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# *In-vitro* comparative anticancer activity of green and black tea extract of *Camellia sinensis* (L).

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**Abstract**
Colon cancer is extensive over the world. While ordinary anticancer therapies can support the influenced patients, cells of essential organs, for example, the kidney, lungs, bladder, and sensory system may experience the ill effects of symptoms of chemotherapeutic medications, with the goal that it is important to look for options. From old occasions, consideration has zeroed in on therapeutic plants and common items. The current examination investigated the cytotoxic impact of green tea and Black tea remove on human colon cancer HCT116 cells was surveyed by the MTT test. The HCT116 cells were treated with an expanding grouping of green tea and Black tea (25-300 µg/ml) for 24 h and the outcomes are communicated as a level of the control an incentive in introducing as a cell cytotoxicity proportion for HCT116 cells utilizing MTT measure. The IC50 of Green tea extricate uncovered by this cytotoxicity measure was around $IC_{50}$ = 175.50µg/ml. The IC50 of Black tea remove uncovered by this cytotoxicity test was around $IC_{50}$ = 220.50 µg/ml. The more prominent rate hindrance was seen in green tea when contrasted and Black tea in a portion subordinate way utilizing MTT test. This examination has featured the way that plant separates can go about as promising anticancer specialists, making them fitting segments to be applied for chemoprevention or malignant growth therapy. This investigation approves the conventional utilization of plants in the administration of the disease.

**Keywords:** anticancer activity, colon cancer cell lines, MTT assay

## Introduction
Tumors with worldwide spreading are conspicuous sicknesses that are described by uncontrolled development and the spread of strange cells and the reason for a great many passing in the current century (Siegel *et al*., 2017) [12]. Cancer is the main source of death in financially created nations and the weight of disease is expanding in monetarily agricultural nations because of populace maturing and development just as, progressively, a selection of malignant growth-related way of life decisions including smoking, actual idleness, and "westernized" slims down [Jemal *et al*., 2011] [7]. Among a wide range of tumors, colorectal cancer (CRC) are the third significant frequency of disease with mortality of almost 9.4% Cancer cases yearly (Grothey *et al*., 2004; Ansari *et al*., 2006) [5, 3]. Additionally, CRC is the third most pervasive malignant growth among the Iranian populace (Ansari *et al*., 2006) [3]. Chemotherapy, radiation treatment, hormonal treatment, and medical procedure are the normal therapies for a wide range of diseases, and because of obstruction and unfavorable or harmful results of these therapies, it has gotten important to look for elective anticancer therapy (Shrivastava *et al*., 2005) [11]. Normal items hold huge pharmacological significance and have been considered as the principal wellspring of possible chemotherapeutic medicines (Roe *et al*., 2016) [10].
Black tea has a long history of utilization going back to China around 5,000 years prior. It is produced using the dried leaves of *Camellia sinensis* (L), a lasting evergreen bush earlier known as Tea. It is local to southeastern Asia. Green tea, Black tea, and oolong tea are completely gotten from a similar plant. In this way, there is a consistent interest to grow new, powerful, and reasonable anticancer medications.
From the beginning of old medication, synthetic mixes got from plants have been utilized to treat human sicknesses. (Coseri, 2009) [4]. Green tea is acquired from the leaves of the plant *Camellia sinensis* (L), and it is one of the most well-known refreshments around the world, especially in Asia. Flavonoids and polyphenol mixes have been accounted for to have ideal properties, for example, hostile to cancer-causing, against mutagenic, antimicrobial, and hostile to oxidant properties (Yıldırım *et al*., 2000) [15]. An investigation led by Ann Beltz *et al.* announced the wide assortment of components by flavonoids and polyphenols of *Camellia sinensis* (L) that forestall malignancy cell endurance (Ann Beltz *et al*., 2016).
According to the adverse and predictable side effects of conventional anticancer treatments and the inaccessibility of these drugs in most developing countries, It is fundamental to use a viable, affordable, and effectively available therapy. Along these lines, this examination planned to assess the anticancer impacts of Green and Black tea concentrate of *Camellia sinensis* (L) HCT116 cell lines using an *in vitro* examination.

## Materials and Methods
### Collection of plants
The new leaves of *Camellia sinensis* (L) were gathered from the dense tea state garden, Ooty, Tamilnadu, South India. The plants were recognized dependent on the morphological plant qualities. Black tea powder was bought from the nearby market at Ooty. The gathered Green tea and Black tea were shipped to the research center. It was conceal dried for 10 days and powdered utilizing a blender processor. The powder was utilized for the extraction of different investigations.

**MTT assay**

The cytotoxicity of Green and Black tea separate on HCT116 cells was controlled by the strategy for Mosmann, (1983).

**Procedure**

Cell viability measure, HCT116 practical cells were accumulated and included utilizing hemocytometer weakened in DMEM medium to a thickness of $1 \times 10^4$ cells/ml was cultivated in 96 well plates for each well and hatched for 24 h to allow the connection. After HCT116 cells were treated with control and the holding various centralizations of Green and Black tea (25 to 300µg/ml) was Applied to each well. HCT116 cells were brooded at 37°C in a soaked 95% air and 5% $CO_2$ incubator for 24 h. After incubation, the medication containing cells wash with a new culture medium, and the MTT (5 mg/ml in PBS) color was added to each well, trailed by hatched for one more 4 h at 37°C. The purple hastened Formosan shape was broken down in 100 µl of concentrated DMSO and the cell practicality was absorbance and estimated 540nm utilizing a multi-well plate peruser. The outcomes were communicated at the level of stable cells concerning the control. The half-maximal inhibitory focus ($IC_{50}$) values were determined and the ideal portion was examined at the distinctive time-frames.

$$\text{Inhibitory of cell proliferation (\%)} = \frac{\text{Mean absorbance of the control} - \text{Mean absorbance of the sample}}{\text{Mean absorbance of the control}} \text{ X } 10$$

The $IC_{50}$ values were settled from the Green and Black tea amount responsive curve where restraint 50% of cell viability was contrasted with control cells.

All analyses were performed at any rate multiple times three-fold.

**Result and Discussion**

In the current examination, the cytotoxic impact of Green and Black tea concentrate of *Camellia sinensis* (L) on the HCT116 malignant growth cell line was resolved to utilize MTT measure at a focus scope of (25 to 300 µg/ml) after 48 h of treatment. Cell viability was evaluated utilizing the MTT test.

**Table 1:** Effect of Green tea on cell viability of human colorectal cancer HCT116 cells was assessed by MTT assay

| Green tea extract | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Control** | **25µg** | **50 µg** | **100 µg** | **150 µg** | **200 µg** | **250 µg** | **300 µg** | **$IC_{50}$ value** |
| 100 | 89.86 | 79.08 | 70.82 | 58.19 | 44.15 | 33.89 | 25.09 | |
| | ±3.34 | ±3.04 | ±2.97 | ±2.04 | ±1.98 | ±1.88 | ±1.37 | **175.50** |

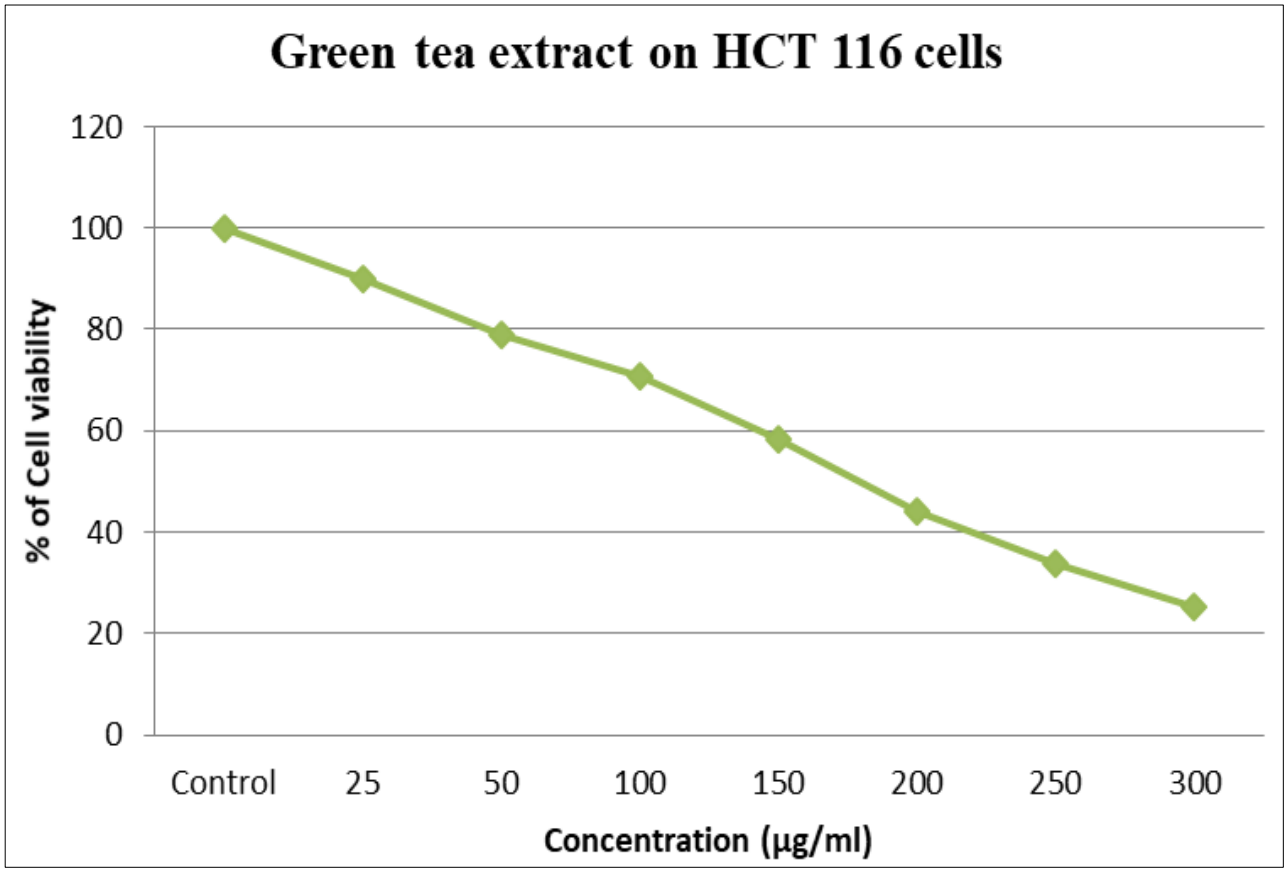


**Fig 1.** $IC_{50}$ concentration of the Green tea extract against HCT116 Cell line

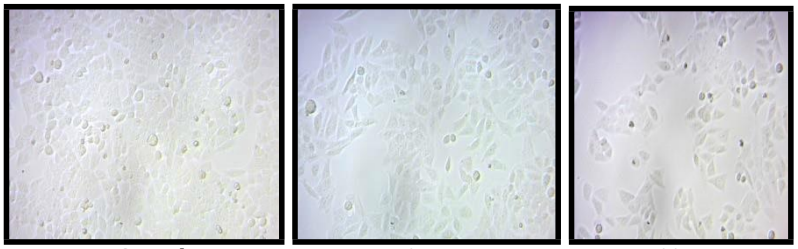


**Fig 2:** Morphological changes in control and Green tea treated colorectal cancer HCT116 cells

**Table 2:** Effect of Black tea on cell viability of human colorectal cancer HCT116 cells was assessed by MTT assay

| Black tea extract | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Control** | **25µg** | **50 µg** | **100 µg** | **150 µg** | **200 µg** | **250 µg** | **300 µg** | **$IC_{50}$ value** |
| 100 | 93.26 ±7.10 | 84.13 ±6.44 | 75.25 ±5.73 | 66.19 ±5.04 | 53.12 ±4.07 | 42.54 ±3.24 | 32.91 ±2.52 | 220.50 |

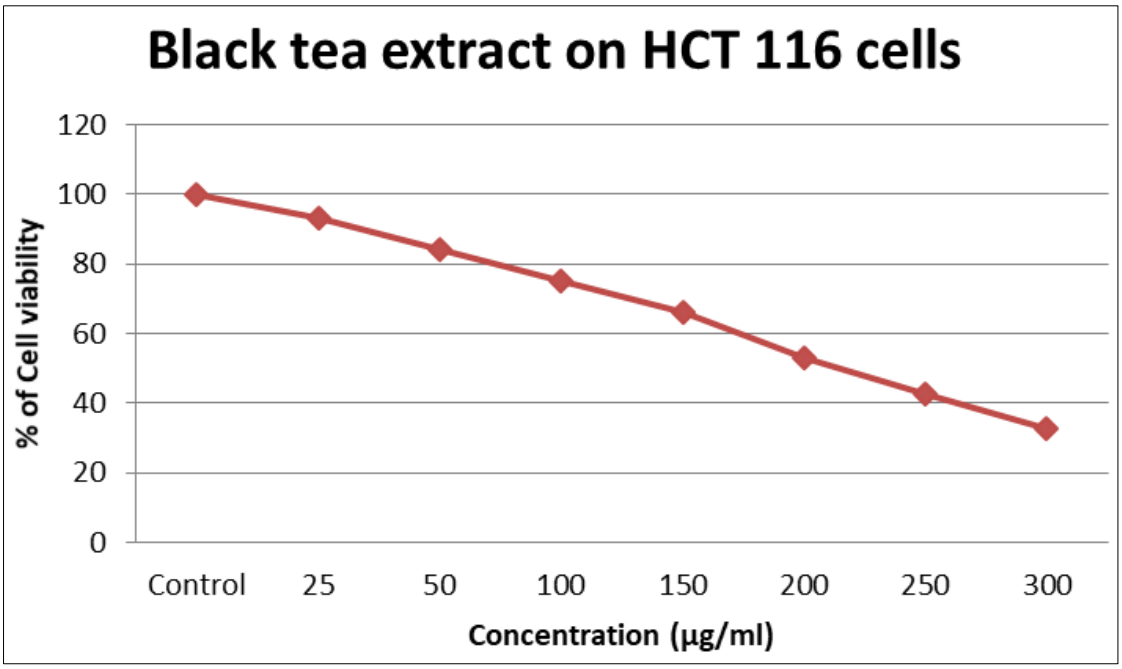


**Fig 3:** $IC_{50}$ concentration of the Black tea extract against the HCT116 Cell line.

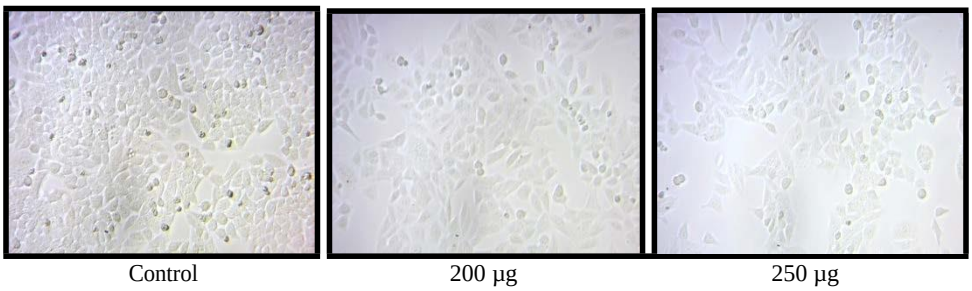


**Fig 4:** Morphological changes in control and Black tea treated colorectal cancer HCT116 cells

The Green and Black tea concentrate of *Camellia sinensis* (L) was assessed for their cytotoxic movement inspected against the human colon malignant growth cell line (HCT-116). The aqueous concentrate of Green tea shows 89.86 % of cell viability at 25µg/ml and Black tea shows 93.26% of cell viability at 25µg/ml independently. The values are condensed in Table 1, and Table 2. The $IC_{50}$ of Green tea separate uncovered by this measure was around $IC_{50}$ = 175.50µg/ml. The $IC_{50}$ of Black tea remove uncovered by this test was around $IC_{50}$ = 220.50 µg/ml. the qualities are shortened in Fig.1 and 3. The impact of fluid concentrates

On apoptotic morphological changes in the HCT 116 cell line was appeared in Fig. 2 and 4. MTT examination was directed to assess the development inhibitory impacts of concentrate from Green tea and Black tea on the cell viability of malignant growth cell line HCT116 cells, which depends on the decrease of MTT at various focuses (25-300 µg/ml). After 48 h of treatment, an aqueous concentrate of Green tea and Black tea displayed a higher inhibitory impact against tumor cells, with changing efficiencies and selectivity while others caused peripheral cell hindrance MTT test depends on the decrease of MTT by mitochondrial

Dehydrogenase by purple Formosan item. It is habitually utilized as an *in vitro* model framework to gauge cytotoxic impacts of an assortment of poisonous substances and plant removes against disease cell lines (Morshed *et al*., 2011) [8]. ROS assumes a significant function in cell senescence preparing to cell passing; consequently, there is a dire requirement for potential therapeutics that may forestall oxidative pressure initiated neurodegeneration. The support of oxidant impact might be answerable for the apoptotic movement of these concentrates, and ROS are key flagging particles to adjust cell passing. Collecting proof shows that malignant growth cells produce elevated levels of ROS that lead to a condition of expanded basal oxidative pressure. The expanded creation of ROS in disease cells was seen *in vitro* examines (Halliwell, 2007) [6]. Ahamad *et al*. uncovered that naringenin prompts cell demise in malignancy cells through initiating ROS age. The disease is described by an uncontrolled expansion in cell multiplication as well as a decrease in cell apoptosis. Restraint of development and enlistment of apoptosis in malignancy cells were considered as the procedures for the disease treatment (Sykiotis and Papavassiliou 2006) [14]. Altogether repressed the development of cells, which was additionally affirmed by the diminished cell thickness. Moreover, Caspase a group of cysteine proteases, are the key proteins that adjust the apoptotic reaction. Caspase-3, a vital killer of apoptosis, is considered as a biomarker for cells going through apoptosis (Sui *et al*., 2016) [13].

## Conclusion

In conclusion, inducing apoptosis by novel bioactive mixes of Green tea and Black tea diminishes Tumor cell multiplication in a portion subordinate path without disabling typical cells. Along these lines, concentrates of Green tea and Black tea might be another restorative alternative in anticancer therapy. Green tea removes additionally demonstrated a higher enemy of a proliferative impact than Black tea extricates it was inferred that green tea showed preferred enemy of proliferative potential over Black tea.

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