# Journal of Pharmacology and Toxicology

ISSN 1816-496X

Academic Journals Inc.

www.academicjournals.com

OPEN ACCESS Journal of Pharmacology and Toxicology

ISSN 1816-496X
DOI: 10.3923/jpt.2017.142.147

# Research Article
# White and Oolong Tea Extracts Inhibition of Fibroblast and Cancer Cell Proliferation Unrelated to the Proanthocyanidins Constituent

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## Abstract

**Background:** Proanthocyanidins are bioactive compounds, found in herbal medicines including, the *Camellia sinensis* plant that exhibited an antioxidant and anticancer activities in both *in vitro* and *in vivo* models. **Objective:** This study aimed to investigate the cytotoxic effect of white and oolong tea against cancer cells and fibroblasts and to link these effects to their content of the proanthocyanidins level. **Materials and Methods:** An aqueous white and oolong tea extracts (10% w/v) prepared by a microwave assisted method. Proanthocyanidins content in each extract is determined and its levels expressed as catechins. The cell lines belonged to HeLa, human rhabdomyosarcoma, mammary (AMN3) adenocarcinoma and primary rat embryo fibroblast cell lines were incubated with each tea extract (equivalent to 2.5 mg of dry weight tea) in RPMI-1640 culture media supplemented with 5% fetal calf serum. Statistical analysis achieved by using Student's t test and difference between percentages test. **Results:** The mean levels of proanthocyanidins in oolong tea found to be four times of that in white tea extract. The extracts of white and oolong tea significantly inhibit the growth of fibroblasts (30.6 and 24%), HeLa (32.7 and 35.3%) and rhabdomyosarcoma cells (66.3 and 59%) of the initial percentage of the cells that did not treat with tea extracts. White and oolong tea extracts did not significantly inhibit the growth of mammary-AMN3 adenocarcinoma cell line (91.7 and 77.8% versus 100%). **Conclusion:** White and oolong tea extracts inhibit the growth of cancer cells and fibroblasts by a mechanism that does not relate to the antioxidant-bioactive substance the proanthocyanidins.

Key words: White tea, oolong tea, HeLa cell, rhabdomyosarcoma cell, mammary adenocarcinoma cell, fibroblast, proanthocyanidins

Received: March 18, 2017 Accepted: May 25, 2017 Published: June 15, 2017

Citation: Marwan Salih Mohamud Al-Nimer, Huda Ghassn Hameed and Nahi Youssif Yaseen, 2017. White and oolong tea extracts inhibition of fibroblast and cancer cell proliferation unrelated to the proanthocyanidins constituent. J. Pharmacol. Toxicol., 12: 142-147.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

## INTRODUCTION

In ancient medicine, tea has been used as a medicinal herb. During the Song Dynasty in China (960 and 1279 AD), the Chinese found that mild tasting and refreshing tea can be obtained from the youngest buds and at that time it was named as "Emperor" and later on known as white tea because of its downy hairs that gave a silvery-gray appearance[1]. White tea (*Bái Chá*) prepared from withering the leaves of *Camellia sinensis* followed by air-or-solar drying of these leaves[2]. Oolong tea or black dragon (Oo means black and Long means Dragon) appeared firstly in Qing Dynasty publications (1644-1911). Oolong tea prepared from crushing the rims of the leaves of *Camellia sinensis* and then this leave subjected to a fermentation process for a short period of time[3]. The phenolic compounds with the antioxidant profile of white tea are similar to those of green tea[4]. The leaves of *Camellia sinensis* contained at least 16 bioactive substances that have a role in the protection of cells against the harmful effects of reactive oxygen species[5,6]. Theasinensin are the polyphenols bioactive compounds in the oolong tea that differed from black tea aflavins and green tea catechins, that suppressed the generation of proinflammatory mediators and reactive nitrogen species[7]. Proanthocyanidins that available in the *Camellia sinensis* plant have antioxidant and anticancer activities in both *in vitro* and *in vivo* studies[8-10]. Dou *et al.*[11] found that 20% of proanthocyanidins of oolong tea is destroying by oxidization during the manufacturing process from fresh leaves. White tea extract protects the DNA of the normal cells against oxidative damage, but inhibits the proliferation of the colorectal cancer cell *in vitro* cell line culture[12]. Herbal medicines with high levels of proanthocyanidins and other flavonoids have antiproliferative effects against various cancer cells[13]. Yu *et al.*[14] attributed the potential anticancer effect of some proanthocyanidins to their chemical structure which characterized by two double inter-flavonoid linkages.

The majority of research concerned with the green tea as it is the herbal medicine that promotes the health, while there is a little information that highlighted the anticancer effect of white and/or oolong tea. The rationale of this study is the antiproliferative effect of the herbal medicine is not restricted to the certain antioxidant ingredient e.g., proanthocyanidins but to the activity of many antioxidants as the anticancer effects of white and oolong tea extracts will be similar despite the difference in the levels of proanthocyanidins in the extracts. Therefore, this study aimed to demonstrate the antiproliferative and cytotoxic effects of white and oolong tea extracts on the cancer and fibroblast cells and to determine the levels of the proanthocyanidins in these herbal tea extracts.

## MATERIALS AND METHODS

This study was conducted in the Department of Pharmacology in the College of Medicine incorporation with National Center of Cancer Research at Al-Mustansiriya University, Baghdad, Iraq from January, 2014 to December, 2014. This study approved by the Institutional Scientific Committee at the College of Medicine.

**Extraction of herbal tea:** The herbal tea, including white and oolong were purchased from local markets in the form of tea bags. An extract prepared by weighing a known weight of herbal tea in a known volume of distilled water to obtain (10% w/v) and using microwave-assisted extraction method (the irradiation power is 600 W and the temperature adjusted at 84°C)[15]. Each extract was preheated for 45 sec followed by a cycle of 10 sec turn off, then a 3 sec irradiation by microwave (Microwave full power 600 W, Samsung, Korea) and this cycle repeated for three times.

**UV-visible scanning of herbal extracts:** Each extract was scanned using UV-visible spectrophotometer ranged between 200-900 nm wavelengths with a maximum absorbance of 3,000 (optical density absorbance) seeking for maximum absorbance at a specific wavelength. Approximately 25 µL of each extract (10% w/v) diluted with distilled water (total volume 4 mL) before scanning. The obtained peak represented the extract concentration equal to 625 µg $mL^{-1}$ (i.e., the dilution factor is 80).

**Determination of proanthocyanidins:** The total amount of proanthocyanidins was determined by mixing 0.5 mL of 0.1 mg $mL^{-1}$ extract tea with 3 mL of 4% vanillin-methanol solution, then adding 1.5 mL hydrochloric acid (0.1N HCl) and allowing to stand for 15 min, then read at 500 nm[16]. The concentration of proanthocyanidins level was calculated using the linear equation based on the calibration curve of catechins (catechins concentration = 0.5825×absorbance). The proanthocyanidins content was expressed as µg catechins equivalent/mg dry weight of tea.

**Cell cultures, reagents and cellular growth:** HeLa, human rhabdomyosarcoma, mammary (AMN3) adenocarcinoma and primary rat embryo fibroblast cell lines (provided by The National Center for Cancer Research in Baghdad, Iraq) were maintained in RPMI-1640 culture media supplemented with 5% fetal calf serum, 0.5% ampicillin and 0.5% streptomycin and maintained in 5% $CO_2$ at 37°C[15]. Several trials of cell reactivation were carried to obtain a monolayer cells in a specific falcon (volume 25 mL) which can be adjusted under

the microscope to look for the presence of monolayer cell. Once the growth of monolayer cells has been formed, the old growth media discarded, washed with Phosphate Buffer Saline (PBS) once, added 0.5-1 mL trypsin-version solution, then added 10 mL sterile growth media (supplemented with 5% fetal calf serum. The cells were plated at $1\times10^5$ cells per well in 200 µL of complete culture medium containing herbal tea extracts (corresponding to 2.5 mg dry weight) and incubated at 37°C for 24 h. The sterilization of the herbal tea achieved by mechanical filtration (using 0.2 µm millipore filter device) prior to the addition.

On the next day (i.e., after 24 h), the viability of cells was determined by the MTT assay. Approximately 30 µL of prepared tetrazolium dye [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazoliumbromide] (5 mg $mL^{-1}$ in phosphate buffer solution) was added to each well in a dark room (to avoid oxidation of the dye) and incubated for 2 h at 37.5°C in the incubator[15]. Then after, whole wells contents were discarded, 100 µL dimethyl sulfoxide added, shaken the microtiter plate for 15 min by using a horizontal shaker before recording the absorbance of each well at 540 nm by ELISA reader.

**Statistical analysis:** The results were expressed as number, percent and whenever possible as Mean±SD. The data were analyzed using Student's t test (unpaired, two tailed) and the difference between percentages test taking the probability of $\leq$0.05 is the lowest limit of significance. All calculations were made using Excel 2003 program for Windows.

## RESULTS

The UV-Visible scan of the white extract showed the presence of a peak of 0.424 absorbance detected at wavelength 355 nm while the oolong tea extract showed two peaks of 1.309 and 0.238 absorbance at wavelengths of 270 and 355 nm, respectively (Fig. 1). The mean level of proanthocyanidins that detected in oolong tea is four times of that detected in white tea extracts (1 µg versus 0.25 µg $mg^{-1}$ dry weight of corresponding tea) (Fig. 2). The cytotoxic effects of herbal tea extracts against fibroblast and three cancer cell lines were illustrated in the Fig. 3. The herbal extract of white and oolong tea significantly ($p<0.001$) inhibits the growth of fibroblast cell compared with cells did not treat with tea extract (30.6±12.8 and 24.0±11.2 versus 100±23%) (Fig. 3a). The inhibitory effect of white and oolong tea extract against the growth of AMN3 mammary cell line did not significantly ($p>0.05$) inhibit from un-treated cells (91.7±41.7 and 77.8±39±39.8 versus 100±18.5%) (Fig. 3b). A significant ($p<0.01$) cytotoxic effects of white and oolong tea

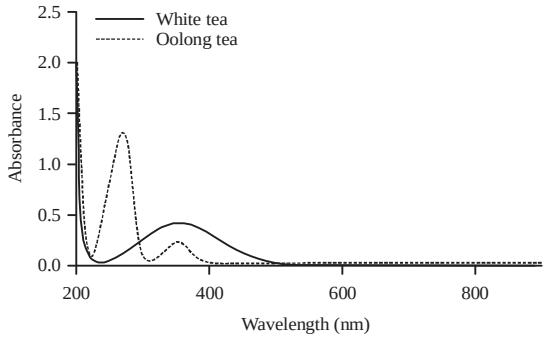


Fig. 1: UV-Visible spectra of aqueous herbal tea extracted by microwave-assisted method

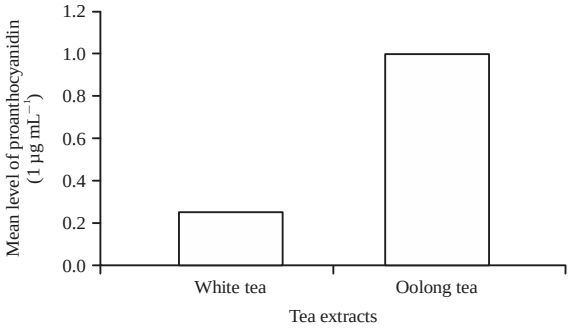


Fig. 2: Mean value of proanthocyanidins level in aqueous herbal tea extracted by microwave-assisted method

extract are observed against rhabdomyosarcoma cell line compared with un-treated cell line (68.3±11.7 and 59.0±13.0 versus 100.0±26.1%) (Fig. 3c). The growth of HeLa cell line is significantly ($p<0.001$) inhibited with white (32.7±11.9%) and oolong (35.3±9.2%) tea extracts compared with un-treated HeLa cell line (100±21.2%) (Fig. 3d).

## DISCUSSION

The results of this study showed that oolong tea extract contained high amount of proanthocyanidins compared with white tea extract by four times. There is a non-significant difference ($p>0.05$) between the antiproliferative effect of white tea and oolong tea extracts against fibroblasts, rhabdomyosarcoma and HeLa cells. Therefore, the antiproliferative effect of tea extracts is independent on the amount of proanthocyanidins contents in the tea.

The absorbance peak wave at wavelength 355 nm that observed in the oolong and white tea extract scans is indicated the presence of the antioxidant rutin[17]. The peak wave absorbance at wavelength 270 nm that observed in oolong tea extract also indicated the presence of the bioactive substance rutin.

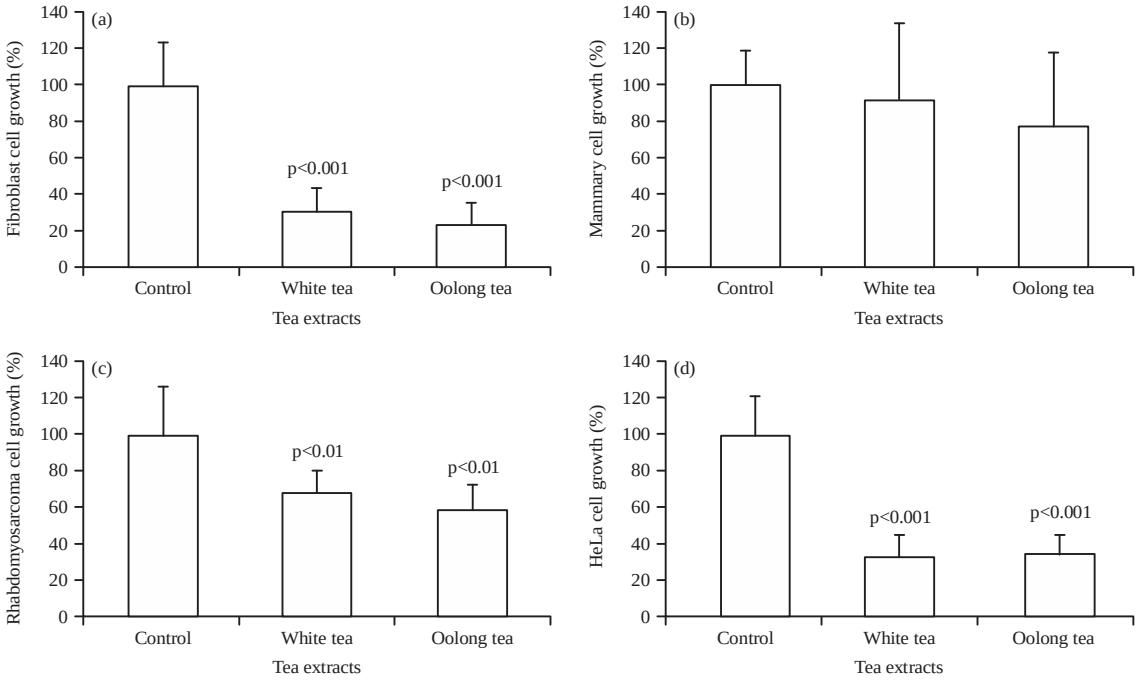


Fig. 3(a-d): Effect of herbal tea extract on the growth of (a) Fibroblast, (b) Mammary, (c) Rhabdomyosarcoma and (d) HeLa cell lines

The results expressed as mean and standard deviation (indicated by bar)

Sikora and Ogonowski[18] demonstrated the UV-Visible spectra of green tea extract was similar to the UV-Visible spectra of rutin, which showed the absorbance maximum, at wavelength 270 nm and less intensive at 355 nm. Therefore, the overall constituent of this bioactive substance was higher in oolong tea than white tea extract.

Biochemical analysis of the tea extracts revealed that the concentration of a proanthocyanidins level in oolong tea extract is four times of the white tea extract. This may be due to the manufacturing process of each tea. One study demonstrated that only 20% of proanthocyanidins is lost during the manufacturing process of oolong tea while the white tea did undergo fermentation process because is made from immature leaves, that is, the buds have fully opened[11].

Both white and oolong herbal tea extracts inhibit significantly the growth of fibroblast ($p<0.001$), HeLa cell ($p<0.001$) and rhabdomyosarcoma cell ($p<0.01$) but the differences between these two extracts did not reach the significant level ($p>0.05$).

The cytotoxic effect of oolong and white tea extracts against the growth of fibroblast indicated that these extracts have antifibrotic effect. There is evidence that epigallocatechin-3-gallate, the most abundant catechin in green tea, inhibits the pulmonary fibrosis that induced by irradiation in rats. Therefore, we can attribute the fibroblast cytotoxicity that observed in this study to the polyphenols contents [19]. In addition, the epigallocatechin-3-gallate caused dysfunction of the mitochondria and through inducing the reactive oxygen species and this could explain the antiproliferative effect of tea extract or its constituents against cancer cells[20]. On the other hand, Thring *et al.*[21] demonstrated the protective effect of white tea on human fibroblast against reactive oxygen species. Otherwise, a literature search did not show the cytotoxic effect of white or oolong tea extract on the growth of fibroblast. There is evidence that the cytotoxic effect of proanthocyanidins exert variable cytotoxic effect against inflammatory and cancer cell[22], which explained our finding that there is no significant difference in the cytotoxic effect against fibroblast cell, between white and oolong tea extracts despite of wide difference in the amount of proanthocyanidins levels in these extracts.

As early as Landau *et al.*[23] reported that black tea extract inhibited the formation of rhabdomyosarcoma in mice. Recently, Okamoto *et al.*[24] reported that a synthetic derivative of procyanidin inhibits the growth of HeLa cells more potent than the green tea polyphenol and they attributed the anti-cancer effect to the phenolic hydroxyl groups. It is possible to attribute the antiproliferative effect of white and oolong tea extracts against HeLa cell growth to the epigallocatechin-3-gallate by binding to the tubulin system and thereby inhibits the cell division[25]. Other mechanisms that lead to cell cycle arrest cannot exclude[26].

## CONCLUSION

It is concluded that no significant difference between white and oolong tea extracts, against the antiproliferative effect against cancer cells and the cytotoxic effect against the fibroblast cell despite there is a significant ($p<0.001$) differences in the proanthocyanidins contents and a variability in the absorbance peaks of active ingredients that detected by UV-visible spectra.

## SIGNIFICANCE STATEMENTS

This study discovered the significant antiproliferative effect of white and oolong tea extracts against fibroblast, HeLa and rhabdomyosarcoma cancer cell line irrespective to their content of proanthocyanidins levels. This study will help the researcher to uncover the critical area of the anticancer effect of *Camellia sinensis*. Thus a new theory on the anticancer effect of white and oolong tea may be not related to the proanthocyanidins levels.

## ACKNOWLEDGMENT

The author would like to thank the College of Medicine, Al-Mustansiriya University and members of the National Center of Cancer Research for their cooperation and providing support for this study.

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