Medicinal & Aromatic Plants
ISSN: 2167-0412

Khan et al., Med Aromat Plants (Los Angeles) 2018, 7:6
DOI: 10.4172/2167-0412.1000322

Research Article Open Access

Purple Tea Composition and Inhibitory Effect of Anthocyanin-Rich Extract on Cancer Cell Proliferation

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Received date: October 27, 2018; **Accepted date:** November 12, 2018; **Published date:** November 22, 2018

Abstract

Tea is commonly used beverage for thousands of years and different forms have been consumed in different parts of the world. Over the time the intake has increased because of proven health benefits, more of green tea. However recently, a modified form of tea named purple tea has claimed to have a high level of anti-oxidants and anti-proliferative properties but these activities of purple tea (PT) are not widely studied. In our study, we checked PT on three different cancer cells NCI-H460 (lung carcinoma), MCF-7 (breast carcinoma) and HeLa (cervical) using the sulforhodamine-B method. We observed that PT extract significantly inhibited cell growth (GI_{50}:165 mg/mL for NCI-H460; 230 mg/mL for MCF-7 and 100 mg/mL for HeLa).

Keywords: *Camellia sinensis*; Tannin; Lung carcinoma; Anti-metastatic

Introduction

Tea is common, non-alcoholic beverage consumed worldwide. Tea is made from leaf of plant *Camellia sinensis*, and can be green, black, oolong and white depending on the processing technique used. Amongst them green tea has gained most of popularity as various studies have associate health benefits like anti-cancer, anti-diabetic with it [1,2]. However, recently purple tea (PT) which is derived from *Camellia sinensis* developed by Tea Research Foundation of Kenya (TRFK) is claimed to have more health benefits than green tea [3]. The health benefits of green tea lie in its composition, mostly polyphenols known as catechins. Catechins is a group of about 30 kind of phenolic compounds mainly including epigallocatechin-3-gallate (EGCG), epigallocatechin (EGC), epicatechin-3-gallate (ECG) and epicatechin (EC). In comparison to green and black tea, purple tea has relatively lesser caffeine content [4]. In addition to common polyphenols with other teas, PT has some unique combination with high levels of special type of polyphenol 1,2-di-O-galloyl-4,6-O-(S)-hexahydroxydiphenoyl-β-D-glucose (GHG), a hydro-lysable tannin and anthocyanidins (malvidin, peralgonodin and cyanidin 3-O-galactoside) [3]. PT is unique among other tea in composition and contains high levels of anthocyanins (135-fold) anthocyanidins (3.5-fold) compared to normal tea. This water-soluble pigment is found in many plants in different percentage including red grapes [5]. berries (blueberry, strawberry, raspberry, blackcurrant, bilberry, cranberry, elderberry) [6]. for different biological purpose such as attracting pollinators and seed dispersers [7]. PT is estimated to contain a 1.5% of anthocyanin compared to 0.1% in blueberries. Anthocyanin has a role in protecting plants against various stresses [8].

PT has high free-radical scavenging rate i.e., 52% compared to 34% for green tea and 28% for black tea [9]. The high level of anthocyanin is seen responsible for its greater antioxidant activity [9]. Preliminary studies have shown some health benefits of anthocyanin rich purple tea [10,11]. This present study investigates the anti-cancer activities of purple tea on three different cell lines, NCI-H460 (lung carcinoma, lung), MCF-7 (adenocarcinoma, breast) and HeLa (uterine cervix).

Materials and Methods

Extract preparation

One gram of PT was dissolved in 100 mL of water and heated at 70°C for 30 minutes. The resulting mixture was kept on a hot plate with constant stirring at 40°C, until water was evaporated, and dry extract was obtained. The extract obtained was dissolved in double distilled water to prepare the stock of 10 mg/mL, which was later used for the experiment.

Cancer cell lines, chemicals and spectral measurements

For Growth inhibitory and cytotoxic activity, human cancer cell lines, NCI-H460 (large cell carcinoma, lung) and MCF-7 (adenocarcinoma, breast) were kindly provided by the National Cancer Institute (NCI), Frederick, MD. HeLa (uterine cervix) were obtained from ATCC (American Type Culture Collection). The cancer cell lines were maintained in culture medium Roswell Park Memorial Institute-1640 medium (RPMI-1640) containing serum (FBS, 10% v/v), gentamycin sulphate, L-glutamine penicillin streptomycin solution (GPSS). sulforhodamine B (SRB), trichloroacetic acid (TCA), tris base, trypan blue, trypsin-EDTA (Sigma Co St. Louis, Mo, USA), acetic acid (Lab scan, Ireland) and doxorubicin (ICN, USA) were purchased from respective suppliers.

Sulforhodamine-B assay

The growth inhibition and cytotoxicity of the extract were performed using sulforhodamine-B assay [6,7] against three human cancer cell lines i.e., breast cancer (MCF-7), large cell lung cancer (NCI-H460), and uterine cervix cancer (HeLa). All three cell lines were trypsinized and seeded in 96-well plate at a density of 7,500 cells/well

(lung) and 10,000 cells/well (breast and cervical cancer cells) in RPMI medium (100 µL). Cells were incubated for 24 hr in CO_2 incubator at 37°C to allow monolayer formation followed by the addition of various concentrations of extracts (10–250 µg/mL) and anticancer drugs doxorubicin (0.001–10 µM) in their appropriate well and incubated for further 48 h. This was followed by fixation of cells with ice-cold trichloroacetic acid (50 µL, 50%) at room temperature for 30 min. The plates were carefully washed five times with distilled water and left for overnight drying in air. Sulforhodamine B dye (100 µL, 0.4% in 1% acetic acid) was introduced in each well and after 30 min the unbound SRB dye was removed using acetic acid (1%) and air-dried overnight. The protein bound SRB dye was solubilized in trisbase solution (100 µL, 10 mm) with gentle shaking on a plate-shaker (Spectra Max for 5 min prior to optical density (OD) measurements at 515 nm in a plate reader. The absorbance values in the presence of the test agents were subtracted from blank values. If the absorbance value of the test well was greater than Tz plates, the % growth was calculated as:

Cell growth (%) =[(T-Tz)/(C-Tz)] × 100

T represents the absorbance after the addition of test agents. To avoid the biasness of time (consumed during the treatment procedure) dependent effect on cells, one of the plates was fixed right after removing from incubator. This was considered as time zero1 plate. One another plate was fixed after addition of treatments and considered as time zero2 plate. Tz was calculated as the mean Tz1 and Tz2.

C is absorbance of control.

The GI_{50} (growth inhibition of 50% of cells) was obtained from dose-response curves prepared by plotting the percentage of cell growth versus the concentrations of test agents.

All the experiments were repeated three times and conducted in triplicates as emphasized by the NCI, Frederick, USA laboratory.

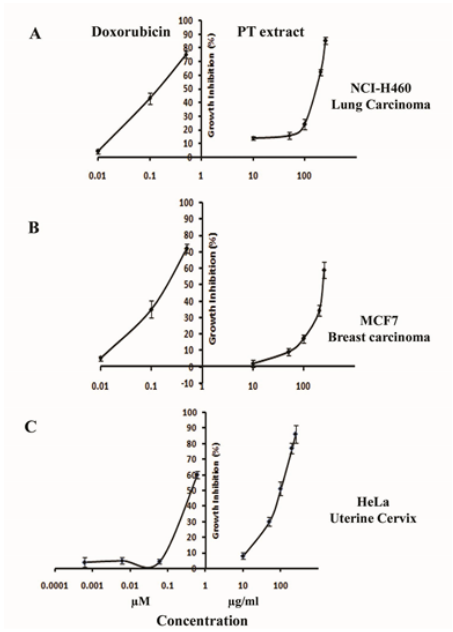

Figure 1: Inhibitory effect of PT extract on growth of different cell lines.

Treatment	Dose (µg/ml)	Growth inhibition (%)	GI_{50} (µg/ml)	LC_{50} (µg/ml)
PT extract	10	+14 ± 1.2	165 ± 3.9	>250
	50	+16 ± 2.6		
	100	+24 ± 3.8*		
	200	+62 ± 2.4***		
	250	+85 ± 2.5***		
	(µM)		(µM)	(µM)
Doxorubicin	0.01	+4.0 ± 1.7	0.25 ± 0.09	9.1 ± 1.8
	0.1	+43 ± 4.1***		
	0.5	+75 ± 2.8***		
	5.0	-19 ± 3.4***		
	10	-51 ± 5.9***		

Table 1: Inhibitory effect of PT extract against growth of human lung cancer cell line NCI (H-460). *Indicates significant (*p<0.05, **p<0.01 and ***p<0.001) growth inhibition and cytotoxicity as compared to respective controls.

Treatment	Dose (µg/ml)	Growth inhibition (%)	GI_{50} (µg/ml)	LC_{50} (µg/ml)
PT extract	10	2 ± 2.1	230 ± 3.1	>250
	50	9 ± 2.0		
	100	17 ± 2.4		
	200	34 ± 3.3***		
	250	59 ± 4.9***		
	(µM)		(µM)	(µM)
Doxorubicin	0.01	+5 ± 1.3	0.4 ± 0.06	10 ± 0.8
	0.1	+35 ± 5.2***		
	0.5	+72 ± 2.6***		
	5.0	-18 ± 3.5***		
	10	-50 ± 3.2***		

Table 2: Growth inhibition induced by PT extract against human breast cancer cell line (MCF-7). *Indicates significant (*p<0.05, **p<0.01 and ***p<0.001) growth inhibition and cytotoxicity as compared to respective controls.

Results

To test the effect of PT extract on cancer cells, we quantify the cells after treatment of PT extract using SRB assay on three different cancer cell lines. PT extract significantly inhibited growth of all three cells lines in concentration dependent manner (Figure 1). Doxorubicin was used as standard anti-cancer drug for comparison. Table 1 shows the effect of PT extract on growth of NCI-H460 cells. 50% of growth

inhibition (GI50) was calculated 165 ± 3.9 mg/ml. Similarly, GI50 against MCF cells was 230 ± 3.1 mg/mL (Table 2) and 100 ± 4.9 mg/mL for HeLa cells (Table 3). Our results indicating that HeLa cells showing high sensitivity to the PT extract than the other two cell lines.

Treatment	Dose (µg/ml)	Growth inhibition (%)	GI_{50} (µg/ml)	LC_{50} (µg/ml)
PT extract	10	8 ± 2.2	100 ± 4.9	>250
	50	30 ± 3.0**		
	100	51 ± 4.3***		
	200	77 ± 3.2***		
	250	86 ± 5.6***		
	(µM)		(µM)	(µM)
Doxorubicin	0.0006	+4.0 ± 3.1	0.5 ± 0.06	5.8 ± 0.2
	0.006	+5.0 ± 1.9		
	0.06	+4.5 ± 1.6***		
	0.6	+60 ± 2.5***		
	6.0	-52 ± 5.9***		

Table 3: Growth inhibition induced by PT extract against human uterine cervix cancer cell line (HeLa). Each value represents mean ± SEM of three independent experiments. Growth inhibition= (+) and cytotoxicity= (–). Concentration causing 50% of cell growth inhibition=GI50. Concentration of drug that killed 50% cells=LC50. *Indicates significant (*$p<0.05$, **$p<0.01$ and ***$p<0.001$) growth inhibition and cytotoxicity as compared to respective controls.

Discussion

Since 2006, numerous studies of the association between tea consumption and cancer risk have been published with results often inconsistent. However, studies have showed reduced risk of cancer (colon, breast, ovary, prostate, and lung) and tea consumption [12-15]. Various studies have shown anti-metastatic effects of catechins which are seen main bioactive components of tea [16-18]. Among the catechins, EGCG is most abundant and regarded responsible for beneficial effects of green tea, as seen by various studies (clinical and animal studies as well as in cell culture) [19-22]. Anti-tumorigenic activities of EGCG have been seen by many *in-vitro* and *in-vivo* studies (animal models) with inhibition at different stages [23-25]. And studies have shown, EGCG inhibit cell proliferation and tumor growth [23,25-28], induction of apoptosis and cell cycle arrest [23,25,29,30], inhibition of invasion and metastasis [24,31-34], and suppression of angiogenesis [35,36]. Various studies have showed the effect of EGCG at molecular level, interfering with VEGF [36] and HGF/Met signaling [37].

Since the extract used in our study is water soluble so is rich in anthocyanin than other phenolic compounds. Anthocyanin have various medicinal properties like antioxidant [38], anti-carcinogenic [39], antiangiogenic [40] and antimicrobial [41]. Various studies have showed anti-cancer effects of berries (rich in anthocyanins) in oral, esophageal and colon [42-44]. Promising results have been shown by anthocyanins in colorectal cancer both *in vivo* and *in vitro* [45]. PT has higher levels of anthocyanin so can be much better in targeting cancer than berries. Recently, anthocyaninin rich plants extracts were analyzed for their anti-proliferative effect in human colon cancer cells and it was seen extracts decreased expression of anti-apoptotic proteins (survivin, cIAP-2, XIAP), induced apoptosis, and arrested cells in G1 [46]. Apart from anthocyanins, studies show anthocyanidins (there aglycones) also arrest the growth of cancer cells [44].

Our study showed PT extract anti proliferative effect in lung, breast and uterine cancer cells. Recent studies showed inhibitory effect of anthocyanin rich PT extract on colorectal carcinoma [11] and on C6 cells [47]. Thus, purple tea seems a new and most likely better alternative both as beverage and nutraceutical product.

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