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Research Article

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Investigation of Cytotoxic Potential of Natural Phenol-Catechin

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ABSTRACT

Background: *Cancer is one of the major causes of death worldwide. It starts when cells grow out of control and crowd out normal cells. Current drug therapy possesses many severe side effects which need safe and effective agent that have the potential to resolve the disease condition.*
Aim: *Evaluation of Cytotoxic potential effect of Catechin on the Cancer cells.*
Materials and method*: Procurement of the pure Catechin Powder from YUCCA Enterprises, Mumbai. Cytotoxic effects of Catechin were evaluated with the standard marketed anticancer drug Methotrexate. Different models like In vitro hemolytic test, Brine Shrimp lethality assay and Allium cepa (onion) model was used to investigate the cytotoxic effect and the result was estimated. To check the significance of data, Statistical tests were performed: using ANOVA test and Tuckey's test.*
Results and discussion: *The effect of Catechin at different concentrations was observed on the cancer cells. Using models like in vitro Hemolytic test the % Hemolysis was observed at the concentration of 150 µg/mL, in Brine Shrimp lethality assay % mortality was calculated at the concentration of 300 µg/mL at the interval of 6 hours and in Allium cepa model, the length and numbers of roots were counted. From this study we can conclude that natural phenol Catechin has the potential cytotoxic effect on the Cancer cells.*
Conclusion: *Present research work emphasizes that the Catechin is having beneficial cytotoxic potential and may have therapeutic potential as anti-cancer agents.*

Keywords: Catechin; Cytotoxic effect; Hemolytic; Brine Shrimp; *Allium cepa*; Anticancer

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INTRODUCTION

Cancer is one of the major causes of death worldwide. Cancer can start any place in the body. It starts when cells grow out of control and crowd out normal cells. This makes it hard for the body to work the way it should [1]. One out of every two men and one out of every three women will have some type of cancer at some point during their lifetime. By the year 2030 the burden is set to more than double: there will be 26.4 million cancer cases; 17 million deaths and 75 million people living with the disease [2]. Cancer is a general term used to refer to a condition where the body's cells begin to grow and reproduce in an uncontrollable way. These cells can then invade and destroy healthy tissue; including organs. Cancer sometimes begins in one part of the body before spreading to other parts [3].

Cancer is caused by changes (mutations) to the DNA within cells. The DNA inside a cell is packaged into a large number of individual genes; each of which contains a set of instructions telling the cell what functions to perform; as well as how to grow and divide. Errors in the instructions can cause the cell to stop its normal function and may allow a cell to become cancerous [2].

Many factors can cause the development of cancer in the body. Some of these factors; such as heredity; lifestyle; use of tobacco is one of the main causes of cancer; especially lung cancer. Tobacco use; whether in the form of smoking; chewing; or exposure to second-hand smoke can also cause cancer of the mouth and larynx; esophagus; throat; and many other parts of the body [4]. Other primary cause of Cancer is Diet/Nutrition; Environment; Exposure to radiation; Hormone therapy.

As mortality of cancer is higher; many advances are been possible both in terms of treatment as well as understanding the mechanism of disease at molecular level. In developing countries herbal medicine is the source of new discoveries for the new drug leads towards various healthcare issues and synthesis of new formulation. Also; herbal medicines as alternative anti-cancer therapy has attracted a great deal of recent attention due to their low toxicity and cost [5].

Synthetic anti-cancer drugs are associated with numerous side effects and worsen the quality of life. The ratio of morbidity and mortality associated with metastatic conditions indicates the quality need for the discovery of new agents with higher therapeutic efficacy.

Catechin is a flavan-3-ol; a type of natural phenol and antioxidant. It is a plant secondary metabolite. The name of the Catechin chemical family derived from catechu; which is the tannic juice or boiled extract of *Mimosa catechu* (*Acacia catechu* L.f). Already reported activities of Catechin are antioxidants in the body; with the potential to combat everything from cardiovascular disease and hypertension to different types of cancer and Alzheimer's disease [6].

Oxidative stress is the key point in linking the toxicity of the multistage cancer process. Reactive Oxygen Species (ROS) are developed in response to both endogenous and exogenous stimuli. Antioxidant rich supplements can reduce the adverse reactions and toxicities in the body [7].

Antioxidants act as a resisting mechanism that protects against oxidative damage and include constituents to remove or repair damaged molecules. It can prevent/retard the oxidation caused by free radicals scavenging.

Hence; the present study is carry out to analyze the effect of secondary metabolite and natural phenol Catechin; present in the various herbs and foods using different concentration to check the Cytotoxic potential activity on the cancer cells.

MATERIALS AND METHODS

The pure Catechin powder was procured from "YUCCA ENTERPRISES" at Mumbai; India. All the quantitative test was carried out for the authentication of the Catechin powder [8-10]. Cytotoxic effects was evaluated using *in vitro* models like Hemolytic test; Brine Shrimp Lethality assay as well as *Allium cepa* (onion) model. In the hemolytic study; hemolytic activity was conducted for study purpose of the cytotoxic effect of Catechin by collecting the blood from the rat by Retro Orital blood collection method [11]. The absorbance of the liberated Hb% was checked using UV Spectrophotometer at the 450 nm. While in the Brine Shrimp Lethality assay the mortality% was noted by counting the viable naupliis at the different concentration of Standard marketed drug Methotrexate and the test drug Catechin with the control group. Dried cyst was procured from the Amazon shopping site. Larvae were considered dead if they do not show any type of locomotion or movement during the observation and counting of naupliis [12,13]. And in the *Allium cepa* model the squash preparation of the root was done using different chemicals. The length and numbers of the roots growth was counted against dose dependent concentration.

Statistical Analysis

To check the significance of data; following statistical tests were performed:

ANOVA: to see the variability within all the groups.

Tuckey's test: For the same purpose mentioned in above test.

P – value: Degree of freedom; Standard deviation; etc.

Data were considered statistically significant at $p<0.05$ and highly significant at $p<0.001$. Statistical analysis was performed using INSTAT statistical software.

RESULTS AND DISCUSSION

In vitro Hemolytic Test

In this test; Serum was separated by cooling centrifuge and different solution of the drug was prepared and

absorbance of sample was taken by the UV spectrophotometer at 450 λmax. As the absorbance of the samples increases there are more chances of haemolysis; which clearly indicates cytotoxic activity of the test sample at different concentrations. (Table 1) (Figure 1)

Table 1: Effect of catechin at different drug concentration

Drug Concentrations	Absorbance (450 nm)	% Hemolysis
Water (+ve)	0.000	-
Phosphate buffer	0.508	-
Blood + PBS(-ve)	1.474	-
Std Drug (20 µg/mL)	0.48	67.43%
Std Drug(40 µg/mL)	0.79	46.40%
Test Drug (5 µg/mL)	0.59	59.97%
Test Drug (150 µg/mL)	0.30	79.64%
Test Drug (500 µg/mL)	0.29	67.43%

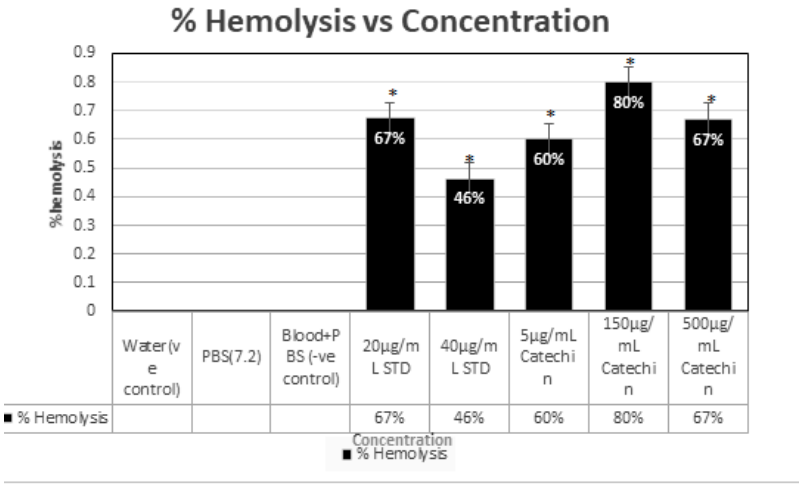


Figure 1: %Hemolysis vs. Concentration

In vitro Hemolytic study for effect of Catechin showed concentration dependent activity in erythrocytes which justifies cytotoxic action of Catechin. As the absorbance of the samples increases there are more chances of haemolysis; which clearly indicates cytotoxic activity of the test sample at different concentrations. The highest cytotoxic was observed at concentration of 150 µg/mL.

Brine Shrimp Lethality Assay

The Brine Shrimp Lethality Assay helps to check the effect of the drugs on the viable naupliis at different time interval. Naupliis can survive without food for 48 hours. This assay is perfect for the analysis of the cytotoxic effect of the Standard as well as test drug. The below Table 2 shows the concentration effect of the drugs at different intervals of time (Figure 2).

The Brine Shrimp lethality assay has been proved convenient method for analysis of biological activities. The concentration dependent death of the naupliis was observed at different time intervals. The highest cytotoxic effect was observed at the concentration of 300 µg/mL at 6 hours of interval**.**

Table 2: % Mortality with Concentration and time interval

Time (hr)	Control	STD 20 µg/mL	STD 40 µg/mL	C1 5 µg/mL	C2 150 µg/mL	C3 500 µg/mL
0 hr	0%	0%	0%	0%	0%	0%
1 hr	0%	10%	20%	20%	20%	40%
2 hr	20%	30%	40%	30%	40%	60%
3 hr	40%	40%	50%	40%	50%	70%
6 hr	50%	60%	70%	60%	70%	90%
8 hr	60%	70%	80%	80%	90%	100%
10 hr	70%	80%	100%	100%	100%	100%
12 hr	90%	90%	100%	100%	100%	100%
14 hr	100%	100%	100%	100%	100%	100%

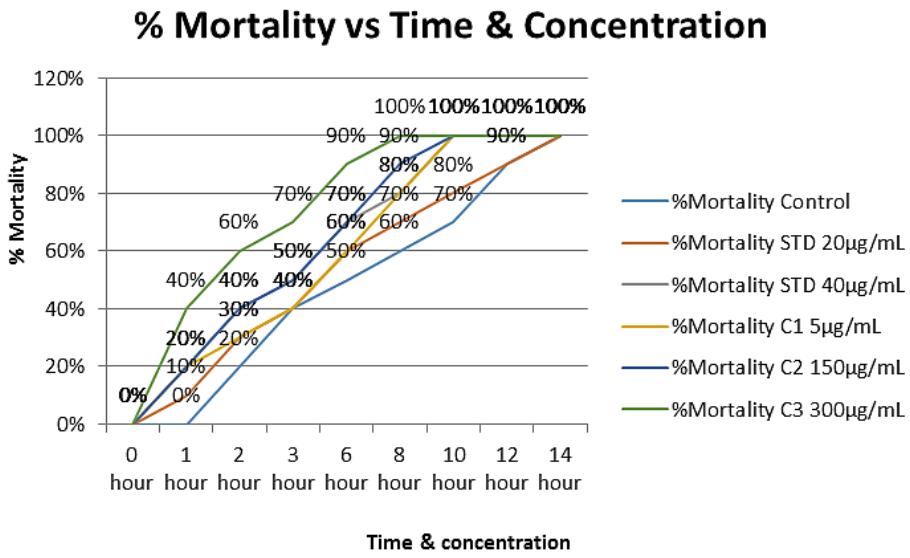
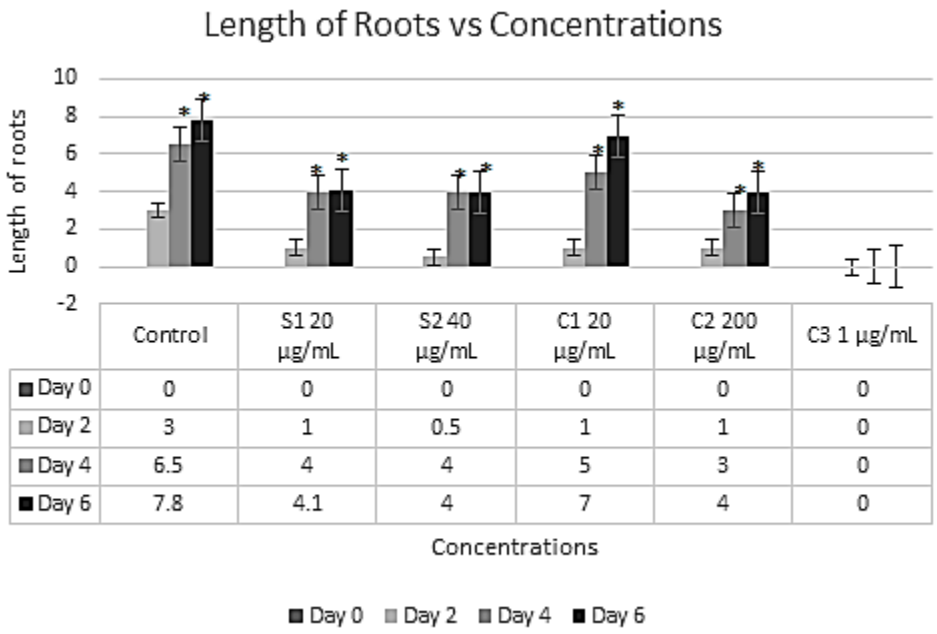


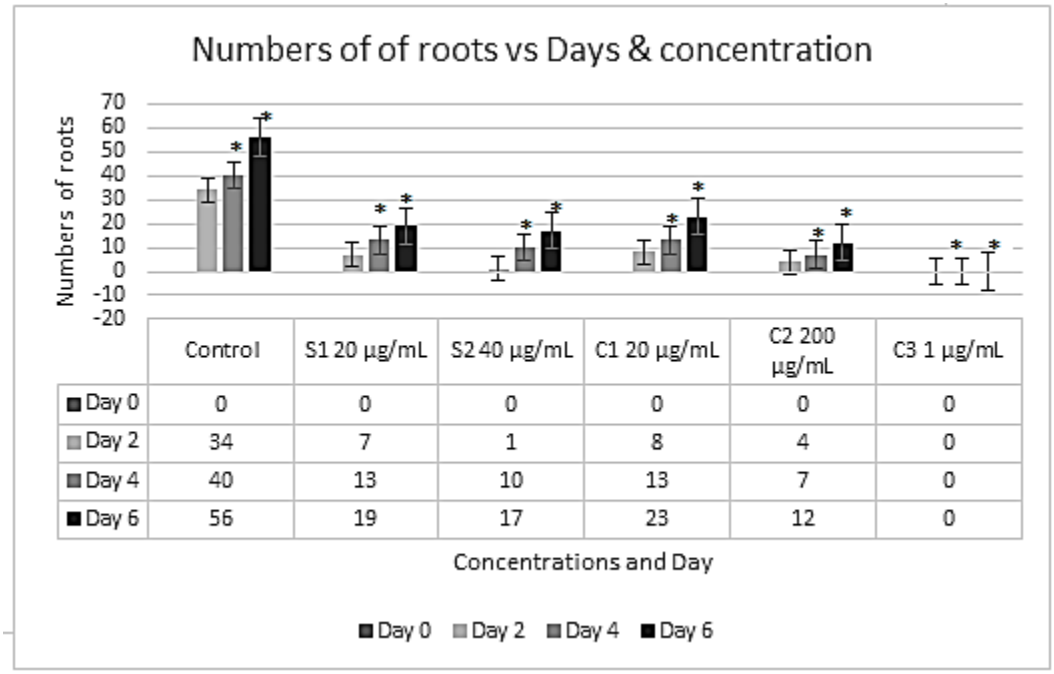
Figure 2: % Mortality vs. Time and Concentration

***Allium cepa* (onion) model**



	Control	S1 20 µg/mL	S2 40 µg/mL	C1 20 µg/mL	C2 200 µg/mL	C3 1 µg/mL
Day 0	0	0	0	0	0	0
Day 2	3	1	0.5	1	1	0
Day 4	6.5	4	4	5	3	0
Day 6	7.8	4.1	4	7	4	0

Figure 3: Length of roots vs. Days and concentrations



	Control	S1 20 µg/mL	S2 40 µg/mL	C1 20 µg/mL	C2 200 µg/mL	C3 1 µg/mL
Day 0	0	0	0	0	0	0
Day 2	34	7	1	8	4	0
Day 4	40	13	10	13	7	0
Day 6	56	19	17	23	12	0

Figure 4: Numbers of roots vs. Days and concentrations

The maximum cytotoxic effect in the *Allium cepa* model was observed at the concentration of 1 µg/mL at the day 6. The length of roots as well as numbers of roots growth was inhibited by the high concentration dose of Catechin. Using *Allium cepa* model inhibition of growth rate and length of roots was observed. From this we are assuming the effect of Catechin on the growth rate of merismetic cells in the *Allium cepa* (onion) then it might also inhibit Cancer

cells and its rapid division with help of different concentration dependent action of the Catechin (Figure 3 and Figure 4).

Statistical Data Analysis
Statistical analysis was performed using INSTAT statistical software.

CONCLUSION

From the investigation of above studies using different models for the potential cytotoxic effect; we can say that the plant secondary metabolites can also use for treating of the Cancer like disease and many others with less side effects compare to synthetic marketed anticancer drugs which have worst side effects and decreases the quality of the life. Considering all these data we can say natural phenol Catechin have cytotoxic effect on cancer cells.

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