronucleus test, or in other in vivo assays, sodium benzoate and benzyl alcohol were not genotoxic (OECD, 2001). Benzoate is known to be converted to uric and hippuric acids for excretion in urine (Bridges et al., 1970; Skinner et al., 2006).

In the present study, the three concentrations (0.016, 0.03125 and 0.0625 mg/ml) of HM were genotoxic and the two highest concentrations were, in addition, also cytotoxic to onion root tip meristem cells compared to the water-treated negative control (p <0.05).

Many herbal extracts have been reported to inhibit mitosis and reduce the mitotic index (MI) (Çelik and Aslantürk, 2007; Akinboro and Bakare, 2007) which is attributed to the inhibition of DNA synthesis, blocking in the G2 phase of the cell cycle or inhibition of protein synthesis (Kim and Bendixen, 1987; Politi et al., 2003). The significance of the cytological and chromosomal aberrations such as chromosomal stickiness, chromosome fragments, lagging chromosomes, binucleated cells and micronuclei (MN) observed in these studies has been discussed by other authors (Patil and Bhat, 1992; Singh, 2003; Krishna and Hayashi, 2000; Çelik and Aslantürk, 2010). Inhibition of cytokinesis and occurrence of binucleated cells following treatment of root tip cells with plant extracts have been reported (Kaussik, 1996; Gömürgen et al., 2005). Levan (1938) described colchicine mitosis (c- metaphase or c-anaphase) as an inactivation of the spindle followed by a random scattering of the condensed chromosomes in the cell. The induction of c-metaphase as shown in the present studies agrees with the results of some other studies which examined the effects of different medicinal herbs (Soliman, 2001; Bidau et al., 2004).

Conclusion

Both HM alone and its mixture with CP or EMS were cytotoxic and genotoxic. No interaction between HM extract and CP could therefore be inferred however there was a synergistic interaction between HM extract and EMS in the induction of genotoxicity.

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CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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