Research Article

The Effect of High Fructose Corn Syrup on SCC-9 Cells Regulated by G0/G1 Cell Cycle Arrest via Apoptosis Induction

Kalimuthu Kavitha[1], Ganapathy Sindhu[1,2,*], Marimuthu Porkodi[1], Kumarasamypillai Radha Thayammal Asha[3], Veeran Veeravarmal[4], Annamalai Vijayalakshmi[5]

[1]Department of Biochemistry and Biotechnology, Faculty of Science, Annamalai University, Annamalai Nagar, Chidambaram, Tamil Nadu, INDIA.
[2]Department of Biochemistry, Government Arts College (Autonomous), Kumbakonam, Tamil Nadu, INDIA.
[3]Department of Biochemistry, Government Arts College, Paramakudi, Tamil Nadu, INDIA.
[4]Department of Oral and Maxillofacial Pathology, Rajah Muthiah Dental College and Hospital, Annamalai Nagar, Tamil Nadu, INDIA.
[5]Plant Head and Director, Galileovasan offshore and Research and Development Private Limited, Nagapattinam, Tamil Nadu, INDIA.

Submission Date: 22-06-2023; Revision Date: 17-07-2023; Accepted Date: 15-09-2023.

ABSTRACT

Aim: The current study's objective was to assess high fructose corn syrup (HFCS) affected oral squamous cell carcinoma (OSCC) SCC-9 cells through apoptosis, DNA fragmentation, and the cell cycle. **Materials and Methods:** The MTT experiment showed that treatment of SCC-9 cells with HFCS causes internucleosomal DNA breakage and loss of cell viability in a concentration-dependent manner, with an IC_{50} of 679.2 µM. PI and Annexin-FITC labelling were utilized to evaluate the apoptotic nuclear characteristics and migration by fluorescence microscope and flow cytometry during HFCS therapy for apoptosis and cell cycle arrest. **Results:** Key findings are HFCS decreased the cell viability (IC_{50} values of 679.2 µm), reduced migration, enhanced DNA fragmentation and decreased colony formation in SCC-9 cells and Caspase 3 and 9 gene expressions. DNA fragmentation assay revealed HFCS might cause oxidative DNA damage in SCC-9 cells significantly ($p < 0.05$). Similarly, we observed apoptotic stage changes in HFCS-treated cells. Furthermore, HFCS-treated SCC9 cells accumulated in the G0/G1 phase compared to the standard drug Colchicine. All things considered, the results suggest that HFCS affects the SCC-9 cell line by causing DNA damage, cell cycle arrest at the G0/G1 phase, and production of caspase 3 and caspase 9. **Conclusion:** Contrary to earlier literature, our study concluded that higher concentrations of HFCS (500 and 1000µM) reduce cell viability, cell migration and enhance apoptosis in oral cancer cells SCC-9. So that these findings may be generalised to human intake equivalent, more research is needed to confirm its effect on predisposition to mouth cancer.

Keywords: HFCS, Oral cancer, MTT, Cell cycle arrest, Apoptosis, Cell migration.

Correspondence:
Dr. Ganapathy Sindhu,
Assistant Professor, Department of Biochemistry and Biotechnology, Faculty of Science, Annamalai University, Annamalai Nagar-608002, Chidambaram, Tamil Nadu, INDIA.

Email: ganapathysindhu@gmail.com, vijirajabio@gmail.com

INTRODUCTION

Since 1970, High Fructose Corn Syrup (HFCS) has been used as a source of fructose, increasing the quantity of fructose consumed by individuals.[1] In the United States, HFCS consumption developed from 64 g/day in 1970 to 81 g/day in 1997. Consumption of monosaccharide increased from lower than 0.5 g/day to higher than 40 g/day.[2] HFCS is essential to fully appreciate the roles that fructose plays in cancer cell metabolism, enter good cells, exit human metabolism and the main enzymes involved in fructose metabolism.[3] Hereafter, because obesity, diabetes, and metabolic syndrome are important risk factors for the development of a wide range of cancer types, associations between fructose consumption and non-cancer manipulators are also considered.

SCAN QR CODE TO VIEW ONLINE

www.ajbls.com

DOI: 10.5530/ajbls.2023.12.51

Consumption of fructose increased in the twenty-first century compared to the nineteenth. High fructose consumption causes metabolic problems such as obesity and insulin resistance, which are linked to the aetiology of pancreatic cancer. Fructose has been linked to tumour development and carcinogenesis. In N-nitrosomorpholine-treated pancreas tissue, fructose induces the growth of acinar-cell tumor nodules and may provide a novel glucose substrate for pancreatic cancer.[4]

A major worldwide health problem, Oral Squamous Cell Carcinoma (OSCC) among human beings causes 145,000 deaths and 247,000 new cases every 5 years. Oral Cancer (OC), the world's eleventh-leading type of cancer, has emerged as the leading cause of mortality in India. When healthy cells are exposed to carcinogenic chemicals, cigarette smoke, and other environmental variables, cancer cells form.[5] The disease's causes are dysregulation of cellular proliferation and disruption of the apoptotic process, which is ultimately critical to tumor organization and expansion.[6]

Cancer cells derived not only provide an important platform for studying the molecular biology of neoplasms, but they are also a source for the investigation of precise therapeutic choices that are similar to cancer traits.[7] The *in vitro* systems agree on both drug dose and experience length in a consistently, chemically, and experimentally unique intermediate. Cancer cells proliferate in an unbalanced manner due to compensatory apoptosis.[8] Apoptosis (programmed cell death) refers to the caspase cascade and endonucleases that are responsible for the protein damage of cellular proteins that are most important in the description of apoptotic characteristics such as plasma membrane blebbing, fragmentation, chromatin condensation, and cell shrinkage.[9] Mitochondria are important organelles that regulate cell death through the expression of apoptosis molecular markers.[10] To ascertain whether or not apoptosis has taken place as a result of the loss of Mitochondrial Membrane Potential (MMP) substances endomembrane produces a transmembrane potential.[11] High-Fructose Corn Syrup (HFCS), a new sweetener, is frequently used as a replacement in the food business. In recent years, HFCS, which is composed of 45% glucose and 55% fructose, has been extensively added to beverages, breads, dairy products, and canned goods.[12] HFCS is already found in 70% of beverages in the United States, and it is becoming increasingly popular in China. As a commonly used food additive, more emphasis is being paid to the health risks of a high HFCS diet.[13] According to certain research, HFCS consumption can contribute to cardiovascular disease, diabetes, and obesity.[14] Significant preclinical evidence indicates that the combination of dietary glucose and fructose, even at low doses, can promote the establishment of intestinal tumours.[15] However, there is minimal research on the carcinogenicity of HFCS, maybe because it is considered that any sucrose alternative would be an improvement.

As a result, we wanted to explore how High Fructose Corn Syrup (HFCS) affected SCC-9 cells from Oral Squamous Cell Carcinoma (OSCC). The current study looks at the effects of HFCS on SCC-9 cells by evaluating cell survival, apoptosis, cell cycle arrest, cell migration, DNA fragmentation, colony formation, and the expression of apoptotic gene marker enzymes Caspase 3 and 9.

MATERIALS AND METHODS

Reagents and chemicals

ATCC SCC-9 cell lines, DMEM media, Foetal Bovine Serum (FBS), Penicillin, MTT 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide, Annexin V/FITS C, Propidium Iodide (PI), RNase, Tween-20, and PBS (Himedia, Mumbai). Acetone, ethanol, and Dimethyl Sulfoxide (DMSO) are all available from Fisher Scientific (India).

Evaluation of cell culture and growth

SCC-9 cells were grown in Dulbecco's Modified Eagle Medium (DMEM) at 37°C in a humidified incubator with 95% air, 5% CO_2, and 10% heat-inactivated Foetal Bovine Serum (FBS), 100 U/mL penicillin, and 100 mg/mL streptomycin. Cells were planted in 12 well plates prior to the addition of HFCS. HFCS was exposed to the SCC-9 cells (0, 15.63, 31.25, 62.5, 125, 250, 500 and 1000 **µM/m**L) for 24 hr.

Cytotoxicity assay

SCC-9 cells were seeded in 96-well plates at a density of $6x10^3$ cells each. The medium was removed after 24 hr and reconstituted with media containing HFCS in a range of concentrations (0, 15.63, 31.25, 62.5, 125, 250, 500, and 1000 µM/mL). Similarly, the medium was dosed with Doxorubicin to Vero cells and SCC-9 cells concentration of (0, 0.5, 1.0, 1.5, 2.0, 2.5 µM/mL). After 48 hr, the medium containing Doxorubicin and HFCS was replaced with a 100 mL media containing MTT (tetrazolium bromide solution in PBS, 5 mg/mL). After 4 hr of dark incubation, living cells dissolved in 100 mL of DMSO shorten MTT's yellow thiazolyl groups to purple tetrazolium crystals. A multi-well plate reader (Bio-Rad) was used to measure absorbance at 540 nm.

Apoptosis and cell cycle flow cytometric analysis

1.5×10^5 cells/mL SCC-9 were treated for 24 hr with control and HFCS at concentrations of 500 and 1000 M/mL for flow cytometric cell cycle assessment. For 24 hr, Doxorubicin and Colchicine (20 µM/mL) were used. At 37°C for 30 min, the cells were stained in the dark with 500 mL of hypotonic PI solution (10 mL sodium citrate, 0.25 mg PI, 0.4 mg RNase, and 0.3 mL tween-20 per 10 mL). An air-cooled Argon laser was used to perform flow cytometry analyses at 633, 488, and 375 nm, recording at least 10,000 events. The DNA profile revealed the relative abundance of the cell population in sub-G1, G0/G1, S, and G2/M phases.

Cell migration assay

SCC-9 cells 1.75×10^6 cells/well in a six-well plate reached confluence after 24 hr. The confluent monolayer was then scraped through using a 200 L tip. The dishes were then cleaned twice before being incubated for 0, 1, and 3 days at 37°C in fresh media containing 10% foetal bovine serum, Doxorubicin (20 M), and two dosages of HFCS (500 and 1000 µM). A 4X inverted microscope (Olympus, Tokyo, Japan) was used to measure the width of the wound at two different random points on the top surface of each well. The wound difference between measurements recorded at time zero and the 2 and 6-hour time periods was computed as the mean SEM of migration.

Assay for DNA Fragmentation

1×10^6 cells incubated for 24 hr at 37°C and 5% CO_2. Confluent cells were treated with doxorubicin 20 µM, HFCS at 500 µM and 1000 µM, and 200 µM H_2O_2 after 24 hr of incubation. Cells were collected by centrifugation at 2000 rpm for 5 min. The cell pellet was suspended in 0.5 mL of lysis buffer (pH 7.8) containing TritonX-100 0.2% or Sodium-N-lauroylsarcosinate, 4 M NaCl, EDTA 20 mM, and Tris-HCl 10 mM. After adding 0.5 CC of phenol, chloroform, and isoamyl alcohol, the lysate was mixed for two to 3 min then centrifuged. The top aqueous layer with twice as much cold 100% ethanol and 3M sodium acetate. After 10 min before being centrifuged at 10,000 rpm for 15 min, removing the supernatant, the DNA pellet air dried, dissolved in TE buffer (10 mM Tris-HCl, pH 7.4, 1 mM EDTA, pH 8.0), and separated by 2% agarose gel electrophoresis at 100 V for 50 min.

Assay for colony development

SCC-9 cells were incubated at 37°C overnight. The following day, cells were exposed to DMSO as the vehicle, 20 µM doxorubicin, 500 µM and 1000 µM doses of HFCS, all at 37C. Every three days, cells were changed with brand-new growth media containing the same medicine. The medium was changed once a week and replaced with new, medication-free media after 7 days. After two weeks of culture, the cells were washed twice with PBS, fixed with cold methanol for 30 min at 4°C, and stained for an hour with 1% crystal violet dye (dissolved in 25% methanol). Using an Epson Perfection V550 Photo Colour Scanner, the plates were cleaned, dried, and scanned.

Assay for Reactive Oxygen Species

The SCC-9 cells were cultivated for 24 hr with doxorubicin (20 M) and two doses of HFCS (500 and 1000 M) before being treated with the fluorescent dye DCFHDA. The cells were only kept in the incubator for 30 min more. To remove the extra color, the cells were rinsed with PBS. The fluorescence intensity was calculated using excitation and emission filters with wavelengths of 488 nm and 530 nm, respectively. During fluorescence microscopy, pictures were taken with blue (450-490 nm) filters.

Real-time PCR

TRIzol solution (Himedia, USA) was used to separate the total RNA from the brain tissues, and RT master mix (Himedia) was used to label the cDNA. Real-time qPCR was used to accomplish the amplification and qPCR measurements utilizing the qPCR master mix SYBR advantage (Himedia) in accordance with the prescribed procedure. GAPDH was used to measure and correct target mRNA levels (Caspase 3 and Caspase 9). Evaluation of expression was conducted using the comparative Cp technique.

RESULTS

The effect of HFCS on Vero and SCC-9 cell viability and growth

Figure 1A depicts the percentage of inhibition in normal Vero cells. HFCS was found in concentrations of (0, 15.63, 1.25, 62.5, 125, 250, 500, and 1000M) that showed a significantly decreased percentage of inhibition (p <0.05) in (0, 0.76, 1.14, 3.03, 5.49, 9.28, 11.36, and 18.94), but the IC_{50} value could not be calculated. (Figure 1 B) But SCC-9 cells showed significantly increased % (0, 1.31, 6.28, 11.24, 18.98, 25.55, 32.70 and 53.43) of inhibition respectively. Standard drug Doxorubicin added Vero cells showed (0, 10.14, 18.18, 22.35, 34.66, 39.02, and 59.47) % inhibition respectively at a concentration of (0, 1.56, 3.13, 6.25, 12.5, 25, 50 and 100) after 24 hr and calculated IC_{50} of 71.96. % of inhibition of standard drug on SCC-9

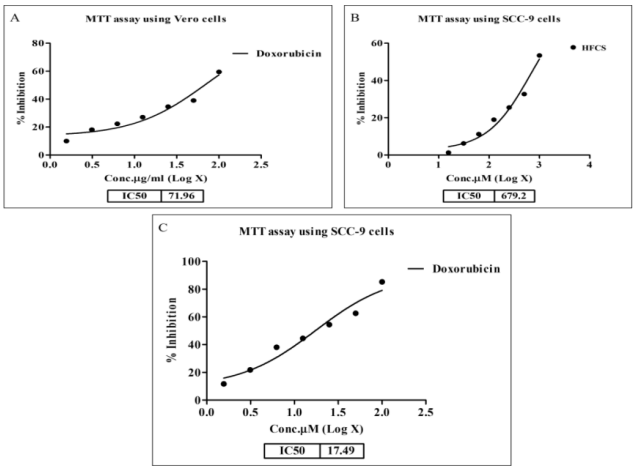


Figure 1: Effect of HFCS on cell viability of Vero cells and SCC-9 cells was assessed by MTT assay. (A) Results are expressed as vero cells treated with Doxorubicin (20 µM). (B) SCC-9 cells treated with HFCS for 24 hr. (C) SCC-9 cells treated with Doxorubicin (20 µM) for 24 hr.

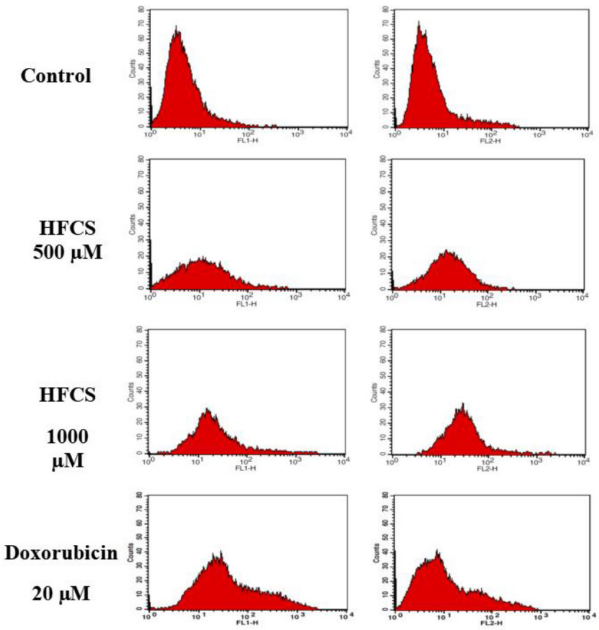
cells was observed for the concentration range of (0, 1.56, 3.13, 6.25, 12.5, 25, 50 and 100**µM**) that showed (Figure 1 C) significantly increased ($p< 0.05$) (0, 11.68, 21.75, 38.10, 44.53, 54.45, 62.63 and 85.26) after 24 hr respectively. At 17.49M, 50% of viable cells were found on SCC-9 cells after 24 hr. Based on this result, the IC_{50} values for HFCS and Doxorubicin in SCC-9 cells were determined to be 679.2 and 17.49M, respectively. For subsequent examination, we used 500 and 1000 M since they significantly (p0.05) encouraged/supported anti-proliferative responses in SCC-9 cells.

Flow cytometry detection of apoptotic cells

Using FITC-conjugated Annexin-V and PI labeling, flow cytometric analysis indicated increased apoptotic cells (PI negative, FITC Annexin-V positive). FITC Annexin-V and PI were used to monitor SCC-9 cells in the presence of HFCS (500 and 1000 **µM**) and Doxorubicin (20 **µM**), as shown in (Figure 2A and 2B). The majority of cells had repressed to late apoptosis/secondary necrosis 48 hr after HFCS treatment, but an early stage of apoptosis was discovered at around 12 hr. In SCC-9, HFCS added at 500M and 1000M was associated with 6.80%, 3.34% early apoptosis and 7.14%, 18.26% late apoptosis, respectively. In SCC-9 cells, standard doxorubicin at 20M triggered 55.37% complete apoptosis.

Effects of HFCS on SCC-9 cell cycle distribution

HFCS on cell cycle distribution in SCC-9 cells were studied after 24 hr of exposure to HFCS at varied doses of 500 and 1000 M/mL. The DNA contents of SCC-9 cells were evaluated by flow cytometric



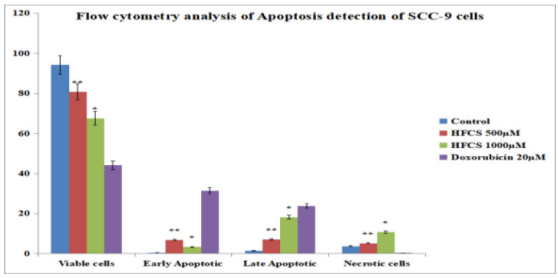


Figure 2: Flow cytometric analysis of HFCS and Doxorubicin alters apoptosis in SCC-9 cells (A) SCC-9 cells were treated with different concentrations of HFCS (500 and 1000 µM) and Doxorubicin followed by Annexin V-FITC staining to analyze the effect of HFCS in apoptosis. (B) This was determined by FACS analysis showing the percentage of early (lower right quadrant) and late (upper right quadrant) apoptotic cells. The data represents the mean ± SD of five independent experiments.

analysis to determine whether a specific disruption of cell cycle-related activities was to blame for the growth effect of HFCS (Figure 3A). SCC-9 cell proliferation was increased following treatment with HFCS (500 and 1000 M/mL) in a concentration-dependent manner. In comparison to the vehicle control and Colchicine 20 M/mL treated SCC-9 cells, we found an increase in the proportion of HFCS-added SCC-9 cells in the S phase (from 15.48 to 22.43%) and in the G2/M phase (9.26 to 13.34%). However, as shown in Figure 3B, HFCS-treated SCC-9 cells consistently tended to accumulate (p 0.02 and p 0.1) in the G0/G1 phase of the cell cycle (from 75.64 to 64.48%). The observed decrease in S and G2M phase accumulation but rise in the standard drug is most likely due to the influence of mild G0/G1 phase

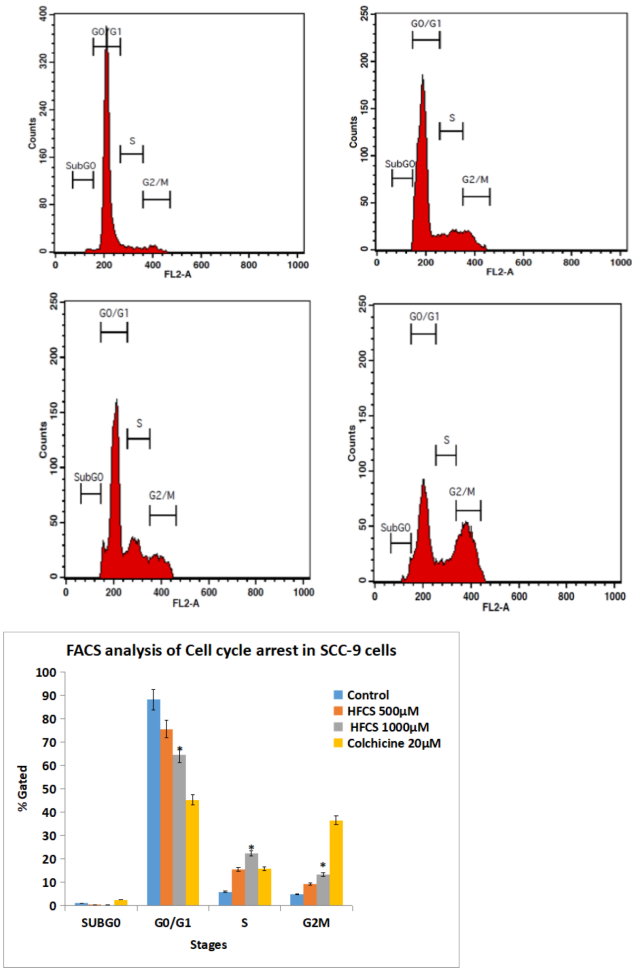


Figure 3: (A) Effect of HFCS on cell cycle distribution in SCC-9 cells was detected by flow cytometry. Cell cycle distributions of vehicle control and HFCS with determinations and where indicated, by *, **, # showed a significant difference $p < 0.01$, 0.02, 0.1 respectively relative to the relevant vehicle control. d SCC-9 cells with various concentrations (500 and 1000 mM) for 24 hr were determined by PI staining in flow cytometry.

accumulation. These data imply that HFCS influences SCC-9 cell proliferation via modulating the G0/G1 and S stages of the cell cycle.

HFCS reduces cancer cell migration and invasion in the mouth

Migration and invasion of cancer cells are critical stages in the metastatic process. SCC-9 cancer cells were treated with Doxorubicin (20 M/mL) and HFCS (500 and 1000 M/mL) and imaged at intervals ranging from 0 to 3 days of incubation to see if HFCS caused cell migration and invasion *in vitro*. Figure 4B shows that SCC-9 oral cancer cell proliferation and migration are time and concentration-dependent. Furthermore, as compared to the conventional treatment of doxorubicin, HFCS did not effectively inhibit SCC-9 cancer cell

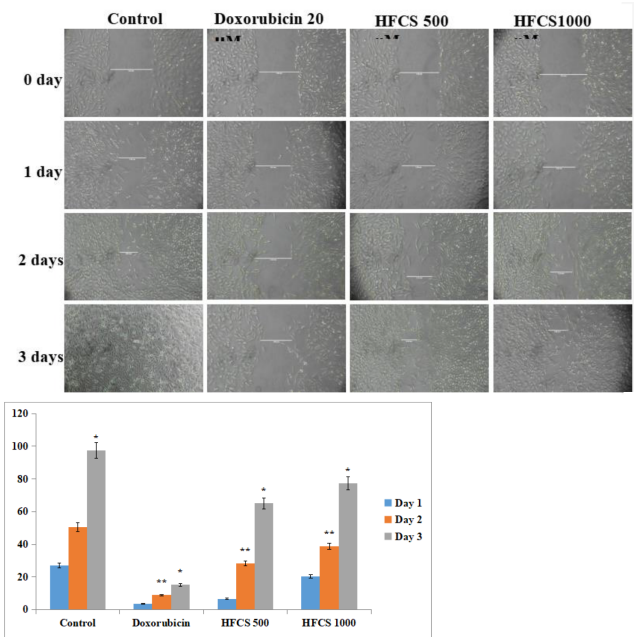


Figure 4: HFCS develops the cellular migration of SCC-9 cells. (A) Confluent monolayers of each cell line were wounded by scratching the surface as uniformly as possible using a 1 µL pipette tip. Cells were treated with Doxorubicin (20 µM/mL) and HFCS (500 and 1000 µM/mL). Every 0 days to 3 days of incubation, wound healing was evaluated by photography. Each figure is representative of three experiments performed in triplicate. Scale bar = 500 µm. (B) The mean denuded zone was measured. The data represent the means ± S.D. of three independent experiments. *$p < 0.05$ and **$p < 0.01$.

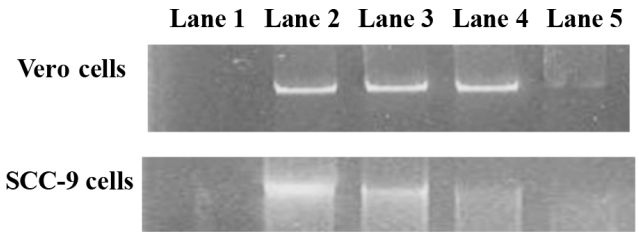


Figure 5: DNA fragmentation of SCC-9 cells exposed to HFCS and Doxorubicin, Fragmentations of genomic DNA in SCC-9 cells were treated with 500 and 1000 µM/mL of HFCS and Doxorubicin 20 µM/mL for 24 hr. DNA laddering formation was viewed on ethidium bromide-stained gel (2%) and photographed by UV illumination. DNA fragmentation studies on Vero and SCC-9 cells. Quick detection of apoptotic DNA ladder in Vero cells (right side) and SCC-9 (Left side) cell line Lane 1: Ladder; Lane 2: Control; Lane 3: HFCS 500 µM; Lane 4: HFCS 1000 µM; Lane 5: H_2O_2 200 µM.

invasion and migration in a concentration-dependent way. The anti-metastatic effect of HFCS was questioned based on this data.

DNA fragmentation studies on Vero and SCC-9 cells

The effect of HFCS on Vero cells and SCC-9 cells was determined in DNA fragmentation assay by agarose

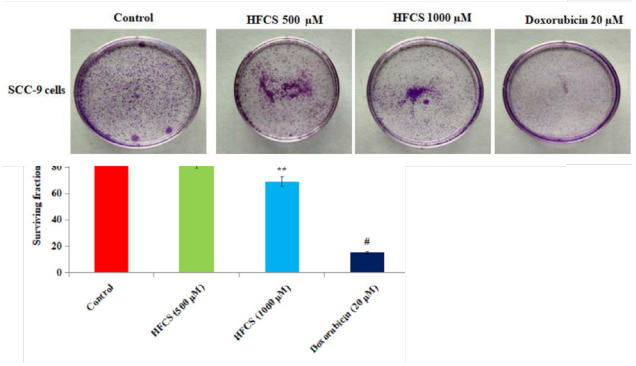


Figure 6: (A) HFCS and Doxorubicin promote colony formation in SCC-9 cells. (A) SCC-9 cells were seeded on 6well plates and treated with HFCS (500 and 1000 µM) and 20 µM doxorubicin to induce DNA damage response and apoptosis. After 2 weeks, the colonies were stained with crystal violet and the number of colonies was counted. (B) Mean denuded zone was measured. The data represent the means ± S.D. of three independent experiments. *$p < 0.05$, **$p < 0.01$ and #$p < 0.001$.

gel electrophoresis. Figures 5A and B When HFCS was applied to SCC9 cells at 500 and 1000 **µM** concentrations, DNA fragmentation was found to be better at higher concentrations. The positive control H_2O_2 treatment at 200M generated significant DNA damage as compared to the Sample. Vero cells treated with HFCS showed no substantial DNA fragmentation, but H_2O_2 treatment at 200**µM** caused DNA damage.

Effect on SCC-9 cells by Colony formation assay

Colonogenic survival assays are used to study the effect of HFCS on SCC-9 cells. Oral Squamous cell lines were added with 500 and 1000 µM of HFSC and Doxorubicin 20 µM. SCC-9 cells showed 100% surviving fraction for Control, 83.82% and 69.02% for 500 µM and 1000 µM of HFCS, and Standard Doxorubicin showed 15.21% recovery at 20 µM treatment respectively. We choose HFCS doses that in single agent experiments resulted in a reduction in viability at higher 500 and 1000 µM in the investigated cell lines. The colony formation of SCC-9 cells is demonstrated to be dose-dependently influenced by HFCS in (Figures 6A and B).

Measuring the amount of ROS inside cells using HFCS

DCFH-DA was utilized in this study to access the ROS produced in SCC-9 cells exposed to varied levels of HFSC (500 and 1000 M) and Doxorubicin (20 M). When compared to untreated cells, the ROS seen in SCC-9 cells subjected to 500 and 1000 M of HFSC and 20 M of doxorubicin were moderately altered respectively (Figure 7). In contrast, HFCS administration (24 hr) reduced ROS production in a dose-dependent manner,

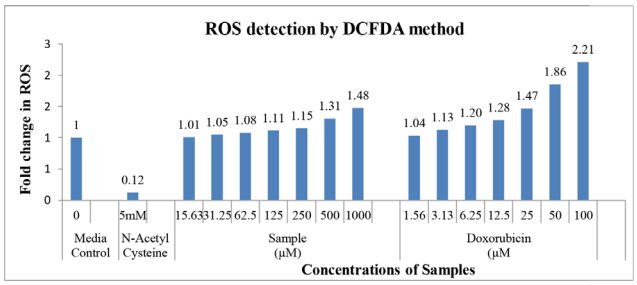


Figure 7: HFCS induces intracellular ROS production in SCC-9 cells by DCFH-DA staining assay. Intracellular ROS measurement by spectrofluorometry. Results in a dose dependent enhance in ROS production as shown by increased% DCF staining ratio.

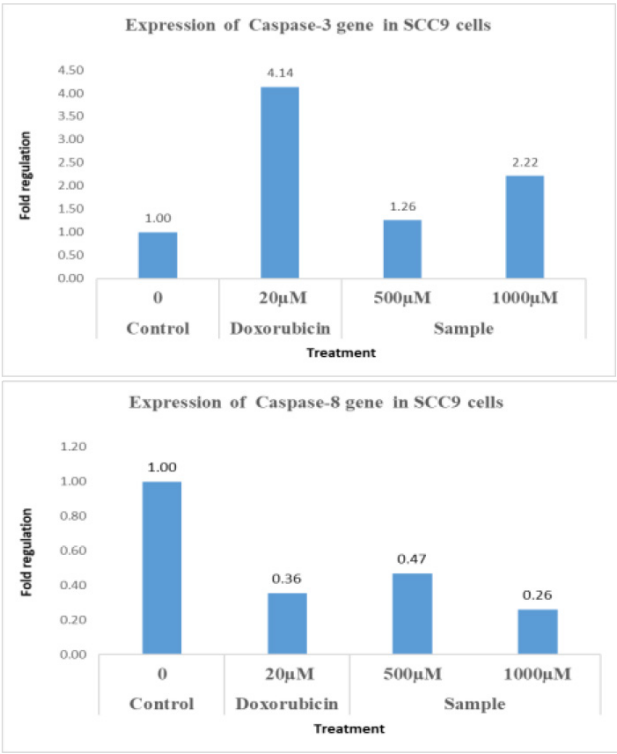


Figure 8: HFCS induces apoptotic marker enzyme analyzed by RT PCR. mRNA Expression Levels of Caspase 3 and 9 in HFCS (500 and 1000 µM) and 20 µM doxorubicin with SCC-9 cells.

as demonstrated by an increase in DCF staining in the nucleus. According to recent findings, HFCS produces ROS in SCC-9 cancer cells, which suggests that HFCS consumption causes the development of cancer.

HFCS was found to increase Caspase-3 and Caspase-9 expression

Caspase-3 and caspase-9 genes were considerably over-expressed on SCC-9 cells in response to HFCS (Figure 8). Doxorubicin 20 M also raised caspase-3 and 9 gene expression. Caspase-3 and caspase-9 gene expression levels in control cells did not differ

significantly. A diagram is shown in Figure 8. Caspase-3 and caspase-9 levels were lower in cells treated with HFCS 1000M than in cells treated with Doxorubicin 20M. In this study, caspase-3 and caspase-9 gene expression levels were revealed to have a substantial positive relationship with HFSC-treated moderately induced apoptosis.

DISCUSSION

There has been no research on the effect of High Fructose Corn Syrup (HFCS) on SCC-9 cell lines from the mouth. The current study hypothesized that the effects HFCS on SCC-9 cells through cytotoxicity, apoptosis, cell cycle arrest, cell migration, DNA fragmentation and colony formation assay in a dose-dependent manner with Doxorubicin standard drug. Due to its powerful sweetening properties and low cost, HFCS, is used in processed foods and beverages, and fructose intake has surged in recent years.[16] According to Chávez-Rodrguez, 2021[17] Fructose is required for cancer cell proliferation and nucleic acid formation, and consuming more fructose promotes cancer development by altering cellular metabolism, increasing reactive oxygen species, increasing DNA damage, and generating inflammation. O'Byrne and Dalgleish state that,[18] the inflammatory process boosts angiogenesis and inflammatory response mediators, which aid in the growth of malignancy. The relationship between fructose consumption and cancer is debatable, and additional research into HFCS consumption and cancer is needed.

Studies on short-term HFCS consumption found no distinctions in outcomes between HFCS and sucrose. HFCS is important to do long-term clinical investigations on the association between fructose consumption and cancer risk.[19] The MTT test is commonly used to assess the effect of HFCS on cancer cell viability and proliferation. Metabolically active cells can transform MTT into a pink product that can be assessed colorimetrically.[20] The effect of HFCS on Squamous oral Cancer Cells (SCC-9) was investigated in this study. Only at higher concentrations (500 and 1000 **µM**) did HFCS inhibit SCC-9 cell growth.

ROS is a harmful species that can cause apoptosis. Overproduction of ROS may cause oxidative stress, while lipid peroxidation may cause double-stranded DNA damage, genotoxicity, and apoptosis. A kind of cell death known as apoptosis is distinguished by morphological modifications and DNA damage. As a result, the effects of HFCS on apoptotic induction were not similar to the conventional medication Doxorubicin. The early, apoptotic, and late apoptotic cells of SCC-9 cells were quantified using Annexin-V and FIT-C labelling, which helped to confirm cell death.[21] Similarly, at lesser dosages, HFCS has less promoted the development of apoptosis. This study found that HFCS had an effect on oral squamous cell cancer.

Several oxidative DNA adducts, such as 8-Oxo-20-deoxyguanosine have been implicated in the tumorigenic process. Only at higher levels did we notice or show that HFCS treatment led to DNA damage, as seen by the development of DNA fragmentation. The DNA fragmentation revealed that the generation of ROS may be the cause of the enhanced DNA damage in HFCS. Our findings showed that HFCS (1000 **µM**) had more DNA fragment breakage as compared to a group that had been injected at a lower dosage of HFCS (500 **µM**), inhibiting apoptosis. In contrast to the H_2O_2 (20 **µM**) supplemented cells, these two concentrations exhibited completely fragmented DNA.[22] Our findings are consistent with prior studies that HFCS causes DNA breakage in colon cancer cells. It has been proposed that HFCS has effects that are dose-dependent.

The cell cycle is stopped in the G0/G1 phase by HFCS. As a result of the effects of HFCS (500 and 1000 M) on SCC-9 cells, there were more cells in the G1/G0 phase, fewer cells entering the S and G2/M phases, and fewer cells returning to the Sub-G0 phase. The G1 and S phase overlaps observed in Colchicine (20 M) could, however, be explained by an apparent early G1 phase accumulation, as evidenced by a broadening of the Sub-G0 phase and an increase in the G1 phase signal. HFCS promotes dose-dependent cell cycle arrest at the G0/G1 phase, according to our most recent research. Our observations, however, indicate that the effect of HFCS on cell cycle arrest during the G0/G1 phase is very mild when compared to the common medication colchicines.

Scratch wound healing experiments were used to investigate the effect of HFCS treatment on tumor cell migration. In a time-dependent manner, administration of HFCS (500 and 1000 **µM**/mL) did not significantly limit the migration of HFCS cells over the denuded area compared to control. After 0-3 days of incubation, the percentage of cellular migration in different concentrations (500 and 1000 **µM**/mL) HFCS compared to the control was 64.2% and 78.98% in SCC-9 cells, respectively.[23] These data suggest that HFCS did not successfully limit the invasive nature of oral cancer cell lines when compared to Doxorubicin (20 **µM**/mL).

As a result, decreased migration, cell-matrix adherence, and invasive potential may help to avoid metastasis. When compared to the traditional medicine

doxorubicin in wound healing experiments, HFCS did not significantly reduce the migration and invasion of oral cancer cells. HFCS inhibited the adherence of oral cancer cells in the cell adhesion assay. The cell is strongly attached to the substrate and is unable to disperse. Our findings suggested that HFCS may have caused oral carcinogenesis. HFCS has the potential to stimulate human oral cancer cell proliferation via a variety of biological mechanisms such as cell cycle regulation, apoptosis, tumorigenesis, adhesion, and invasion. In this context, we demonstrated that HFCS was capable of promoting SCC-9 cell migration via a wound-healing experiment.

To confirm the effect of HFCS, we used the colony growth assay to test its potential to affect the growth capacity of pre-formed colonies. SCC-9 cells were cultivated for 0 days, 1 day, 2 days, and 3 days to form colonies before being exposed to two concentrations of HFCS for 3 days. HFCS (500-1000 M) treatment reduced the number of colonies in a concentration-dependent way.

CONCLUSION

We detected DNA damage, apoptotic stage alterations, induced cytotoxicity, ROS measurement, induced cell motility, colony-forming capacity, and caspase 3 and 9 apoptotic gene expression. Furthermore, HFCS applied to SCC9 cells accumulated more cells in the G0/G1 phase than Colchicine, the gold standard. According to the study's findings, HFCS likely influences the SCC-9 cell line's produced oxidative stress, resulting in increased DNA damage and cell cycle arrest in the G0/G1 phase. Caspase-3 and 9 genes were shown to be moderately upregulated by HFCS. The findings also shed light on how HFCS may act as a causal or additive factor in the carcinogenesis process. So that these findings may be generalized to human intake equivalent, more research is needed to confirm its effect on predisposition to mouth cancer.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Bray GA, Nielsen SJ, Popkin BM. Consumption of high-fructose corn syrup in beverages may play a role in the epidemic of obesity. Am J Clin Nutr. 2004;79(4):537-43. doi: 10.1093/ajcn/79.4.537, PMID 15051594.
2. Vos MB, Kimmons JE, Gillespie C, Welsh J, Blanck HM. Dietary fructose consumption among US children and adults: the Third National Health and Nutrition Examination Survey. Medscape J Med. 2008;10(7):160. PMID 18769702.
3. Krause N, Wegner A. Fructose metabolism in cancer. Cells. 2020;9(12):2635. doi: 10.3390/cells9122635, PMID 33302403.
4. Pereira RM, Botezelli JD, da Cruz Rodrigues KC, Mekary RA, Cintra DE, Pauli JR, *et al*. Fructose consumption in the development of obesity and the effects of different protocols of physical exercise on the hepatic metabolism. Nutrients. 2017;9(4):405. doi: 10.3390/nu9040405, PMID 28425939, PMCID PMC5409744.
5. Bugshan A, Farooq I. Oral squamous cell carcinoma: metastasis, potentially associated malignant disorders, etiology and recent advancements in diagnosis. F1000Res. 2020;9:229. doi: 10.12688/f1000research.22941.1, PMID 32399208, PMCID PMC7194458.
6. Mansoori B, Mohammadi A, Davudian S, Shirjang S, Baradaran B. The different mechanisms of cancer drug resistance: A brief review. Adv Pharm Bull. 2017;7(3):339-48. doi: 10.15171/apb.2017.041. PMID 29071215.
7. Rycaj K, Tang DG. Cell-of-Origin of Cancer versus Cancer Stem Cells: assays and Interpretations. Cancer Res. 2015;75(19):4003-11. doi: 10.1158/0008-5472.CAN-15-0798. PMID 26292361.
8. Khazaei S, Esa NM, Ramachandran V, Hamid RA, Pandurangan AK, Etemad A, *et al*. *In vitro* antiproliferative and apoptosis inducing effect of *Allium atroviolaceum* bulb extract on breast, cervical, and liver cancer Cells. Front Pharmacol. 2017;8:5. doi: 10.3389/fphar.2017.00005, PMID 28197098.
9. Elmore S. Apoptosis: a review of programmed cell death. Toxicol Pathol. 2007;35(4):495-516. doi: 10.1080/01926230701320337, PMID 17562483.
10. Tait SW, Green DR. Mitochondrial regulation of cell death. Cold Spring Harb Perspect Biol. 2013;5(9):a008706. doi: 10.1101/cshperspect.a008706, PMID 24003207.
11. Adams DS, Masi A, Levin M. H+ pump-dependent changes in membrane voltage are an early mechanism necessary and sufficient to induce Xenopus tail regeneration. Development. 2007;134(7):1323-35. doi: 10.1242/dev.02812. PMID 17329365.
12. White JS. Straight talk about high-fructose corn syrup: what it is and what it ain't. Am J Clin Nutr. 2008;88(6):1716S-21S. doi: 10.3945/ajcn.2008.25825B, PMID 19064536.
13. Bray GA, Nielsen SJ, Popkin BM. Consumption of high-fructose corn syrup in beverages may play a role in the epidemic of obesity. Am J Clin Nutr. 2004;79(4):537-43. doi: 10.1093/ajcn/79.4.537. Erratum in: Am J Clin Nutr. 2004;80(4):1090. PMID 15051594.
14. Meyers AM, Mourra D, Beeler JA. High fructose corn syrup induces metabolic dysregulation and altered dopamine signaling in the absence of obesity. PLOS ONE. 2017;12(12):e0190206. doi: 10.1371/journal.pone.0190206, PMID 29287121.
15. Goncalves MD, Lu C, Tutnauer J, Hartman TE, Hwang SK, Murphy CJ, *et al*. High-fructose corn syrup enhances intestinal tumor growth in mice. Science. 2019;363(6433):1345-9. doi: 10.1126/science.aat8515, PMID 30898933.
16. Keshava R, Muniyappa N, Gope R, Ramaswamaiah AS. Anti-cancer effects of *Imperata cylindrica* Leaf extract on human oral squamous carcinoma cell line SCC-9 *in vitro*. Asian Pac J Cancer Prev. 2016;17(4):1891-8. doi: 10.7314/apjcp.2016.17.4.1891, PMID 27221872.
17. Chávez-Rodríguez L, Escobedo-Calvario A, Salas-Silva S, Miranda-Labra RU, Bucio L, Souza V, *et al*. Fructose consumption and hepatocellular carcinoma promotion. Livers. 2021;1(4):250-62. doi: 10.3390/livers1040020.
18. O'Byrne KJ, Dalgleish AG. Chronic immune activation and inflammation as the cause of malignancy. Br J Cancer. 2001;85(4):473-83. doi: 10.1054/bjoc.2001.1943, PMID 11506482, PMCID PMC2364095.
19. Sigala DM, Hieronimus B, Medici V, Lee V, Nunez MV, Bremer AA, *et al*. Consuming sucrose- or HFCS-sweetened beverages increases hepatic lipid and decreases insulin sensitivity in adults. J Clin Endocrinol Metab. 2021;106(11):3248-64. doi: 10.1210/clinem/dgab508, PMID 34265055.

20. Nakagawa T, Lanaspa MA, Millan IS, Fini M, Rivard CJ, Sanchez-Lozada LG, *et al*. Fructose contributes to the Warburg effect for cancer growth. Cancer Metab. 2020;8:16. doi: 10.1186/s40170-020-00222-9, PMID 32670573, PMCID PMC7350662.
21. Ghasemi M, Turnbull T, Sebastian S, Kempson I. The MTT assay: utility, limitations, pitfalls, and interpretation in bulk and single-cell analysis. Int J Mol Sci. 2021;22(23):12827. doi: 10.3390/ijms222312827, PMID 34884632, PMCID PMC8657538.
22. Zorov DB, Juhaszova M, Sollott SJ. Mitochondrial Reactive Oxygen Species (ROS) and ROS-induced ROS release. Physiol Rev. 2014;94(3):909-50. doi: 10.1152/physrev.00026.2013, PMID 24987008, PMCID PMC4101632.
23. Pandey S, Wang E. Wang E. Cells en route to apoptosis are characterized by the upregulation of c-fos, c-myc, c-Jun, cdc2, and RB phosphorylation, resembling events of early cell-cycle traverse. J Cell Biochem. 1995;58(2):135-50. doi: 10.1002/jcb.240580203, PMID 7673322.

Cite this article: Kavitha K, Sindhu G, Porkodi M, Asha KRT, Veeravarmal V, Vijayalakshmi A. The Effect of High Fructose Corn Syrup on SCC-9 Cells Regulated by G0/G1 Cell Cycle Arrest via Apoptosis Induction. Asian J Biol Life Sci. 2023;12(2):386-94.