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# Modulation of Mutagen-Induced Genotoxicity by two Lesotho Medicinal Plants in *Allium cepa* L

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**Abstract**

Methanolic extracts (mg $mL^{-1}$) of *Dicoma anomala* (0.0625, 0.125, 0.25), *Chenopodium album* (0.078, 0.156, 0.313), used in traditional medicine in Lesotho, and *Camellia sinensis* (0.1094, 0.2085, 0.44) were assessed for cytotoxicity, genotoxicity and modulation of Cyclophosphamide (CP 1.25 mg $mL^{-1}$) - and EMS (0.25 mg $mL^{-1}$)-induced genotoxicity using the *Allium cepa* assay following 24 hours treatment. Cytotoxicity was measured by the mitotic index (MI). Genotoxicity (GT) was expressed as the number of aberrant mitotic cells per 100 mitotic cells. The MI and GT of test groups (triplicates), were compared with the negative (water) control group using t-test. Modulatory effect (ME) was calculated as, ME(%) = (1-(A-B)/(A-C) × 100) -1. A positive ME indicated an increase (synergism or potentiation) while a negative ME indicated a reduction (anti-genotoxicity) of mutagen-induced genotoxicity. The concentrations of *D. anomala* extract were cytotoxic and genotoxic. Mixtures of CP or EMS with the lower concentrations of *D. anomala* extract were more genotoxic than CP (250.50% increase) or EMS (149.74 and 157.37 % increase) or extracts alone. Only 0.313 mg $mL^{-1}$ of *C. album* extract was cytotoxic but none of the three concentrations was genotoxic. Mixtures of CP with extracts of *C.album* were cytotoxic. CP-induced genotoxicity was reduced (55.18, 68.36, 57.40 %) and EMS-induced genotoxicity was also reduced by low concentrations (50.72, 61.13 %) of *C.album* extract. However, 0.313 mg $mL^{-1}$ *C. album* extract increased (236.75%) EMS-induced genotoxicity. *C. sinensis* extracts and their mixtures with CP were not cytotoxic or genotoxic. CP-induced genotoxicity was reduced (63.61, 66.62, 78.64 %) but EMS-induced genotoxicity was increased (124.97, 4.48, 110.52 %) by *C. sinensis* extract.

**Keywords**: *Dicoma anomala, Chenopodium album, Camellia sinensis,* anti-genotoxicity, Cyclophosphamide, ethyl methanesulphonate.

## 1. Introduction

Different herbs and plants have been used for millennia by different cultures and peoples in traditional medicine to treat numerous diseases. It has been suggested in more recent times, that diets that have high fruit and vegetable content may help protect against cataracts, diabetes, Alzheimer's disease (Willett, 2002; Ames et al., 1993) and asthma (Woods et al., 2003). It was found in one study for instance, that people who consumed the highest amount of vegetables and fruits had a 20% lower risk of coronary heart disease and the lowest risks were observed in people who consumed more green leafy vegetables and fruits rich in vitamin C (Joshipura et al., 2001). Much of the protective effect of vegetables and fruits has been attributed to phytochemicals such as the carotenoids, flavonoids isoflavonoids and phenolic acids whose major role is protection against oxidation (Boyer & Liu, 2004; Wong et al., 2012). Phytochemicals are thought to act as a proton sink that synergistically bring about the antioxidant and free radical scavenging potentials observed (Alisi et al., 2011; Ndhlala et al., 2013). Many studies have also shown that the consumption of naturally occurring compounds can modify the mutagenic and carcinogenic effects of environmental contaminants (Debisri & Archana, 1996; Gimmler-Luz et al., 1999).

The benefits of vegetables and fruits not withstanding however, recent investigations have revealed that many plants used as food or in traditional medicine have mutagenic, cytotoxic and genotoxic effects in *in vitro* and *in vivo* assays (Higashimoto et al., 1993; Schimmer et al., 1994; Kassie et al., 1996; Çelik & Aslantürk, 2007). Medicinal herbs have also been shown to cause adverse effects or have the potential to interact with other medications (Zink & Chaffin, 1998). Many plants contain mutagenic and/or carcinogenic substances (Ames, 1986; de Sá Ferreira & Ferrão Vargas, 1999) and their use has been correlated with high rate of tumor formation

in some human populations (Wynder et al., 1983; Ames, 1986; Nagao et al., 1986; Nguyen et al., 1989; Brito et al., 1990), which raises concern about the potential mutagenic or genotoxic hazards that could result from the long-term use of such plants as food or medicine. For many of the plants used in traditional medicine, not enough information is available on their potential risk to health (Basaran et al., 1996).

The extracts of some plants have been observed to induce both mutagenic and antimutagenic effects on known mutagens in different test systems (Debisri & Archana, 1996). The natural plant products that induce mutations and modify the action of other known mutagens on the living organisms do so by either, inactivating the existing mutagens within the cell (desmutagens), inhibiting the production of mutagens in the cell (bioantimutagens), synergizing the activity of existing mutagens (comutagens) or activating the promutagens within the cell into mutagens (Debisri & Archana, 1996).

Two plant species commonly used in traditional medicine in Southern Africa are *Dicoma anomala* Sond (Hloenya in Sesotho) and *Chenopodium album (Seruoe* in Sesotho). *D. anomala*, common name fever bush or stomach bush, is an erect, sub-erect or prostrate perennial herb belonging to the family, Asteraceae. It bears aromatic semi-woody underground tubers at the base of a woody subterranean stem. Two subspecies of D. *anomala* occur in South Africa: *D. anomala* sub sp *anomala* and *D. anomala* subsp. *gerrardii* (Becker et al., 2011). Phytochemical investigations of *D. anomala* have identified several classes of secondary metabolites; including acetylenic compounds, phenolic acids, flavonoids, sesquiterpene lactones, triterpenes and phytosterols (Becker et al., 2011). *Dicoma anomala* is used as a root decoction for blood disorders, colic, diarrhoea, dysentery, toothache, and purgative for intestinal worms (Kazembe & Chinyuku, 2012). *Chenopodium album* L. (family Chenopodiaceae, genus Chenopodium) is an annual shrub widely grown in Europe, North America, Asia, and Africa. It is commonly known as WhiteGoose foot, pigweed, fat hen or lamb-quarters' (Bailey, 1977; GRIN Database, 2005). Phytochemical analyses revealed the presence of alkaloids (Horio et al., 1993; Cutillo et al., 2004), apocarotenoids (DellaGreca et al., 2004), flavonoids (Gohar & Elmazar, 1997), phytoecdysteroids (Dinan, 1992; Dinan et al., 1998; DellaGreca et al., 2005a) and an unusual xyloside (DellaGreca et al., 2005b) in the plant. Concoctions of the leaves are used in Lesotho for the treatment of abdominal pain, worm infection, pulmonary and nervous obstruction (Shale et al., 1999). *Camellia sinensis* (family Theaceae) is a plant that is native to mainland South and Southeast Asia, especially India and China (Ming, 1992). The leaves are used in the production of green tea. Green tea (non-fermented) is derived directly from drying and steaming of fresh tea leaves (Zuo et al., 2002). Green tea contains mainly polyphenolic catechins such as (-)-epicatechin, (+)-gallocatechin, (+) catechin as well as gallic acid and caffeine, to which some of its beneficial effects have been attributed (Gupta et al., 2002). *Camellia sinensis* was included in the study so that it could be used for comparative analysis since its phytochemistry, antioxidant activity and biochemistry have been extensively studied (Buřičova & Reblova, 2008).

Studies on agents that modulate carcinogen-induced genotoxic effects in experimental animals provide end points that can be used for assessing the antimutagenic or anticarcinogenic properties of putative chemopreventive compounds and for predicting their protective efficacy in humans (Khaidakov et al., 2001).

Cyclophosphamide (CP) is an antineoplastic drug that belongs to the nitrogen mustered family of alkylating agents which include chlorambucil and busulpfan which are used for the treatment of various cancers and some autoimmune diseases. These drugs work by interrupting the cell cycle by forming DNA cross linking, DNA lesions and mediates G0/G1 and S phase arrest (Baumann & Preiss, 2001). Cyclophosphamide is an indirect-acting agent (promutagen) that is first oxidized by the microsomal cytochrome P450-linked enzyme to be further converted into its biologically reactive ultimate metabolites, acrolein, phosphoramide mustard and nornitrogen mustard. Phosphoramide mustard alkylates DNA (Mohn & Ellenberger, 1976; Hales, 1982). At chromosomal level, CP causes chromosomal aberration and increase in frequency of sister chromatid exchange. It also causes apoptosis and cell toxicity (Panigrahy et al., 2011).

Ethyl methanesulphonate (EMS) is a colorless liquid used experimentally as a mutagen, tetratogen, and brain carcinogen and as a research chemical (Stubbs et al., 1997). EMS has been used in a wide variety of biological test systems in studies of mutation effects (Sega, 1984; Platzek et al., 1995; Bökel, 2008). EMS induces DNA damage by a direct mechanism, acting at various sites as a monofunctional ethylating agent of nucleotides (Müller et al., 2009).

The *Allium cepa* L assay is one of the established plant bioassays, validated by the international programme on chemical safety (IPCS, WHO), as an efficient and standard test for chemicals screening and *in situ* monitoring of the genotoxicity of environmental substances (Leme & Marin-Morales, 2009). This plant bears a few chromosomes (2n = 16) that are large, which facilitates the evaluation of chromosome damages and/or

disturbances in cell division cycle, including eventual aneuploidy risks (Grant, 1982; Fiskesjö, 1985; Leme & Marin- Morales, 2008). *Allium cepa* is used to study basic mechanisms as well as the effects of environmental contaminants on cell division and chromosome. The results obtained from such studies, using the *Allium cepa* assay have been shown to be similar with those of mammalian and non-mammalian test systems (Constantin & Owen 1982; Fiskesjö, 1985; Cauhan et al., 1999; Aydemir et al., 2008). Finally, plants have monooxygenase enzyme systems that are to a certain degree similar to the mammal monooxygenase enzyme complex, although the plant enzyme complex possesses a number of distinguishing characteristics (Plewa & Gentile, 1982; Higashi, 1988). Onion root cells also have the ability to activate promutagens (the MFO-system) (Fiskesjö, 1985).

We are not aware of any published data on the genotoxic and antimutagenic effects of *Dicoma anomala* and *Chenopodium album* despite the fact that different parts of these plant species are widely used in traditional medicine. The purpose of this study was to assess the methanolic extracts of *Dicoma anomala*, *Chenopodium album* and, the refence *Camellia sinensis* (green tea) for cytotoxicity, genotoxicity and modulation of the genotoxicity induced by cyclophosphamide (CP) and Ethyl methanesulphonate (EMS) using the *Allium cepa* root meristem cells chromosome aberration assay system.

## 2. Materials and Methods

*Test organism***:** Onion (*Allium cepa*) seeds: variety of Texas Grano 502 P.R.R. was a product of Sakata seeds, Lanseria 1748, Republic of South Africa and were purchased from Maseru garden centre, Lesotho, Southern Africa.

*Mutagens and chemicals:* Cyclophosphamide (CP) was a product of Sigma Chemical Company and Ethylmethane sulphonate was a product of Fluka. Methanol (Absolute) was a product of Associated Chemical Enterprises (PTY) LTD of The Republic of South Africa; Hydrochloric acid and Glacial acetic acid were products of UNILAB of The Republic of South Africa; Aceto-carmine stain from Carolina Biological Supply Company, USA.

Plant material and preparation of crude extracts: The plant parts used were as follows: Dicoma anomala (root), Chenopodium album (leaves) and Camellia sinensis (leaves). Dicoma anomala and Chenopodium album were collected from the outskirts of the National University of Lesotho, and authenticated by the curator of the Herbarium at the Department of Biology of university, where the voucher specimens of these plants have been deposited. Camellia sinensis (leaves) were purchased as green tea from a local Chinese shop as EVE'S GREEN TEA, LOT 13T-TO15, which was packed in China for Evergrow Import & Export CC, T/A EVE'S TEA HOUSE, Emmarentia, Johannesburg, South Africa. The crude extracts were prepared according to the method of Adedapo et al. (2009), with slight modifications. Briefly, chopped and dried roots of Dicoma anomala were dried in an oven at 37$^{o}$ C for 48 hours and ground into powder. The leaves of Chenopodium album and Camellia sinensis were also dried and ground. The powders were stored in the refrigerator at 4$^{o}$ C until use. Extraction was carried out at 1g 50mL$^{-1}$ of 98% methanol. The mixture was then shaken on the orbital shaker for 48h. The extract was filtered through No.1 Whatman filter paper (Whatman Ltd., England) using suction. The filtrates obtained were concentrated under vacuum in a rotary evaporator and oven dried at 35ºC for 12 hours to constant weight and stored at 4$^{o}$ C in the refrigerator till used.

### *2.1 Experiments to Select Concentrations of Plant Extracts to Use*

A preliminary dose selection experiment was conducted to determine the doses of test compounds i.e. CP, EMS and plant extract to be used in the actual experiments according to the method of Asita & Makhalemele (2008). The $EC_{50s}$ (mg/mL) values of the test compounds were approximately, CP (2.5); EMS (1.00); *Dicoma anomala* (1.5); *Chenopodium album* (1.25) and *Camellia sinensis* (1.75). The EC50s of the test compounds proved to be too toxic in trial experiments. The following concentrations (mg mL$^{-1}$) of the mutagens and crude plant extracts were therefore used in the genotoxicity tests; CP (1.5); EMS (0.25), *Dicoma anomala* (0.0625, 0.125 and 0.25); *Chenopodium album* (0.078, 0.156 and 0.313) and *Camellia sinensis* (0.1094, 0.2085 and 0.44).

### *2.2 Genotoxicity Assay*

The method used was similar to the method of Asita & Makhalemele (2008). *Alium cepa* (onion) seeds were spread on water moistened filter paper in a petri dish, at room temperature (22 ± 2 °C), until they germinated (about 72 to 96 hours) and the radicles reached a length of about 2 cm. Ten seedlings were transferred onto a filter paper kept moistened in a petri dish with CP (1.5 mg/mL), EMS (0.25 mg/mL), extracts of *Dicoma anomala* (0.0625, 0.125 and 0.25); *Chenopodium album* (0.078, 0.156 and 0.313) and *Camellia sinensis* (0.1094, 0.2085 and 0.44) or mixtures of CP or EMS with each of the three different concentrations of each plant extract, for 24 hours at room temperature. Distilled water was used as a negative control.

In one study, the methanolic extract of *Trigonella foenum-graecum* was more effective at reducing $CdSO_4$-induced mutagenicity in the *Allium sepa* assay when roots were treated simultaneously with extracts and $CdSO_4$ compared to separate treatments with extract first before mutagen or mutagen first before extract (Mekki, 2014).

*2.3 Root Harvest and Slide Preparation*

After 24 hours of exposure, three root tips from three seeds per dose were collected at random and assessed as described by Asita & Makhalemele (2008). The slides were coded, viewed, using oil immersion, under the light microscope (Olympus CX 21) at 1000 x magnification and scored blind for normal and aberrant cells in the different stages of the cell cycle. The most representative ones for each structural aberration class were photographed using a Zeiss PrimoStar microscope mounted with Canon camera model, Power Shot A640.

*2.4 Scoring of Slides and Data Analysis*

2.4.1 Scoring of Slides

On each of three slides (n = 3) per treatment, a total of 2000 cells, classified into interphase or dividing cell i.e. Prophase (Normal, N or aberrant, ABN); Metaphase (N or ABN); Anaphase (N or ABN) or Telophase (N or ABN) were scored, i.e. a total of 6000 cells each for the control and treatment groups.

2.4.2 Cytotoxicity

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

$$MI = \text{Number of dividing cells/Total number of cells scored x } 100. \quad (1)$$

The MI was used as a measure of cytotoxicity (CT). The MI of each treatment group was compared with that of the negative control group using t-test at a probability level of 0.05, using the SPSS for windows, version 11.0 software.

2.4.3 Genotoxicity

Genotoxicity (GT) was expressed as the number of aberrant mitotic cells (AMC) per 100 mitotic cells [i.e AMC + normal mitotic cells (NMC)] scored according to the formula:

$$\text{Frequency of AMC (\%)} = AMC/(AMC + NMC) \times 100 \quad (2)$$

The percentage of aberrant mitotic cells (AMC) in each group of three slides per concentration of test agent was compared with that of the negative control group using t-test. P values less than 0.05 ($P < 0.05$) were considered as indicative of significance.

2.4.4 Modulatory Effect (ME) of Plant Extracts on Mutagen-Induced Genotoxicity

The modulatory effect (ME) of plant extract on CP- or EMS-induced genotoxicity (GT) was calculated using a modified formula of Akeem et al. (2011):

$$ME(\%) = (1-(A-B)/(A-C) \times 100) -1 \quad (3)$$

Where, ME is the percentage reduction (ME is negative) or increase (ME is positive) of genotoxicity (GT); 'A' is the frequency of GT induced by CP or EMS alone; 'B' is the frequency of GT induced by each mixture of plant extract and CP or EMS and 'C' is the frequency of GT induced by tap water alone.

A positive (+) value of ME indicated that the mixture of mutagen and plant extract was more genotoxic than mutagen alone but may be more (synergism: if both extract and mutagen were mutagenic) or less (mutagen potentiation: if extract was not mutagenic) genotoxic than the plant extract alone. A negative (-) value of ME indicated that the mixture of mutagen and plant extract was less genotoxic than mutagen alone (anti-genotoxicity) but may be less (antagonism: if both extract and mutagen were genotoxic but mixture was not) or more (extract potentiation by mutagen if extract was not genotoxic) genotoxic than the plant extract alone.

2.4.5 Data Analysis

Data were expressed as mean ± S.D. of three values. Differences between controls and treatment groups were determined using Student's t-test. P values of less than 0.05 were considered statistically significant.

## 3. Results

*3.1 Chromosome Aberrations*

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 aberrant dividing cells observed and scored contained the following types of aberrations: For treatments with *D. anomala* were sticky

chromosomes (S) and c-mitosis (C-Mit); sticky chromosomes (S), c-mitosis (C-Mit), chromosome largards (L)., anaphase and telophase bridges (A.B) and chromosome breaks for treatments with *C. album*; sticky chromosomes (S), c-mitosis (C-Mit), chromosome largards (L)., anaphase and telophase bridges (A.B) and chromosome breaks for treatments with *C. sinensis* and EMS.

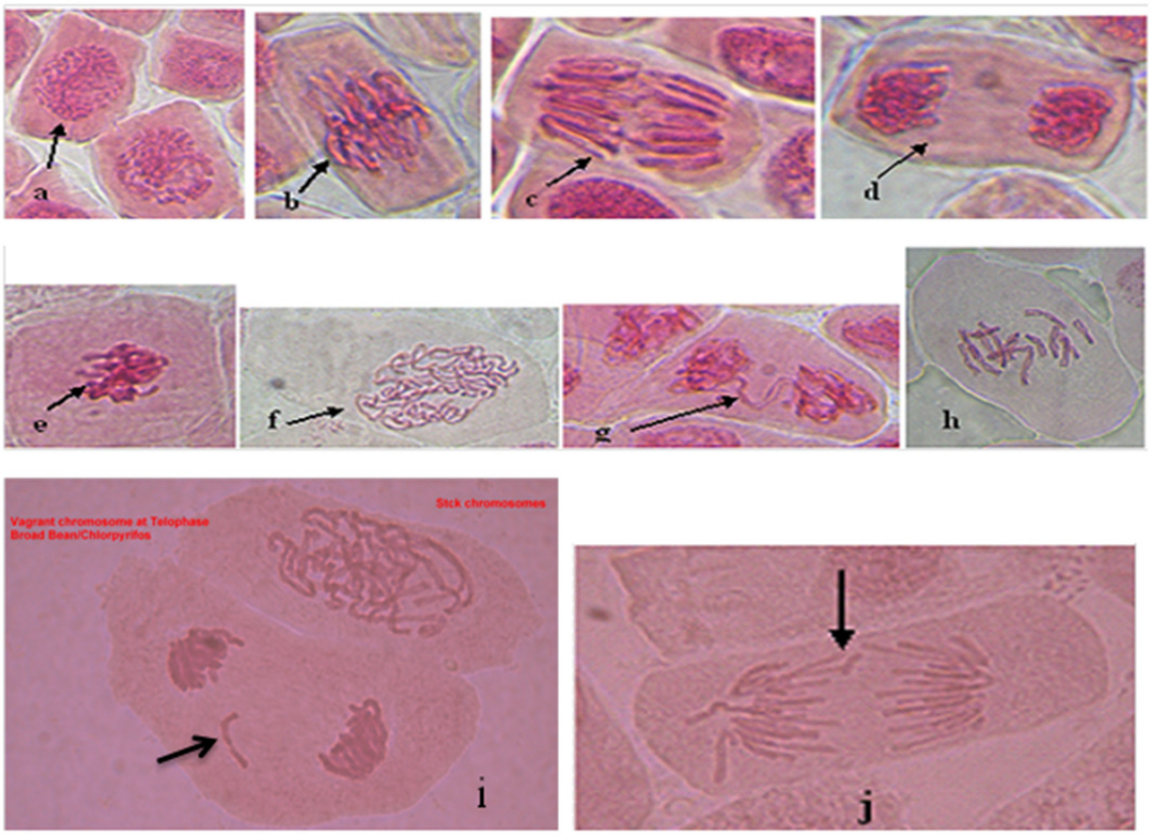


Figure 1. Photographs of cells of *Allium cepa* showing untreated cells in normal division stages and cells treated with mutagen, plant extract and mixtures of plant extract and mutagen showing the different types of chromosomal abnormalities observed. Magnification is 1000 X. (a) Normal prophase cell (b) Normal metaphase (c) Normal anaphase (d) Normal telophase (e) Sticky metaphase (f) sticky prophase cell (g) Chromosome bridge at telophase (h) C-mitosis (i) Vagrant or laggard chromosome (j) lagging chromosome fragment

### *3.2 Cytotoxicity and Genotoxicity*

The results of the cytotoxicity and genotoxicity experiments with the extracts of the three different plants and the mutagens are presented in tables 1, 2 and 3. In table 4 is presented a summary of the information in Tables 1, 2 and 3.

#### 3.2.1 Cytotoxicity and Genotoxicity Analysis of *D. Anomala* Extract

In Table 1 are the results of cytotoxicity and genotoxicity experiments with extract of *D. anomala* and the mutagens, CP and EMS.

*(P+M)/ (A+T) Ratio:* Examination of the (P+M)/(A+T) ratio in column 8 of Table 1 shows that the test agents, i.e. CP, EMS or different concentrations of *D. anomala* extract alone or their separate mixtures with CP or EMS, did not induce any 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 (1.25 mg $mL^{-1}$) or EMS (0.25 mg $mL^{-1}$) used was not toxic to the root meristem cells of *A. sepa* when compared to the water treated negative control ($P>0.05$). The two highest concentrations of *D. anomala* extract (0.125 and 0.25 mg $mL^{-1}$) were toxic ($P<0.05$). The mixture of CP separately with each of the three different concentrations of *D. anomala*

extract was toxic to the root meristem cells ($P < 0.05$). The mixture of EMS separately with the lowest (0.0625 mg $mL^{-1}$) or highest (0.25 mg $mL^{-1}$) concentration of *D. anomala* extract was toxic to the root meristem cells ($P < 0.05$). The mixture of EMS separately with the middle (0.125 mg $mL^{-1}$) concentration of plant extract was not toxic to the root meristem cells ($P > 0.05$).

*Genotoxicity (GT)*: Examination of induction of genotoxicity in column 10 of Table 1 shows that the concentration of CP (1.25 mg $mL^{-1}$) or EMS (0.25 mg $mL^{-1}$) used was genotoxic to the root meristem cells of *A. sepa* when compared to the water treated negative control ($P<0.05$). All three concentrations of *D. anomala* extract were genotoxic ($P<0.05$) and the genotoxicity was concentration dependent. The mixture of CP separately with each of the three different concentrations of *D. anomala* extract was genotoxic to the root meristem cells ($P <0.05$). Each mixture of EMS separately with each of the three different concentrations of plant extract was also genotoxic to the root meristem cells ($P<0.05$).

Table 1. Cytotoxicity and Genotoxicity of extract of *D .anomala* (root), EMS and CP to meristem cells of onion root tip and the Modulatory effects (ME) of *D .anomala* extracts (PE) on EMS- and CP-induced Genotoxicity

| | | Cells Scored | | | | | | | | Modulatory Effect on Genotoxicity | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Cells in mitosis | | | | | | | | |
| TC concentration (mg $mL^{-1}$) & Treatment | Statistics | Cells in interphase | N | ABN | Total cells in mitosis | Total Number of Cells Scored | (P+M)/ (A+T) | MI | Genotoxicity | Plant Extract on CP | Plant Extract on EMS |
| | MEAN | 1855.00 | 145.00 | 0.00 | 145.00 | 2000 | 3.02 | 7.25 | 0.00 | | |
| Water | SD | 42.46 | 42.46 | 0.00 | 42.46 | 0 | 1.47 | 2.12 | 0.00 | | |
| | MEAN | 1894.33 | 83.67 | 22.00 | 105.67 | 2000 | 3.83 | 5.28 | 22.05# | | |
| CP (1.25) | SD | 38.03 | 34.93 | 5.57 | 38.03 | 0 | 3.50 | 1.90 | 5.95 | | |
| | MEAN | 1892.00 | 80.67 | 27.33 | 108.00 | 2000 | 3.34 | 5.40 | 27.22# | | |
| EMS (0.25) | SD | 24.76 | 34.02 | 13.80 | 24.76 | 0 | 0.26 | 1.24 | 18.27 | | |
| | MEAN | 1852.00 | 115.67 | 32.33 | 148.00 | 2000 | 2.38 | 7.40 | 24.25# | | |
| PE (0.0625) | SD | 34.18 | 49 | 14.84 | 34.65 | 0 | 0.10 | 1.71 | 15.65 | | |
| | MEAN | 1952.33 | 19.67 | 28.00 | 47.67 | 2000.00 | 3.67 | 2.38* | 62.30# | | |
| PE (0.125) | SD | 32.88 | 17.39 | 21.93 | 32.88 | 0.00 | 1.37 | 1.64 | 37.16 | | |
| | MEAN | 1947.00 | 0.67 | 52.33 | 53.00 | 2000.00 | 9.96 | 2.65* | 99.05# | | |
| PE (0.25) | SD | 17.00 | 1.15 | 16.01 | 17.00 | 0.00 | 9.72 | 0.85 | 1.65 | | |
| CP + PE | MEAN | 1961.33 | 10.00 | 28.67 | 38.67 | 2000.00 | 1.95 | 1.93* | 77.29# | 251.50+† | |
| (0.0625) | SD | 21.39 | 15.62 | 19.43 | 21.39 | 0.00 | 1.50 | 1.07 | 27.89 | | |
| CP + PE | MEAN | 1939.33 | 30.67 | 30.00 | 60.67 | 2000.00 | 3.30 | 3.03* | 51.74# | 135.62† | |
| (0.125) | SD | 36.02 | 20.50 | 15.52 | 36.02 | 0.00 | 0.58 | 1.80 | 5.92 | | |
| | MEAN | 1980.00 | 0.67 | 19.33 | 20.00 | 2000.00 | 3.43 | 1.00* | 92.00# | 318.21† | |
| CP + PE (0.25) | SD | 13.23 | 0.58 | 13.61 | 13.23 | 0.00 | 0.99 | 0.66 | 10.58 | | |
| EMS + PE | MEAN | 1974.67 | 12.33 | 13.00 | 25.33 | 2000.00 | 2.06 | 1.27* | 67.98# | | 150.74+† |
| (0.0625) | SD | 16.44 | 18.01 | 2.00 | 16.44 | 0.00 | 0.27 | 0.82 | 38.68 | | |
| EMS + PE | MEAN | 1946.33 | 11.67 | 42.00 | 53.67 | 2000.00 | 2.28 | 2.68 | 70.06# | | 158.37+ † |
| (0.125) | SD | 61.78 | 10.02 | 53.02 | 61.78 | 0.00 | 0.62 | 3.09 | 27.19 | | |
| EMS + PE | MEAN | 1949.33 | 20.00 | 30.67 | 50.67 | 2000.00 | 3.35 | 2.53* | 45.88# | | 69.55† |
| (0.25) | SD | 47.18 | 9.54 | 42.10 | 47.18 | 0.00 | 0.59 | 2.36 | 34.82 | | |

TC = Test compound; N = Normal mitotic cells (comprising prophase, metaphase, anaphase and telophase); ABN = Aberrant mitotic cells; SD = Standard deviation; CP = Cyclophosphamide; EMS = Ethylmethane sulphonate; PE = Plant extract; MI = Mitotic index; * = 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); † = PE + Mutagen mixture more genotoxic than mutagen alone but more or less than PE alone; + † = PE + Mutagen mixture more genotoxic than mutagen or PE alone; ‡ = PE + Mutagen mixture less genotoxic than mutagen alone (reduction of genotoxicity) but more or less than PE alone; +‡ = PE + Mutagen mixture less genotoxic than mutagen or PE alone.

*Modulatory effect (ME) of plant extract on CP-induced Genotoxicity (GT):* Examination of the ME in column 11 of Table 1 shows that the mixture of each concentration mg $mL^{-1}$) of *D. anomala* extract with CP produced a positive value of ME (0.0625 = 250.50; 0.125 = 134.62 and 0.25 = 317.21). The mixtures were more genotoxic than the CP alone. In addition, the genotoxicity of the mixture of CP and the lowest concentration of *D. anomala* extract was greater than that of CP or plant extract alone. This observation suggests a synergistic effect of CP and *D. anomala* extract at low plant extract concentrations. However, the genotoxicity of the separate mixture of CP and the middle or top concentration of the plant extract was greater than that of CP alone but less than that of *D. anomala* extract alone.

*Modulatory effect (ME) of plant extract on EMS-induced Genotoxicity (GT):* Examination of the ME in column 12 of Table 1 shows that the mixture of each concentration (mg $mL^{-1}$) of *D. anomala* extract and EMS produced a positive value of ME (0.0625 = 149.74; 0.125 = 157.37 and 0.25 = 68.55). The mixtures were more genotoxic than the EMS alone. In addition, the genotoxicity of the mixture of EMS with the lowest or middle concentration of *D. anomala* extract was greater than that of EMS or *D. anomala* extract alone. This observation suggests a synergistic effect of EMS and the plant extract at low plant extract concentrations. However, the genotoxicity of the mixture of EMS and the highest concentration of the *D. anomala* extract was greater than that of EMS alone but less than that of the plant extract alone. It has to be noted that the middle and highest concentrations of *D. anomala* extracts and all *D. anomala* extract and mutagen mixtures were all toxic with low mitotic indices (MI).

3.2.2 Cytotoxicity and Genotoxicity Analysis of *C. album* Extract

In Table 2 are the results of cytotoxicity and genotoxicity experiments with extract of *C. album* and the mutagens, CP and EMS.

*(P+M)/ (A+T) Ratio:* Examination of the (P+M)/(A+T) ratio in column 8 of Table 2 shows that the test agents, i.e. CP, EMS, or different concentrations of *C. album* extract alone or their separate mixtures with CP or EMS, did not induce any 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 2 shows that the concentration (mg $mL^{-1}$) of CP (1.25) or EMS (0.25) used was not toxic to the root meristem cells of *A. sepa* when compared to the water treated negative control ($P>0.05$). Only the highest concentration (mg $mL^{-1}$) of *C. album* extract (0.313) was toxic ($P<0.05$). Each separate mixture of CP with each of the three different concentrations of *C. album* extract was toxic to the root meristem cells. Each mixture of EMS separately with each of the three different concentrations of *C. album* extract was toxic to the root meristem cells ($P<0.05$).

*Genotoxicity (GT)*: Examination of the induction of genotoxicity in column 10 of Table 2 shows that the concentration (mg $mL^{-1}$) of CP (1.25) or EMS (0.25) used was genotoxic to the root meristem cells of *A. sepa* when compared to the water treated negative control ($P<0.05$). None of the three different concentrations of *C. album* extract was genotoxic ($P>0.05$) when compared to the water treated negative control group. None of the mixtures of CP separately with each of the three different concentrations of *C. album* extract was genotoxic to the root meristem cells ($P > 0.05$). Only the mixture of EMS separately with the highest concentration of *C. album* extract (0.313 mg $mL^{-1}$) was genotoxic to the root meristem cells ($P<0.05$).

*Modulatory effect (ME) of plant extract on CP-induced Genotoxicity (GT):* Examination of the ME in column 11 of Table 2 shows that the mixture of each concentration (mg $mL^{-1}$) of *C. album* extract separately with CP produced a negative value of ME (0.078 = -55.18; 0.156 = -68.36 and 0.313 = -57.40). Thus the mixtures were less genotoxic than CP alone. Because all the three concentrations of *C. album* extract and their individual mixtures with CP were not genotoxic, though CP alone was genetoxic, the results suggest the *C. album* extracts abolished the genotoxic effect of CP and was therefore exhibited anti-genotoxic effect to CP-induced genotoxicity.

*Modulatory effect (ME) of plant extract on EMS-induced Genotoxicity (GT):* Examination of the ME in column 12 of Table 2 shows that the mixture EMS with the lowest and middle concentrations (mg $mL^{-1}$) of *C. album* extract were less genotoxic than EMS alone thereby producing negative ME values (0.078 = -50.72; 0.156 = -61.13). However, the mixture of EMS with the highest concentration of *C. album* extract was more genotoxic than EMS alone thereby producing positive ME value (0.313 = 236.75). The mixture of EMS and the lowest or middle concentration of *C. album* extract was not genotoxic when compared with the negative control in the t-test statistical analysis. Therefore the lowest and middle concentrations of *C. album* extract abolished the genotoxic effect of EMS in the mixtures and were therefore anti-genotoxic against EMS-induced genotoxicity. The mixture of EMS and the highest concentration of *C. album* extract was more genotoxicity than EMS or *C. album* extract alone, which suggested a synergistic effect of EMS and the *C. album* extract at high concentrations.

Table 2. Cytotoxicity and Genotoxicity of extract of *C. album* (leaves), EMS and CP to meristem cells of onion root tip and the Modulatory effects (ME) of *C. album* extracts (PE) on EMS- and CP-induced Genotoxicity

| | | Cells Scored | | | | | | | | Modulatory Effect on Genotoxicity | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Cells in mitosis | | | | | | | | |
| TC concentration (mg mL$^{-1}$) & Treatment | Statistics | Cells in interphase | N | ABN | Total cells in mitosis | Total Number of Cells Scored | (P+M)/ (A+T) | MI | Genotoxicity | Plant Extract on CP | Plant Extract on EMS |
| | MEAN | 1855.00 | 145.00 | 0.00 | 145.00 | 2000.00 | 3.02 | 7.25 | 0.00 | | |
| Water | SD | 42.46 | 42.46 | 0.00 | 42.46 | 0.00 | 1.47 | 2.12 | 0.00 | | |
| | MEAN | 1894.33 | 83.67 | 22.00 | 105.67 | 2000.00 | 3.83 | 5.28 | 22.05# | | |
| CP (1.25) | SD | 38.03 | 34.93 | 5.57 | 38.03 | 0.00 | 3.50 | 1.90 | 5.95 | | |
| | MEAN | 1892.00 | 80.67 | 27.33 | 108.00 | 2000.00 | 3.34 | 5.40 | 27.22# | | |
| EMS (0.25) | SD | 24.76 | 34.02 | 13.80 | 24.76 | 0.00 | 0.26 | 1.24 | 18.27 | | |
| | MEAN | 1880.33 | 117.67 | 2.00 | 119.67 | 2000.00 | 7.48 | 5.98 | 2.94 | | |
| PE (0.078) | SD | 45.37 | 48.79 | 3.46 | 45.37 | 0.00 | 7.38 | 2.27 | 5.09 | | |
| | MEAN | 1893.67 | 86.33 | 20.00 | 106.33 | 2000.00 | 3.05 | 5.32 | 19.77 | | |
| PE (0.156) | SD | 21.20 | 40.27 | 29.60 | 21.20 | 0.00 | 1.46 | 1.06 | 28.49 | | |
| | MEAN | 1973.00 | 27.00 | 0.00 | 27.00 | 2000.00 | 7.73 | 1.35* | 0.00 | | |
| PE (0.313) | SD | 14.73 | 14.73 | 0.00 | 14.73 | 0.00 | 8.08 | 0.74 | 0.00 | | |
| | MEAN | 1966.33 | 31.33 | 2.33 | 33.67 | 2000 | 13.78 | 1.68* | 9.88 | -54.18‡ | |
| CP + PE (0.078) | SD | 22.85 | 23.44 | 2.52 | 22.85 | 0 | 10.02 | 1.14 | 14.33 | | |
| | MEAN | 1972 | 25 | 3 | 28 | 2000 | 9.71 | 1.4* | 6.98 | -67.36‡ | |
| CP + PE (0.156) | SD | 17.35 | 13.89 | 5.2 | 17.35 | 0 | 4.97 | 0.87 | 12.08 | | |
| | MEAN | 1988 | 11 | 1 | 12 | 2000 | 8.78 | 0.6* | 9.39 | -56.40‡ | |
| CP + PE (0.313) | SD | 2.65 | 3.46 | 1 | 2.65 | 0 | 5.34 | 0.13 | 9.11 | | |
| | MEAN | 1943.33 | 48.00 | 8.67 | 56.67 | 2000.00 | 13.65 | 2.83* | 13.41 | | -49.72‡ |
| EMS + PE (0.078) | SD | 52.94 | 45.21 | 8.50 | 52.94 | 0.00 | 18.50 | 2.65 | 12.89 | | |
| | MEAN | 1974.00 | 22.67 | 3.33 | 26.00 | 2000.00 | 10.00 | 1.30* | 10.58 | | -60.13‡ |
| EMS + PE (0.156) | SD | 22.65 | 20.40 | 2.89 | 22.65 | 0.00 | 10.39 | 1.13 | 10.88 | | |
| | MEAN | 1996.00 | 0.67 | 3.33 | 4.00 | 2000.00 | 3.67 | 0.20* | 91.67# | | 237.75+† |
| EMS + PE (0.313) | SD | 3.61 | 1.15 | 2.52 | 3.61 | 0.00 | 3.79 | 0.18 | 14.43 | | |

TC = Test compound; N = Normal mitotic cells (comprising prophase, metaphase, anaphase and telophase); ABN = Aberrant mitotic cells; SD = Standard deviation; CP = Cyclophosphamide; EMS = Ethylmethane sulphonate; PE = Plant extract; MI = Mitotic index; * = 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); † = PE + Mutagen mixture more genotoxic than mutagen alone but more or less than PE alone; + † = PE + Mutagen mixture more genotoxic than mutagen or PE alone; ‡ = PE + Mutagen mixture less genotoxic than mutagen alone (reduction of genotoxicity) but more or less than PE alone; +‡ = PE + Mutagen mixture less genotoxic than mutagen or PE alone.

### 3.2.3 Cytotoxicity and Genotoxicity Analysis of *C. sinensis* Extract

In Table 3 are the results of cytotoxicity and genotoxicity experiments with extract of *C. sinensis* and the mutagens, CP and EMS.

*(P+M)/ (A+T) Ratio:* Examination of the (P+M)/(A+T) ratio in column 8 of Table 3 shows that the test agents, i.e. CP, EMS, *C. sinensis* extracts and separate mixtures of CP or EMS with either of the three different concentrations of *C. sinensis* extract, did not induce any significant reduction in the (P+M)/ (A+T) ratio, when compared with the water treated negative control group ($P>0.05$).

Table 3. Cytotoxicity and Genotoxicity of extract of *C sinensis* (leaves), EMS and CP to meristem cells of onion root tip and the Modulatory effects (ME) of *C sinensis* extract (PE) on EMS- and CP-induced Genotoxicity

| TC concentration (mg $mL^{-1}$) & Treatment | Statistic | Cells Scored | | | | | (P+M)/ (A+T) | MI | Genotoxicity | Modulatory Effect on Genotoxicity | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Cells in mitosis | | | Total Number of Cells Scored | | | | | |
| | | Cells in interphase | N | ABN | Total number | | | | | Plant Extract on CP | Plant Extract on EMS |
| Water | MEAN | 1855.00 | 144.67 | 0 | 144.67 | 2000 | 3.02 | 7.23 | 0.00 | | |
| | SD | 42.46 | 42.77 | 0 | 42.77 | 0 | 1.47 | 2.14 | 0.00 | | |
| CP (1.25) | MEAN | 1894.33 | 83.33 | 22 | 105.33 | 2000 | 3.83 | 5.27 | 22.20# | | |
| | SD | 38.03 | 35.47 | 5.57 | 38.59 | 0 | 3.5 | 1.93 | 6.13 | | |
| EMS (0.25) | MEAN | 1892.00 | 80.33 | 27.33 | 107.67 | 2000 | 3.34 | 5.38 | 27.40# | | |
| | SD | 24.76 | 34.53 | 13.8 | 25.15 | 0 | 0.26 | 1.26 | 18.59 | | |
| PE (0.1094) | MEAN | 1880.67 | 117.33 | 2 | 119.33 | 2000 | 3.08 | 5.97 | 2.13 | | |
| | SD | 17.01 | 20.43 | 3.46 | 17.01 | 0 | 0.12 | 0.85 | 3.69 | | |
| PE (0.2085) | MEAN | 1914.67 | 68.67 | 16.67 | 85.33 | 2000 | 4.14 | 4.27 | 2.25 | | |
| | SD | 33.65 | 33.13 | 0.58 | 33.65 | 0 | 0.96 | 1.68 | 2.23 | | |
| PE (0.44) | MEAN | 1936.67 | 56.67 | 6.67 | 63.33 | 2000 | 5.05 | 3.17 | 7.70 | | |
| | SD | 50.85 | 51 | 9.87 | 50.85 | 0 | 2.65 | 2.54 | 7.21 | | |
| CP + PE (0.1094) | MEAN | 1881.00 | 108.67 | 10.33 | 119 | 2000 | 4.23 | 5.95 | 8.02 | -62.61‡ | |
| | SD | 10.44 | 25.81 | 15.37 | 10.44 | 0 | 1.13 | 0.52 | 11.82 | | |
| CP + PE (0.2085) | MEAN | 1877.67 | 113.67 | 8.67 | 122.33 | 2000 | 4.17 | 6.12 | 7.36 | -65.62‡ | |
| | SD | 15.50 | 7.23 | 8.62 | 15.5 | 0 | 0.29 | 0.78 | 6.97 | | |
| CP +PE (0.44) | MEAN | 1919.67 | 77 | 3.33 | 80.33 | 2000 | 4.61 | 4.02 | 4.71 | -77.64‡ | |
| | SD | 39.63 | 38.43 | 2.89 | 39.63 | 0 | 1.20 | 1.98 | 5.03 | | |
| EMS + PE (0.1094) | MEAN | 1958.67 | 27.33 | 14 | 41.33 | 2000 | 3.80 | 2.07* | 61.24# | | 125.97† |
| | SD | 24.01 | 11.93 | 14 | 24.01 | 0 | 1.97 | 1.20 | 26.70 | | |
| EMS + PE (0.2085) | MEAN | 1924.67 | 59.33 | 16 | 75.33 | 2000 | 4.17 | 3.77 | 28.44# | | 5.48† |
| | SD | 53.82 | 45.52 | 12.53 | 53.82 | 0 | 2.52 | 2.69 | 15.49 | | |
| EMS + PE (0.44) | MEAN | 1956.67 | 32.33 | 11 | 43.33 | 2000 | 5.94 | 2.17* | 57.31# | | 111.52† |
| | SD | 39.55 | 35.28 | 4.58 | 39.55 | 0 | 2.66 | 1.98 | 39.55 | | |

TC = Test compound; N = Normal mitotic cells (comprising prophase, metaphase, anaphase and telophase); ABN = Aberrant mitotic cells; SD = Standard deviation; CP = Cyclophosphamide; EMS = Ethylmethane sulphonate; PE = Plant extract; MI = Mitotic index; * = 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); † = PE + Mutagen mixture more genotoxic than mutagen alone but more or less than PE alone; + † = PE + Mutagen mixture more genotoxic than mutagen or PE alone; ‡ = PE + Mutagen mixture less genotoxic than mutagen alone (reduction of genotoxicity) but more or less than PE alone; +‡ = PE + Mutagen mixture less genotoxic than mutagen or PE alone.

*Cytotoxicity:* Examination of the MI in column 9 of Table 3 shows that the concentration (mg $mL^{-1}$) of CP (1.25) or EMS (0.25) used was not toxic to the root meristem cells of *A. sepa* when compared to the water treated negative control ($P>0.05$). All three concentrations (mg $mL^{-1}$) of *C. sinensis* extract (i.e. 0.1094, 0.2085 and 0.44) were not toxic ($P>0.05$). Each separate mixture of CP with each of the three different concentrations of *C. sinensis* extract was not toxic to the root meristem cells ($P>0.05$). The mixture of EMS separately with the lowest (0.1094 mg $mL^{-1}$) and the highest (0.44 mg $mL^{-1}$) concentration of *C. sinensis* extract were cytotoxic ($P<0.05$).

*Genotoxicity (GT)*: Examination of the induction of genotoxicity in column 10 of Table 3 shows that the concentration (mg $mL^{-1}$) of CP (1.25) or EMS (0.25) used was genotoxic to the root meristem cells of *A. sepa* when compared to the water treated negative control ($P<0.05$). All the three concentrations (mg $mL^{-1}$) (0.1094, 0.2085 and 0.44) of *C. sinensis* extracts were not genotoxic ($P<0.05$) when compared to the water treated negative control group. The mixtures of *C. sinensis* extract with CP separately were also not genotoxic to the

root meristem cells (P > 0.05). However, the mixtures of EMS separately with each of the three concentrations of *C. sinensis* extract were genotoxic to the root meristem cells (P<0.05).

*Modulatory effect (ME) of plant extract on CP-induced Genotoxicity (GT):* Examination of the ME in column 11 of Table 3 shows that the mixture of each concentration (mg $mL^{-1}$) of *C. sinensis* extract separately with CP produced a negative value of ME (0.1094 = -63.61; 0.2085 = -66.62 and 0.44 = -78.64). Thus the mixtures were less genotoxic than CP alone. Because all the three concentrations of *C. sinensis* extract and their individual mixtures with CP were not genotoxic, though CP alone was genetoxic, the results suggested that the *C. sinensis* extract abolished CP-induced genotoxicity and therefore exerted an anti-genotoxic effect on CP-induced genotoxicity.

*Modulatory effect (ME) of plant extract on EMS-induced Genotoxicity (GT):* Examination of the ME in column 12 of Table 3 shows that the mixtures were more genotoxic than EMS alone thereby producing positive ME values (0.1094 mg $mL^{-1}$ = 124.97; 0.2085 mg $mL^{-1}$ = 4.48 and 0.44 mg $mL^{-1}$ = 110.52). Because the mixtures were more genotoxic than EMS or *C. sinensis* extract alone, it suggested a potentiation of EMS-induced genotoxic effect since *C. sinensis* extracts were not genotoxic.

## 4. Discussion

The interest in plant-based pharmaceuticals that intensified due to the development of methods of screening for anticarcinogenic drugs (Hartwell, 1967) resulted in the extension of the survey to include plant extracts and plant products able to modify the process of mutagenesis, because of the observation of a correlation between carcinogenesis and mutagenesis (Debisri & Archana, 1996). In this study the methanolic extracts of two plants used in traditional medicine in Lesotho, namely, *Dicoma anomala* (root), family**,** Asteraceae and *Chenopodium album* (leaves), family Chenopodiaceae, were evaluated for cytotoxicity, genotoxicity and modulatory effects on the genotoxicity induced by the direct acting mutagen, Ethyl methanesulphonate (EMS) and the indirect acting mutagen, Cyclophosphamide (CP), using the onion (*Allium cepa* L.) root tip meristem chromosome aberration assay system. The methanolic extract of *Camellia sinensis* (green tea) were also evaluated for comparison.

The results of the present study are summarized inTable 4.

The concentrations of CP (1.25 mg $mL^{-1}$) and EMS (0.25 mg $mL^{-1}$) used in the present study reduced the mitotic index (MI) of treated roots but the decreases were not significant (P>0.05) and were adjudged not cytotoxic. They were however genotoxic to the root meristem cells of *A. sepa* when compared to the water treated negative control (P<0.05). EMS at a concentration of $2x10^{-2}$M (0.2484 mg $mL^{-1}$) was however both toxic and mutagenic to root meristem cells of *Allium cepa* (Çelik & Aslantürk, 2010). CP at a concentration of 1% (1 mg $mL^{-1}$) was also both toxic, i.e. significantly reduced the mitotic index (MI) and clastogenic, significantly induced chromosome aberrations, in treated onion root meristem cells (Akeem et al., 2011).

The three concentrations (0.0625, 0.125, 0.25 mg $mL^{-1}$) of *Dicoma anomala* tested were cytotoxic and genotoxic to the onion root meristem cells. Many plant extracts including betel and tobacco leaf extracts demonstrated cytotoxicity to root-tip cells of *Allium* cepa (Sopova et al., 1983; Abraham & Cherian, 1978). Methanolic extracts of another Asteraceae species, *Schkuhria pinnata* (Lam.) used in traditional medicine to treat eye infections, Pneumonia and diarrhea in South Africa was not mutagenic in the Salmonella microsome assay against *Salmonella typhimurium* tester strain TA98 but showed antibacterial activity (Luseba et al., 2007). The mixture of CP (1.25 mg $mL^{-1}$) with each concentration of extract of *Dicoma anomala* separately was cytotoxic and genotoxic to the root tip meristem cells. The mixture of CP with each of the three concentrations of *D. anomala* was more genotoxic than CP alone (250.50, 134.62, and 317.21 % increase). In addition, the mixture of CP with the lowest concentration was also more genotoxic than the *Dicoma anomala* extract alone. These observations suggested a synergistic interaction between CP and *Dicoma anomala* extract at low concentration but not at the higher concentrations. Crude methanolic extract (1.2 mg $mL^{-1}$) of *Dicoma anomala* moderately inhibited cytochrome P450 (CYP) isoforms in *in vitro* tests using human liver microsomes (Gwaza et al., 2009). However, the plant monooxygenase enzyme complex is slightly different from the animal one (Plewa & Gentile, 1982; Higashi, 1988). Therefore, although cyclophosphamide is a promutagen (Mohn & Ellenberger, 1976; Hales, 1982), onion root cells have the ability to activate promutagens (Fiskesjö, 1985), which may have partly contributed to the results observed in the present study. The mixture of EMS (0.25 mg $mL^{-1}$) with each concentration of extract of *Dicoma anomala* separately was genotoxic. The mixtures of EMS with the lowest and highest concentrations were in addition, also cytotoxic. The cytotoxicity was therefore not dose dependent. The mixture of EMS with each of the three concentrations of *D. anomala* extract was more genotoxic than EMS alone (149.74, 157.37 and 68.55 % increase). In addition, the mixture of EMS with the lower concentrations of *D anomala* extract was also more genotoxic than *Dicoma anomala* extract alone. These observations suggested a synergistic interaction between EMS and the *Dicoma anomala* extract at low concentrations but not at higher concentrations.

Table 4. Summary of the cytotoxic, genotoxic and modulatory effects on CP- & EMS-induced genotoxicity by *D. anomala*, *C. album* and the reference *C. sinensis* extracts in tests with *Allium cepa* root meristem cells

| Test Compound | Concentration (mg $mL^{-1}$) | (P+M)/A+T) | MI | Genotoxicity | Modulatory Effect on Genotoxicity (% reduction or increase of mutagen-induced GT) | |
|---|---|---|---|---|---|---|
| | | | | | Plant Extract on CP | Plant Extract on EMS |
| CP | 1.25 | ns | nt | # | | |
| EMS | 0.25 | ns | nt | # | | |
| *D. anomala* | 0.0625 | ns | nt | # | | |
| *D. anomala* | 0.125 | ns | * | # | | |
| *D. anomala* | 0.25 | ns | * | # | | |
| *D. anomala* + CP | 0.0625 + 1.25 | ns | * | # | 251.50+ † | |
| *D. anomala* + CP | 0.125 + 1.25 | ns | * | # | 135.62† | |
| *D. anomala* + CP | 0.25 + 1.25 | ns | * | # | 318.21† | |
| *D. anomala* + EMS | 0.0625 + 0.25 | ns | * | # | | 150.74+† |
| *D. anomala* + EMS | 0.125 + 0.25 | ns | nt | # | | 158.37+† |
| *D. anomala* + EMS | 0.25 + 0.25 | ns | * | # | | 69.55† |
| *C. album* | 0.078 | ns | nt | ngt | | |
| *C. album* | 0.156 | ns | nt | ngt | | |
| *C. album* | 0.313 | ns | * | ngt | | |
| *C. album* + CP | 0.078 + 1.25 | ns | * | ngt | -54.18‡ | |
| *C. album* + CP | 0.156 + 1.25 | ns | * | ngt | -67.36‡ | |
| *C. album* + CP | 0.313 + 1.25 | ns | * | ngt | -56.40‡ | |
| *C. album* + EMS | 0.078 + 0.25 | ns | * | ngt | | -49.72‡ |
| *C. album* + EMS | 0.156 + 0.25 | ns | * | ngt | | -60.13‡ |
| *C. album* + EMS | 0.313 + 0.25 | ns | * | # | | 237.75+† |
| *C. sinensis* | 0.1094 | ns | nt | ngt | | |
| *C. sinensis* | 0.2085 | ns | nt | ngt | | |
| *C. sinensis* | 0.44 | ns | nt | ngt | | |
| *C. sinensis* + CP | 0.1094 + 1.25 | ns | nt | ngt | -62.61‡ | |
| *C. sinensis* + CP | 0.2085 + 1.25 | ns | nt | ngt | -65.62‡ | |
| *C. sinensis* + CP | 0.44 + 1.25 | ns | nt | ngt | -77.64‡ | |
| *C. sinensis* + EMS | 0.1094 + 0.25 | ns | * | # | | 125.97† |
| *C. sinensis* + EMS | 0.2085 + 0.25 | ns | nt | # | | 5.48† |
| *C. sinensis* + EMS | 0.44 + 0.25 | ns | * | # | | 111.52† |

TC = Test compound; GT = genotoxicity; (P+M)/A+T) = (Prophase + Methaphase)/(Anaphase + Telophase); ns = (P+M)/A+T) of treatment not significantly different from negative control; CP = Cyclophosphamide; EMS = Ethylmethane sulphonate; PE = Plant extract; MI = Mitotic index; * = TC is toxic (MI treatment significantly different from negative control, $P<0.05$ in the t-test, n = 3); nt = TC is not toxic; # = TC is genotoxic (significant difference from negative control, $P<0.05$ in the t-test, n = 3); † = PE + Mutagen mixture more genotoxic than mutagen alone but more or less than PE alone; +† = PE + Mutagen mixture more genotoxic than mutagen or PE alone; ‡ = PE + Mutagen mixture less genotoxic than mutagen alone (reduction of genotoxicity) but more or less than PE alone; +‡ = PE + Mutagen mixture less genotoxic than mutagen or PE alone.

In Table 4, of the three concentrations (0.078, 0.156, 0.313 mg $mL^{-1}$) of *Chenopodium album* tested, the lower concentrations were not cytotoxic nor genotoxic when compared to the negative control. The highest concentration was cytotoxic but not genotoxic to the onion root meristem cells. Ethanolic extracts of leaves of *C. album* showed antimicrobial activities against *Escherichia coli, Salmonella typhimurium, Staphylococcus aureus, Proteus vulgaris* and *Pseudomonas aeruginosa* (Singh et al., 2011) and *Bacillus subtilis* with 13mm of inhibition zone (Korcan et al., 2013). In one study, methanolic extracts of *C. album* and eight other South African medicinal plant species were not mutagenic to *S. typhimurium* (TA 98) in the Ames test (Mudzwiri, 2007). In a study by Khoobchandani et al. (2009), methanolic extract of leaves of *C. album* demonstrated anticancer activity

against the estrogen dependent human breast cancer cell line (MCF-7) with an IC (50) of 27.31 mg $mL^{-1}$. In the present study, it was the highest concentration (0.313 mg $mL^{-1}$) that was cytotoxic but not genotoxic to the onion root meristem cells. The mixture of CP (1.25 mg $mL^{-1}$) with each of the three concentrations of extracts of *C. album* separately was cytotoxic to the onion root tip meristem cells. However, none of the mixtures was genotoxic even though CP alone was genotoxic. By comparison, the mixures of CP with the *C. album* extracts were less genotoxic than CP alone (55.18, 68.36 and 57.40 % reduction) respectively. These observations suggested a reduction of CP-induced genotoxicity (antigenotoxic effect) by the extracts of *C. album*. In a study *in vitro*, the aqueous and methanolic extracts of *C. album* (3,6,9 mg $mL^{-1}$) reduced the frequency of chromosome aberrations induced by CP (100 μg $mL^{-1}$) in a dose dependent manner in human lymphocytes but the extracts alone were not genotoxic (Panigrahy et al., 2011). The present results thus corroborate the results of Panigrahy et al. (2011) cited above. In mice, an increase in the anticlastogenic activity of CP-induced clastogenicity by β-carotene at lower doses and an absence of a protective effect at higher concentrations were observed (Salvadori et al., 1992). Salvadori et al. (1992) interpreted the observations to mean different mechanisms of β-carotene modulation and a possible alteration of the balance of CP activation/detoxification mechanism of the promutagen. The mixtures of EMS (0.25 mg $mL^{-1}$) with the extracts of *C. album* were cytotoxic with the mixture of EMS and the highest concentration being also genotoxic. By comparison the mixures were less genotoxic than EMS alone (50.72 and 61.13 % reduction) respectively. However, the mixture of EMS with the highest concentration was more genotoxic than EMS alone (236.75% increase). These observations suggested a reduction of EMS-induced genotoxicity (antigenotoxic effect) by low concentrations of *C. album* extract but a synergistic interaction between *C. album* extract and EMS at high concentrations. These kinds of complex interactions between plant extracts and known mutagens have been reported. For instance, Rhizome juice of ginger was found to be antimutagenic against 6-gingerol-induced mutagenesis (Nakamura & Yamamoto, 1982). However, when the juice was added to known mutagens such as AF-2 and MNNG, mugagenesis was increased by the ginger juice, and the potent mutagen identified in this case was 6-gingerol (Nakamura & Yamamoto, 1982). The interpretation was that ginger juice containd antimutagenic substances that can suppress the activity of 6-gingerol and that, in the presence of certain specific mutagens like AF-2 and MNNG, 6-gingerol is able to express its mutagenicity (Nakamura & Yamamoto, 1982). We are not inferring that a compound of like nature is present in the *C. album* extract only that the kinetics of action are similar.

Examination of the results of tests with *Camellia sinensis* in Table 4 shows that, the three concentrations (0.1094, 0.2085, 0.44 mg $mL^{-1}$) of *C. sinensis* tested did not significantly reduce the MI ($P>0.05$) when compared to the negative control and were therefore adjudged not cytotoxic. The concentrations did not also induce significant increases in the frequency of chromosome aberrations and were adjudged not genotoxic, when compared to the negative control. The mixture of CP (1.25 mg $mL^{-1}$) with each of the three concentrations of extract of *C. sinensis* separately was not cytotoxic and not genotoxic to the onion root tip meristem cells. By comparison, the mixures of CP with each of the three different concentrations of *C. sinensis* extracts were less genotoxic than CP alone (63.61, 66.62 and 78.64 % reduction) respectively. These observations suggested a reduction of CP-induced genotoxicity (antigenotoxic effect) by the tested concentrations of extracts of *C. sinensis*. The inhibition of the mutagenicity of known mutagens by plant extracts, which by themselves are not mutagenic, is a well-known phenomenon. For instance the spice, turmeric (*Curcuma longa* L) and its yellow pigment, curcumin, were non-mutagenic but a diet that included 1% turmeric reduced benzo(a)pyrene (BaP)-and DMBA-induced stomach tumours and spontaneous mammary tumours in mice (Nagabhushan & Bhide, 1985; Nagabhushan et al., 1987a; Nagabhushan et al., 1987b) and curcumin inhibited the mutagenicity of benzo[a]pyrene and dimethyl benzo[a] anthracene in a dose-dependent manner in *Salmonella typhimurium* strain TA98 in the presence of S-9 mix (Nagabhushan et al., 1987a). The observations were interpreted to mean that curcumin may alter the metabolic activation and detoxification of mutagens (Nagabhushan et al., 1987a). Extracts of green and black tea leaves decreased MNNG –induced mutagenicity in *E. coli* WP2 in vitro (Jain et al., 1987). Constituents of complex mixtures of plant origin, including tea and coffee, namely, caffeic, chloregenic, ellagic, ferulic, gallic and tannic acids and vitamin C have been shown to inhibit the mutagenicity of direct-acting N-nitroso compounds, and vitamin A inhibited metabolic activation of promutagenic nitrosamines (Ames, 1982; Gichner & Veleminsky, 1988). The mechanisms by which crude vegetable and fruit extracts reduce the cytotoxic effects of various clastogens may be different for different plants. The extracts of cruciferous plants such as Brussels sprouts, cabbage, cauliflower and broccoli have been shown to be capable both of activating enzymes such as arylhydrocarbon hydroxylase and of detoxifying enzymes such as the cytosolic GSH S-transferase (Debisri & Archana, 1986). It has been reported that intake of diets containing powdered preparations of Brussels sprouts, cabbage, coffee beans, or tea leaves increased the activity of glutathione-S-transferase (GST), which catalyses the binding of electrophiles to glutathione (GSH) (Sparnins et al., 1982). It has therefore been suggested that

GST activated by vegetables juices may be involved in the anticlastogenic activity of the vegetables (Debisri & Archana, 1996) and that the mechanisms of the inhibition of metabolic activation of indirectly acting mutagens may be by the inactivation of metabolizing enzymes or interaction with promutagens to make them unavailable for the enzymatic process (Hayatsu et al., 1988). The mixtures of CP and extracts of *C. sinensis* were not genotoxic in the present study. The mixture of EMS (0.25 mg $mL^{-1}$) with 0.1094 mg $mL^{-1}$ (lowest concentration) or 0.44 mg $mL^{-1}$ (highest concentration) of extract of *C. sinensis* reduced the mitotic index of onion root tip cells significantly (P<.05) compared to the negative control and were adjudged to be cytotoxic. The mixture of EMS (0.25 mg $mL^{-1}$) with 0.2085 mg $mL^{-1}$ (middle concentration of *C. sinensis* extract) did not significantly reduce the MI the root tip cells (P>05) compared to the negative control and was adjudged not cytotoxic. The cytotoxicity was therefore not concentration dependent. However, the mixtures of EMS with the different concentrations of extracts of *C. sinensis* were more genotoxic than EMS alone (124.97, 4.48 and 110.52 % increase). This observation suggested a potentiation of the genotoxic activity of EMS by extracts of *C. sinensis.* Such potentiating effects of activities of mutagens by plant extracts, which alone are not mutagenic, are common. For example, while small doses of *Angelicae radix* and Cnidii rhizome extracts enhanced the mutagenicity of benzo(a)pyrene (BaP), at higher doses a decreasing effect was observed (Sakai et al., 1988). In the present study, extracts of *C. sinensis* were not genotoxic but when mixed with the direct acting mutagen EMS, the mixtures were more genotoxic than the EMS alone.

MI measures the proportion of cells in the M-phase of the cell cycle and therefore its inhibition could be interpreted as cellular death or a delay in the cell mitotic activities (Rojas et al., 1993), which, according to Sudhakar et al. (2001) could be due to inhibition of DNA synthesis or a blocking in the G2 phase of the cell cycle, thereby preventing the cell from entering mitosis. Many herbal extracts have been reported to inhibit mitosis (Çelik & Aslantürk 2006; Çelik & Aslantürk 2007; Akinboro & Bakare, 2007). In the present study, methanolic extracts of *D. anomala* and the highest concentration of *C. album* decreased the MI in treated *A. cepa* roots. The present results therefore suggested that the tested concentrations of extracts of *Dicoma anomala* (root) and *Chenopodium album* (leaves) exerted mito-depressive or cytotoxic effects on cell division of *A. cepa* root meristem cells which could be attributed the effects of compounds present in the extracts.

The aberrant dividing cells observed and scored in the present study were sticky chromosomes (S) and c-mitosis (C-Mit) following treatments with *D. anomala*; sticky chromosomes (S), c-mitosis (C-Mit), chromosome largards (L)., anaphase and telophase bridges (A.B) and chromosome breaks following treatments with *C. album* or *C. sinensis* mixture with EMS. The levels of inductions of the aberrant cells were however not significant when compared to the negative control, following treatments with extracts of *C. album* or *C. sinensis*.

Sticky chromosomes, lose their normal appearance, and seen with sticky "surface," which cause cluster (Babich et al., 1997). Stickiness has been attributed to the effect of pollutants and chemical compounds on the physical and chemical properties of DNA, protein or both, on the formation of complexes with phosphate groups in DNA, on DNA condensation or on formation of inter- and intra-chromatid cross links (Shahin & El-Amoodi, 1991; Rencüzoğullari, et al., 2001; El-Ghamery et al., 2003; Gömürgen, 2005; T¨urkoglu, 2007). The presence of chromosome fragments is an indication of chromosome breaks, and can be a consequence of anaphase/telophase bridges (Sharma & Sen, 2002; Singh, 2003). 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. According to Mustafa & Arikan (2008), a large number of laggard chromosomes and c-anaphases indicate that a test compound acted as a potent spindle inhibitor. The induction of vagrant chromosomes according to Elghamery et al. (2003), leads to the separation of unequal number of chromosomes in the daughter nuclei and subsequently formation of daughter cells with unequal sized or irregularly shaped nuclei at interphase. In the present study, the treatments of root tips with *D. anomala* and to, insignificant level, *C album* and *C. sinensis* induced c-mitosis in the onion root tip meristem cells. Thus the frequencies of total chromosome aberrations increased significantly upon exposure to *D. anomala*, mixtures of *D. anomala* with CP or EMS, the mixture of highest concentration of *C. album* with EMS and mixtures of *C. sinensis* with EMS. These results are in agreement with the results of some other studies which examined the effects of different medicinal herbs (Soliman, 2001; Bidau et al., 2004).

The ratio of dividing cells in prophase and metaphase to those in anaphase and telophase i.e. (P+M)/(A +T) has been used as indicator of metaphase arrest. 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 the relative proportions of P+M and A+T types did not differ significantly when compared to the negative control following treatment of the onion root tips with extracts of the individual plant species, the individual mixtures of the extracts with the mutagens or the mutagens

alone. The observation suggested that the plant extracts did not contain appreciable concentrations of spindle poisons. However, very few c-mitosis (C-Mit) cells were observed on the prepared slides. In a study of Parry et al. (1999), treatment of a pulmonary cell line derived from the Chinese hamster and designated DON: Wg3H resulted in decrease in the MI with increased dose but, the relative proportions of P+M and A+T types remained the same. In the present study too, the MI of treated root meristem cells decreased following certain treatments but the relative proportions of P+M and A+T types remained the same. The observation was taken as a typical indicator of cytotoxic damage (Parry et al., 1999).

Working with crude extracts means working with complex mixtures of biologically active compounds, some of which can be cytotoxic and/or genotoxic; others can be cytoprotective and/or antigenotoxic. The results suggest that, although *D. anomala* and *C album* have beneficial effects as medicinal plants, they can cause serious problems and damage on cells if they are improperly used.

## 5. Conclusion

The tested concentrations of *D. anomala* (0.0625, 0.125, 0.25 mg $mL^{-1}$), were cytotoxic and genotoxic. Mixture of CP with the lowest concentration of *D. anomala* was more genotoxic than CP (250.50% increase) or *D. anomala* extract alone. The mixtures of EMS with the lower concentrations of *D. anomala* extract were more genotoxic than EMS (149.74 and 157.37 % increases) or *D. anomala* extract alone. The lower concentrations of *C. album* extract were not cytotoxic and all the three concentrations (0.078, 0.156, 0.313 mg $mL^{-1}$), were not genotoxic. The mixtures of CP with extracts of *C. album* were cytotoxic. CP-induced genotoxicity was reduced (55.18, 68.36 and 57.40 % reductions) by the *C. album* extracts. EMS-induced genotoxicity was reduced (50.72 and 61.13 % reductions) by low concentrations but increased by the highest concentration (236.75% increase) which suggested antigenotoxicity and synergism respectively. The tested concentrations of *C. sinensis* and the mixtures of CP with *C. sinensis* extracts were not cytotoxic and not genotoxic. However, CP-induced genotoxicity was reduced (63.61, 66.62, 78.64 %) while EMS-induced genotoxicity was increased (124.97, 4.48 and 110.52 %) by extracts of *C. sinensis*. The frequencies of total chromosome aberrations increased significantly upon exposure to *D. anomala*, mixtures of *D. anomala* with CP or EMS, the mixture of 0.313 mg $mL^{-1}$ concentration of *C. album* with EMS and mixtures of *C. sinensis* with EMS. The (P+M)/(A +T) ratios did not differ significantly ($P>0.05$) following the treatments.

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