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# CYTOGENETIC EFFECTS OF MEDICINAL PLANTS FOR DIABETES (AQUEOUS PLANT EXTRACT OR TABLET) ON HUMAN LEUKOCYTES TREATED IN VITRO

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## ABSTRACT

The cytogenetic effects of aqueous extracts from bark of "duhat" (*Syzygium cumini* L.), leaves of "damong maria" (*Artemesia vulgaris* L.) and periwinkle (*Catharanthus roseus* L.) and tablet of "ampalaya" (*Momordica charantia* L.) were determined using human leukocytes cultured in vitro. Three to four drops of blood from five to seven volunteers aged 20-21 were cultured in chromosome medium with 0, 3% and 5% sterile extracts. Mitotic index was obtained by counting 500 cells per treatment while chromosomal aberrations were observed in at least 50 cells. Mean mitotic indices using the three medicinal plant extracts were reduced significantly as the concentrations of treatments were increased. For Artemesia, the control showed a mean mitotic index of 16.86% and 22.95 and 25.96% for periwinkle leaves and "duhat" bark, respectively. "Ampalaya" tablets did not significantly affect the mitotic index. Loose sister chromatids, gaps and breaks were the chromosomal abnormalities observed. The frequency of cells with no chromosomal aberration was reduced from 66.67 to 36.00% for Artemesia leaves, 97.00 to 79.00% for periwinkle and 94.00 to 81.00% for "duhat" bark. Artemesia, periwinkle and "ampalaya" significantly induced chromosome condensation. Mean frequency reached 45.00%. Results indicated that all four medicinal plants are possible mutagens. "Duhat" bark is a mitotic inhibitor and as such can be tapped as possible tumor growth suppressor.

***Key words:*** leukocytes, mitotic index, cytogenetic effects, mitotic inhibitor, periwinkle, mutagenic, *Syzygium cumini* L., *Catharanthus roseus* L., *Momordica charantia* L., *artemesia vulgaris* L.

## INTRODUCTION

About 35,000 of third world plants are medicinal. Here in the Philippines, medicinal plants have been part of our cultural heritage (de Padua, 1990). Considering the vast number of species with medicinal potentials, in 1969, the National Institute of Science and Technology now DOST (Department of Science and Technology) initiated the program called "Drugs from Plants." It aims to develop safe, effective and low cost drugs from local sources. Manuals and pamphlets were published, seminar workshops have been conducted and herbal gardens were set up all over the country.

*Diabetes mellitus* is known to affect approximately 120 million people worldwide and in the Philippines 3.1M. It is a metabolic disorder manifested by loss of weight despite an overly good appetite. Cells are depleted of fats and proteins to fulfill the cellular energy requirement not supplied through glucose metabolism. The cells of the pancreas of diabetics are unable to produce enough insulin, which allows glucose derived from food to enter the cells to be broken down as a source of energy. Blood sugar rises and glucose appears in the urine, carrying water with it. These results in increased urine output and thirst. Fat metabolism may be enhanced leading to the accumulation of ketoacid, which if unchecked can result to coma or death.

### *Medicinal Plants for Diabetes*

There are plants, which possess medicinal values and are known to be included in folklore list of insulin substitutes. Morales (1996) conducted a survey of medicinal plants in Olongapo City and eight (8) towns of Pangasinan used to treat *Diabetes mellitus*. A total of 79 plant species were identified and number one in the list is the bark of "duhat" (*Syzygium cumini* L.). Also included are "damong maria" (*Artemesia vulgaris* L.), periwinkle (*Catharanthus roseus* L.) and "ampalaya" (*Momordica charantia* L.). "Duhat" bark as a decoction in water is taken three times a day. The medicinal value of "duhat" bark for diabetes was first studied by Garcia (1987). A light brownish white, amorphous, odorless and slightly bitter powder was isolated from "duhat". One gram of powder has 440 units of insulin. *Artemesia* is used as expectorant, antiseptic, can treat dysmennorhea, stomach pain, dysentery, pulmonary tuberculosis and many others (Co, 1989). Periwinkle or locally known as chichirica can cure stomachache, toothache, indigestion, and dysentery. It contains vincristine and viblastine used in treating Hodgkin's lymphoma (Pratt, 1994). "Ampalaya", a vegetable famous for its bitter taste, is used in China as anti-tumor, anti-infective agent in the US, against HIV infection, stomachache, and constipation (Paño, 1999). Worlwide it is accepted as a strong anti-diabetic because it contains polypeptide p, a plant insulin with less side effects compared to the insulin obtained from the pancreas of animals. In the Philippines, the potential of "ampalaya" as antidiabetic drug was noted by Zamora

(1992). The Department of Health's Institute for traditional Medicine and the U.P. College of Pharmacy are continuously doing research on the antidiabetic potential of "ampalaya". Decoction from "ampalaya", eggplant (*Solanum melongena* L.) and rose apple or "makopa" has been discovered to remedy diabetes and is now owned by a US Company (Bengwayan, 1999). The initial sale in the first year is expected to reach 500 Million dollars. Patent for "ampalaya" has been applied and it is now privately owned by US National Institute of Health, US Army and New York University.

The Philippine government with its increasing population must provide the health needs of the Filipinos. In the event that the of cost synthetic medicine is continuously increasing, the popularity of herbal medicine to cure different diseases rises tremendously. Any new medicine must be subjected to proper screening. Cytogenetic analysis of somatic cells is one of the major methods of testing. The most common source of cells for clinical cytogenetic study is the peripheral blood lymphocytes (Life Technologies, 1998). Routine cytogenetic studies performed on peripheral blood lymphocytes include detection of chromosomal aberrations associated with numerical and structural changes. Knowing that Filipinos are using aqueous medicinal plants and tablets for diabetes, it is but necessary to assess how safe it is to use these medicinal plants.

This study was conducted to determine the cytogenetic effects of aqueous medicinal plant extracts and tablets for diabetes on human leukocytes treated in vitro. Medicinal plants include *Artemesia* (Fig. 1a) and periwinkle (Fig. 1b) leaves, "duhat" bark and "ampalaya" tablet. Specific objectives are to determine the effects of the medicinal plant extracts on mitotic index and evaluate if they can induce chromosomal aberrations.

The study was conducted at the Genetics and Molecular Biology Division, Institute of Biological Sciences, College of Arts and Sciences, University of the Philippines Los Baños College, Laguna.

## MATERIALS AND METHODS

Three to four drops of blood from 5-7 male donors (20-21 years old) were cultured on chromosome medium. The blood was treated separately with different concentrations (0, 1%, 3%, 5%) of sterile aqueous medicinal plant extracts and tablet on the 48$^{th}$ hour of culture. Colcemid was added to the culture (on the 71$^{st}$ hour) to arrest the chromosomes at the metaphase plane. After one hour and fifteen minutes of colcemid treatment, cells were fixed and harvested. Smears were prepared for each treatment. Five hundred cells were observed to score for mitotic index (number of dividing cells/total number of cells observed). Fifty cells per treatment were scored for chromosomal aberrations. Analysis of variance was done and means were compared using LSD.

a

b

Figure 1. Pictures of (a) periwinkle *(Catharanthus roseus* L.) and (b) *Artemesia vulgaris* L.

## RESULTS AND DISCUSSION

### *Effects on Cell Division*

Photomicrographs of dividing leukocytes arrested at metaphase (from the control group) are shown in Fig. 2. Chromosomes are clearly visible. The centromere location of each chromosome is distinct.

Analysis of variance on mean mitotic index revealed that the treatments are significantly different. The number of dividing cells decreased as the concentrations of treatments increased. For *Artemesia* (Table 1), comparison of means revealed that the mean mitotic index of control and 1% (16.86 and 16.80, respectively) is significantly different from 5% (14.53). Using periwinkle leaves, mean mitotic indices are significantly different except for 3 and 5%. Using "duhat" bark, the mitotic index was significantly reduced compared with the control (25.91). A very low proportion of dividing cells [5% (14.53%)] was noted. "Duhat" bark is a mitotic inhibitor and as such can be tapped as a possible tumor growth suppressor. The story of the discovery of the antitumor value of periwinkle extract is worth repeating. Periwinkle, being cited in folk medicine as an aid in treating diabetes was included in the survey to identify oral insulin substitute by Noble and Beer at the University of Western Ontario and Svoboda at Eli Lily in Indianapolis. Studies showed that crude extract of periwinkle drastically reduced white blood cell counts especially granulocytes. It tremendously decreased bone marrow activity in rats. The effects of the active leukopenic extract against leukemia cells were further tested *in vitro*. This led to the isolation of vincaleukoblastine (Lewis and Elvir-Lewis, 1977).

### *Effects of Medicinal Plants on Chromosomal Morphology*

Changes in chromosome structure offer readily scored morphological evidence of damage to the genetic material (Evans, 1984). These include gaps, breakages,

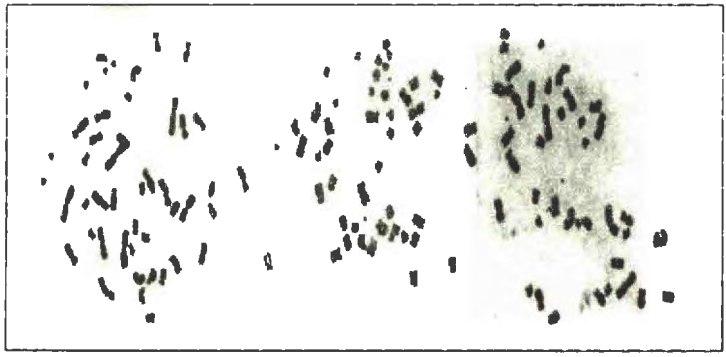

Figure 2. Chromosomes of untreated (control) human leukocytes arrested at metaphase plane. Magnification (4,000X).

Table 1. Mean mitotic index of human leukocytes treated in vitro with different concentrations of aqueous medicinal plant extract for diabetes.

| Treatment Concentrations | Mean Mitotic Index (%) Medicinal Plants | | |
|---|---|---|---|
| | *Artemesia leaves* | *Periwinkle leaves* | *"Duhat" bark* |
| Control | 16.86a | 22.95a | 25.96a |
| 1% | 16.80a | 19.28b | 9.96b |
| 3% | 15.66ab | 16.36bc | 8.60b |
| 5% | 14.53b | 14.32c | 0.04c |

loose sister chromatids and condensed chromosome sets. These chromosomal aberrations are gross structural changes, which can be detected without detailed karyotype analysis (Engle *et al*, 1986).

The mean frequency of chromosomal aberrations noted is presented in Table 2. Using Artemesia leaves, the number of normal cells decreased as treatment concentration increased. The number of cells with loose sister chromatid (characterized by early sister chromatid separation) increases from 23.33 in control to 32.00% in 5%. Figure 3 shows loose sister chromatid observed using 1 to 3% *Artemesia* and 5% periwinkle. The frequency of gap (defined as the separation in the chromatid arm without loss of alignment or dislocation of the distal fragment) also increased with increasing concentrations of treatment. A similar observation was made with the frequency of break, a discontinuity of the chromatid arm with either loss of alignment or dislocation of the distal fragment (Ford, 1973). Figure 4 shows a break observed using 3% *Artemesia*. Compared to *Artemesia*, the number of normal cells is higher when periwinkle leaves are used (97.00-79.00%). The frequency of other chromosomal aberrations was noted to be low as well. A low frequency of cells with chromosomal aberrations was observed using "duhat" bark. This would explain why higher percentage of cells with normal chromosomes was noted (94.00-81.00%).

### *Effects on Chromosome Condensation*

*Artemesia* and periwinkle leaves induced chromosome condensation (Fig. 5). This is when chromosomes contracted to at least half the normal size as those found in the control. The chromosomes could no longer be classified according to groups. The frequency of cells that showed condensed chromosomes as treatment increases is highly significant. Mean frequencies when compared are significantly different (Table 3). Both for *Artemesia* and periwinkle, the frequency of cells with condensed chromosomes increased from 5 to 44.00% and 9 to 45.00%, respectively. Shortening of the chromosomes could possibly be an indication of chromatin

Table 2. Mean frequency of chromosomal aberrations observed on human leukocytes treated *in vitro* with different concentrations of aqueous medicinal plant extract for diabetes.

| Medicinal Plant Used | Chromosomal Aberrations Observed | Mean Frequency (%) | | | |
|---|---|---|---|---|---|
| | | Treatment Concentration | | | |
| | | Control | 1% | 3% | 5% |
| *Artemesia leaves* | Normal | 66.67 | 48.34 | 39.99 | 36.00 |
| | Loose sister chromatid | 23.33 | 26.67 | 33.33 | 32.00 |
| | Gap | 10.00 | 23.33 | 21.68 | 23.00 |
| | Break | 0.0 | 1.66 | 5.00 | 9.00 |
| Periwinkle leaves | Normal | 97.00 | 92.00 | 86.60 | 79.00 |
| | Loose sister chromatid | 20.00 | 6.60 | 10.66 | 19.5 |
| | Gap | 1.0 | 0.66 | - | - |
| | Break | - | 0.66 | 2.66 | 1.5 |
| "Duhat" bark | Normal | 94.00 | 92.00 | 81.00 | - |
| | Loose sister chromatid | 1.33 | 2.66 | 3.00 | - |
| | Gap | 4.66 | 4.66 | 8.00 | - |
| | Break | - | 0.66 | 8.00 | - |


Figure 3. Loose sister chromatids observed when human leukocytes were treated *in vitro* with 1 and 3% *Artemesia* and 5% periwinkle aqueous leaf extract. Magnification (4,000X).
(a-b) treated with 1 and 3% Artemesia;
(c) treated with 5% periwinkle.

Figure 4. Break observed when human leukocytes were treated *in vitro* with 3% *Artemesia* aqueous leaf extract. Magnification (4,000X).

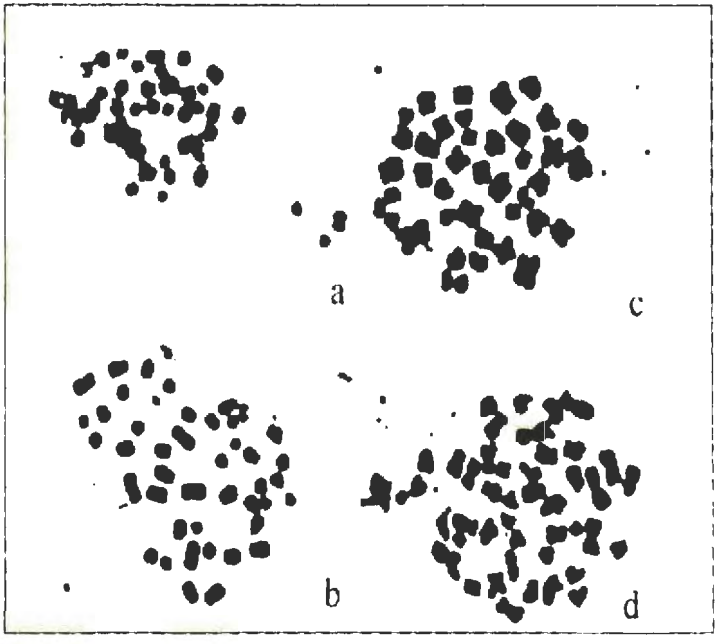


Figure 5. Chromosome condensation observed when human leukocytes were treated *in vitro* with Artemesia (a, b) and periwinkle (c,d) leaf extracts. Magnification (4,000X).

Table 3. Mean frequency of chromosome condensations observed on human leukocytes treated *in vitro* with different concentrations of aqueous medicinal plant extracts for diabetes.

| Treatment Concentration (%) | Mean Frequency (%) of Chromosome Condensation | |
|---|---|---|
| | *Artemesia leaves* | *Periwinkle leaves* |
| Control | 5.00a | 9.00a |
| 1 | 16.00b | 21.00b |
| 3 | 32.00c | 33.00c |
| 5 | 44.00c | 45.00d |

condensation (Engle *et al,* 1986). Polyamines could be present in the extract, which can induce chromosome condensation because of their ability to form stable complexes with DNA proteins (Davidson and Anderson, 1960; Du Praw, 1970).

### *Effects of "Ampalaya" Tablets on Cell Division*

The different concentrations of "ampalaya" tablet did not significantly affect mean mitotic index (Table 4). The control has 13.00% while 3 and 5% have 11.30 and 10.90%, respectively. "Ampalaya" tablets do not show toxicity to the cells at the concentration tested, thus allowing for normal cell division to occur.

### *Effects of "Ampalaya" Tablets on Chromosome Morphology and Condensation*

As shown in Table 5, "ampalaya" tablets are not potent chromosome breaking agents. The frequency of cells with normal chromosomes was high (88.70-95.10%) at 0% to 5%. The frequency of gaps, breaks and loose sister chromatids was low at all concentrations of ampalaya.

However, "Ampalaya" tablets significantly affected chromosome condensation (Table 6). At 3 and 5%, chromosome condensation was 2.3 x and 1.7x , respectively. There was no difference between control and 1% treatment.

## SUMMARY AND CONCLUSION

The three medicinal plant extracts affected cell division of human leukocytes cultured *in vitro*. "Duhat" bark is a probable inhibitor of cell division and as such can be tapped as a tumor growth suppressor. The depressive effects of the different extracts on mitotic index may also indicate the ability of the extracts to hamper the production of new cells in the body such as blood cells and sex cells. The chromosomal aberrations observed like gaps, breaks and loose sister chromatids

Table 4. Mean mitotic index of human leukocytes treated in vitro with different concentrations of "ampalaya" (*Momordica charantia* L.) tablet.

| Treatment Concentration (%) | Mean Mitotic Index (%) |
|---|---|
| Control | 13.00 |
| 1 | 12.30 |
| 3 | 11.30 |
| 5 | 10.90 |

Table 5. Mean frequencies (%) of chromosomal aberrations observed on human leukocytes treated *in vitro* with different concentrations of "ampalaya" (*Momordica charantia* L.) tablet.

| Chromosomal aberrations | Treatment Concentration | | | |
|---|---|---|---|---|
| | Control | 1% | 3% | 5% |
| Normal | 94.30 | 95.10 | 88.70 | 92.50 |
| Loose sister chromatid | 2.60 | 2.90 | 5.60 | 3.10 |
| Gap | 2.00 | 1.10 | 3.10 | 3.00 |
| Break | 1.10 | 0.90 | 2.60 | 1.40 |

Table 6. Mean frequencies (%) of chromosome condensation observed on human leukocytes treated *in vitro* with different aqueous concentrations of "ampalaya " (*Momordica charantia* L.) tablet.

| Treatment Concentration (%) | Chromosome Condensation (%) |
|---|---|
| Control | 8.6a |
| 1% | 8.6a |
| 3% | 20.6b |
| 5% | 15.1bc |

according to Evans (1984) are morphological evidences of damage to the genetic material. All four treatments significantly induced chromosome condensation. Highly condensed chromosomes are not available for transcription. Thus, synthesis of important proteins or enzymes necessary for the cells' primary processes may be inhibited and this condition is detrimental to a dividing cell.

Considering that the medicinal plants tested produced visible cytogenetic effects, caution must be exercised against the excessive and frequent oral intake to minimize the possibility of detrimental effects.

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