

CYTOTOXICITY OF STRAWBERRY EXTRACT ON ORAL CANCER CELL LINE

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ABSTRACT

Objective: Berries including strawberry may have beneficial effects against oxidative stress-mediated diseases such as cancer. The purpose of the present study was to investigate the cytotoxic effects of strawberry extract on oral cancer cell line.

Methods: Strawberry methanolic extract (SBE) was prepared, and cytotoxic activity of different concentration of SBE on KB cell lines was determined by (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay and neutral red dye incorporation test.

Results: Strawberry extract exhibits cytotoxic activity over the oral cancer cell lines. On administration of about 100 µg/ml of strawberry extract, about 50% of cell viability could be observed and assessed from the cell lines.

Conclusion: Strawberries have a cytotoxic effect on oral cancer cell line due to the presence of anticancer constituents in the berries. These berries can be used as a natural medicine for cancer sufferers.

Keywords: Apoptosis, Berries, Cancer, Cytotoxic effect, (3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide), Neutral red.

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INTRODUCTION

Reactive oxygen species (ROS) are tumorigenic by their ability to increase cell proliferation, survival, and cellular migration. Ethnobotany, the study of traditional human uses of plants, is recognized as an effective way to discover future medicines [1]. Antioxidants have the potential to suppress cancer and to reduce the risk of cancer development by scavenging ROS [2]. Intake of Vitamin E, C, and A has been reported to reduce lung cancer risk because of their roles as regulators of cell differentiation (Vitamin A), antioxidants (Vitamins C and E), and modulators of DNA synthesis, methylation, and repair [3]. Berries are said to be vital sources of natural chemopreventive agents comprising Vitamins A, C, and E, selenium, carotenoids, anthocyanins, flavonols, proanthocyanidins, ellagitannins, and phenolic acids that have anticancer effects [4].

Berry fruits have been widely consumed in our diet and have attracted much attention due to their potential health benefits [5]. Berries contain a diverse range of phytochemicals with properties of antioxidant, anticancer, and anti-inflammatory works [6]. Berries possess many cancer-preventive substances in them including vitamins such as A, C, and E, folic acid, as well as minerals, such as calcium, selenium, and zinc [7]. Berry bioactives are known to have many roles in cancer prevention. Inhibition of the formation of carcinogen-induced DNA adducts, inhibition of carcinogen-induced tumorigenesis in animals, and modulation of signaling pathways involved with cellular proliferation, apoptosis, inflammation, angiogenesis, and cell cycle arrest include in the many roles it plays [8,9].

Cancer till today remains the leading cause of death in both developed and developing countries [10]. Plants have been beacon of therapeutic sources for curing diseases from times immemorial [11]. Strawberry, one of the most commonly berries consumed in the Mediterranean diet, is a rich source of antioxidants and phenolic compounds that exert beneficial effects on human health [12]. However, to date, there is a lack of investigation about its cytotoxic effects [13,14]. Berries

including strawberry, Korean raspberry, and mulberry are noted to have beneficial effects against diseases such as cancer [15]. Hence, the present study aimed at evaluating the cytotoxic activity of strawberry extract on oral cancer cell line.

METHODS

Extract preparation

For the preparation of strawberry methanolic extract (SBE), Indian strawberry fruits were purchased from the local markets, cut into small pieces and dried in shadow. The powdered strawberry was then extracted with methanol using Soxhlet extraction techniques. Following evaporation, crude methanolic extracts were stored at room temperature under sterile conditions until further use.

Maintenance of cell lines

KB cell lines were procured from National Centre for Cell Sciences, Pune. The cells were maintained in Minimal Essential Medium enhanced with 10% FBS, streptomycin (100 µg/ml), and penicillin (100 U/ml) in a humidified atmosphere of 50 µg/ml CO₂ at 37°C. The vial containing the KB cell lines acquired from ATCC (CCL-17) was removed from liquid nitrogen freezer and immediately placed in a 37°C water bath. It was agitated continuously until the medium thawed. Then, it was centrifuged for 10 min at 150–200× g room temperature. Supernatant was discarded and cells were washed with fresh medium to remove residual dimethyl sulfoxide (Table 1).

(3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) (MTT) assay

MTT is a water-soluble tetrazolium salt, which on cleavage by succinate dehydrogenase is converted to an insoluble purple formazan by cleavage of the tetrazolium ring within the mitochondria. The formazan product thus formed is impermeable to the cell membranes, and therefore, it accumulates in healthy cells [16]. The KB cells were routinely grown and subcultured as monolayers in Dulbecco's Minimal Essential Medium supplemented with 10% newborn calf serum. The experiments in this

Table 1: Cell viability assay of SBE-treated KB cell lines

S. No	Treatment	Concentration ($\mu\text{g/ml}$)	Absorbance 540 nm	% Cell viability
1	KB untreated cells		0.318	100 \pm 5.9
2	SBE treated	25	0.267	83.9 \pm 7.1*
3		50	0.221	69.4 \pm 5.8*
4		100	0.205	64.4 \pm 4.1*
5	Cyclophosphamide	100	0.097	50.5 \pm 2.1*

Results are expressed as Mean \pm SEM (n=3). *p<0.001 statistically different in comparison with KB-untreated cells. SEM: Standard error of the mean, SBE: Strawberry methanolic extract

investigation were conducted with cells that had been initially batch cultured for 10 days. At this stage, the cells were harvested and plated at approximately 30,000 cells/well in 96-well microtiter plates and left to rest for 24 h at 37°C in a humidified atmosphere of 5% CO₂. The cells were then exposed to with/without various concentrations of the extract or the medium alone (as normal). Concentrations of the extract ranging of 25–100 $\mu\text{g/ml}$ were used.

At the end of the period, cytotoxicity was assessed by estimating the viability of the KB cells by the MTT reduction assay. After 1 h incubation, the test solution from each well was removed by aspiration and replaced with 50 μl of MTT prepared in minimum essential media (MEM) without phenol red (MEM). The plates were gently shaken and incubated for 3 h at 37°C in a humidified 5% CO₂ atmosphere. The supernatant was removed and 50 μl of propanol was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at 540 nm.

In the cell viability, reduction of MTT can occur only in metabolically active cells, the level of cell activity is a measure of the viability of the cells. This is measured by absorbance of the spectrophotometer and the intensity is compared and the means were compared. The solution transferred to centrifuge tubes and that was centrifuged at top speed 2 min to precipitate cell is calculated by the following formula:

$$\% \text{ cell viability} = \frac{\text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

Cytotoxicity assay by neutral red (NR) incorporation

The NR incorporation method, described by Borenfreund and Puerner (1985), was used to evaluate cytotoxicity through lysosome viability. Different concentrations of extracts were obtained by dissolution in maintenance medium (MM). They were tested in a range from 0 to 2.5 mg/ml of SBE. After exposure for 24 h, the assay was performed according to Trintinaglia *et al.* (2015). Cell monolayers grown in were incubated for 24 h at 37°C with different concentrations of extracts, in triplicate. Then, medium was removed and 500 μl of NR solution (30 $\mu\text{g/ml}$ in MM) was added to each well. The plates were incubated once more for 3 h at 37°C to promote the uptake of the dye by cells. Subsequently, the supernatant was removed. The monolayers were washed with PBS, and 500 μl of extraction solution (H₂O:acetic acid:ethanol) (49:1:50) was incorporated in each well. After gently shaking the plates, the absorbance was read on a spectrophotometer at 540 nm. Monolayers incubated only with MM were used as control.

RESULTS AND DISCUSSION

On testing, the oral cancer cell lines with strawberry extract for any noticeable cytotoxic activity after incubation both the MTT assay and the NR incorporation tests did show effective results with low cell viability percentage on application of strawberry extract, thereby giving path to apoptosis-related activity on the cancerous oral mucosa layer. Cytotoxic activity may also be carried out using the 2,3-bis-(2-methoxy-4-nitro-5-sulphophenyl)-2H-tetrazolium-5-carboxanilide, resulting in a water-soluble product, in other words, the MTS assay [17]. The conversion of cellular macromolecules to specific ROS during normal

cellular metabolism is the causation for many chronic diseases which include cardiovascular diseases, arthritis, diabetes, and many types of cancer [18].

NR incorporation

Major causes of cellular injury in an increasing number of diseases including cancer are the ROS. ROS are created in the cell mostly through the normal cellular metabolism [19]. Environmental factors such as ultraviolet light and toxic chemicals, as well as the inflammatory process, result in their production. ROS produced by sources alternatively include carcinogens, by ionizing radiation and by phagocytic cells involved in the inflammatory response contributing to these diseases [20]. Antioxidants play a major role in the interception of ROS or limiting their cellular effects. Natural remedies such as treating cancer by analyzing the cytokines activity *in vitro* of sweet potato also have been of budding areas of research owing to its properties of antidiabetic, antimutagenicity, immunostimulatory, and memory-enhancing properties [21].

Berries exhibit high antioxidant potential, exceeding that of many other foodstuffs mainly due to their comprisal phenolic and flavonoid compounds. Berry constituents also influence cellular processes associated with cancer progression including signaling pathways associated with cell proliferation, differentiation, apoptosis, and angiogenesis [22]. Strawberry, Korean raspberry, and mulberry extracts are known to have anticancer effects on cervical and breast cancer cell lines [23] which support this present evidence for the anticancer action of SBE. Phenolic compounds of strawberry have been found to have effectively inhibited the growth of human oral (CAL-27 and KB), colon (HT29 and HCT-116), and prostate (LNCaP and DU145) cancer cells [24-26]. Moreover, also strawberry extracts inhibited the growth of human colon (HCT-116), lung (A549), stomach (SNU-638), and fibrosarcoma (HT-1080) cancer cells [27-29]. In addition, previous studies have reported that dietary freeze-dried strawberries and Korean raspberries have the ability to inhibit N-nitrosomethylbenzylamine-induced tumorigenesis in the rat [30,31]. Therefore, all these strong evidences support the findings of the current study which proves that strawberry is a strong anticancer agent which induces apoptosis of KB cells, so the cell viability percentage decreases (Fig. 1).

CONCLUSION

Even though there exist many chemical drugs for the treatment of oral carcinoma, they have adverse side effects which make the patient feel he would have been dead rather than being walking dead person with immense side effects. Hence, there are alternative forms of drugs given by nature which has bioactive substance which acts against oral cell lines and has immense promising therapeutic uses which can be used to treat cancer patients in medical field.

AUTHOR'S CONTRIBUTION

Ramya G has performed the review of literature, biochemical analysis, and compilation of paper. Dr. Vishnu Priya V has majorly provided with the literature survey, research proposal, and paper writing. Gayathri R has performed statistical analysis and phytochemical work.

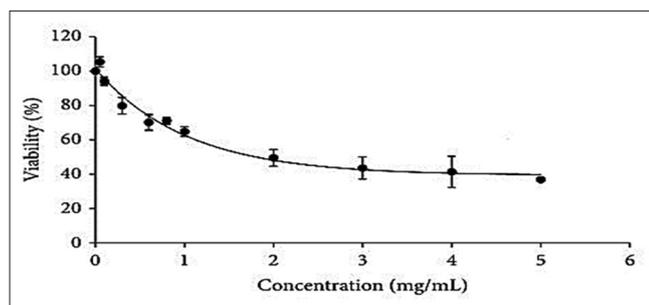


Fig. 1: Cell viability in strawberry methanolic extract-treated KB cells using neutral red incorporation. The results are expressed as mean±standard error of the mean (n=3)

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this article.

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