

## Cytotoxic effect of green tea leaf extract on tumor cell line

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### Summary

The study was conducted to evaluate antitumor effects of green tea (*Camellia sinensis*) extracts (aqueous and methanolic) on Rhabdomyosarcoma; cell line and a normal cell line; mouse embryo fibroblast; Chemical detections of green tea extracts revealed that the aqueous and methanolic extracts were positive for flavonoids, alkaloids, phenol and glycosides. The percentage growth inhibition of five plant concentrations (50, 100, 250, 500 and 1000 µg/ml) were assessed *in vitro* using tumor cell lines Rhabdomyosarcoma and normal cell line mouse embryonic fibroblasts. The results revealed that the five concentrations of the plant extracts showed anti-tumor properties in a concentration-dependent manner, and the methanolic extract recorded better values of percentage growth inhibition than the aqueous extract in Rhabdomyosarcoma cell lines, while, less percentage growth inhibition values were recorded in the mouse embryo fibroblast cell line.

**Keywords: Green tea extract, Antitumor, Rhabdomyosarcoma.**

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### Introduction

Plants are source of different compounds. They contain and can produce a variety of chemical substances that have different biological actions, with a special reference to their medical importance. Therefore, they are employed by herbalists of different cultures, anciently and recently, to remedy peoples of their sicknesses (1). Green tea is a type of tea made solely from the leaves of *Camellia sinensis*, that has undergone minimal oxidation during processing. Many varieties of green tea have been created in countries where it is grown, these varieties can differ substantially due to variable growing conditions, processing and harvesting time (2 and 3). The phytochemical screening of tea revealed the presence of alkaloids, saponins, tannins, catechin and polyphenols. The cardinal antioxidative ingredient in the green tea extract is green tea catechins which comprise four major epicatechin derivatives; namely, epicatechin (EC), epigallocatechin, (EGC), epicatechingallate (ECG), and epigallocatechingallate (EGCG) (4). Among their many biological activities, the predominant polyphenols in green tea-EGCG, EGC, ECG, EC, the theaflavins and thearubigins in black tea have antioxidant activity these chemicals, especially EGCG and ECG, have substantial free radical scavenging activity and may protect cells from DNA

damage caused by reactive oxygen species. Tea polyphenols have also been shown to inhibit tumor cell proliferation and induce apoptosis in laboratory and animal studies (5). In other laboratory and animal studies, tea catechins have been shown to inhibit angiogenesis and tumor cell invasiveness (6 and 7). In addition, tea polyphenols may protect against damage caused by ultraviolet (UV) B radiation, and they may modulate immune system function (8). Furthermore, green teas have been shown to activate detoxification enzymes, such as glutathione S-transferase and quinone reductase that may help protect against tumor development (9). Although many of the potential beneficial effects of tea have been attributed to the strong antioxidant activity of tea polyphenols, the precise mechanism by which tea might help prevent cancer has not been established (10). Therefore this study was conducted to evaluate antitumor effects of green tea (*Camellia sinensis*) extracts (aqueous and methanolic) on tumor cell line and a normal cell line.

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### Materials and Methods

The plant (*Camelli sinensis*) Leaves were collected from local market then grounded into fine powder by using grinding machine (11). Preparation of water and methanolic extracts of *Camellia sinensis* leaves for antimicrobial activity: Water extract: Twenty five grams of

the leaf powder were extracted for three hours in 250 ml of the distilled water using the soxhlet apparatus and the source of heating was water bath (70°C). The filtrate was then evaporated at 70°C using a rotary evaporator, and the resultant crude extract was frozen at -20°C until use to prepare the required concentrations (12).

**Methanolic extract:** Twenty five grams of leaf powder were extracted for six hours in 250 ml of 95% methanol. The filtrate was concentrated using rotary evaporator at 40 °C until dryness and the resultant crude extract was frozen at -20°C until use to prepare the required concentrations (13).

**Detection of some active compounds in water and methanolic leaf extracts of green tea:** The detection of alkaloid was done by thoroughly mixed aliquot of 0.2 ml of the water or methanolic extract with 5 ml of 1% HCl in a steam bath, and then 1 ml was treated with Mayer's reagent; the appearance of white precipitate was an evidence for the presence of alkaloid (14).

The flavonoids detected by macerating a quantity of 10 g of the powdered explants in 95% ethanol then filtered and mixed with aliquot of 10 ml of 50% ethanol added to 10 ml of 50% aqueous KOH, the appearance of yellow color was an evidence for the presence of flavonoids (15).

The detection of phenol was performed by mixing an equal quantity of aqueous ferric chloride 1% with potassium iron cyanide 1%. Equal quantity of the reagent and aqueous or methanolic plant extract were mixed. The appearance of blue-green color indicated the presence of phenol (16).

**Detection of glycosides:** Equal amounts of the water and methanolic extract was mixed with Fehling's reagent in a test tube, and then boiled in a water bath for 10 min. The formation of red precipitate indicated the presence of glycosides (17).

The *in vitro* anti-tumor activity of aqueous and methanolic extracts was carried out at the Iraqi Center for Cancer and Medical Genetic Research (Al-Mustansiryah University). In this study, the cytotoxic activity of the two plant extracts was evaluated against two tumor cell lines (human rhabdomyosarcoma; RD and a normal cell line (mouse embryonic fibroblasts;

MEF). The percentage of growth inhibition was calculated according to an equation presented by (2001) (18):

$$\text{Growth inhibition}\% = \left\{ \frac{\text{control absorbance} - \text{treated absorbance}}{\text{control absorbance}} \right\} \times 100$$

**Growth Inhibition Assessment in RD, MEF Cell Lines:** The RD cell line was on passage number 195 and MEF was the normal cell line on passage number 54. The laboratory assessment of growth inhibition was carried out according to a method adopted by (2000) (19). The cells (RD) were supplemented as a monolayer attached cells in Falcon culture flasks (25 ml) containing RPMI-1640 medium. The cells were washed with PBS, and then 1 ml of trypsin–versine solution was added with a gentle shaking until the cells were detached from the flask surface. Such manipulation was carried out with the aid of phase contrast inverted microscope. Then, the contents of the flask were transferred to another flask and incubated at 37°C for 15 min. (sub-culture), then the cells were counted and their number was adjusted to  $1 \times 10^6$  cell/ml. At the same time, viability was assessed using a dye-exclusion test (trypan blue stain), and it was always greater than 96%. The cells were seeded in the wells of 96-well tissue culture plate, which was carried out by pipetting 150 µl of the cell suspension into each well, and the plate was incubated overnight at 37°C. The day after, the wells were examined to inspect the formation of cell monolayer, and then five concentrations (50, 100, 250, 500 and 1000 µg/ml) of each plant extract were prepared and the solvent was RPMI-1640 medium. In each well, 50 µl of each concentration was pipetted and the plate was wrapped with a cling film and incubated at 37°C. In this regard three plates were prepared to cover three incubation periods; 24, 48 and 72 hrs. At the end of each incubation period, the medium in each well was discarded and the wells were washed once with PBS, and then 0.8 ml of neutral red solution was added to each well. The plate was re-incubated at 37°C for 2 hrs. After incubation, the neutral red solution was discarded and each well was washed once with PBS, and in each well, 0.1 ml of phosphate buffered-ethanol (0.1M NaH<sub>2</sub>PO<sub>4</sub>-ethanol; 1:1)

was added. Then the wells were read using ELISA reader at wave length of 492 nm, and the absorbance was recorded. The growth inhibition of the tested materials was calculated using the following equation:

$$\text{Growth inhibition\%} = \left\{ \frac{\text{control absorbance} - \text{treated absorbance}}{\text{control absorbance}} \right\} \times 100$$

The values of the investigated parameters were expressed in terms of mean ± standard error, and differences between means were assessed by analysis of variance (ANOVA), least significant difference (LSD) and Duncan test, using the computer program SPSS version 11.5. The difference was considered significant when the probability value was equal or less than 0.05.

### Results and Discussion

The phytochemical screening of green tea revealed the presence of alkaloids, flavonoids, phenols and glycosides (Table, 1). One of the major antioxidant ingredients in the green tea extract is green tea catechins (GTC), which comprise four major epicatechin (EC) derivatives; namely, epigallocatechin (EGC), epicatechingallate (ECG) and epigallocatechin gallate (EGCG). The amount of catechin in various tea extract was in the order green tea (26.7%), oolong tea (23.2%), pouching tea (15.8%) and black tea (14.3%) (3 and 4) The Results revealed that the 5th concentration (1000 mg/ml) of the aqueous and methanolic extract was recorded the highest percentage growth inhibition in Rhabdomyosarcoma cell lines (87 and 90) % respectively. However, the first four concentrations contradicted such scope, in which the PGI recorded significantly (P ≤ 0.05) higher values in methanolic extract (68, 69, 70 and 74) % compared with aqueous extract (39, 33, 29 and 19) % respectively (Table, 2). The aqueous and methanolic extracts recorded an approximated range of PGI against the mouse embryonic fibroblast cell line (aqueous extract: 8-14%; methanol extract: 14-19% with the exception of the concentrations 1000 and 500 µg/ml, in which the methanolic extract demonstrated a significantly (P ≤ 0.05) increased PGI as compared with the PGI of the corresponding aqueous extract concentration (19 vs. 14%) (Table, 3).

**Table, 1: Some secondary metabolites detected in *Cammellia sinensis* water and methanolic leaf extracts.**

Type of secondary metabolite	Result of detection (leaves)	
	Water extract	Methanolic extract
Alkaloids	+ve	+ve
Flavonoids	+ve	+ve
Phenols	+ve	+ve
Glycosides	+ve	+ve

+ve indicates the presence of secondary metabolite.

**Table, 2: Growth inhibition effects of green tea aqueous and methanol extract on RD cell lines.**

Extract Concentration (µg/ml)	Percentage of Growth Inhibition (Mean ± S.E.)*	
	Aqueous extract	Methanol extract
50	19.50 ± 0.24 <sup>c</sup>	68.00 ± 7.94 <sup>b</sup>
100	29.00 ± 2.08 <sup>b</sup>	69.00 ± 6.56 <sup>b</sup>
250	33.33 ± 0.66 <sup>b</sup>	70.00 ± 2.00 <sup>b</sup>
500	39.00 ± 1.00 <sup>b</sup>	74.00 ± 1.00 <sup>b</sup>
1000	87.00 ± 3.15 <sup>a</sup>	90.00 ± 2.65 <sup>a</sup>

Different letters indicates Significant (P ≤ 0.5) difference between means of each columns.

**Table, 3: Growth inhibition effects of green tea extracts (aqueous and methanolic) on MEF cell line.**

Extract Concentration (µg/ml)	Percentage of Growth Inhibition (Mean ± S.E.)*	
	Aqueous extract	Methanol extract
50	8.80 ± 0.72 <sup>b</sup>	14.50 ± 1.44 <sup>b</sup>
100	10.90 ± 1.30 <sup>b</sup>	15.63 ± 0.73 <sup>b</sup>
250	12.40 ± 1.56 <sup>b</sup>	14.13 ± 2.92 <sup>b</sup>
500	14.00 ± 1.53 <sup>a</sup>	19.70 ± 1.24 <sup>a</sup>
1000	14.00 ± 1.15 <sup>a</sup>	19.53 ± 0.74 <sup>a</sup>

Different letters indicates significant (P ≤ 0.5) difference between means of each columns.

The Cytotoxic effect of green tea leaves was mentioned by (20) who reported that six compounds were isolated and purified from the aqueous-alcoholic extract of green tea leaves, including gallic acid (GA), (-)-epicatechin (EC), (-)-epigallocatechin (EGC), (-)-epicatechingallate (ECG), (-)-epigallocatechingallate (EGCG) and caffeine. Together with (+)-catechin, these compounds were tested against each of four human tumor cell lines (MCF-7 breast carcinoma, HT-29 colon carcinoma, A-427 lung carcinoma and UACC-375 melanoma), the three most potent green tea components against all four tumor cell lines were EGCG, GC and EGC. EGCG was the most potent of the seven green tea components against three out of the four cell lines (MCF-7 breast cancer, HT-29 colon cancer and UACC-375 melanoma).

Researchers (21) tested that the inhibitory effects of green tea components were tested on the prostate cancer cell lines LNCaP, PC-3 and DU145. (-)-Epigallocatechin-3-gallate (EGCG) proved to be the most potent catechin at inhibiting cell growth. The inhibition induced by EGCG was found to occur via apoptotic cell death as shown by changes in nuclear morphology and DNA fragmentation. Another study by (22) proved that the treatment with 50  $\mu$ M and 100  $\mu$ M EGCG increased the percentages of cells in the G2-M phase from 13.8% to 15.6% and 24.1%, respectively and the DNA histogram after treatment with 100  $\mu$ M EGCG was similar to that after treatment with genistein, suggesting that EGCG induces G2-M arrest in PC-9 cells. Cancer-related research is conducted worldwide every day, since cancer is a leading cause of death. These studies often involve the investigation of the effects of biologically active substances on cancer cells, and they frequently originate from plants. In conclusion, the present study demonstrated that methanolic extract of green tea is a potent anti-cancer compound inducing growth inhibition in Rhabdomyosarcoma. Further research based on animal models may resolve in vivo efficacy of green tea.

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### التأثير السمي لمستخلص أوراق الشاي الأخضر في الخلايا السرطانية

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#### الخلاصة

صممت الدراسة لتقييم التأثير السمي للمستخلص الكحولي والمائي لأوراق الشاي في خط خلايا سرطان العضلات المخططة البشري وخط خلايا الفأر الجنينية الطبيعية المولدة للألياف. وأظهرت الفحوصات الكيميائية للمستخلصين المائي والكحولي للنبات وجود مركبات الفلافونويد، القلويدات، الفينول والجليكوسيدات. وقيمت نسبة تثبيط النمو لخمسة تراكيز (50 و 100 و 250 و 500 و 1000 ميكروغرام/مل) للمستخلصين الكحولي والمائي للنبات في المختبر باستعمال خط الخلايا السرطانية وخط الخلايا الطبيعية وكشفت النتائج أن التراكيز الخمسة للمستخلصين الكحولي والمائي للنبات أظهرت خصائص مضادة للورم بطريقة تعتمد على التركيز، وسجل المستخلص الكحولي للنبات قيم تثبيط للنمو أفضل من المستخلص المائي في خط خلايا سرطان العضلات المخططة البشري، في حين سجلت أقل قيم لتثبيط النمو في خط خلايا الفأر الجنينية الطبيعية المولدة للألياف.

الكلمات المفتاحية: مستخلص الشاي الأخضر، مضاد للورم، سرطان الخلايا المخططة البشري.