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# Kitre Sakızının Farklı Kanser Hücreleri Üzerine Proliferatif Etkisi

Sevgi IRTEGUN KANDEMIR[1, 2*], Israt JAHAN[3], Fatma TEKE[4]

**Öne Çıkanlar:**
- Kitre sakızı
- Kanser hücresi
- Proliferasyon

**Anahtar Kelimeler:**
- Kitre sakızı
- Kanser hücresi
- Sitotoksisite
- MTT test
- Proliferasyon

**ÖZET:**

Kitre sakızı (GT) *Astragalus* cinsinin Asyatik türlerinin dallarından ve gövdelerinden boşaltılan doğal bitki eksüdasıdır. Heterojen bir polisakkarit olan GT büyük biyouyumluluk, termal kararlılık, biyolojik olarak parçalanabilirlik, hidrofiliklik ve antioksidan aktivite gibi ayırt edici fizikokimyasal ve biyolojik özellikleri nedeniyle çeşitli biyomedikal alanlarda ve geleneksel olarak etnotıpta kullanılmaktadır. Bu çalışmada, GT'nin çeşitli kanser hücre dizileri üzerinde sitotoksik etkilerinin olup olmadığını araştırmayı amaçladık. Bu amaçla insan kolorektal adenokarsinomu (CACO-2), glioblastoma multiforma tümörü (T98G), yumurtalık sarkomu (SKOV-3) ve meme kanseri (MCF-7) hücreleri gibi dört farklı kanser hücre dizisi kullanıldı. GT, solvent olarak hem %5 DMSO hem de $dH_20$ kullanılarak 200 µg/mL, 100 µg/mL, 50 µg/mL, 25 µg/mL ve 12.5 µg/mL konsantrasyonunda hazırlandı. *İn vitro* sitotoksisite çalışması için MTT (3-(4, 5-dimetiltiazol-2-il)-2,5-difeniltetrazolyum bromür) kolorimetrik deney kullanıldı. Hücre canlılığı yüzdeleri tüm uygulamalar için %80'in üzerinde bulunduğundan, GT'nin bu kanser hücreleri üzerinde sitotoksik etkisi olmadığı bulundu. Ancak, belirli konsantrasyonlarda GT'nin doza bağlı olarak dikkate değer hücre proliferasyonu etkinliği, MCF-7 dışındaki tüm kanser hücrelerinde gözlendi. Sonuç olarak, bu çalışma GT'nin kanser hücrelerinin proliferasyonunu arttırıcı etkisinden dolayı kanser hastalarının GT veya GT içeren ürünlerin kullanımı açısından dikkatli olunması gerektiğini önermektedir.

# Proliferative Effect of Gum Tragacanth on Different Cancer Cells

**Highlights:**
- Gum tragacanth (GT)
- Cancer cell
- Cell proliferation

**Keywords:**
- Gum tragacanth (GT)
- Cancer cell
- Cytotoxicity
- MTT assay
- Cell proliferation

**ABSTRACT:**

Gum tragacanth (GT) is a natural plant exudate discharged from the twigs and stems of Asiatic species of the Astragalus genus. GT is a heterogeneous polysaccharide which has been utilized in various biomedical fields and traditionally in ethnomedicine because of its distinctive physicochemical and biological properties, such as great biocompatibility, thermal stability biodegradability, hydrophilicity and antioxidant activity. The aim of this study was to examine whether GT has cytotoxic effects on various cancer cell lines. For this aim, four cancer cell lines i.e., human colorectal adenocarcinoma (CACO-2), glioblastoma multiforme tumor (T98G), ovarian sarcoma (SKOV-3), and breast cancer (MCF-7) cells were used. GT was prepared at the concentration of 200 µg/mL, 100 µg/mL, 50 µg/mL, 25 µg/mL and 12.5 µg/mL, using both 5% DMSO and $dH_2O$ as solvent. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) colorimetric assay was used for *in vitro* cytotoxicity study. GT had no cytotoxic effect on these cancer cells since cell viability percentages were found to be above 80% for all the treatments. However, remarkable dose-dependent cell proliferation efficiency of GT at certain concentrations was observed on all cancer cells except MCF-7. In conclusion, this study suggests that cancer patients should be careful about the use of GT or products containing GT due to the increasing effect of GT on the proliferation of cancer cells.

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**Ethics Committee Approval:** "Ethics Committee Approval" is not required since no survey, interview, focus group work (collecting samples from patients), observation, experiment on animals, interview techniques were taken place for this study.

## INTRODUCTION

Natural gums are characterized as a special type of polysaccharides of natural origin, which are mainly exuded by shrubs, tubers, trees, and seeds, often by macro algae (brown and red seaweeds) or other microbial sources particularly due to any kind of mechanical injuries or pathological attacks, gums as sticky secretions are released by many shrubs and trees in order to give protection to a wound (Taghavizadeh Yazdi et al., 2021). Moreover, unfavorable conditions like drought also influence the secretion of gums, which is the result of oozing of saps after disruption of cell walls (Nussinovitch, 2009). Gum tragacanth (GT) as heterogeneous polysaccharide is most abundant plant exudates, attained from the twigs and stems of the *Astragalus* genus, which widely exists in the drought-prone (Taghavizadeh Yazdi et al., 2021) and rocky terrains of south west Asia, precisely in Türkiye, Pakistan, Syria, Iran, and also in Greece (Mohammadifar et al., 2006; Nejatian et al., 2020). It is an anionic polysaccharide gum, chemically rich in carbohydrate (83.81–86.52% w/w) preciously glucose, galactose, arabinose, fucose, xylose, galacturonic acid, and traces of rhamnose. It is also comprised of protein (0.31–3.82% w/w), moisture (8.79–12.94% w/w) and minerals (total ash, 1.8–3.2% w/w); however, its composition diverges because of the diversities at species level as well as geographical and agronomical variations (Balaghi et al., 2011).

Since GT as renewable sources is biocompatible and moderately inexpensive, it has been consumed industrially as thickener, stabilizer, emulsifier, gelling, and moisture-controlling agent in waste water management, textiles, agro-chemicals, food processing and packaging, cosmetic industries, pharmaceutical, along with health care sectors for wound healing and carriers of drug delivery (Saha & Bhattacharya, 2010; Maity & Saxena, 2016; Nazarzadeh Zare et al., 2019; Nejatian et al., 2020). Powdered GT has been broadly utilized historically in ethnomedicine for treating dysentery, toothache, stomachache, cough, fatigue and diabetic wounds (Mosaddegh et al., 2012). As herbal remedy moreover it has been applied in the form of mucilage or paste or lotion for healing topical burn and curing lip fissures and tightening bone fractures (Nazarzadeh Zare et al., 2019). GT has also taken an important place in Iranian traditional medicine, intensively utilized as a general tonic, laxative and analgesic agent for anthelmintic and jaundice therapy (Verbeken et al., 2003). Additionally, GT demonstrates antibacterial and antifungal activities after proper functionalization or modification. For instance, antibacterial activities against both Gram-negative and Gram-positive bacteria were observed by functionalized-GT hydrogels i.e., GT/poly (acrylic acid) and GT/poly (acrylamide) hydrogels, which were modified by quaternary ammonium salt (QACs) (Ranjbar-Mohammadi et al., 2016). Since GT shows diverse therapeutic potentials, it is also necessary to assess whether or not GT exhibits any anticancer activity so that its utility can be extended in the pharmaceutical industry. For this aim, this study was designed to screen anticancer activity of GT on four different human cancer cell lines, where *in vitro* MTT cell-proliferation assay was used for cytotoxicity study by assessing cell metabolic activity.

## MATERIALS AND METHODS

### Equipment and Chemicals

GT as powdered form was commercially purchased from Sigma-Aldrich. Human colorectal adenocarcinoma (CACO-2), glioblastoma multiforme tumor (T98G), ovarian sarcoma (SKOV-3), and breast cancer (MCF-7) cell lines were selected for this study, which were acquired from the American Type Culture Collection (ATCC). All the experimental steps of this study were conducted in the Cell Culture Laboratory of Dicle University Scientific Research Centre.

### *In vitro* cytotoxicity of GT

For *in vitro* MTT assay, both CACO-2 and SKOV-3 cells were cultured with the Roswell Park Memorial Institute (RPMI) 1640 medium (Sigma-Aldrich) whereas T98G and MCF-7 cells were cultured with Dulbecco's Modified Eagle's Medium (DMEM) (Sigma-Aldrich). The flasks with cells in medium were incubated at optimized growth conditions i.e., 37°C, with 5% $CO_2$, 95% humidity. Using a hemocytometer, cells were counted after they had reached their 80% confluency, and later on, 90 µL suitable medium comprising 7 x $10^3$ cells of MCF-7 and 1 x $10^4$ cells of CACO-2, SKOV-3 and T98G were seeded into each well of 96-well plates and incubated overnight at optimized growth conditions (Kandemir & Ipek, 2023; Atalar et al., 2023).

GT powder was meticulously suspended in two different solvents: 5% DMSO (Dimethyl sulfoxide) and ultra-purified water ($dH_2O$). This process was employed to generate distinct concentrations of GT (200 µg/mL, 100 µg/mL, 50 µg/mL, 25 µg/mL, and 12.5 µg/mL) for the various treatments. Following the designated incubation period, 10 µL of each GT concentration was introduced into each well and incubated for an additional 24 hours. Similarly, both 5% DMSO (Dimethyl sulfoxide) and $dH_2O$ were employed as control groups for each respective cell line in the same manner. Four replicates were designed for each treatment. After finishing the incubation period with treatments, each well of the plates was added with 10 µL of MTT (5 µg/mL) reagent, which was incubated for 3 hours (Çınar & Nazıroğlu, 2023). Afterwards, the medium was aspirated gently, and 100 µL of DMSO was added per well and incubated at low-pitched shaking for 15 min using orbital shaker. Afterwards, the absorbance was measured at 540 nm wavelength using microplate reader (Multi ScanGo, Thermo) (Buranaamnuay, 2021). Absorbance values were used for calculating percentage of cell viability using the following formula:

$$\% \text{ viability} = \frac{Absorbance\ of\ treated\ cells}{Absorbance\ of\ untreated/control\ cells} \quad \%100 \qquad (1)$$

### Statistical analysis

All replicated data were used for analysis of graphical representation by means of the t-test using GraphPad Prism 7 software. $P < 0.05$ was regarded as significant differences.

## RESULTS AND DISCUSSION

### Results

Cellular metabolic activity was observed and measured through *in vitro* MTT assay for detecting cellular toxicity and/or proliferation effect of four cancer cell lines (CACO-2, T98G, SKOV-3 and MCF-7) in response to GT. Control groups were distinctly established, with DMSO and water employed as individual control groups. Cell viability percentages for all cancer cells treated with GT at determined concentrations (200, 100, 50, 25 and 12.5 µg/mL; with both DMSO and $dH_2O$) were found to be above 80%, (Fig. 1, 2, 3 and 4). The findings suggest that GT does not induce significant cytotoxic effects on these cancer cells, in accordance with ISO 10993-5, which defines cell viability percentages exceeding 80% as non-cytotoxic (López-García et al., 2014).

Surprisingly, GT exhibited remarkable dose-dependent proliferation on all cancer cells except MCF-7 cells. Overall, greater proliferation rates were observed in the GT treatments prepared with DMSO, as opposed to those prepared with water. For Instance, cell proliferation was observed in CACO-2 cells treated with GT concentrations of 12.5, 25, and 50 µg/mL with DMSO, with corresponding cell viability rates of 126%, 124%, and 106% (Figure 1a). Conversely, treatment with GT prepared in water did not produce any observable proliferative effects (Figure 1b).

GT concentrations of 100 and 200 µg/mL, in both DMSO and $dH_2O$, were found to induce proliferation in T98G cell (Figure 2). Notably, at 100 µg/mL GT, cell viability rates were higher when it was dissolved in DMSO (111%) compared to $dH_2O$ (106%) while at higher concentrations (200 µg/mL), cell viability rates were almost similar between these two solvents, with rates of 106% and 109% in DMSO and $dH_2O$, respectively (Figure 2a and 2b). Moreover, a proliferative effect with a cell viability rate of 104.6% was observed in response to a GT concentration at 50 µg/mL when it was only dissolved in DMSO (Figure 2a).

Like CACO-2, GT concentrations at 12.5, 25, and 50 µg/mL (dissolved in DMSO were found to increase proliferation in SKOV-3 cell, with corresponding cell viability rates of 129%, 108.5%, and 118.5% (Figure 3a). On the other hand, treatment prepared by $dH_2O$ showed less cell proliferation in this cell line, only at 12.5 µg/mL GT concentration with a cell viability rate of 102.5% (Figure 3b). Unlike the other cells however, GT did demonstrate neither toxicity nor proliferation effects on MCF-7 cell (Figure 4). Notably, no significant differences were detected among the control groups (DMSO and dH2O) for each cell line (Figure 5).

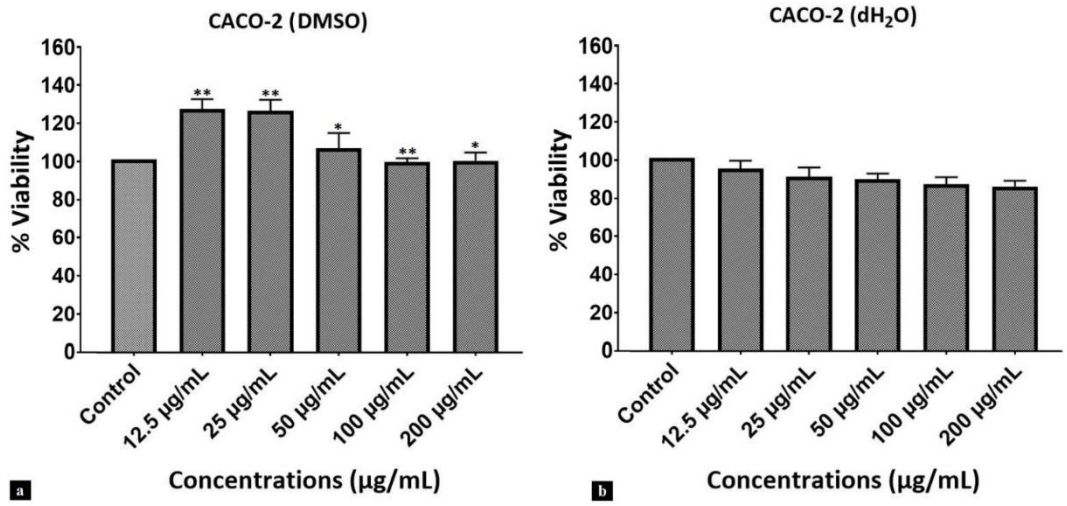


**Figure 1.** Proliferative effect of GT on human colorectal adenocarcinoma (CACO-2) cell. Cell viability studies were conducted using different concentrations of GT prepared by (a) DMSO, * $p \leq 0.016$ and ** $p \leq 0.006$ compared to their control counterparts; and (b) $dH_2O$, no significant differences compared to their control counterparts. Four replicates were performed for each treatment (n=4), mean ± standard deviation is also included for each treatment. Control cells were treated with both DMSO and $dH_2O$ in equivalent volumes as those used in the treatment groups

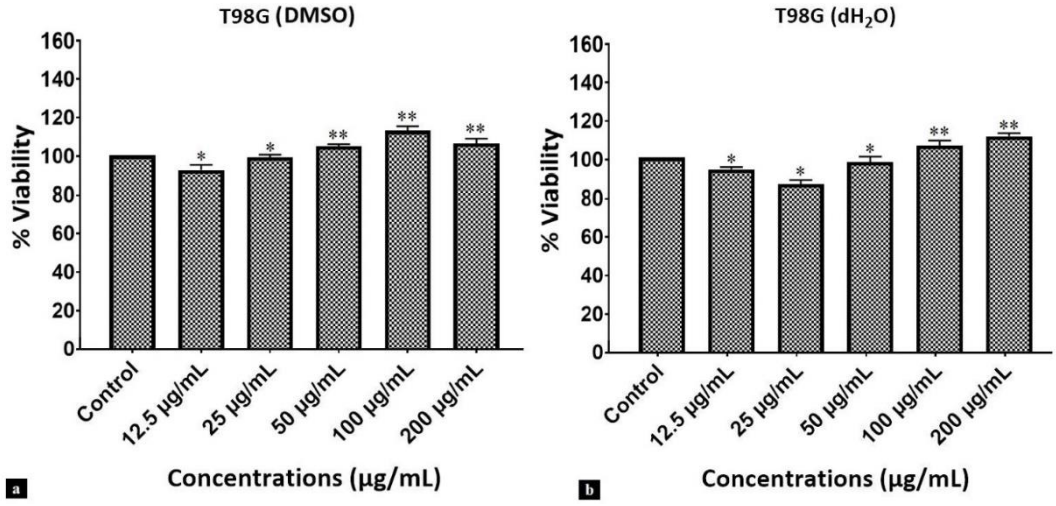


**Figure 2.** Proliferative effect of GT on human glioblastoma multiforma tumor (T98G) cell. Cell viability studies were conducted using different concentrations of GT prepared by (a) DMSO, * $p \leq 0.03$ and ** $p \leq 0.0095$ compared to their control counterparts; and (b) $dH_2O$, * $p \leq 0.0218$ and ** $p \leq 0.0025$ compared to their control counterparts. Four replicates were performed for each treatment (n=4), mean ± standard deviation is also included for each treatment. Control cells were treated with both DMSO and $dH_2O$ in equivalent volumes as those used in the treatment groups

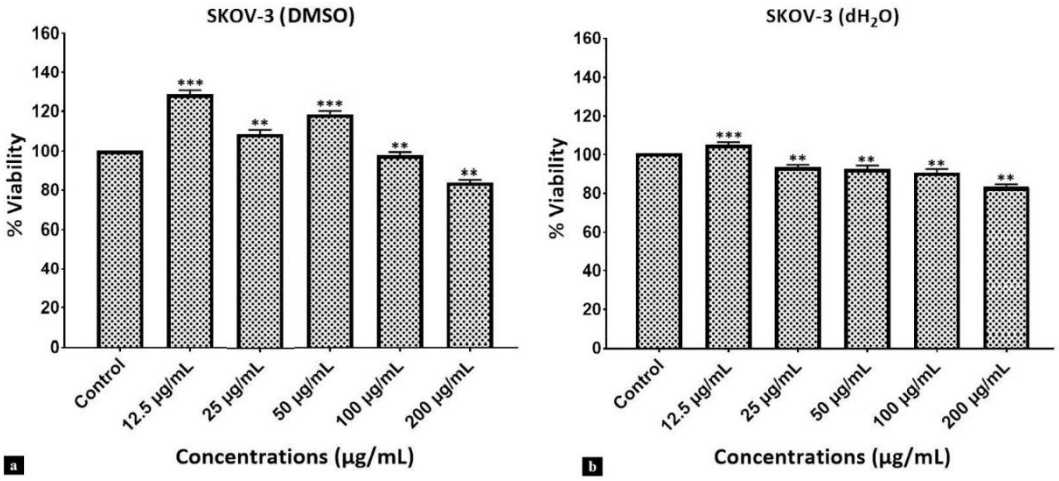


**Figure 3.** Proliferative effect of GT on human ovarian sarcoma (SKOV-3) cell. Cell viability studies were conducted using different concentrations of GT prepared by (a) DMSO, ** $p \leq 0.004$ and *** $p \leq 0.0002$ compared to their control counterparts; and (b) $dH_2O$, ** $p \leq 0.0039$ and *** $p \leq 0.0004$ compared to their control counterparts. Four replicates were performed for each treatment (n=4), mean ± standard deviation is also included for each treatment. Control cells were treated with both DMSO and $dH_2O$ in equivalent volumes as those used in the treatment groups

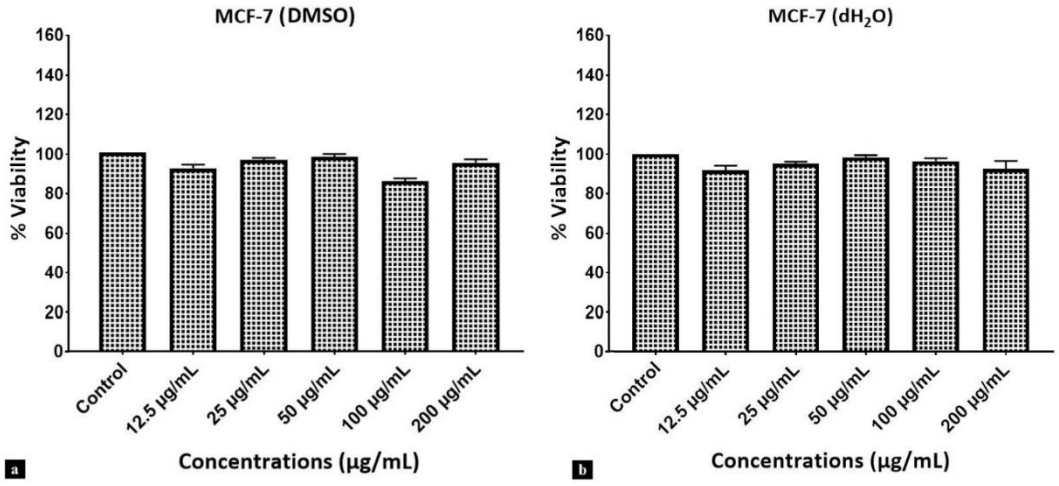


**Figure 4.** GT has no proliferative or cytotoxic effect on human breast cancer (MCF-7) cell. Different concentrations of GT were prepared using both DMSO (a) and $dH_2O$ (b) and used for proliferation studies. Four replicates were performed for each treatment (n=4), mean ± standard deviation is also included for each treatment; no significant differences were observed compared to their control counterparts. Control cells were treated with both DMSO and $dH_2O$ in equivalent volumes as those used in the treatment groups

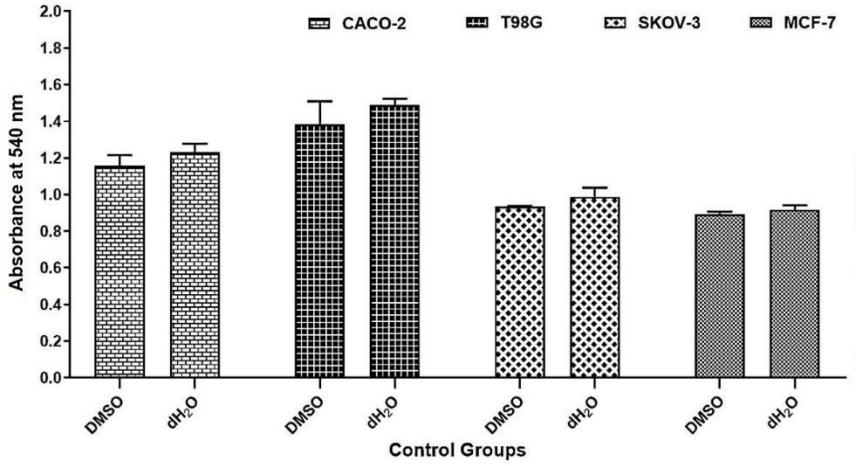


**Figure 5.** Absorbance comparison of both the control groups, DMSO and $dH_2O$. Each group was subjected to four replications (n=4), and the mean ± standard deviation is provided; no significant differences were observed among the control groups for each cell line

## Discussion

In this study, none of the cell lines showed any significant inhibitory effects against GT. Similarly, in a preceding investigation, water-soluble tragacanth (WST) prepared by de-esterification of this natural plant exudate was used for *in vitro* cytotoxicity study on cervical cancer (Hela), hepatocellular carcinoma/ primary liver cancer (HepG2), and healthy mouse fibroblast (L929) cell lines, which demonstrated the presence of non-toxic effects on these cells in spite of at relatively high concentrations (500 µg/mL WST) (Fattahi et al., 2013). GT was also supplemented to B6C3F1 mice at dietary levels to observe oral toxicity, and the result was concluded to be negligible (Hagiwara et al., 1991). In case of acute toxicity studies GT similarly exhibited its non-toxic effects on rats, hamsters, and rabbits after oral consumption (Collins et al., 1987). Furthermore, additional *in vivo* screening was conducted to evaluate the impact of dietary GT on humans. This analysis revealed that ingested GT had had minimal effects on parameters such as triglycerides and phospholipids, serum cholesterol, glucose tolerance, and other factors (Nazarzadeh Zare et al., 2019).

In the present study furthermore, it was observed that GT treatments at the concentration of 12.5, 25 and 50 µg/mL suspended by DMSO showed noticeable proliferation effects on CACO-2 and SKOV-3 cells while treatments prepared with $dH_2O$ did not have such effect on these two cell lines. T98G cell however showed proliferative effects at the concentration of 50, 100, 200 µg/mL and 100, 200 µg/mL GT in DMSO and $dH_2O$, respectively. Additionally, it is essential to emphasize that there were no noteworthy distinctions detected between the control groups (DMSO and dH2O) for each specific cell line. This finding indicates that the observed dose-dependent proliferation can be attributed to the presence of GT.

The higher proliferation rate observed in the GT treatments with DMSO as compared to water may be attributed to a significant portion of the active components in GT, such as bassorin, being insoluble in water. This property likely hampers the effective proliferative potential of GT when it is dissolved in water (Mohammadifar et al., 2006; Azarikia & Abbasi, 2016).

In terms of its proliferative effects, there have been a few studies conducted on the potential of GT to promote the cell growth. This natural gum has been found to have antioxidant properties, which may contribute to its ability to promote cell growth and repair (Taghavizadeh Yazdi et al., 2021). One study also found that GT increased the proliferation of human fibroblasts, which are cells that play a crucial role in wound healing and tissue repair (Dixit et al., 2022). Similarly, another study showed that GT enhanced the growth of liver cells in rats, which suggested that this gum could potentially be used to promote liver regeneration in humans (Kitchin & Brown, 1989). However, unappealing proliferative effects of this natural gum on tumor cells, supportive to the findings of present study, were also observed in an earlier investigations, where longer treatments (24 hours) with GT from 80 to 200 mg/L concentration was found to be capable of stimulating some specific physical changes of Landschütz ascites tumor cells collected from Balb/C mice (Galbraith et al., 1963). According to Galbraith et al. (1963), GT is associated with a significant increase in the solid concentration and dry mass of the cytoplasm of ascites tumor cells, as well as a slight change in mean cell volume (Galbraith et al., 1963). Taken together, these findings suggest that GT may play a role in the proliferation of intracellular material in tumor or cancer cells.

Unlike the other cells, GT did not exhibit any proliferation stimulus on MCF-7 cells. Regarding the proliferation responses against GT, the discrepancy among these four cancer cells might be their variation in cancer development strategies since it has already been proven that asymmetric and abnormal proliferation of cells induces cancer cells, subsequently produces more than a hundred

distinctive forms of cancer, and they can differ expressively in their behavior as well as in a response to certain treatments (Bertram, 2000).

Utilization of GT has been rising in various biomedical fields because of its distinctive physicochemical and biological properties i.e., great biocompatibility, thermal stability, excellent biodegradability (through microorganisms and enzymatic activity or physical force like ultrasonic waves), amazing hydrophilicity and antioxidant activity, low cost and natural availability (Fattahi et al., 2013). Moreover, different GT-based constructs such as nanofibers, hydrogels, nanogels as carrier were used for loading and delivering anti-cancer, anti-inflammatory, antioxidant and antibacterial agents to targeted localities via oral or other administration routes (Gupta et al., 2018; Sheorain et al., 2019; Verma et al., 2020; Sayadnia et al., 2021). However, very few screening has been piloted to evaluate the effect of GT on cancer cells following either by *in vitro* techniques or animal model, which should be the vital steps before exploiting this heterogeneous polysaccharide gum in any aspect of biomedical applications, especially in drug delivery and tissue engineering.

## CONCLUSION

Based on the present report, it can be concluded that GT does not retain any significant cytotoxicity on many cancer cell lines, rather it can induce cell proliferation on certain cancer cells as dose-dependent manner. At particular concentrations, GT demonstrated notable efficacy in promoting cell proliferation in all cancer cell lines, except for MCF-7. In future studies, conducting precise molecular level examinations, such as western blotting and real-time PCR, will be invaluable for elucidating the mechanisms underlying the proliferation effects of GT on specific cancer cells. Finally, in light of the observed *in vitro* proliferative effects on certain cancer cells, it is highly recommended to exercise caution when it comes to the consumption of GT containing products, especially for individuals who are cancer patients. This is particularly crucial considering that GT has recently been incorporated into food and pharmaceutical products.

### Conflict of Interest

The article authors declare that there is no conflict of interest between them.

### Author's Contributions

SİK, IJ, FT Concept: SİK, FT; Design: SİK, FT; Material supplying: SİK, FT, Execution: SİK, IJ; Data acquisition: IJ, Data analysis/interpretation: IJ; Literature Search: IJ; Writing: IJ; Critical revision: SİK

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