

COMPARATIVE STUDY OF THE CYTOTOXICITY AND APOPTOTIC EFFECT OF BEET ROOT, AND SILICA-BEET NANOPARTICLES WITH 5-FU NANOPARTICLES AGAINST SCC-090 CELL LINE

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ABSTRACT

Objective: Nanoparticle delivery systems can be used to target anticancer drugs to tumor tissues. Beet root appears to be a powerful dietary source of antioxidant agent with anti-cancer effect due to ability to scavenge free radicals. Application of silica-nanoparticles (SiNP) in cancer treatment are promising, with increased data suggesting anti-proliferative effects in cancer cells. The aim of our study was to compare the cytotoxic effect of the beet root NP, silica-beet NP with 5fu (flourarcil) NP, a chemotherapeutic agent against tongue carcinoma cell line scc-090.

Methods: Cell Line cells were obtained from American Type Culture Collection , cells were cultured using DMEM (Invitrogen/Life Technologies). Beet root nanoparticles Si-beet NP and 5FU drug NP were used. The effect of the tested materials were investigated by MTT assay and DNA fragmentation (DPA method).

Results: The MTT cytotoxicity assay revealed that beat root (nano) and Si-beat roots (nano) have obvious cytotoxicity on SSC-090 cancer cell lines at low significant IC50 value as 0.73 μ g/ml and 2.94 μ g/ml respectively. While 5FU drug revealed obvious cytotoxicity on SSC-090 cancer cell lines at higher value IC50 8.83 μ g/ml. these results denote that low doses of beat root (nano) may exert remarkable cell death on tongue cancer cells line scc-090.

Conclusion: Our findings demonstrate that beet root NP could be capable of inducing cancer cell death with prominent apoptosis at very low ic50 value 0.73 μ g/ml against oral tongue carcinoma SCC-090 cell lines.

KEYWORDS: Beet root, silica nanoparticles, 5-FU drug and Apoptosis.

INTRODUCTION

Nanoparticle delivery concept can be used to target anticancer drugs to tumor tissues by either passive or active targeting. Passive targeting refers

to the accumulation of a drug or drug carrier system at a desired depending on the inherent size of the nanoparticles, while, active targeting involves the attachment of a moiety, like a monoclonal antibody

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or a ligand, to deliver a drug to pathological sites or to cross biological barriers based on molecular recognition processes^[1]

The antioxidant, anti-inflammatory and vascular-protective effects offered by beetroot and its constituents have been investigated by several *in vitro* and *in vivo* human and animal studies; hence it might be considered for treatment of several pathological disorders^[2].

Red beet (*Beta vulgaris* var. *rubra* L., BVR) and green beet (*B. vulgaris* var. *ciela* L., BVC) belong to the same plant family (Amaranthaceae–Chenopodiaceae). *Beta vulgaris* var. *rubra* L. is valued for its root, while BVC is grown for its leaves. The most important bioactive phytochemicals in BVR are betalains, pigments derived from betalamic acid and grouped into yellow betaxanthins (BX) and red betacyanins (BC) with its powerful antioxidant and anti-inflammatory effect^[3].

Human diseases such as cancer, could result from oxidative stress produced by continual and excess production of reactive oxygen and nitrogen species (RONS) that induce long-term cellular disruption [4]. Therefore many antioxidant food sources have been evaluated for their ability to scavenge RONS and prevent oxidative stress^[5].

In a study conducted by Wootton-Beard, P.C. et al, 2011, they found that found the betanin, the most abundant betalain found in beetroot was the most effective inhibitor of lipid peroxidation. Betanin has high antioxidant activity from its strong electron donating capacity and ability to defuse highly reactive radicals targeting cell membranes. They concluded that the main mechanism that beet root juice exerts its antioxidant effects is by scavenging free radical species^[6].

5-FU is an analogue of uracil with a fluorine atom at the C-5 position in place of hydrogen. It rapidly enters the cell. 5-FU is converted intracellularly to several active metabolites:

fluorodeoxyuridine monophosphate (FdUMP), fluorodeoxyuridine triphosphate (FdUTP) and fluorouridine triphosphate (FUTP). These active metabolites disrupt RNA synthesis and the action of thymidylate synthase (TS)^[7].

More than 80% of administered 5-FU is normally catabolized primarily in the liver, where dihydropyrimidine dehydrogenase (DPD) is abundantly expressed causing unwanted side effects such as gastrointestinal symptoms, alopecia, cardiotoxicity, neutropenia, dermatitis, cardiac toxicity, and damage of the central nervous system result in limiting its applications^[8,9].

Application of silica-nanoparticles (SiNP) in cancer treatment are promising, with increased data suggesting anti-proliferative effects in cancer cells compared to normal cells. SiNPs present favorable characteristics, including wide bio-distribution, chemical stability, cellular internalization, and tumor penetration^[10].

In addition, studies report that SiNPs can trigger cytotoxic and genotoxic effects, causing generation of ROS, aberrant aggregation of nucleoplasmic proteins, DNA damage, and finally apoptotic death in treated cells.^[11]

Far to our knowledge, no studies were performed on the cytotoxic nanoparticle effect of beet root against cancer cells, so the aim of the present study was to compare the cytotoxic effect of the red beet root nanoparticles (NPs), silica-beet NPs with 5fu (flourarcil) NPs, a widely used chemotherapeutic agent against tongue carcinoma cell line scc-090.

MATERIALS AND METHODS

Cell Line Scc-090 (human tongue squamous cell carcinoma). cells were obtained from American Type Culture Collection, cells were cultured using DMEM (Invitrogen/Life Technologies) supplemented with 10% FBS (Hyclone.), 10 ug/ml of insulin (Sigma), and 1% penicillin-streptomycin.

All of the other chemicals and reagents were from Sigma, or Invitrogen.

Cell culture protocol was done according to standard procedures at *confirmatory diagnostic unit, VACSERA-Egypt*. Plate cells (cells density 1.2 – 1.8 × 10,000 cells/well) in a volume of 100µl complete growth medium + 100 ul of the tested compound per well in a 96-well plate for 24 hours before the MTT assay .

The cultures were incubated at 37°C for 24 hrs. After treatment of SCC-090 cells with the serial concentrations of the beet NPs, Si-beet NPs and 5FU NPs drug (100, 25, 6.25, 1.56 and 0.39 ug/ml). Incubation is carried out for 48 h at 37°C, then the plates are to be examined under the inverted microscope and proceed for the MTT assay.

Nanoparticles preparation:

Beet root, Si-beet and 5FU nanoparticles were prepared at *National research center, Egypt*. The plant extract was used to develop nano-suspension with poly acrylic acid (PAA) and calcium chloride. PAA solution was used at a concentration of 0.05% (in water) with NaOH to pH 8 and 0.1% calcium chloride solution in water. In addition to silica -beet nano-suspension^[12].

The stability of nanoparticles were observed for 5 days in term of color, turbidity and sediment. Evaluation of nanoparticle included particles size (400-500 nm) using a Malvern Particle sizer and examination of the zeta potential using a Malvern zeta potential measuring device.^[13]

Fluorouracil Encapsulated Chitosan Nanoparticles preparation

Chitosan, derived from crab shell, in the form of flakes was purchased from Sigma-Aldrich (Medium Molecular Weight, Catalogue no. 448877).

The degree of deacetylation and molecular weight for the medium-molecular-weight chitosan (MWM chitosan) is 75–85% and 190–310 kDa based on viscosity, respectively.^[14]

Sodium tripolyphosphate (TPP) (purity: 85%), 5-fluorouracil (5-FU) (purity: 99%), and phosphate-buffered saline (PBS) tablets (pH 7.4), were maintain chitosan concentration at 0.75 (mg/mL). Prepared chitosan solutions were mixed with 5-FU solutions (5-FU dissolved in water), and 1.0mg/mL 5-FU containing chitosan solutions were maintained. Tween 80 (Sigma,Germany) (0.5% (v/v)) was added to chitosan solutions, and pH was arranged as 4.6–4.8. Prepared 5-FU-containing chitosan solutions were flushed mixed with 0.5mg/mL TPP solutions with a ratio of volume ratio of (2 : 1) (v/v) (chitosan : TPP). The nanoparticle suspension was gently stirred for 20 min at room temperature to allow excess 5-FU adsorption on the nanoparticles to reach isothermal equilibrium. 5-FU encapsulated chitosan nanoparticles were centrifuged at 12000 g for 30 min, re-suspended in water and freeze-dried used for further analyses. Prepared 5-FU encapsulated chitosan nanoparticles were analyzed by Zetasizer Nano S (Malvern,UK) in order to determine mean average particle size distributions.^[15]

All nanoparticle solutions used for the current study were freshly prepared and sonicated before treatment at *confirmatory diagnostic unit, VACSERA-Egypt*. Nanoparticles were sterilized with UV exposure prior to the treatment

Cytotoxicity assay protocol (MTT)

The MTT method of monitoring in vitro cytotoxicity is a mean to measure the activity of living cells by mitochondrial dehydrogenases. The key component is (3-[4,5dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) or MTT. Solutions of MTT, dissolved in medium or balanced salt solutions without phenol red, are yellowish in color. Mitochondrial dehydrogenases of viable cells cleave the tetrazolium ring, yielding purple formazan crystals which are insoluble in aqueous solutions.^[16]

The crystals are dissolved in acidified isopropanol. The resulting purple solution is spectrophotometrically measured.^[17] An increase or decrease in cell number results in a concomitant change in the amount of formazan formed, indicating the degree of cytotoxicity caused by the test material using BIOLINE ELIZA READER. *IN VITRO TOXICOLOGY ASSAY KIT MTT BASED Stock No. TOX-1. 7H258*, Sigma-Aldrich, Inc. the MTT assay was done according to the standard procedure at *confirmatory diagnostic unit, VACSERA-Egypt*. Spectrophotometrically measure absorbance at a wavelength of 570 nm for spectrophotometric measurement using BIOLINE ELIZA READER.

DNA fragmentation assay [Diphenylamine (DPA) method]

This method is based on that extensively fragmented double-stranded DNA can be separated from chromosomal DNA upon centrifugal sedimentation. The protocol includes the lysis of cells and the release of nuclear DNA, a centrifugation step with the generation of two fractions (corresponding to intact and fragmented DNA, respectively), precipitation of DNA, hydrolysis and colorimetric quantitation upon staining with diphenylamine (DPA), which binds to deoxyribose^[18,19].

The Kit used for enzyme-assay: *SIGMA chemical* using BIOLINE ELIZA READER w/ 450nm Solvent DMSO following the standard procedure at *confirmatory diagnostic unit, VACSERA-Egypt*.

Measurement of growth by the diphenylamine colorimetric method

Diphenylamine reagent (Burton 1956) for colorimetric assay was prepared by dissolving 1.5g of reagent-grade diphenylamine in 100 ml of glacial acetic acid and then adding 1.5 ml concentrated H₂SO₄. Prior to use, 1.6 % of aqueous acetaldehyde was added to 100 ml of diphenylamine reagent. It is notable that the agent must be colorless and stored in the dark. For growth quantification by the

diphenylamine colorimetric reaction, cell pellets were harvested from 1 ml cultures by centrifugation at 10,000×g for 10 min and then washed twice with appropriate buffer. Then, the cell pellets were re-suspended with 2 ml diphenylamine reagent and incubated at 60 °C for 1 h. The supernatants from the diphenylamine reactions were collected after centrifugation and transferred to 96-well micro titer plates measured by a multi-functional microtiter plate reader (Synergy Hybrid Reader, BioTek, USA). The readings measured by this reaction system are all within the standard error of the optical density measurement.^[20]

RESULTS

MTT cytotoxicity assay

The MTT cytotoxicity assay revealed that beet root NP and Si-beet root NP have obvious cytotoxicity on SSC-090 cancer cell lines at low significant IC₅₀ value as 0.73 μg/ml and 2.94 μg/ml respectively after incubation period 48 hours. While 5FU drug revealed obvious cytotoxicity on SSC-090 cancer cell lines at higher value IC₅₀ 8.83 μg/ml after incubation period 48 hours table (1,2) (fig. 1,2). These results denote that low doses of beet root NP may exert remarkable cell death on tongue cancer cells line scc-090.

Combining Si-beet NP revealed obvious cytotoxic effect on cancer cells but at a higher significant IC₅₀ value 2.93 μg/ml. While beet NP without silica, produced obvious cytotoxic effect on cancer cells at very lower significant IC₅ value 0.73 μg/ml. this may denote that addition of silica did not enhance the cytotoxic effect of beet root NP. (fig. 1,2)

DNA fragmentation assay

Our results showed that Beet root nanoparticles when added to tongue cancer cell line ssc-090 revealed a highly significant DNA fragmentation of cancerous cells in comparison to non- treated

TABLE (1) Showing the comparison of IC50 values of the beet NP, si-beet NP and 5fu NP drug against tongue carcinoma cell line (scc-090) ANOVA t-test.

Ser	Sample code	IC50-uM
		UPCI:SCC090
1	Beet Roots NPs	0.73 ±0.02
2	Si-Beet NPs	2.94 ±0.12
3	5FU NPS	8.83±0.51
	p-value	0.000

TABLE (2). Showing highly significant difference between control untreated cancer cells and the treated cancer cells with either beet NP, Si-beet NP and 5FU NP drug ANOVA test.

Groups	Control	Beet NP	Si-beet NP	5FU NP	p-value (ANOVA)
Mean±SD	0.44167±8.643E-03	0.191667±4.506E-03	0.241667± 3.507E-03	0.255 ±4.025E-03	0.000

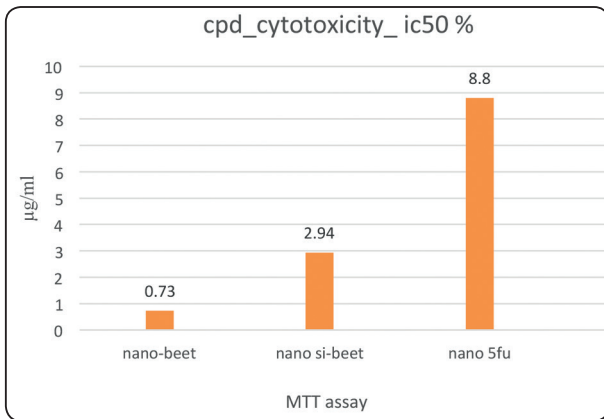


Fig. (1): Bar chart showing comparison of cytotoxicity-ic50 value of beet root NPSi-beet NP and 5fu NP drug against tongue carcinoma cell line (scc-090). Notice that beet root NP produced the cytotoxic effect on cancer cells at very low significant value of ic50 0.73µg/ml denoting the strong cytotoxic effect of this plant.

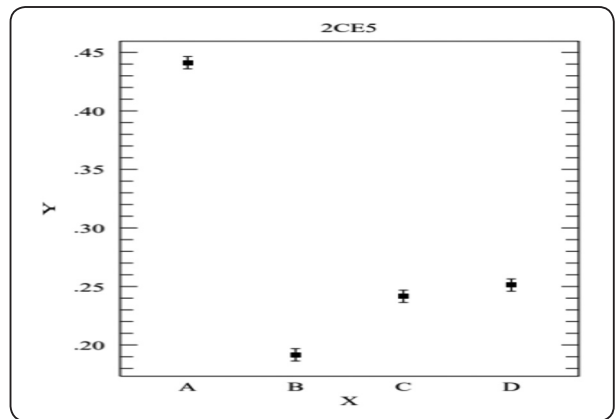


Fig. (2) Pot graph showing the cytotoxic effect of beet NP (B) , Si-beet NP (C) and 5FU NP (D) on cancer cell viability in comparison to control non-treated cancer cells scc-090 (A). ANOVA test with plot output variance. Notice that the viability of scc-090 cancer cells highly decreased when treated by beet NP (B)

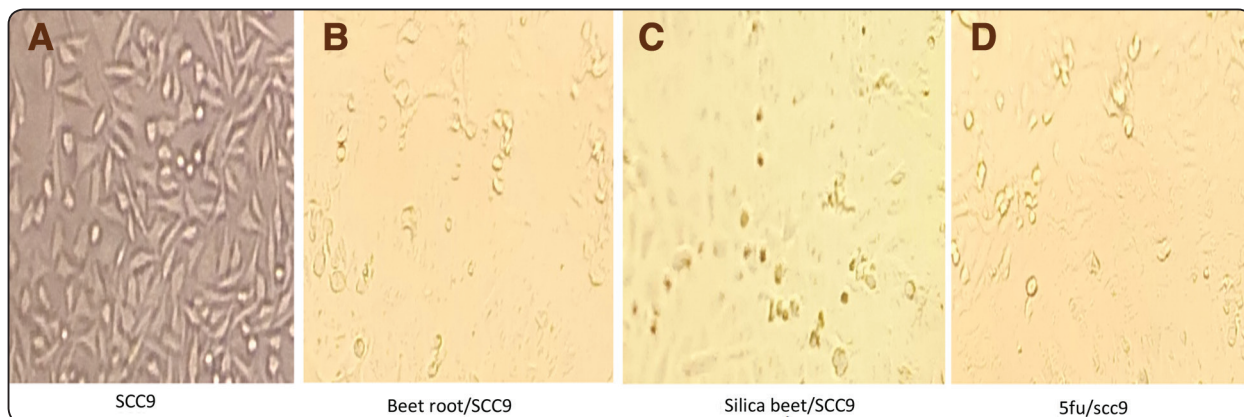


Fig. (3) A- SCC-090 cell line before treatment, beet NP against scc090. C-Si-beet NP against scc090. D-5fu NP against scc090. After 48hs incubation period. cell culture images by inverted microscope.

cancer cells denoting the strong apoptotic effect of nano-beet particles. While, the si-beet nanoparticles revealed lower value of DNA fragmentation of scc-090 cells compared to nano-beet group. However, si-beet nanoparticles produced also a highly significant apoptotic effect in comparison to non-treated cancer cells table (3) fig (4).

Moreover, 5fu nanoparticles revealed a highly significant DNA fragmentation 45% of cancerous scc-090 cell line in comparison to non- treated cancer cells denoting the strong apoptotic effect of nano-5fu particles. Our previous observations may reveal that nano-beet particles have the highest DNA fragmentation and so strong apoptosis of cancer cells scc-090 table (3) fig. (4).

TABLE (3) Showing highly significant difference between control untreated cancer cells SCC-090 and the treated cancer cells with the nano-beet, silica loaded nano-particle and 5FU-nano in reference to DNA fragmentation ANOVA test.

S r	Sample code	DPA
		UPCI:SCC090
		% DNA fragmentation
1	Beet Root NPs	60.88496
2	Si-Beet NPs	39.87539
3	5FU NPs	45.22145
4	Control SCC090	9.965035
	p-value	0.000

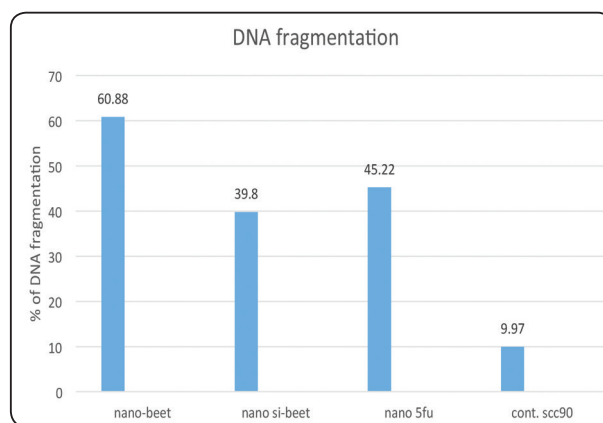


Fig. (4) bar chart showing the prominent apoptotic effect of beet root NPs , si- beet NPs and 5FU NPs drug against SCC-090 tongue cancer cell lines. Notice that the highest value apoptosis was revealed by beet root NP.

DISCUSSION

For cancer therapy using nanotechnology, the nano-therapeutic agents are supposed to be released into the interstitial fluid, tumor surface or directly into the intracellular space. Where, endocytosis needs to occur from plasma membrane to the cytosol as well as lysosomes, where the particles degrade and release their payloads^[21].

In the present study, the MTT cytotoxicity assay revealed that beet root np and Si-beet NP have obvious cytotoxicity on SSC-090 cancer cell lines at low significant IC₅₀ as 0.73 $\mu\text{g/ml}$ and 2.94 $\mu\text{g/ml}$ respectively 48hrs compared to non-treated cancer cells after incubation period 48 hours. Moreover, 5FU NP drug revealed obvious cytotoxicity on SSC-090 cancer cell lines at higher significant value IC₅₀ 8.83 $\mu\text{g/ml}$ after incubation period 48hrs compared to beet NP and Si-beet NP treated cancer scc-090 cells

The previous observation may denote that very low doses of nano-beet may have a prominent cytotoxic effect on tongue cancer cells (scc-090) and consider beet root plant as pharmaceutical active agent since the US Food and Drug Administration (FDA) and national cancer institute USA, considered that plant extract with IC₅₀ values $\leq 20\mu\text{g/ml}$ following incubation between 48 and 72 h, are considered pharmaceutically effective agent and can be used as bench mark for suitable screening cancer drugs from plants and herbs.^[22]

The previous observation in accordance with Paluszczak, J et al, 2010. They showed that the beet root extract, betaine may act as a cytotoxic agent by methylating DNA in cancer cells. They explained that betanin could inhibit the DNA methyltransferase activity in human breast cancer MCF-7 cells^[23]

A study conducted by Govind J. Kapadia¹, they used two cancer cell lines of human origin (prostate PC-3 and breast MCF-7), the beetroot extract was shown to exhibit consistent cytotoxic activity.^[24]

An interesting observation in our study that combining of silica NP to beet root NP revealed obvious cytotoxic effect on cancer cells but at higher significant iC₅₀ value 2.93 $\mu\text{g/ml}$ after incubation period 48hrs. While beet root NP without silica, produced obvious cytotoxic effect on cancer cells at very low significant IC₅₀ value 0.73 $\mu\text{g/ml}$. this may denote that addition of silica did not enhance the cytotoxic effect of beet root NP.

The previous observation could be explained on the basis that molecular cell response to NP treatment are strictly dependent on the cellular uptake of these particles. It was found that SiNPs can easily enter cells by endocytosis, localizing to the cytoplasm or cellular organelles, such as the endoplasmic reticulum (ER) and mitochondria.^[25]

Meanwhile, NP size is an important factor influencing NP-dependent cellular effects. Lu et al showed that decreasing NP size (7-20 nm) increased their diffusivity intracellular, while other studies have suggested that the size of inorganic NPs (100 nm or 70 nm) accumulate at the tumor site with different degree of intracellular diffusion.^[26]

In the current study, the growth inhibitory effect and apoptosis of both Beet root NP and Si-beet NP were evident in the two groups on cancer cells line scc-090 but the beet root NP revealed significant higher apoptotic effect at lower significant IC₅₀ value.

The previous observation in accordance with Farabegoli F, et al, 2017. They found that cytotoxicity of BX and BC (beet root active metabolites) showed a high cytotoxicity at ic₅₀ 0.25–0.35 $\mu\text{g/mL}$ against CaCo-2 colorectal cancer cells. They found the cytotoxic effect was exerted through the activation of apoptosis^[27]

In a study conducted by Lu et al, when using SiNP treatment, they found increased expression of p53 and caspase 3 and decreased expression of Bcl2 and procaspase 9 in human HEPG2 hepatoma cells,

while none of these effects were observed in normal human L02 hepatocytes^[28].

Scarpa et al., 2017 examined the anti-proliferative effect vitexin-2-O-xyloside a major constituent of green beet against CaCo-2 colon cancer cells and HepG2 liver cancer cells, 30- μ M vitexin-2-O-xyloside was able to induce a remarkable increase in the endogenous ROS levels, in both CaCo-2 and HepG2 cancer cells, leading to the reduction of their proliferation rate[29]. Harris and Brugge, 2015 concluded that vitexin-2-O-xyloside could act as a pro-oxidant when it enters cancer cells in certain concentrations, and it may kill the cancer cell by exacerbating oxidative stress and interfering with cell signaling pathways at different levels.^[30]

Farabegoli et al., 2017 found that, BX and BC were able to reduce oxidative damage caused directly by H₂O₂ in CaCo-2 colon cancer cells. They concluded that betalains exert their antioxidant effect at a genomic level by increasing the expression and transcriptional activity of the redox-sensitive transcription factor nuclear factor (erythroid-derived 2)-like 2 (Nrf2) (Na and Surh, 2014), which is normally bound to the cytosolic protein Kelch-like ECH-associated protein 1 (Keap1)^[31]. When betalains react with the redox-reactive cysteine residues of Keap1, the connection Nrf2-Keap1 is disrupted and Nrf2 translocates into the nucleus, where it binds to the antioxidant responsive elements and initiates the gene transcription of phase-II and antioxidant enzymes so betalain behave as antioxidants in both human erythrocytes and cancer cells^[32,33].

An animal study conducted by Lechner et al, 2010. They investigated if long term (35 weeks) treatment with a betacyanin containing extract (78 μ g-mL-day⁻¹ of E162, red food colour prepared from beetroot) would inhibit tumour incidence in rats exposed to a potent tumour promoter (N-nitrosomethylbenzylamine). In comparison to control, the beetroot extracts markedly inhibited

cell proliferation, angiogenesis and tumorigenesis in oesophageal lesions. They revealed that beet root effect could be attributed to its radical scavenging and anti-inflammatory activity.^[34]

Another animal study presented by Szaefer, H et al, 2014, where the experimental rats were fed beetroot juice (8 mL-kg-bm-day⁻¹ for 28 days) and treated with the carcinogen 7,12-dimethylbenz[a]anthracene (DMBA). On day 27 and 28 of the beetroot juice-feeding period study. Markers of liver damage and inflammation were significantly increased following the DMBA treatment; however, these were markedly reduced in the rats pre-treated with beetroot juice compared to the control group that received water only. They explained the results that beetroot extracts appeared to maintain endogenous antioxidant activity (reduced glutathione, glutathione peroxidase and catalase enzymes) at normal cellular concentrations following the oxidative insult, beetroot may exhibit indirect antioxidant effects that act to up regulate antioxidant defence mechanisms.^[35]

In the current study, the DNA fragmentation assay revealed that Beet root NP when added to tongue cancer cell line scc-090 showed a highly significant DNA fragmentation of cancerous cells in comparison to non- treated cancer cells denoting the strong apoptotic effect of nano-beet particles. While, the si-beet NP revealed lower value of DNA fragmentation of scc-090 cells compared to beet NP group. However, si-beet nanoparticles produced also a highly significant apoptotic effect in comparison to non- treated cancer cells.

In addition, our study revealed that 5fu nanoparticles revealed a highly significant DNA fragmentation of cancerous scc-090 cell line in comparison to non- treated cancer cells denoting the strong apoptotic effect of nano-5fu particles. Our previous observations may reveal that beet NPs have the highest DNA fragmentation and so higher apoptotic effect against tongue cancer cell line scc-090.

The previous findings are in accordance with a study conducted by Nowacki et al., 2015. They investigated the antiproliferative effect of beet root bioactive BC agent in MCF-7 breast cancer cells, they showed that a mixture of betanin/isobetanin is able to induce apoptosis in these cancer cells, through the activation of p53, increase of the protein levels of pro-apoptotic factors Bcl-2-associated death promoter (Bad), TNF-related apoptosis-inducing ligand receptor 4 (TRAILR4), Fas and so induction of autophagic cell death^[36].

The anti-apoptotic effect of 5fu anti -cancer drug was revealed by DNA microarray screen of 5-FU-inducible target genes was FAS.^[37] FAS is a member of the tumour necrosis factor (TNF) receptor superfamily, which, when bound by its cognate ligand FASL, recruits caspase-8 zymogens via the adaptor protein FADD (FAS-associated death domain). The FASL/FAS/FADD/caspase-8 complex is known as the death-inducing signalling complex (DISC). Caspase-8 is activated at the DISC and subsequently initiates a caspase cascade that results in apoptosis^[38]. We could conclude that beet root and 5fu drug have similar apoptotic effect on cancer cells by activation of FAS pathway

The chemotherapeutic agents, daunorubicin and its analogs, doxorubicin, epirubicin and idarubicin.^[39] appear to exert their lethal effects on cancer cells via intercalation with DNA bases.^[40] and by inhibition of DNA topoisomerase I and II enzyme activities.^[41] An interesting observation conducted by the betanin red beetroot extract, there is a striking similarity in the chemical structure and configuration with the anticancer compounds, e.g., doxorubicin, both have a planar aromatic chromophore and a six-membered sugar molecule which produce intercalation with DNA in cancer cells causing cancer cell death^[42]. This suggests that betanin may play a major role in the observed cytotoxic effect of the red beetroot extract through a possible mechanism of action common with doxorubicin and related anthracycline chemotherapeutic drugs.^[43]

CONCLUSION

The beet root and silica –beet nanoparticle might be capable of inducing cancer cell death against SCC-090 cell lines by induction of apoptosis.

RECOMMENDATION

Further studies should be performed to investigate the beet root effect on molecular level of cell pathways and it should be screened against more cancer cell lines for the possible use of this plant for chemotherapy in the future.

Compliance with ethical standards

Conflicts of interest. The authors declare that they have no conflict of interest.

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