THE CURATIVE EFFECTS OF POLYPHENOLIC COMPOUNDS AGAINST CHROMOSOMAL ABNORMALITIES INDUCED IN MICE-BEARING SOLID TUMOR

ABSTRACT:
Many plant polyphenolic compounds have been shown to have cancer-preventing activities. Induction of chromosomal abnormalities was investigated in the bone marrow of mice 39 days post transplantation of solid tumor in mice treated with Ehrlich ascites carcinoma (EAC) cells only, tannic acid (0.5, 1, 1.5 mg/100g b.w.), catechin (2, 4, 6 mg/100g b.w.) and epicatechin (0.5, 1, 1.5 mg/100g b.w.) after tumor growth (0.3 mm3) via their subcutaneous injection and normal control group. The obtained results denoted that treatment with 1 mg tannic acid, 4 mg catechin and 1 mg epicatechin /100 gm mouse; induced more regression in the tumor volume. Moreover, it was found that the induced chromosomal aberrations during experimentation (breaks, fragments and deletions) recorded very highly significant frequency (P < 0.001) in mice-bearing solid tumor. These aberrations were sharply declined with the effective doses of the tested drugs. Catechin and epicatechin were very powerful in reducing the frequency of different chromosomal aberrations more than the tannic acid. Regarding the mitotic index (MI) in all groups, the catechin and epicatechin induced more inhibition in its elevated percentage in mice-bearing solid tumor more than the tannic acid.

Key words: Solid tumors- Polyphenolic compounds- Chromosomes.

INTRODUCTION
The first observations pointing to a connection between chromosomal aberrations and cancer were made about a century ago. In 1890, the German pathologist David Von Hansemann observed abnormal chromosomal arrangements and segregation in dividing cancer cells (Cornelisse, 2003). Acquired chromosome abnormalities may occur in various types of haematologic malignancies and solid tumors. These are chromosome rearrangements in somatic cells that can alter the position of various genes and subsequently, alter the gene product, leading to malignancy (Horwitz, 2000). Gisselsson (2001) reported that all malignant tumor types have been shown to contain a chromosomal aberration; which varies greatly between malignancies, ranging from simple balanced rearrangements to complex abnormalities affecting both chromosome structure and number. It was reported also that the majority of malignant solid tumors exhibit a complex pattern of chromosomal abnormalities (Gorunova et al., 1998). Attila et al. (2003) mentioned that cancer cells are characterized by having aberrant chromosomes. The number of aberrations and the specific chromosomes affected are correlated with tumor progression. They reported also, that a hallmark in of cancer development is the accumulation of genetic lesions. Some of these may be seen in the form of chromosomal aberrations when the cells enter mitosis. The cytogenetic aberrations are now considered to be of great importance as an initial step in tumorigenesis not only in hematological neoplasias but also in solid tumors (Mitelman et al., 2004). Donna et al. (2003) concluded that chromosomal aberrations can be analyzed in solid tumors using different techniques and these analyses identify a broad range of chromosomal abnormalities.

Green tea, *Camellia sinensis*, contains high levels of polyphenols, including catechin, epicatechin, gallate, and epigallocatechin gallate (Frankel, 1999). Polyphenols have been shown to inhibit tumorigenesis at different organ sites in animal models, including skin, lung, forestomach, stomach,
duodenum and small intestine, colon, pancreas, liver, and mammary gland (Yang et al., 1998). Catechins are a group of compounds that naturally occur in the plant kingdom. These compounds are called tea polyphenols and are rich in tea beverages that are consumed daily by most people. Green tea is rich with catechins which have inhibitory effects on carcinogenesis in rodent models (Yang and Wang, 1993). Tannic acid which is plant-derived polyphenolic compound exerts chemopreventive activity on hepatocarcinogenesis in male mice (Taitzoglou et al., 2000). Tea catechins have the ability of markedly reduction of tumor size (Wange et al., 1990). Also oral, subcutaneous or intraperitoneal administration of catechins in mice resulted in significant suppression of the growth of implanted tumor cells (Oguni et al., 1988; Hara et al., 1989; Yan and Wang, 1992). Ito et al. (1989) investigated that the green tea has the ability to induce suppression in the chromosomal aberrations produced by aflatoxin B1 in rat bone marrow cells. Halder et al. (2005) reported the antimutagenic and anticlastogenic effects of black tea polyphenols in Salmonella assay in vitro and in vivo in bone marrow cells of mice thus it was found an apparent reduction in different chromosomal aberrations induced with a carcinogen. The present study aimed to investigate the possible effects of tumor cells on the chromosomes of mice-bearing solid tumor before and after treatment with tannic acid, catechin, and epicatechin, respectively.

MATERIALS AND METHODS

Experimental animals:

This study was carried out on white adult female Swiss albino mice, weighting 20±2gm obtained from the breeding unit of the National Cancer Institute (Cairo). Animals were housed 10 mice /cage and maintained on a stock diet formulated to meet mice nutrient requirements and free water supply.

Tumor transplantation:

A line of Ehrlich Ascites Carcinoma (EAC) was supplied from the National Cancer Institute. Each mouse was injected subcutaneously in the right thigh with 0.3 ml of Ehrlich Ascites Carcinoma (EAC), which contained 3x106 cells. The control mice received an equal volume (0.3ml) of normal saline only.

The animals were used when their tumor had grown to about 3 mm3 in volume after 9 days from tumor inoculation such period was believed to permit the development of the tumor without causing death of inoculated animals (Shibamoto et al., 1986).

Drugs and their effective doses:

Tannic acid is a polyphenolic compound has a yellow color and was purchased from Sigma (St. Louis, MO), it dissolved in distilled water in concentrations (0.5mg, 1mg, 1.5mg/100gm b.w.). Twenty female mice were divided into 4 groups and injected subcutaneously with the selected doses after 9 days from tumor transplantation and continued twice a week for 30 days.

(+)- Catechin which is a polyphenolic compound that purchased from Sigma Chemical Company was dissolved in a drop of ethyl alcohol at the following concentrations (2mg, 4mg, and 6mg/100gm b.w.). Twenty female mice were divided as the same in case of tannic and at the same conditions.

(-)- Epicatechin that is a polyphenolic compound which was purchased from ICN Biomedicals Inc. was dissolved in a drop of ethyl alcohol in the selected concentrations (0.5mg, 1mg and 1.5mg/100gm b.w.). Also, 20 mice were divided into 4 groups as mentioned before and at the same conditions of the previous 2 drugs.

The effective dose of tannic, catechin and epicatechin is the dose that induced more regression in the tumor volume at the end of experimentation.

Experimental design:

Female mice were divided into five groups, each group consisted of 10 mice placed in individual cages and classified as follows:

Group I : Control mice treated with saline.
Group II: Mice -bearing tumor.
Group III: Mice-bearing tumor treated with subcutaneous injection of the effective dose of tannic acid twice a week, starting from the ninth day of tumor inoculation for thirty days.
Group IV: Mice-bearing tumor treated with subcutaneous injection of the effective dose of catechin with the same previous conditions.
Group V: Mice-bearing tumor treated with subcutaneous injection of the calculated dose of epicatechin with the same previous conditions.

All animals belonging to different groups were sacrificed after 30 days of treatment

Preparation of chromosomes:

Preparation of chromosomes from the bone marrow of mice was done according to the method described by Hus and Patton (1969) and modified by Zambrano et al. (1982). The preparation of chromosomes in different groups occurs as follows: the mice belonging to different groups were injected intraperitoneally 2-hour before sacrifice with 0.5 ml of 0.0012% colchicines/20gm b.w. (3mg/kg b.w.). Bone marrow cells were collected from the femur in phosphate buffered solution (0.8gm NaCl, 0.02gm KCl, 0.217gm Na2Hpo4) were dissolved in 100ml distilled water and PH was adjusted to be 7 using not more than 0.02 gm KH2po4 and centrifuged for 5 minutes at 1500 r.p.m discarding from the supernatant. To swell the cell volume 8ml of hypotonic solution (0.075M KCl) was added and
incubated for 20 minutes at 37°C. Centrifugation occurred for 5 minutes at 1500 r.p.m., discarding most of the supernatant. For fixation 2-3ml of absolute methanol and glacial acetic acid (3:1) was added to each tube and centrifuged, removing the supernatant. This was repeated three times for complete fixation. It was known that the used fixative must be cold and freshly prepared. About three drops of fresh cell suspension were dropped on a clean slides dipped in 70% ethyl alcohol and flamed. The slides were stained with 10% Giemsa and mounted in DPX. For each animal 50 examined metaphase spreads were analyzed for chromosomal aberrations. The mitotic index (number of dividing cells per 1000 cells) was also determined.

Statistical analysis:

The results of tumor size are presented as mean ±SD; statistical analysis was performed using ANOVA test followed by multiple comparisons post-hoc analysis (Tukey), with a P value of less than 0.05 considered significant.

Chromosomal aberrations and the mitotic index in the present work were represented in tables as percentages (%). For statistical analysis; Chi-Square (χ²) test was applied.

Results

Tumor size significance:

Data shown in figure 1 and table 1 indicated that different doses of tannic acid, catechin and epicatechin induced regression in the tumor volume, the chosen doses which induced more regression in the tumor volume and recorded a very highly significant decrease (P < 0.001); were 1mg tannic acid, 4mg catechin and 1mg epicatechin for (100gm b.w.).

Table 1. Volume of Solid Tumor (mean ± SD) in Mice Bearing EAC before or after the treatment with different doses of catechin, epicatechin and tannic acid.

<table>
<thead>
<tr>
<th>Animal groups</th>
<th>Tumor Volume mm³ (Mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive Control</td>
<td>2.094 ± 0.3</td>
</tr>
<tr>
<td>0.5 mg tannic acid</td>
<td>1.94 ± 0.045277</td>
</tr>
<tr>
<td>1 mg tannic acid</td>
<td>1.466 ± 0.052</td>
</tr>
<tr>
<td>1.5 mg tannic acid</td>
<td>1.73 ± 0.064031</td>
</tr>
<tr>
<td>Positive Control</td>
<td>2.07 ± 0.15</td>
</tr>
<tr>
<td>2 mg catechin</td>
<td>1.83 ± 0.044721</td>
</tr>
<tr>
<td>4 mg catechin</td>
<td>1.04 ± 0.197864</td>
</tr>
<tr>
<td>6 mg catechin</td>
<td>1.612 ± 0.094181</td>
</tr>
<tr>
<td>Positive Control</td>
<td>2.21 ± 0.180278</td>
</tr>
<tr>
<td>0.5 mg epicatechin</td>
<td>1.958± 0.086429</td>
</tr>
<tr>
<td>1 mg epicatechin</td>
<td>1.014 ± 0.158</td>
</tr>
<tr>
<td>1.5 mg epicatechin</td>
<td>1.682± 0.075961</td>
</tr>
</tbody>
</table>

Fig. 1. Volume of Solid Tumor (mean ± SD) in Mice Bearing EAC before or after the treatment with different doses of catechin, epicatechin and tannic acid (P.C.: Positive Control, Cat.: Catechin, Epi: Epicatechin, Tan.: Tannic acid).

Cytogenetic study:

The recorded chromosomal aberrations included chromatid break (any unstained region in the chromatid with dimeter larger than its width), deletion (when one chromatid was markedly shorter than its sister chromatid) and fragment (when a part of chromatid was observed without an evident centromere. Some chromosomal aberrations were also found in figure 3(c&d) such as end to end association, ring chromosome and others but neglected statistically because they were very rare when calculated.

Inoculation of mice with Ehrlich cancer cells induced a very highly significant (P < 0.001) rise in breaks, fragments and total chromosomal aberrations (TCA). On the other hand, cancer cells induced a highly significant (P < 0.005) rise in chromosomal deletions. Tannic acid has the ability to induce great inhibition in the breaks and deletions (6%, 4.8%) chromosomal aberrations, thus the percentage of breaks and deletions becomes near to normal values (1.6%, 2%) with no significant change. On the other hand, tannic acid reduced the percentage of fragments from a very highly significant (P < 0.001) to a highly significant (P < 0.005) only. As a result of tannic acid treatment, the total number of recorded aberrations (TCA) was still very highly significant (P < 0.001) although the percentage of (TCA) was reduced. Catechin and epicatechin have the great ability to reduce the percentage of different chromosomal aberrations (breaks, fragments and deletions) from a very highly significant (P < 0.001) in case of breaks and fragments and from a highly significant (P < 0.005) in case of deletions to no significant and near to normal values. They also have the ability to reduce the percentage of (TCA) from a very highly significant (P < 0.001) to significant values (P < 0.01). This proves that catechin and epicatechin...
have powerful abilities in reducing different types of chromosomal aberrations (Table 2 and Figs 2&3).

Table 2. Chromosomal aberrations in bone marrow of control mice, mice-bearing solid tumor (Positive Control) and mice-bearing tumor treated with tannic acid, catechin and epicatechin, respectively.

<table>
<thead>
<tr>
<th>Animal groups</th>
<th>No. of Mice</th>
<th>No. of exam. Cells</th>
<th>Structural Chromosomal Aberrations %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Breaks</td>
</tr>
<tr>
<td>Control</td>
<td>5</td>
<td>250</td>
<td>1.6%</td>
</tr>
<tr>
<td>Positive Control</td>
<td>5</td>
<td>250</td>
<td>12.4%***</td>
</tr>
<tr>
<td>Tannic acid</td>
<td>5</td>
<td>250</td>
<td>8%</td>
</tr>
<tr>
<td>Catechin</td>
<td>5</td>
<td>250</td>
<td>3.6%</td>
</tr>
<tr>
<td>Epicatechin</td>
<td>5</td>
<td>250</td>
<td>4%</td>
</tr>
</tbody>
</table>

Fig. 2. Chromosomal aberrations in bone marrow of control mice, mice-bearing solid tumor (Positive Control) and mice-bearing tumor treated with tannic acid, catechin, and epicatechin, respectively.

The mitotic index (number of dividing cells/1000 cells) was very highly significant (P < 0.001) as a result of mitotic inducing agent of Ehrlich cancer cells. The mitosis was significantly (P < 0.01) decreased as a result of tannic acid treatment. On the other hand, catechin and epicatechin have the powerful inhibitory effect against mitosis from a very highly significant (P < 0.001) to no significant (P > 0.01) and this proves about the possible curative reducing effect of catechin and epicatechin against elevated induction in mitotic index as a result of Ehrlich cancer cells (Table 3 & Fig. 4).

Table 3: Mitotic index in bone marrow of control mice, mice-bearing solid tumor and mice-bearing solid tumor treated with tannic acid, catechin and epicatechin, respectively.

<table>
<thead>
<tr>
<th>Animal groups</th>
<th>Mice</th>
<th>No. of Exam. Cells</th>
<th>Mitotic Index (MI) %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5</td>
<td>1000</td>
<td>4.2%</td>
</tr>
<tr>
<td>Positive Control</td>
<td>5</td>
<td>1000</td>
<td>7.8%***</td>
</tr>
<tr>
<td>Tannic acid</td>
<td>5</td>
<td>1000</td>
<td>6.9%*</td>
</tr>
<tr>
<td>Catechin</td>
<td>5</td>
<td>1000</td>
<td>5.6%</td>
</tr>
<tr>
<td>Epicatechin</td>
<td>5</td>
<td>1000</td>
<td>5.9%</td>
</tr>
</tbody>
</table>

Fig. 4. Mitotic index in bone marrow of control mice, mice-bearing solid tumor and mice-bearing solid tumor treated with tannic acid, catechin and epicatechin, respectively.

Discussion

Cancer is one of the leading causes of death in the world, particularly in developing countries. Thus, one of the goals of cancer research has been and continues to be the discovery of natural products for cancer prevention and/or treatment. In the first part of the study Ehrlich carcinoma tumor cells used because they have been shown to be a good model for fast growing in vivo, developing of ascites tumor through intraperitoneal injection or developing solid tumor through subcutaneous injection (Gabai et al., 1995). The present study was designed aiming at highlighting the effects of tannic acid, catechin and epicatechin as chemopreventive agents on the growth of Ehrlich ascites carcinoma cells. The data obtained from this investigation revealed that administration of 1mg tannic acid, 4mg catechin and 1mg epicatechin at the ninth day of tumor inoculation induced a reduction of tumor size which extended to the end of
experimental period. This reduction is in agreement with that obtained by (Vucenik et al., 1992; Vucenik et al., 1998; Vucenik and Shamsuddin, 2003). They found that mice injected with phytic acid (IP6) every other day resulted in a significant inhibition of tumor size. Katiyar et al. (1997) found that green tea polyphenols protected against the induction and subsequent progression of papillomas to squamous cell carcinomas in experimental animals. Wang et al. (1992a&b) showed that green tea polyphenols inhibited the growth of established skin tumors induced chemically. Oral, subcutaneous, or intraperitoneal administration of green tea polyphenols in mice resulted in significant suppression of the growth of implanted tumor cells (Yan, 1992) and this is the same finding in the present study. Ehrlich ascites carcinoma (EAC) induced different structural chromosomal aberrations in bone marrow of mice. This is in agreement with (Solmon et al., 1991) who concluded that EAC induced chromosomal genetic changes through aneuploidy which plays a key role in tumor development and progression. The level of structural chromosomal aberrations had the range of 4-fold to 8.5-fold higher than the control, the percentage of different chromosomal aberrations found in this work is in concomitance with (Valentina et al., 1999) who found that the level of structural chromosomal aberrations was nearly 10 fold higher than control in hepatic tumor. According to previous mentioned results about the anticarcinogenic effects of tannic acid, it has the ability to ameliorate the percentage of different chromosomal aberration by reducing them near to normal values. The percentage of chromosomal aberrations was reduced from 1.6 fold to 2 folds in relation to positive control mice and this proves the curative effect of tannic acid against chromosomal aberrations. Catechins have the powerful reducing effect against different chromosomal aberrations; catechin inhibits the level of different chromosomal aberrations in a range from 2.4 folds to 3.1 folds and epicatechin also reduced the percentage of chromosomal aberration in the same pervious range when compared with these aberrations in positive control group. Also, catechin and epicatechin inhibit the mitotic index to be as found in normal untreated mice, tannic acid is less effective in amelioration of the percentage of mitotic index near to normal values (Table 3). These results are in agreement with (Zhang et al., 2000); who recorded that polyphenols have the ability to induce apoptosis and cell cycle arrest in cancer cells in vivo study.

References


http://www.egyptseb.org

الأثر الإلاجي لمركبات البوليفينول ضد النشوبات الكروموموسية المحدثة في الفئران الحاملة للأورام

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تمت دراسة النشوبات الكروموموسية المحذرة من التخاذ العظمي للفئران الحاملة للأورام السلبية عن طريق حقن الخلايا السرطانية (إرتش) تحت الجلد للفئران القبر مع علاج النشوبات بحمض الثاني ببروتيون (0,5 جم/ 100 جم من وزن الجسم) والمجموعات الثلاثة والرابعة حاملة للأورام السلبية مع علاج بالكاسثين بجرعات (1,5 جم/ 100 جم) و من الأبيكاثين بجرعات (1.0, 1.5 جم / 100 جم) بعد أن يعمر الفئران 3 شهور من قبل الجلد الفئران في الفئران ذاتها. أما المجموعة الأخيرة فيوفرت المجموعة المصابة التي لم ت巰ض للامة بالورم أو أي علاج بل حقن بالحوت ملح و. أثناء التجربة، تم تتذكير الفئران بالورم 3 مرات على خلفية الجلد الفئران. في الوقت نفسه، تم تقديم الفئران للمجموعات الثلاثة والرابعة حاملة الأورام السلبية مع علاج بالكاسثين، وتعرض الفئران للفئران المصابة. كما، تم تقديم الفئران للمجموعات الثلاثة والرابعة حاملة الأورام السلبية مع علاج بالكاسثين، وتعرض الفئران للفئران المصابة. كما، تم تقديم الفئران للمجموعات الثلاثة والرابعة حاملة الأورام السلبية مع علاج بالكاسثين. وجميع الفئران كانت مثالية في إنخفاض النشوبات الكروموموسية الناتجة عن حقن الخلايا الازف تحت جلد فئران التجربة.

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