DERLEME / Review

Review: In Vitro Study Revealed Sugar As Anticancer Constituent

İn vitro Çalışmalar Şekeri Bir Antikanser Bileşeni Olarak Gösteriyor; Literatür Derleme

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Lokman M, Ramli N, Ismail IS, Shahbuddin M, Yusof A, Ramli R. Review: In Vitro Study Revealed Sugar as Anticancer Constituent. J Biotechnol and Strategic Health Res. 2017;2:39-44.

Abstract

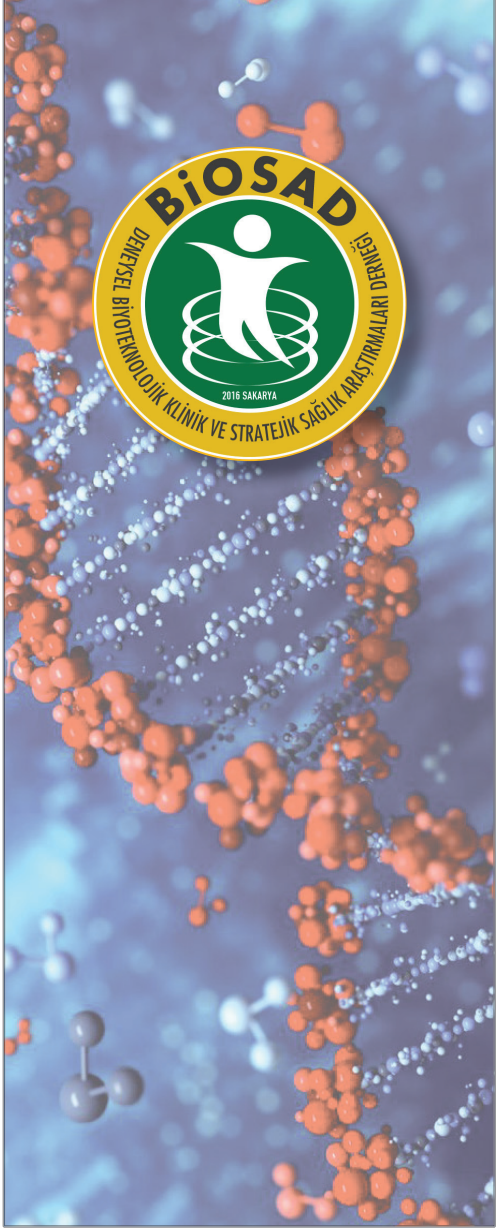
The majority of deaths among cancer patients are attributed to the metastatic spread of cancer cells to vital organs instead of primary tumor outgrowth. Plus, cancer therapeutics which focusing on conventional chemotherapy is often toxic not only to tumor cells but also to normal cells and caused their therapeutic use is limited. In this review, aberrant glycosylation is proposed to be a method of cancer diagnosis in order to identify cancer at its earliest and more treatable stage. Aberrant glycosylation is a universal feature of cancer cells. Glycosylation, the attachment of sugar moieties to protein, is a major form of post-translational modification (PTM) that is observed in approximately half of all proteins. Certain glycan structures are well known markers for tumor progression and its availability and composition in the microenvironment may affect glycosylation of the cell. In order to understand the biological application of simple sugars as anticancer agents, it is vital to fundamentally investigate the relationship between simple sugars and cell proliferation using living cell lines in vitro. Various simple sugars have been studied previously in a number of cancer cell lines representing different evolutionary stages of cancer. It has been examined that there is a selective effect of individual simple sugars on the pattern of growth and metabolism of certain cell lines in tissue culture. The results from previous studies reported that certain simple sugars were able to alter the growth pattern and morphology of several cancer cell lines which indicate that sugars have antiproliferative effect on cancer cells.

Key words: sugar, anticancer, in vitro

Özet

Kanser hastalarında ölümlerin çoğunluğu, kanser hücrelerinin primer tümör gelişimi yerine hayati organlara yayılımı ile ilgilidir. Ayrıca, konvansiyonel kemoterapiye odaklanan kanser tedavileri, genellikle sadece tümör hücreleri için değil aynı zamanda normal hücrelere toksiktir ve terapötik kullanımının neden olduğu sınırlıdır. Bu derlemede, anormal glikozilasyon, kanseri en erken ve tedavisi mümkün olan aşamada tanımlamak için bir kanser tanısı yöntemi olarak önerilmiştir. Aşikâr glikosilasyon, kanser hücrelerinin evrensel bir özelliğidir. Glikosilasyon, şekere yarımların proteine bağlanması, tüm proteinlerin yaklaşık yarısında gözlemlenen post translasyonel modifikasyonun (PTM) önemli bir şeklidir. Belirli glikan yapıları, tümör ilerlemesi için iyi bilinen belirteçlerdir ve mikro ortamdaki mevcut durumu ve bileşimi hücrenin glıkosilasyonunu etkileyebilir. Basit şekerlerin kanser önleyici ajanlar olarak biyolojik olarak uygulanmasını anlamak için basit şekerler ile hücre proliferasyonu arasındaki ilişkiyi in vitro canlı hücre dizileri kullanarak araştırmak hayati önem taşımaktadır. Çeşitli basit şekerler daha önce kanserin farklı evrim safhalarını temsil eden bir dizi kanser hücre dizisinde incelenmiştir. Doku kültüründe bazı hücre çizgilerinin büyüme ve metabolizma modelleri üzerinde bireysel basit şekerlerin seçici bir etkisi olduğu incelenmiştir. Önceki çalışmaların sonuçları, bazı basit şekerlerin, şekerlerin kanser hücreleri üzerinde antiproliferatif etkisi olduğuna işaret eden birkaç kanser hücre çizgisinin büyüme modelini ve morfolojisini değiştirebildiğini bildirmiştir.

Anahtar Kelimeler: şeker, antikanser, in vitro



Geliş Tarihi / Received : **04.06.2017**
Kabul Tarihi / Accepted : **22.08.2017**

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Introduction

To date, cancer patients are often diagnosed at advanced stages due to lack of distinct for early symptoms. There are patients with primary tumors that could be cured by surgical resection but for those who are in later stages, they receive chemotherapy and radiotherapy for cancer treatment. However, the majority eventually succumb to metastases[1]. The majority of death among cancer patients are attributed to the metastatic spread of cancer cells to vital organs instead of primary tumor outgrowth[2]. Plus, cancer therapeutics which focusing on conventional chemotherapy is often toxic not only to tumor cells but also to normal cells and caused their therapeutic use is limited. In regard to advance the current treatment of cancer, there is an increasing interest in developing an effective diagnostic method to inhibit proliferation and restrain the metastatic capability of cancer cells. In this review, aberrant glycosylation is proposed to be a method of cancer diagnosis in order to identify cancer at its earliest and more treatable stage. Thus, the underlying mechanisms and functions of sugars, proteins and glycoproteins in cellular biological response have to be understood. In the first stage, we want to investigate the antiproliferative effects of simple sugars on different types of living cell lines in vitro and eventually to evaluate the anticancer potential of specific sugars on cancer cells. This review will highlight the current status of the use of simple sugars that are being evaluated as potential anticancer agents.

Abberant Glycosylatıon

Aberrant glycosylation is a universal feature of cancer cells[3,4]. Glycosylation, the attachment of sugar moieties to protein, is a major form of post-translational modification (PTM) that is observed in approximately half of all proteins[5]. Certain glycan structures are well known markers for tumor progression and its availability and composition in the microenvironment may affect glycosylation of the cell. Tumor initiation and progression are associated with alterations in the structures of the glycan that is covalently attached to glycoproteins and glycolipids patterns at the cell surface[3]. Aberrant expression of glycan structures may affect ligand-receptor interactions and thus interfere with regulation of cell adhesion, migration and proliferation[6]

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Dietary sugars play a regulatory roles in enzymatic glycosylation reactions in human[7]. Sugars are the major source of metabolic energy in animal cells. Cells need carrier or transporter proteins for the introduction of sugars through their plasma membrane because of the hydrophilic nature of the cells[8]. Cancer cells are well known to display an enhanced sugar uptake and consumption. In fact, sugar transporters are deregulated in cancer cells thus they incorporate higher amount of sugar than normal cells. Previous in vitro studies have consistently reported that exogenous sugars would affect cell differentiation and cell-surface glycan structures[9,10,11]. The ability to distinguish the differences in the glycosylation of proteins between cancer and control patients emphasizes glycobiology as a promising field to introduce new potential method for cancer diagnosis[12].

Latest Research Findings of Its Potentil

In order to understand the biological application of simple sugars as anticancer agents, it is vital to fundamentally investigate the relationship between simple sugars and cell proliferation using living cell lines in vitro. Various simple sugars have been studied previously in a number of cancer cell lines representing different evolutionary stages of cancer. It has been examined that there is a selective effect of individual simple sugars on the pattern of growth and metabolism of certain cell lines in tissue culture[13,14,16,17,19]. The addition of simple sugars to a certain cell lines can caused an alteration in the morphology of the cells, a change in the pattern of cell association and a decrease in the rate of cell growth[13,14,16,17,19]. Different specific sugars did not cause similar effects on the same cell line even with its closely related sugar. For example, L-fucose is able to alter the morphological characteristics of 3T3 mouse culture but not D-fucose although they are stereoisomers[13]. From here, it is found that the effect of specific simple sugar differs distinctly on different cell lines.

Fructose on MDA-MB-468 Human Breast Cancer Adenocarcinoma Cell Lines (Monzavi-Karbassi et al, 2010)[14]

This research demonstrated that substituting fructose for glucose as the carbon source in cell culture media for MDA-MB-468 human breast tumor cell line induces phenotypic changes which are associated with tumor progression and metastasis. The cell-surface glycosylation phenotype was modified in cells grown in

glucose/fructose and fructose media where the cells displayed multiple changes in lectin binding compared to the cells grown in glucose media. These results suggested that adding fructose induced modification in carbohydrate epitopes. Alteration in glycan structures can affect proliferative, migratory, invasive and adhesive properties of tumor cells. The attachment of tumor cells to vascular endothelial cells is a main step in metastasis. Human umbilical vein endothelial cell (HUVEC) adhesion assay was done to examine the effect of carbon source and there was significantly increased binding when cells were grown at higher fructose concentration compared to glucose. The expression of sialylated structures was increased in fructose-treated cells. In this research, the Promega Cell Titer 96 Aqueous One Solution Cell Proliferation Assay was performed to study the cell growth of MDA-MB-468 cell line treated with fructose, glucose and fructose/glucose. The solution combines PES, a chemically stable electron coupling reagent, and MTS, a novel tetrazolium compound that is soluble in tissue culture medium. From the results, it showed that growth rate of fructose-fed cells was significantly inhibited compared to glucose and glucose/fructose. However, the addition of fructose promoted the migratory ability of the cells where faster wound closure was seen and it was mostly due to an increase in cell migration and not cell proliferation. This cellular motility is an ex vivo measure for cell metastatic potential[15]. After being adapted with fructose, the tumor cells lose their epithelial features and took on a fibroblast-like shape. The cells displayed a more dispersed growth pattern than cell propagated in other media. There were distinctive changes in the shape of the cells if compared to the cells cultured in either glucose or glucose/fructose where they maintained their rounded, cluster conformation. Presence of fructose in the medium also mediated tumor cell invasiveness.

D-allose on HuH-7 human adenocarcinoma cancer cell lines (Yamaguchi et al., 2008)[16]

The effect of D-allose on the proliferation of cancer cells was examined in this study as well as the underlying molecular mechanism of the action. The HuH-7 hepatocellular carcinoma cells were treated for 48 hours with various concentration of D-allose, D-psicose, D-glucose and D-fructose. Proliferation of the cells were measured by using MTT method and D-allose shown to inhibit cell growth by 40% in a dose-dependent manner. Meanwhile, other sugars did not show any growth inhibitory effect on the cells. Flow cytometric analysis was performed to examine the cell cycle of HuH-7 cells. D-allose significantly increased the percentage of cells in G1 phase and decreased the percentage in S phase. D-allose inhibited the HuH-7 cell growth by inducing the G1 cell cycle arrest but not apoptosis. D-allose was revealed to upregulated thioredoxin interacting protein (TXNIP) gene expression in the microarray analysis and from Western blot analysis, its increase at protein level was confirmed. The overexpression of TXNIP also induced G1 cell cycle arrest. p27kip1, a key regulator of G1/S cell cycle transition, showed to be increased at the protein but not the transcriptional level based on the analysis of cell cycle regulatory genes. Yamaguchi et al. (2008) suggested that D-allose may act as a novel anticancer agent via specific TXNIP induction and subsequent p27kip1 protein stabilization.

D-allose on HeLa, HepG2, HuH-7 cancer cell lines (Sui et al, 2005)[17]

D-allose was one of the rare sugars investigated by Sui et al. (2005) apart from D-psicose, D-allose, D-altrose and D-talitol. By the discovery of Izomoring[18], an effective strategy for mass production of rare sugars from inexpensive D-glucose and D-fructose has been developed to put an end to the laborious, time-consuming and inefficient chemical production of rare sugars. The research focused on the effect of rare sugars on the cell growth of HeLa (human cervical cancer), HepG2 (human hepatocarcinoma), HuH-7 (human hepatocarcinoma) and immortalized cell line HaCaT (human skin keratinocyte) cell lines in vitro. The viability of the cells was evaluated by MTT assay after 24-, 48- and 72-h treatment. The result showed that D-allose had a significant inhibitory effect on cancer cell proliferation in a dose-dependent manner plus it was proportional to the length of treatment. Another rare sugar, D-altrose also showed a definite inhibitory effect in HeLa, HepG2 and HaCaT cells. Cell cycle analysis by flow-cytometry, TUNEL assay and Western blot analysis were performed in HepG2 cells in order to understand the potential molecular mechanisms of the cell. After being treated with D-allose for 5 days, the accumulation

of cells in the G2/M phase was observed where the distribution of in G2/M phase cells was increased compared in control cultures. Morever, there were found decrement of S phase cells in D-allose-treated cells if compared with control cells. In the TUNEL assay analysis, the percentage of apoptosis of D-allose-treated HepG2 cells was increased significantly higher compared to untreated control HepG2 cells. These results proved that D-allose inhibits cell growth, and might correlate with the moderate G2/M arrest, modification of cell cycle regulatory proteins and induction of apoptosis.

D-Glucosamine on Ascites Tumor Cells (Bekesi et al, 1969)[19]

Bekesi et al (1969) reported that incubation in vitro with D-glucosamine showed an inhibitory effect on Sarcoma 37, and Sarcoma 180 ascites tumors and Ehrlich ascites carcinoma cells. The cells viability was examined by the trypan blue staining method, where only unstained cell were considered viable. The viability of the control Ehrlich ascites cells incubated in Krebs-Ringer phosphate buffer (containing glucose or pyruvic acid, but no glucosamine) was maintained. However, incubation of the three ascites tumor cells lines in the same buffer system with addition of D-glucosamine resulted in rapid loss of viability and after 5 hours less than 5% of the cell population was unstained. It was apparent that all the cell lines were equally susceptible to the treatment of glucosamine. The effect of 19 different sugars were also been studied in this research on the viability of Sarcoma 180 ascites tumor cells with addition of same concentration for each sugar. Based on the results, D-mannose was the only neutral sugar that showing a powerful cytotoxic effect and caused the loss of viability of the cells although it was presumed that exogenous D-mannose, like D-glucose and D-fructose which can be utilized by the cells[20]. It was also reported that the loss of viability of the ascites tumor cells was progressively decreased with the increasing concentration of D-mannose added in the incubation media. However, after being tested with L-mannose, the cells only showed a minor change in the viability which proved the specificity of the sugar treatment. Other than D-mannose, nanoacetylated amino sugars such as D-glucosamine, D-mannosamine and D-galactosamine also showed inhibitory effects on the tumor growth.

L-fucose and D-mannose on 3T3 mouse fibroblast and BS-C-1 monkey kidney cell line (Cox, et al. 1965)[13]

In this research study, the sugars that were used for treatment were D-glucose, D-galactose, D-mannose, L-fucose, N-acetyl-D-glucosamine, N-acetyl-D-galactosamine and N-acetyl-D-mannosamine. The cellular morphology of three types of mouse lines were examined daily for any alteration, cell detachment from the surface, growth pattern and tendency for the cultures to become confluent and overlap. L-fucose was the only sugar which appeared to alter the cells morphology and the growth pattern for all three mouse lines. The most striking effect was observed on 3T3 embryonic mouse fibroblast as the culture did not achieve confluent pattern with less dividing cells. The morphology of the cells were also altered to be more spindle-shaped. Similar but less striking effect was seen in cultures of 3T3 primary mouse embryo fibroblast. On the other hand, effect of L-fucose on the embryonic mouse line 3T6 was minimal if compared to the two types of cells stated before, where the cells exhibited little contact inhibition and formed a multilayered cell sheet in the culture. On top of that, D-mannose also appeared to inhibit the proliferation of African green monkey kidney cell line (BS-C-1), three types of human skin fibroblast and also heteroploid epithelial human cell line (HeLa Ch). The cells became less confluent and exhibit less cell overlapping. The morphology of the cells were also altered in response to D-mannose except for HeLa cell line. The cells viability after treated with sugars at 48-72 hour was calculated by the total cell protein per bottle. For 3T3 cultures and BS-C-1 green monkey kidney cell cultures, L-fucose and D-mannose were the only sugars that able to reduce the growth rate of the cells respectively. Even the mechanism of this effect was not clear, they suggested that L-fucose and D-mannose may alter the morphology of the cells by binding to specific sites on the cell surface.

Conclusion

The results from previous studies reported that certain simple sugars were able to alter the growth pattern and morphology of several cancer cell lines which indicate that sugars have antiproliferative effect on cancer cells. However, all cell lines did not respond in the same way. The mechanism which account for the

specificity of the sugar reactions are still unclear. However, it is the most intriguing aspect of the modification observed. It may seems possible that certain sugars interfere directly with the intermediary metabolism of some cell lines but not others. It is implausible since most mammalian cell lines have similar metabolic pathways and nutritional demands. An alternative yet attractive explanation is that the specificity of the effects is due to binding of the sugars with complementary sites on the cell surface leading to subsequent morphologic and metabolic changes. Many cell functions are arbitrated by the sugar moieties present on the cell surface and that addition and treatment of sugar to the culture medium specifically alters some of these functions.

Acknowledgement

The authors are grateful to the Ministry of Higher Education Malaysia for funding this research under FRGS15-253-0494 grant.

Table 1 : Overview of sugar studies on antiproliferative effect in vitro

Type of Sugars	Type of Cells	Method	Outcome	Reference
• Glucose • Glucose + fructose • Fructose	MDA-MB-468 (human breast adenocarcinoma)	Cell Titer 96 Aqueous One Solution Proliferation Assay (ELISA reader)	Addition of fructose inhibits higher rate of cellular proliferation compared to glucose and glucose/fructose.	Monzavi-Karbassi et al (2010)
• D-allose • D-psicose • D-glucose • D-fructose	HuH-7 (human hepatocarcinoma)	MTT assay Flow cytometric analysys cDNA microarray analysis RTqPCR Western blot and immunoprecipitation analysis	D-allose inhibit cell growth in a dose dependent manner but not other sugars D-allose induced G1 cell cycle arrest D-allose inhibit the cell growth by specific TXNIP induction and p27kip1 protein stabilization	Yamaguchi et al (2008)
• D-psicose • D-allose • D-altrose • D-talitol	HeLa (human cervical cancer) HuH-7 (human hepatocarcinoma) HepG2 (human hepatocarcinoma) HaCaT (human skin keratinocyte)	MTT assay Flow cytometric analysis Western Blot analysis TUNEL assay	D-allose inhibits the proliferation of cells in a dose and time dependent manner. D-altrose also showed a definite inhibitory effect on cells. D-allose increased G2/M phase distribution and also decreased S phase population in HepG2 cells. Significant increment in percentage of apoptosis of D-allose-treated HepG2.	Li Sui et al (2005)
• D-glucosamine • N-acetylglucosamine • D-mannosamine • N-acetylmannosamine • D-galactosamine • N-acetylgalactosamine • D-glucose • -methyl-D-glucoside • 2-deoxy-D-glucose • D-galactose • D-fructose • D-mannose • L-Mannose • D-fucose • L-fucose • L-rhamnose • -D-ribose • D-xylose • L-arabinose	Sarcoma 37 ascites tumor cells Sarcoma 180 ascites tumor cells Ehrlich ascites tumor cells	Trypan blue staining method	D-glucosamine and D-mannose reduce viable cell after incubation in dose-dependent manner	Bekesi et al (1969)
• D-glucose • D-galactose • L-fucose • D-fucose • D-mannose • N-acetyl-D-glucosamine • N-acetyl-D-galactosamine • N-acetyl-D-mannosamine	3T3 (Heteroploid Embryonic Mouse Fibroblasts) 3T3 (Primary Mouse Fibroblast) 3T6 (Embryonic Mouse Line) BS-C-1 (African green monkey kidney cell line) Human skin fibroblast HeLa Ch (heteroploid epithelial human cell line)	Total cell protein measurement per bottle Protein and radioactivity assay	3T3 cells grown in L-fucose had a slow growth while cells grown in medium supplemented with equal amounts of the other sugars became confluent. D-mannose inhibit the growth rate of BS-C-1, human skin fibroblast and HeLa cells.	Cox & Gesner (1967)

References

1. Yamashina, T., Ishihara, R., Nagai, K., Matsuura, N., Matsui, F., Ito, T., Fujii, M., Yamamoto, S., Hanaoka, N. & Takeuchi, Y. (2013). Long term outcome and metastatic risk after endoscopic resection of superficial esophageal squamous cell carcinoma. Am J Gastroenterol, 108, 544 551.
2. Häuselmann, I., & Borsig, L. (2014). Altered tumor-cell glycosylation promotes metastasis. Frontiers in oncology, 4, 28.
3. Hakomori, S. (2001). Tumor-associated carbohydrate antigens defining tumor malignancy: Basis for development of anti-cancer vaccines. Adv Exp Med Biol, 491, 369–402.
4. Chandrasekaran, E.V., Xue, J., Neelamegham, S., & Matta, K.L. (2006). The pattern of glycosyl- and sulfotransferase activities in cancer cell lines: A predictor of individual cancer-associated distinct carbohydrate structures for the structural identification of signature glycans. Carbohydr Res, 34, 983–994.
5. Apweiler, R., Hermjakob, H., Sharon, N. (1999). On the frequency of protein glycosylation, as deduced from analysis of the SWISS-PROT database. Biochim Biophys Acta, 1473, 4–8.
6. Fuster, M.M., & Esko, J.D. (2005). The sweet and sour of cancer: Glycans as novel therapeutic targets. Nat Rev Cancer, 5, 526–542.
7. Berger, V., Perier, S., Pachiaudi, C., Normand, S., Louisot, P., & Martin, A. (1998). Dietary specific sugars for serum protein enzymaticglycosylation in man. Metabolism, 47, 1499-1503.
8. Calvo, M. B., Figueroa, A., Pulido, E. G., Campelo, R. G., & Aparicio, L. A. (2010). Potential role of sugar transporters in cancer and their relationship with anticancer therapy. International journal of endocrinology.
9. Delhotal, B., Lemonnier, F., Couturier, M., Wolfrom, C., Gautier, M., & Lemonnier, A. (1984). Comparative use of fructose and glucose in human liver and fibroblastic cell cultures. In Vitro, 20, 699-706.
10. Wolfrom, C., Loriette, C., Polini, G., Delhotal, B., Lemonnier, F., & Gautier, M. (1983). Comparative effects of glucose and fructose on growth and morphological aspects of cultured skin fibroblasts. Exp Cell Res , 149, 535-546.
11. Wolfrom, C., Kadhom, N., Raulin, J., Raynaud, N., & Gautier, M. (1994). Fructose-induced enhanced mitogenicity of diploid human cells: possible relationship with cell differentiation. In Vitro Cell Dev Biol Anim, 30A, 263-268.
12. Tuccillo, F. M., de Laurentiis, A., Palmieri, C., Fiume, G., Bonelli, P., Borrelli, A., & Scala, G. (2014). Aberrant glycosylation as biomarker for cancer: focus on CD43. BioMed research international.
13. Cox, R. P., & Gesner, B. M. (1965). Effect of simple sugars on the morphology and growth pattern of mammalian cell cultures. Proceedings of the National Academy of Sciences of the United States of America, 54(6), 1571.
14. Monzavi-Karbassi, B., Hine, R. J., Stanley, J. S., Ramani, V. P., Carcel-Trullols, J., Whitehead, T. L. & Saha, R. (2010). Fructose as a carbon source induces an aggressive phenotype in MDA-MB-468 breast tumor cells. .International journal of oncology, 37(3), 615.
15. Albini, A., Benelli, R., Noonan, D.M., & Brigati, C. (2004). The 'chemoinvasionassay': a tool to study tumor and endothelial cell invasion of basement membranes. Int J Dev Biol 48, 563-571.
16. Yamaguchi, F., Takata, M., Kamitori, K., Nonaka, M., Dong, Y., Sui, L., & Tokuda, M. (2008). Rare sugar D-allose induces specific up-regulation of TXNIP and subsequent G1 cell cycle arrest in hepatocellular carcinoma cells by stabilization of p27kip1. International journal of oncology, 32(2), 377-386.
17. Sui, L., Dong, Y., Watanabe, Y., Yamaguchi, F., Hatano, N., Tsukamoto, I. & Tokuda, M. (2005). The inhibitory effect and possible mechanisms of D-allose on cancer cell proliferation. International journal of oncology, 27(4), 907-912.
18. Izumori, K. (2002). Bioproduction strategies for rare hexose sugars. Naturwissenschaften 89, 120-124.
19. Bekesi, J. G., Molnar, Z., & Winzler, R. J. (1969). Inhibitory effect of D-glucosamine and other sugar analogs on the viability and transplantability of ascites tumor cells. Cancer research, 29(2), 353-359.
20. Fare, G., Sammons, D. C. H., Seabourne, F. A., & Woodhouse, D. L. (1967). Lethal Action of Sugars on Ascites Tumor Cells in vitro. Nature, 214, 308-309.
21. Baba, T., Yoshida, T., Yoshida, T. & Cohen, S. (1979). Suppression of cell-mediated immune reactions by monosaccharides. J Immunol, 122, 838-841.
22. Stutman, O., Dien, P., Wisun, R.E. & Lattime, E.C. (1980). Natural cytotoxic cells against solid tumors in mice: blocking of cytotoxicity by D-mannose. Proc Natl Acad Sci USA, 77, 2895-2898.