Cytotoxic effect of camellia sinensis on the normal myeloid and cancer cells of patients with chronic myelogenous leukemia

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ABSTRACT

Objective: The present study was designed to determine in vitro the cytotoxic effect of green tea on the normal and leukemic myeloid stem cells of patients with chronic myelogenous leukemia. Methods: The serial dilution of aqueous green tea extraction in the concentrations (250, 125, 62.5, 31.25, 15.625, 7.8125 µg/ml) were added to the cells culture. Using MTT assay. Results: The results show the cytotoxic effect (inhibition rate of growth) of green tea (viable cell count was 52x10⁵) was in dose and time dependent. There were significant differences (P≤0.05) between concentrations and time. The highest inhibition rate on the leukemic cells was 71.283% in concentration 250 µg/ml and the lowest was 38.370% in concentration 7.8125 µg/ml for exposure time 72 hr. While the highest inhibition rate on the normal myeloid cells was 35.772% inconcentration 250 µg/ml and the lowest was 11.016% in concentration 7.8125 µg/ml for exposure time 72 hr. Conclusions: Green tea showed high effect on the leukemic cells and less effect on the normal myeloid cells culture.

KEYWORDS: Green tea extraction, normal and leukemic myeloid cells.

INTRODUCTION

Chronic myelogenous leukemia (CML) is a type of leukemia in about 20% of all types of leukemia.[1] It is a malignant clonal, myeloproliferative disorders of the pluripotent hematopoietic stem cells.[2,3,4] Characterized by formation of specific abnormal chromosome...
such as Philadelphia chromosome in 95% of patients \(^{(5,6)}\). It occurs mostly in adults and rarely in children.\(^{(7)}\)

Camellia sinensis (green tea) is a plant which belongs to the family Theacaceae. The medical parts of the plant are the very young downy leaves from which green or black tea is produced. It represents approximately 20% of world tea consumption.\(^{(8)}\) It was originally cultivated in China and is grown as a tea plant today in India, China, Sri Lanka, Japan, Indonesia, Kenya, Turkey, Pakistan, Malawi and Argentina.\(^{(9,10)}\)

**MATERIALS AND METHODS**

Fifteen patients with CML and 15 persons (both of normal and leukemic were: 6 males and 9 females and their ages ranged between 35-70 years) with normal hematopoietic stem cells (myeloid cells) who attended to the National Center of hematology/ Al – Mustansiyria University / Baghdad, Al – Kadhimyia Teaching Hospital / Baghdad, Iraq and the private clinic in the period from April 2014 to April 2015 and this work was carried out in the Iraqi Center for Cancer and Medical genetics research.

Human bone marrow was obtained from the posterior iliac crest by an aspiration needle under local anesthesia (10 ml xylocaine), the specimen was taken in EDTA anticoagulant tube for culture.\(^{(11)}\) Sterile PBS was added to the bone marrow specimen for dilution at a ratio of (2 :1), then ficoll- opaque was added slowly in a ratio of ficoll to the diluted blood was (4 : 2) for the isolation of myeloid cells as buffy coat layer. This layer was taken by micropipette gently and put in other tube, then centrifuged at 2000 rpm for 20 minutes and 18° C in cold centrifuge. The cells were taken and removed the supernatant, they washed twice in 10 ml of RPMI-1640 (US Biological, USA) serum free medium with centrifugation at 1000 rpm for 10 minutes at 18° C.\(^{(12,13)}\)

Later, the cells were placed into 25 cm falcon after adding 10 ml of RPMI – 1640 (20% FCS), this medium was prepared by dissolving 16.35g powder of RPMI – 1640 with HEPES buffer and L – glutamine in approximately 600 ml of triple distilled water (TDW) and then added 2 g of sodium bicarbonate powder, 1 ml of ampicillin, 0.5 ml of streptomycin and 200 ml of fetal calf serum (20% FCS) were added. The volume was completed to one liter with triple distilled water and the medium was sterilized, then incubated at 37° C.\(^{(14)}\) After 1 –2 days, the cells culture was examined under inverted microscope to be sure that the cells were confluent monolayer. The subculture was done by adding 10 ml of RPMI-1640 (20% FCS)
to the falcon and dividing it into another falcon after rocking gently for a redistribution into culture falcon, then incubated at 37° C.\[15] The same procedure was used for the maintenance of cells culture in normal and leukemic myeloid cells culture with the viable cells count was $52 \times 10^5$ Cells.

Dried green tea leaves (China) were obtained from the Medical Plant Center/ Ministry of Iraqi Health. The extraction of green tea was done by the conventional hot water extraction method (CWE), in which 1.25g of dry green tea leaves was infused in 100 ml of distilled water at 100 °C for 40 mints to obtain soluble polyphenols dissolved in the aqueous extract. The solution was freshly prepared on daily use, and filtered by a filter paper (Whatman No.1) to obtain 1.25% of aqueous green tea extraction.\[16]

Green tea was prepared in the concentrations (250, 125, 62.5, 31.25, 15.625, 7.8125 µg/ml) by using the maintenance medium (RPMI-1640 serum free medium), two-fold serial dilution were prepared starting from 500 µg / ml as a stock ending with 7.8125 µg /ml. After the growth of myeloid cells (normal and leukemic cells) in falcon at 37° C and reaching to confluent monolayer that was examined under the inverted microscope.

200 µl of cell suspension was transferred to each well in micro titration plate of 96 wells flat bottom (each well contain approximately $52 \times 10^5$ cells for normal and leukemia, then the plate was covered with a sterile adhesive film and incubated at 37 °C. The plate was centrifuged by a cooling centrifuge (1000 rpm for 10 mints) and the medium was gently removed, then 200µl of serially dilution of green tea with concentrations 250, 125, 62.5, 31.25, 15.625, 7.8125 µg / ml were added to the cells. Four replicates were used for each concentration. Others well were used for control (cells treated only with SFM of RPMI-1640). The plate was covered by the sterile adhesive parafilm and re– incubated at 37 °C for a selected time 24, 48 and 72 hr.\[17]

MTT (Methyl thiazolyltetrazolium) assay was used to calculate cells viability after 24, 48, 72hr by removing the medium gently though a micropipette after centrifugation by a cooling centrifuge(1000 rpm for 10 minutes), then 28 µl of MTT solution (2 mg /ml) was added and incubated for 2 hr at 37° C. After removing the MTT solution, the crystals remaining in the wells were dissolved by adding 100µl of dimethylsulfoxid (DMSO). The plate was shaken for 15 minutes and read at 550 nm by ELISA reader.\[18] The end point parameters were
calculated for each normal and leukemic cells culture as follows: Percentage of inhibition rate for cells growth was calculated as: Percentage of inhibition rate = (A – B) / A X 100.

A : is the mean of optical density for untreated wells (control).
B: is the mean of optical density for treated wells.\textsuperscript{19}

**Statistical analysis**
The descriptive data of the results was demonstrated as ranges, percentages , means, standard errors and LSD at P≤0.05 for comparison.\textsuperscript{20}

**RESULTS**
This study showed that the inhibition of growth rate (cytotoxic effect) of green tea on the leukemic myeloid stem cells was in dose and time dependant. There were significant differences ((P≤0.05) between concentrations and time (24, 48,72hr) as in the table (1). While on thenormal myeloid cells depending on the concentration, thus increased the effect with the increased concentration at 72hr, the inhibition rates were 11.016%, 19.264%, 22.262%, 26.501%, 31.686%, 35.772% for 7.8125, 15.625, 31.25, 62.5, 125, 250 µg/ ml, respectively in comparison to inhibition rate on leukemic cells as in figure (1).

Table (1): Inhibition rate of green tea on leukemic cells at 24,48,72 hr.

<table>
<thead>
<tr>
<th>Conc. (µg/ml)</th>
<th>Mean ± SE</th>
<th>LSD value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 hr.</td>
<td>48 hr.</td>
</tr>
<tr>
<td>7.8125</td>
<td>11.02 ± 1.81</td>
<td>24.88 ± 1.28</td>
</tr>
<tr>
<td>15.625</td>
<td>21.84 ± 1.49</td>
<td>34.54 ± 1.53</td>
</tr>
<tr>
<td>31.25</td>
<td>28.80 ± 1.66</td>
<td>40.89 ± 0.48</td>
</tr>
<tr>
<td>62.50</td>
<td>39.29 ± 0.68</td>
<td>49.17 ± 2.45</td>
</tr>
<tr>
<td>125</td>
<td>45.28 ± 1.21</td>
<td>55.74 ± 1.22</td>
</tr>
<tr>
<td>250</td>
<td>59.69 ± 1.81</td>
<td>61.97 ± 0.56</td>
</tr>
<tr>
<td>LSD value</td>
<td>4.621 *</td>
<td>4.374 *</td>
</tr>
</tbody>
</table>

SEM* = standard error of mean.
* = significant differences.
DISCUSSION

In this study showed highly effect of green tea on the leukemic cells and less effect on the normal myeloid cells. The classical method for evaluating the effect of herb on cells is based on proportion of inhibition that indicates the rate of inhibition of the cell growth or percentage of toxicity.

Tea is the most beverage consumed in the world. The relationship between tea and cancer incidence is an important concern. Several studies of green tea showed a protective effect of tea consumption against certain types of cancer. In this study, the green tea activity contributed to presence of polyphenols.\(^{[21]}\)

There are four major polyphenols found in the leaves such as epigallocatechingallate (EGCG), epigallocatechin (EGC), epicatechin (EC) and epicatechingallate (ECG). The EGCG is the most powerful catechin and the active constituent is epigallocatechin – 3-gallate that account for 40% of the total polyphenols\(^{[22,23]}\) and the antioxidant capacity is about 100 times greater than vitamin C and 25 times greater than vitamin E which protects the DNA from the free radical damage that is suggested to increase the risk of cancer. The EGCG has a critical role in inhibiting the DNA synthesis and cell replication which is important for the survival of cancer cells. It inhibits the dangerous activity of the cell leading to an uncontrol growth.\(^{[24,25]}\) It selectively induces apoptosis in human carcinoma, inhibits telomerase and methyltransferase and protects cells from lipid peroxidation. Therefore, the
effect depends on the causative factor on a specific cancer. There is no available data for the effect of green tea on the CML and normal myeloid cells.

CONCLUSIONS
Green tea showed high effect on the leukemic cells and less effect on the normal myeloid cells culture. This is a guide for choosing any drug or herb with high cytotoxic effect on the leukemic cells and less cytotoxic effect on the normal cells.

REFERENCES