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## **CROCUS SATIVUS L. PREVENTS PROGRESSION OF CELL GROWTH AND ENHANCES CELL TOXICITY IN HUMAN BREAST CANCER AND LUNG CANCER CELL LINES**

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### **ABSTRACT**

#### **Keywords:**

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The cytotoxicity activity of *Crocus sativus* was screened against human breast cancer cell lines (MCF-7), Lung cancer cell lines (A549) and normal cancer cell lines (L929) with increasing concentration (2-10 $\mu$ g/ml) for 24hrs by MTT bioassay. Percent inhibition of CS extract were calculated for MCF-7, A549 and L929 (Figure 17). CS extract shows maximum inhibition 87.43% against MCF-7 Breast cancer cell lines and for Lung cancer cell lines maximum inhibition 75.38% was achieved at the concentration of (10 $\mu$ g/ml) while on normal cell lines CS extract did not showed any marked cytotoxicity activity. Our study concluded *Crocus sativus* extract have potential *invitro* anti- proliferative activity against cancer cell lines and is least sensitive against normal cell lines. However before coming to conclusive statement more research is needed.

## INTRODUCTION

Efforts to find any therapeutic options for cancers have guided the investigators to consider herbal medicine to be tested. Treatment of cancer was the subject of our interest that led us to study alternative therapies such as the use of herbs. We sought to investigate saffron and its potential effect on cancerous cells. Saffron (*Crocus sativus* L) is one of the worthiest perennial flowers with a violet color and usually 3 golden petal stigma in the Iridaceae family<sup>1</sup>. It has been used as a food spice since the ancient times. Anti-carcinogenic activity of saffron was reported in the beginning of 1990 and research on this subject has increasingly continued during the past decade<sup>2</sup>. Saffron and its main ingredients have shown antitumor and anticarcinogenic activities both *in vitro* and *in vivo*.<sup>3,4</sup> In the present study we investigated *in vitro* anti proliferative effect of saffron (*Crocus sativus*) in two cancer cell lines.

## MATERIALS AND METHODS

### Preparation of Saffron Extract

Saffron harvested from saffron farms of pulwama (a city in the northeast of Kashmir ) was used in this study. Aqueous extract was prepared with 10 g of its ground petal stigma and 400 mL of distilled water in a maceration extractor for 18 hours. The prepared extract was concentrated to 100 mL with a rotatory evaporator in low pressure and filtered through a 0.2-mm filter to be sterilized. The resultant solution was stored at 4°C to 8°C. Various concentrations of saffron (50 µg/mL, 100µg/mL, 200 µg/mL, 400 µg/mL, 800 µg/mL, 1000 µg/mL, 2000 µg/mL, and 4000 µg/mL) and a control solution without saffron extract were prepared immediately and refrigerated before the experiments.

### Chemical Required

96-well plate ,Plate reader-spectrophotometer ,Sonicator, Special reagent , MTT ,Buffer Solutions, PBS (phosphate buffered saline ), 137 mM NaCL, 2.7mM KCL, 8.5 mM Na<sub>2</sub>HPO<sub>4</sub>, 1.5 mM KH<sub>2</sub>PO<sub>4</sub>, Sterile filters , Organic solution (Isopropanol).

### Cell culture

Human cancer cell lines were procured from National Center for cell science (NCCS) pune. Cells were grown in tissue culture flasks in complete growth medium (RPMI-1640 medium with 2mM glutamine, pH 7.4, supplemented with 10% fetal calf serum, 100 µg/ml streptomycin and 100 units/ml penicillin) in a carbon dioxide incubator (37°C, 5% CO<sub>2</sub>, 90% RH). The cells at subconfluent stage were harvested from the flask by treatment with trypsin [0.05% in PBS (pH 7.4) containing 0.02% EDTA]. Cells with viability of more than 98% as determined by trypan blue exclusion, were used for determination of cytotoxicity The cell suspension of 1 x 10<sup>5</sup> cells/ml was prepared in complete growth medium.

### MTT Assay<sup>5</sup>

After cell incubation with the potential Extract for the desire time in a 24-cell culture plate (with 1ml of culture medium), add 100  $\mu$ l of MTT stock solution (final concentration  $\sim$ 0.5 mg/ml) to each well. Incubate at 37°C for 2 hours in the humidified CO<sub>2</sub> incubator. At the end of the incubation period, take off the medium and add 1 ml of acidic isopropanol to solubilise the purple formazan dye. Carefully sonicate each well to solubilise completely the converted dye. Transfer 200  $\mu$ l of the dye solution of each well for a 96-well plate in duplicate. Read the absorbance at 570 nm using 690 nm as reference in a plate reader spectrophotometer. Express the results as % of sample absorbance in relation to the absorbance in the negative control.

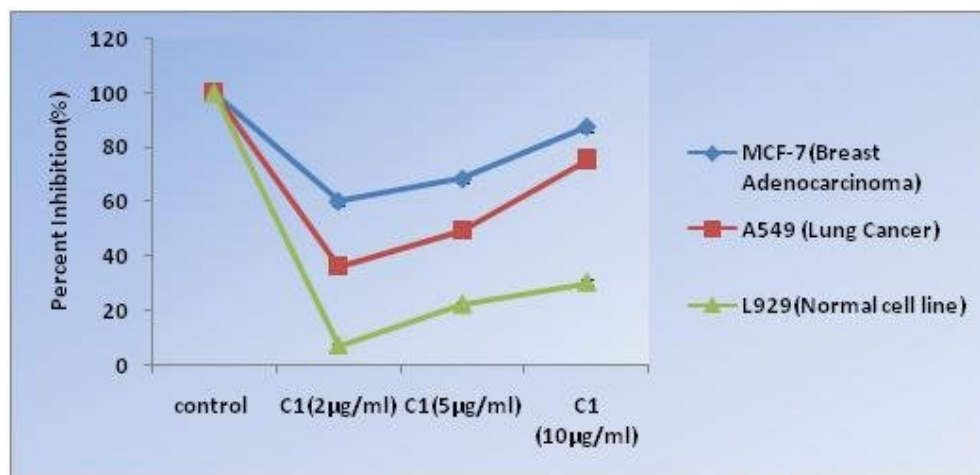
### Statistical analysis

The experimental data were expressed as mean  $\pm$  SEM. The significance of difference among the various treated groups and control group were analyzed by means of one-way ANOVA. The level of significance was set at  $p < 0.05$

### RESULTS

The cytotoxicity activity of *Crocus sativus* was screened against human breast cancer cell lines (MCF-7), Lung cancer cell lines (A549) and normal cancer cell lines (L929) with increasing concentration (2-10 $\mu$ g/ml) for 24hrs by MTT bioassay. Percent inhibition of CS extract were calculated for MCF-7, A549 and L929 (Figure 17). CS extract shows maximum inhibition 87.43% against MCF-7 Breast cancer cell lines and for Lung cancer cell lines maximum inhibition 75.38% was achieved at the concentration of (10 $\mu$ g/ml) while on normal cell lines CS extract did not showed any marked cytotoxic activity.

**Figure 1: Cytotoxicity activity of CS Extract against Cancer cell lines and Normal cell lines**



Data are presented as Mean $\pm$ SEM,  $P < 0.05$  was considered Statistically Significant

## DISCUSSION

In the present Study the cytotoxicity activity of *Crocus sativus* was screened against human breast cancer cell lines (MCF-7), Lung cancer cell lines (A549) and normal cell lines (L929) with increasing concentration (2-10µg/ml) for 24hrs by MTT bioassay. Percent inhibition of CS extract were calculated for MCF-7, A549 and L929 (Figure 1). CS extract shows maximum inhibition 87.43% against MCF-7 Breast cancer cell lines and for Lung cancer cell lines maximum inhibition 75.38% was achieved at the concentration of (10µg/ml) while on normal cell lines CS extract did not showed any marked cytotoxicity activity. Our study confirmed the study of (Escribano *et al*)<sup>6</sup> who reported that the inhibitory activity on the *invitro* growth of HeLa cells produced by saffron extract was mainly due to Crocin, where as picrocrocin and safranal played a minor role in the cytotoxicity of saffron extracts. So our study suggested that sugars might play a role in saffron's cytotoxic effects. These findings are in accordance with the results of our previous study on pancreatic cancer cell line (Hamid *et al*)<sup>7</sup> and (Abdullaev)<sup>8</sup> who reported no effect of crocetin on colony formation in HeLa cells and two other solid tumor cell lines, but are, However, in disagreement with (Tarantilis *et.al* and Nair *et.al*)<sup>9,10</sup> who reported cytotoxicity for crocetin against a cell lines derived from non solid tumors and various tumor cell line and human primary cells from surgical specimens. So our study concluded that Saffron could be better target for novel anticancer lead. However before coming to conclusive statement more research is needed.

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