The Association of Sugar and Sugar Substitutes to Breast, Lung, and Oral Cancer Cell Lines: A Scoping Review

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ABSTRACT

Cancer which synonymously known as neoplasia is a genetic disorder of cell growth that is triggered by acquired or less commonly inherited mutations affecting a single cell and its clonal progeny. The aims of this scoping review was to investigate the role of sugar and sugar substitutes in breast, lung, and oral cancers with a hypothesis that sugar promoted carcinogenesis. Three databases (EBSCO, PubMed, and Scopus) were searched from January 2010 to December 2021 to identify the preclinical studies eligible for this scoping review. The review was performed according to the Preferred Reporting Items for Systematic reviews and Meta-Analyses for Scoping Reviews (PRISMA-ScR) guidelines. A total of 361 articles were reviewed and the qualitative synthesis used 12 of these articles. Based on the qualitative synthesis, four studies reported dietary sugar (glucose- and/or sucrose) induced cancer progression, one study revealed sugar substitute (aspartame) induced cancer proliferation, seven studies reported that sugar substitutes inhibit cancer proliferation, and one study reported that sucrose promotes cancer while xylitol inhibits cancer. In addition, it was reported that D-allose and cisplatin have a synergistic effect in treating cancer. In conclusion, simple sugar intake is associated with an increased risk of carcinogenesis. In contrast, sugar substitutes inhibit cancer cell line progression, subsequently acting as a potential cancer therapy, thus supporting the study’s hypothesis.

Keywords
Cancer risk factors, sugar, sugar substitutes, carcinogenesis

INTRODUCTION

Worldwide statistical cancer trends are rapidly growing. Cancer is synonymously known as tumour or neoplasia. It is defined as a "genetic disorder of cell growth that is triggered by acquired or less commonly inherited mutations affecting a single cell and its clonal progeny". As reported by GLOBOCAN, breast cancer is ranked as the 1st most common cancer worldwide, followed by lung cancer. Meanwhile, oral cancer is ranked the 11th most common cancer worldwide. Most of the oral cancer cases have been reported from Asian countries. The majority of OSCC cases are identified at advanced stages. Cancers are a broad group of complicated diseases that potentially metastasise. The aetiology can either be inherited or acquired or both. Acquired aetiology, such as diet, is a modifiable factor seen as a worthy target to be focused on to maximise cancer survival rate. Indeed, the worldwide cancer trend is expected to increase due to adopting an unhealthy lifestyle.

The association between sugar and tumorigenesis remains unclear. Fundamentally, cells require energy to maintain their integrity and growth. This energy is generated within mitochondria in the presence of substrate such as glucose through a biochemical process which eventually produces adenosine triphosphate (ATP). Glucose is the simplest form of carbohydrates, a type of sugar that the body uses to produce energy. The dietary sugar consumed daily is a
source of cell-ATP supply. However, natural sugar substitutes (SS) such as xylitol, erythritol, tagatose, and trehalose, and synthetic SS such as saccharin, aspartame, sucralose, and acesulfame potassium are the non-ATP source.

Cancerous cells require a massive supply of ATP for biomolecule production. Angiogenesis increases the delivery of energy fuels to the cells. Therefore, high dietary sugar may spike the proliferation of cancerous cells. It is also suggested that carcinogenesis may be promoted by a high sugar diet, which stimulates the synthesis of insulin and insulin-like growth factor-I (IGF-I), inducing oxidative stress and promoting weight gain. Other than the obesity and adiposity pathways, mechanisms underlying a link between sugary drinks and cancer might involve insulin resistance caused by their high glycaemic index, which has been related to breast cancer, biliary tract cancer, hepatocellular cancer, and diabetes-related carcinomas. In addition, various pathway has also been linked, which associate sugar and cancer, such as provoking inflammation cascade.

Sugar-sweetened beverages (SSBs) are the most consumed caloric beverages and the driving source of added sugars. It potentially increases the risk of obesity, which is perceived as a potent cancer risk factor. Studies have shown that sugar promotes cancer progression rather than cancer development. Aspartame, a synthetic SS found in fruit juices, might play a role in cancer development. Whilst in other articles showed contradictory results. Sugar consumption is rising worldwide, yet its definite association with cancer is mainly unknown. A proper study is required to investigate the potential of sugar or SS to promote carcinogenesis. Evidence of the association between sugar and cancer risk in preclinical models has been acknowledged as a reliable tool to provide a clear answer with solid reasoning and justification. Hence, a scoping review that properly summarised and synthesised all related evidence on sugars and their effect on cancer is needed.

This scoping review highlighted three types of cancer: breast, lung and oral cancer. The justification for selecting these cancers is to map, identify knowledge gaps and clearly describe the differences in indications between the top rank epidemiological cancer (breast and lung) research trend with less common cancer (oral cancer) on the impact of sugar and sugar substitutes on carcinogenesis potential. This study focuses on the cancer parameters assessing the DNA damage, cancer cell viability, and proliferation.

**MATERIALS AND METHODS**

The scoping review was conducted according to Preferred Reporting Items for Systematic reviews and Meta-Analyses for Scoping Reviews (PRISMA-ScR) guidelines (Figure 1). It aims to improve the quality of scoping review protocols, similar to the impact achieved by other reporting guidelines.

**Formulation of Review Question**

The question of this scoping review was formulated according to the PICO formulation. PICO stands for the population of the studies (P), intervention or exposure (I), comparison of intervention or exposure (C), and outcome of the interest (O).

For the present study, PICO is formulated as follows where P: breast, lung, and oral cancer population, I: sugar and sugar substitute, C: the activity of the sugar and sugar substitute on the normal cell and cancer cell, and O: the effects of sugar and sugar replacement on the cancer cell. This formulation has been suggested in the Cochrane Handbook for Systematic Reviews for Interventions as a potential model for developing review questions and search terms. Therefore, the formulated question for this review is "What are the effects of sugar and sugar substitutes against breast, lung, and oral cancer?"

**Search strategy**

Scopus, PubMed, and EBSCO were used throughout this scoping review. The articles were restricted to the English language. The search terms were set as ["in vitro" OR "tissue" OR "cells" OR "ex vivo" OR "animal"] AND
Identification of studies via databases and registers

Records identified from*:
- Databases (n = 361)
- Scopus (n = 16)
- PubMed (n = 189)
- EBSCO (n = 156)

Records removed before the screening:
- Duplicate records removed (n = 4)
- Records removed for other reasons (review articles, n = 2)

Records screened by title (n = 355)

Records excluded** (n = 328)

Reports sought for retrieval (n = 27)

Reports not retrieved (n = 0)

Reports assessed for eligibility (n = 27)

Full-text articles excluded with reasons (n = 15):
- Article does not focus on breast, lung, or oral cancer.
- Study did not discuss the association of sugar or sugar substitute on breast, lung or oral cancer.
- No significant laboratory parameter selected (DNA damage, cancer cell viability, and proliferation) mentioned in the articles.

Studies included in review (n = 12)

Figure 1: Flowchart of the data selection process in accordance with PRISMA-ScR guidelines.

Eligibility Criteria

The inclusion criteria of this scoping review are (i) publication type is an original article with the criteria of implementing experimental research on the association of the sugar and sugar substitute with breast, lung, and oral cancer, and (ii) the article was published between January 2010 up to December 2021 and excluded the studies dated before and after, (iii) article was published in the English language, (iv) the studies is worldwide (no restriction to any region or country) and (v) the participants included in the published article are preclinical studies both in-vitro and in-vivo. Studies that did not

("sugar" OR "artificial sugar" OR "artificial sweeteners") AND ("cancer" OR "breast cancer" OR "HER2" OR "MCF7" OR "MDA-MB-231" OR "lung cancer" OR "lung carcinoma" OR "oral cancer" OR "OSCC") AND ("DNA" OR "Comet Assay" OR "cell proliferation" OR "Ki-67" OR "MTT" OR "cell viability"). The identical search strategy was applied in all databases. The search aimed to identify all randomised controlled trial studies design, which later, the effects of sugar and sugar substitutes on breast, lung, and oral carcinogenesis were summarised.
focus on PICO formulation were excluded from this review. Grey studies were also excluded. Case reports, letters, conference abstracts, and review papers were also excluded.

Data extraction

The three screening stages (title, abstract and full text) were done independently by four authors (NA, NF, MZ, and HS). Any disagreements were resolved by discussion among authors. The characteristics of the included studies in this review (author, year, country of study, type of study, samples, and laboratory methods), described the association of sugar to breast, lung, and oral cancer progression were analysed by (MZ, HS, WNH, and NIS) and summarised in Table I.

RESULTS

Studies included

Initially, 361 records (Scopus: 16, PubMed: 189, EBSCO: 156) were found from the database search. The studies were then checked for duplicates and article types, resulting in 355 potentially eligible studies for inclusion. The study titles and abstracts were screened to select relevant studies for inclusion in the review, leaving only 27 studies qualified for full-text screening. Only 12 studies were eligible to be included in the review based on the inclusion criteria. Kappa score among the authors showed a high level of agreement (K>0.90).

Study characteristics

All studies included in this scoping review discussed the association of sugar with cancer progression. Five studies were reported dietary sugars, namely glucose- and sucrose-induced cancer progression. Whilst five studies revealed that sugar substitutes inhibit cancer proliferation, subsequently acting as potential cancer therapy. However, a survey conducted by Alleva et al. 2011 showed a sugar substitute, aspartame-induced angiogenesis, indirectly promotes cancer proliferation. Another study reported the significant suppression proliferation of oral cancer upon xylitol or sucrose plus xylitol supplementation. The synergistic effect of D-allose/cisplatin on cancer was also mentioned in a study. The analyses of the articles were summarised in Table I.

The Association of Sugar with Cancer Progression and Proliferation

The association of sugar with cancer progression and proliferation depends on the types of sugar tested. In this scoping review, the selected paper eligible for analysis revealed glucose and/or sucrose induce cancer cell proliferation. In contrast, two other studies revealed sucrose in combination with artificial sugar demonstrated anti-tumour effects against cancer progression and proliferation. Regarding artificial sugar, the result showed cancer inhibition, except for aspartame. To evaluate the anti-tumour properties, apoptosis and cell proliferation assays were utilised. The laboratory output results showed good evidence, either quantitatively or qualitatively. This is a reliable experimental tool for observing apoptotic cell development and evaluating cell proliferation suppression.

An in-vitro study reported the partial substitution of glucose by xylitol, a type of sugar alcohol, notably suppressed proliferation and ATP generation of oral cancer cells. Suggested that xylitol is likely to play a role in suppressing oral cancer development.

Another study pointed out that particular sugar may elicit an anti-tumour effect in combination with existing anticancer drugs. This study revealed that D-allose, a rare monosaccharide inhibited non-small cell lung cancer (NSCLC) cell proliferation in-vitro and tumour growth in-vivo. Together with cisplatin, a widely available chemotherapeutic drug for lung cancer, D-allose synergistically possessed growth inhibitory potential in NSCLC, suggesting that the sugar could be a new supplement to treat lung cancer. D-allose showed a more significant anti-proliferative effect on squamous cell carcinoma than adenocarcinoma.

A previous investigation demonstrated that polysaccharides from the marine alga Gracilaria lemaneiformis (PGL) inhibited cell proliferation, altered cell
<table>
<thead>
<tr>
<th>Ref. no.</th>
<th>First Author, year</th>
<th>Title</th>
<th>Type of Study</th>
<th>Cell lines and sugar tested</th>
<th>Laboratorial Method</th>
<th>Impact on cancer cell line</th>
</tr>
</thead>
</table>
| (38) | Icsei et al. 2020 | Trans-Pt/Pt (2I) Saccharinates with a phosphine ligand: Synthesis, cytotoxicity and structure-activity relationship | In-vitro experimental study | • Cancer cell lines: Breast (MCF-7), and lung (A549) human cancer cell lines.  
• Normal cell line: BEAS-2B human bronchial epithelial cell line.  
• Tested sugar compound: Artificial sweetener (saccharin) complexes  
• Control tested compound: Cisplatin | 1. ATP method – cell viability assay  
2. DNA binding  
3. DNA groove binders  
4. Molecular docking  
5. Enzyme inhibition study  
6. Cytotoxicity study | Inhibit the proliferation of cancer cell |
| (44) | Alleva et al. 2011 | In vitro effect of aspartame in angiogenesis induction | In-vitro experimental study | • HUVEC, human umbilical vein endothelial cells  
• IMR-90, human cell line  
• Sugar tested: Artificial sweetener (aspartame) | 1. In vitro angiogenesis assay (AngioKit model)  
2. Cell viability assay (MTT)  
3. Inflammatory mediator and growth factor determination  
4. Intracellular ROS assay by flow cytometry (FACS)  
5. Western blot analysis | Induce the proliferation of cancer cell |
• Animals and xenotransplantation: EBC1 cells were inoculated into BALB/c-nu mice.  
• Sugar tested: Sugar (monosaccharide) D-allose  
• Drug used: Cisplatin | 1. MTT assay  
2. Comet assay  
3. Flow cytometry | Inhibit the proliferation of cancer cell |
| (33) | Tchounwou PK, Yedjou CG, Fantah I, Tchounwou PB. 2014 | D-Glucose-Induced Cytotoxic, Genotoxic, and Apoptotic Effects on Human Breast Adenocarcinoma (MCF-7) Cells | In-vitro experimental study | • Cell culture: Human breast adenocarcinoma (MCF-7) cells  
• Sugar tested: D-glucose | 1. Cytotoxic assay (MTT) assay  
2. Annexin V-FITC, apoptosis assay  
3. Flow cytometry - Cell cycle analysis | Induce the proliferation of cancer cell |
| (40) | Akter et al. 2015 | A New Cytotoxic Steroidal Glycoside from Gracilarioopsis lemaneiformis. | In-vitro experimental study | • Normal cell line: NIH 3T3 fibroblast cell and VERO epithelial cell  
• Cancer cell line: MCF-7 and MDA-MB231  
• Sugar tested: Artificial sweetener (sugar moiety of analogous Steroidal Glycoside)  
• Drug used: Cisplatin | 1. CCK-8 assays - Cell viability analysis  
2. Annexin-FITC/PI Apoptosis analysis – Flow cytometry  
3. Fas/Fasl. Expression Analysis – Quantitative Real-Time PCR (RT-qPCR) & NANO DROP 2000 spectrophotometer  
4. Western Blot Analysis  
5. siRNA and transfection  
6. Cell Invasion and Migration Analysis | Inhibit the proliferation of cancer cell |
| (43) | Kang et al. 2017 | Characterisation and Potential Antitumor Activity of Polyacetylated Glucoside from Gracilaria lemaneiformis. | In-vitro experimental study | • Human non-small cell lung cancer (NSCLC) cell line A549  
• Sugar tested: galactose | 1. CCK-8 assays - Cell viability analysis  
2. Annexin-FITC/PI Apoptosis analysis – Flow cytometry  
3. Cytotoxic assay (MTT) assay  
4. Flow cytometry - Cell cycle analysis | Inhibit the proliferation of cancer cell |
| (34) | Rao et al. 2015 | O-GlcNAcylation of G6PD promotes the pentose phosphate pathway and tumor growth | In-vitro and in-vivo experimental study | • Normal cell lines: 293T  
• Cancer cell lines: A549 (lung cancer), MCF7 (breast cancer), H661 (lung cancer),  
• Immuno-compromised mice (BALB/c-nude, male, 5 – 6-week-old, Charles River Laboratories)  
• Sugar tested: N-acetylglucosamine | 1. Bicinchoninic Acid protein assay  
2. Modified coupled enzyme assay  
3. NADPH/NADP Quantitation Kit  
4. Glutathione Assay Fluorometric Kit  
5. Lactate dehydrogenase (LDH)-based toxicity assay kit  
6. Cell proliferation assays | Induce the proliferation of cancer cell |
| (35) | Trachootham et al. 2017 | Partial Substitution of Glucose with Xylitol Suppressed the Glycolysis and Selectively Inhibited the Proliferation of Oral Cancer Cells | In-vitro experimental study | Normal cell lines: 293T  
• Cancer cell lines: A549 (lung cancer), MCF7 (breast cancer), H661 (lung cancer),  
• Immuno-compromised mice (BALB/c-nude, male, 5 – 6-week-old, Charles River Laboratories)  
• Sugar tested: N-acetylglucosamine | 1. Bicinchoninic Acid protein assay  
2. Modified coupled enzyme assay  
3. Glucose Uptake Fluorometric Assay Kit  
4. NADP+ / NADPH Quantitation Kit  
5. Glutathione Assay Fluorometric Kit  
6. Lactate dehydrogenase (LDH)-based toxicity assay kit  
7. Cell proliferation assays | Induce the proliferation of cancer cell |

**Table I:** Summary of studies assessing the Association of Sugar and Sugar Substitutes to Solid Tumours
maturity sensor OGT regulates Hipk stability and tumorigenic-like activities in Drosophila. Therefore, these macromolecules are the building blocks that form the physical function. By contrast, cancer cells upregulate the fuel usage to make more of the information (RNA and DNA) is critical for cell growth and proliferation. Cells use sugar as their fuel.

Figure 2: The sugar fuels in cancer biology. Cells, either normal or cancerous, can utilise sugar as their fuel. Sugar is taken up by cells, converted and processed to make molecules essential for providing energy (ATP and also for generating molecules that contribute to the essential building blocks of macromolecules (DNA, RNA, and proteins) and form lipid bilayers for the configurations of membranes around cells which is vital for cell life. Therefore, these macromolecules are the building blocks that form the physical components of a cell. A cell that proliferates needs a lot of these macromolecules. Genomic information (RNA and DNA) is critical for cell growth and proliferation. Cells use sugar as fuel to generate energy and maintain homeostasis, and that’s important to their specialised function. By contrast, cancer cells upregulate the fuel usage to make more of the macromolecules needed for the building blocks to make the cancer cell’s mass and physical form. Ultimately it contributes to the growth and proliferation of the cancerous cell.

**DISCUSSION**

This scoping review provides evidence on the association of sugar with cancer progression and proliferation. The general theoretical hypothesis pathway of sugar promotes carcinogenesis is drawn in the diagram below.

**Sugar promotes cell proliferation**

Some studies revealed natural sugar substitutes demonstrated anti-tumour effects against cancer progression and proliferation. On the other hand, several studies showed a possible association between prolonged intake of heavy synthetic artificial sugar and cancer. The link between artificial sweeteners and cancer is still debated. A recent meta-analysis and case-control study discovered that artificial sweetener usage was not linked to...
an increased risk of cancer when all types of cancers were analysed intensively except for urinary system cancer in women.\textsuperscript{43}

In cancer cells, glucose metabolism shifts from using pyruvate to feed oxidative phosphorylation toward the use of lactate in aerobic glycolysis (the Warburg effect).\textsuperscript{44} In the transition to Warburg metabolism, the lactate dehydrogenase enzyme is crucial. Cancer cells' growth is thought to be enhanced by diverting glucose to generate building blocks for increased biomass in the form of amino acids at the cost of ATP production efficiency through the tricarboxylic acid (TCA) cycle. Thus, the Warburg effect metabolism acts as a co-factor for the epidermal growth factor receptor (EGFR). A Drosophila model requires this growth factor for epithelial neoplasia and metastasis. This has been verified that lactate dehydrogenase (LDH) fuelled up neoplasia, and increased glucose consumption promotes neoplasia in the study.\textsuperscript{37}

Another finding is an indirect link between sugar metabolism and cancer cell development. Aspartame (APM), a sugar substitute, stimulated blood vessel formation (angiogenic agent) at low supplementation doses. It induces regenerative cytokine production, leading to the activation of MAPKs and subsequently forming new blood vessels. The formation of new blood vessels from pre-existing capillaries (angiogenesis) is required for tumour development. Angiogenic signalling pathways are crucial in various diseases, including cancers, cardiovascular diseases, etc. The toxic effects of APM have been primarily based on its ability to cause cell transformation over the years. If a malignant cell has developed itself, it needs factors to help it evolve, supported by angiogenesis. On top of that, APM increases the levels of inflammatory mediator IL-6, VEGF, and their soluble receptors released from endothelial cells into the medium. This sugar substitute can induce VEGF-pathway activation by erk1/2 and p38 phosphorylation.\textsuperscript{44}

Additionally, a study has shown that glucose consumption and cell proliferation indirectly relate to protein O-GlcNAcylation of glucose-6-phosphate dehydrogenase (G6PD and pentose phosphate pathway (PPP)). An in vitro enzymatic assay done by a previous study showed an increase of OGT (an enzyme responsible for adding O-GlcNAcylatation onto proteins) activity in cell lysate under hypoxia.\textsuperscript{34} Hypoxic therapy improved glucose uptake rate significantly, which was associated with an increase in O-GlcNAcylation level. As a result, O-GlcNAcylation of G6PD appears to accelerate cellular biosynthesis and induce cell proliferation.\textsuperscript{34}

**The Association of Sugar with Cancer Induced-DNA Damage**

The association of sugar with cancer-induced-DNA damage was investigated by Icsel et al., 2020.\textsuperscript{38} In this experimental study, breast (MCF-7), colon (HCT116), and lung (A549) human cancer cell lines were used to evaluate the cytotoxicity of New trans-[Pd(sac)2(PPhMe2)(DMSO)]·H2O (Pd) and trans-[Pt(sac)2(PPhMe2)2]·H2O (Pt) complexes (sac=saccharinate and PPhMe2= dimethyl phenyl phosphine) in-vitro. ATP viability assay was used, and it showed that Pd was biologically inert, whilst Pt showed significant anticancer potency on MCF-7 cancer cells, similar to cisplatin. Based on the results, Pt appeared to target DNA, while Pd had a higher affinity for human serum albumin (HSA). Pt activity mechanism studies revealed that apoptotic cell death was caused by a significant increase in intracellular ROS (reactive oxygen species) levels, mitochondrial damage, and the formation of DNA double-strand breaks.

To assess the complexes' DNA binding in-vitro, plasmid pBR322 DNA was incubated. The DNA cleavage ability and the amount of DNA forms were determined. Pd complex showed no DNA cleavage at low concentrations, but single-strand breaks (SSBs) on the plasmid DNA were observed at high concentrations. This tested configuration of the substitute sugar complex induces tumour single-strand DNA (ssDNA) damage. On the other hand, the Pt complex is more potent than the Pd complex since Pd at high concentration induces ssDNA while the Pt complex demonstrates a concentration-dependent DNA cleavage. This was confirmed when they performed a nuclease activity assessment; the nuclease activity of the Pt complex is much higher than the Pd complex.
upon exposure of plasmid pBR322 DNA to the DNA minor groove binders; DAPI (4',6-diamidino-2-phenylindole) and major groove binders; MG (methyl green). The results suggest that Pd and Pt complexes selectively bind to DNA's major groove.

The molecular docking method was used to analyse the trans-configured Pd and Pt complexes' DNA binding affinity. The docking data gained from the study showed the formation of strong NH···O hydrogen bonds between both complexes with the adenine moiety of DNA, as well as the construction of electrostatic interactions between the sac's phenyl ring and the oxygen of DNA's phosphate backbone. The binding energies of both Pd and Pt docked with DNA are higher than of cisplatin as the complexes bind to DNA non-covalently while cisplatin binds to DNA non-covalently.45

The findings from the study proposed that on several cancer lines, both trans-configured Pd and Pt complexes demonstrate different cytotoxic activity, with Pt being higher cytotoxicity than Pd, which is biologically inert.

CONCLUSION
Our scoping review revealed that dietary sugar could proliferate cancer cells model in vitro through the production of ATP. Our findings also highlighted that sugar substitutes might potentially be an anti-tumour with cancer type-dependent, thus supporting the study's hypothesis.

LIMITATIONS OF THE STUDY
This study highlighted the scope within the in-vitro study model and covered three types of cancer: breast, lung, and oral. Therefore, further studies should be conducted on animals before a clinical trial involving humans to resemble the exact human pathophysiology of cancer.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

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