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THE UTILIZATION OF DIOSGENIN FOR MANAGING THE GENETICALLY TRANSMITTED OPTIC NERVE SHEATH MENINGIOMAS (ONSMS)

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Abstract
Diosgenin is known as a steroidal saponin which was found to have effective oncological properties. However, in the past research, insufficient number foci have been given on diosgenin's anti-proliferative effects within the context of "optic nerve sheath meningioma (ONSM) cells." ONSMs are often asymptomatic and can be transmitted genetically. Therefore, the present research focuses on the hereditarily transferred ONSM, focusing on the role of diosgenin in influencing cell migration and invasion, autophagy, cell death (apoptosis), and progression of cell cycle in this regard. For this purpose, the WTS-1 assay was utilized for evaluating HBL-52 ONSM cells viability, while western blot and electron microscopy were used for detecting autophagy. Moreover, cell invasion and migration of the associated cells were also evaluated, using transwell as well as wound healing assays. Finally, fluorescence microscopy, flow cytometry and western blot were used for determining the apoptotic cell death. The results obtained have shown that HBL-52 ONSM viability is decreased by diosgenin due to autophagy activation. However, Beclin 1 and LC3-II expressions were also upregulated by autophagy and diosgenin resulted in arrest of cell cycle at G1 sub-phase. Moreover, the cell invasion as well as migration of HBL-52 ONSM cells were decreased by diosgenin, triggering the apoptosis which depended on mitochondria. In conclusion, diosgenin is found to have effective anti-tumoral characteristics within the ONSM cell within the in vitro situations. Thus, the present study has also been effective in providing important implications to promote the utilization of diosgenin for managing the genetically transmitted ONSM.

Keywords: *Optic Nerve Sheath Meningioma; Genetically transmitted ONSM; ONSM; Diosgenin; HBL-52 ONSM; The treatment of ONSM.*

1. Introduction

Optic nerve sheath meningiomas (ONSMs) are benign non-metastatic but local invasive neoplasms or tumors that originate in the optic nerve, particularly in the location of meninges. The optic nerve sheath is regarded as an unusual location for meningioma and it accounts for only 1 to 2 per cent of meningiomas and approximately 2 per cent of all orbital tumors (Parker et al., 2018). These meningiomas are the prevailing causes of tumors in primary optic nerve, which exhibits a variety of clinical symptoms and prognoses among people. ONSMs usually affect people between 30 and 50 years old, and female are more affected than males, exhibiting a gender ratio of 3:1. Nonetheless, ONSMs may appear in person of any age, including children as quoted in the study conducted by (Horowitz et al., 2023). ONSMs cause morbidity in the patients. Though the mortality rate associated with this condition is practically zero, these neoplasms commonly lead

to blindness or disfigurement in patients. Considering the variability of the clinical course and the potential of the treatment to lead to morbidity in patient, the ONSM is a disorder that comes with various challenges (Parker et al., 2018). Nearly 95 per cent of ONSMs are unilateral, although rare bilateral tumors can affect people with neurofibromatosis type-2 more commonly than the general population (NF2) (Parker et al., 2018; Patel et al., 2017). NF2 is a rare genetic disorder, which is characterized by the proliferation of tumors in the nervous system, and it is associated with neural benign tumors including ONSMs. Furthermore, inherited predisposition is linked with the loss of DNA on chromosome 22q12, which has been found in 40 per cent of ONSM cases. Several cytogenetic studies have reported this heritable abnormality as the most commonly reported genetic mutation. Monosomy of chromosome 22 has been diagnosed in almost 70 to 80 per cent of ONSM cases (Gossman, 2021). Studies have shown that, despite being a benign tumor, meningioma can be quite belligerent during pregnancy (Carbone et al., 2022; Chakravarthy et al., 2018). Patients suffering from ONSMs usually receive either radiotherapy or chemotherapy. Moreover, the ONSM accounts for 13 to 19 per cent of all intracranial tumors. The overall survival rate varies depending on the age of patients as patients at 5 and 10 years have 87 per cent and 58 per cent chance of survival, respectively. In addition, the prevalence of the ONSMs among different ethnicities which includes Caucasians, Africans, African Americans, and Asian countries including Turkey, exhibits a variability. Furthermore, the incidence of ONSMs increases with age, indicating 2 to 7 cases among ever 100,000 women, and 1 to 5 amongst ever 100,000 men. The highest rate of incidence in the group of women in their 60s and men in their 70s (Gossman, 2021). The essay seeks to explore the chromosomal transmitted disorder, the ONSMs in the context of Turkey, which remains a rarely explored region in terms of the incident of genetically induced ONSMs. Therefore, the dissertation is quite significant. Moreover, it explores the existing treatments of ONSMs and their efficacy. In addition, that paper examines the potential of diosgenin as an anti-tumoral agent for treating ONSM. Thus, it is immensely significant for examining the genetic basis of ONSMs in the context of Turkey and the role of diosgenin for inhibiting the growth of tumor process. Furthermore, this study helps to fill the existing research gap in cytogenetics literature by highlighting the role of diosgenin as a potential molecule for curing ONSM.

2. Literature Review

2.1. Genetic Basis of ONSMs

Cytogenetic studies revealed the mutations in meningioma sample almost half a century ago. These studies revealed the loss of genetic material on the left arm of 22nd chromosome as a pathogenic somatic mutation in meningioma cells. Later, the researchers discovered the role of a suppressor gene that worked for NF2 tumor on the chromosome 22q. Meningioma is developed in almost 50 per cent of individuals containing dominant NF2 in autosomal cells. Furthermore, somatic NF2 genetic mutation leads to more than 50 per cent of sporadic adult meningiomas (Kaidonis et al., 2022). Thus, NF2 is strongly related to the proliferation of neural tumors in general and ONSMs in particular. A heritable mutation in the suppressor gene for NF2 found on chromosome 22q12 is responsible for the ONSMs. The genetic mutation is hereditarily conveyed in an autosomal-

dominant fashion and the rate of spontaneous mutation is very high (Ardern-Holmes et al., 2017). Additionally, a high rate of mosaicism in such patients with sporadic gene mutations problematically provide the diagnosis (Asthagiri et al., 2009). The analysis of the NF2 can be challenging, therefore, children receive a delayed diagnosis. In addition, the recent studies on the next-generation sequencing have led to significant discoveries regarding various somatic mutations responsible for ONSM. A novel research conducted by Clark et al. (2013) on genomic analysis has delineated four genes answerable for meningioma. These genes include TRAF7, KLF4, AKT1, and SMO. Several other studies have identified other somatic mutations responsible for meningioma. Youngblood et al. (2019) performed targeted sequencing of established meningioma driver genes and reported strong association of genomic subgroups with tumor locations. The study revealed the enrichment of NF2 meningiomas in male patients, and it helped expand the previously established correlations between genetic drivers and clinical features. Furthermore, Kaidonis et al. (2022) made a significant contribution to the research in the field of ONSM by presenting the first report on ONSM in a patient who had a mutation in the TRAF7 gene. The case examined in the article focused on a 15-year-old girl who was suffering from bilateral ONSM, diffuse meningiomatosis, and other syndromic features. The hereditary examination of the meningioma sample years after having performed the biopsy revealed a pathogenic p. R641C variant inside the WD40 domain of the TRAF7 gene. A further examination of other tissues was also performed, which revealed that the same variant was there but at a reduced allele frequency.

2.2. Pathology and Clinical Features

ONSMs are marked by the proliferations of meningothelial cells, originating from the arachnoid villi of the arachnoid matter. They form rounded masses, which compress the adjacent tissues. Usually, a circumferential growth of ONSMs surrounding the optic nerve is observed and they rarely invade the nerve tissue, however they may occupy the length of the nerve and end up entering the intracranial space. ONSMs rarely become malignant (Lee et al., 2021). The studies done on ONSM reveals a gradual and painless visual loss in the affected eye of the patients. In case of delayed treatment, the tumor can lead to complete blindness. Moreover, patients with ONSM usually struggle with optic atrophy and optociliary shunt vessels. In addition, patients exhibit proptosis and resistance to retropulsion as symptoms of ONSM. Furthermore, patients also report gaze-evoked amaurosis. The early stages of ONSM are marked by a chronic optic nerve edema because of the tumor compression intra-orbital optic nerve region (Lee et al., 2021; Patel et al., 2017).

2.3. Clinical Management of ONSMs

The slow growing nature of ONSM makes their diagnosis quite challenging. Besides, the appearances of optic nerves may vary which can range from atrophy to swelling, leading to errors in diagnosis. The clinical features of ONSMs may vary, making the diagnosis challenging (Parker et al., 2018). The diagnostic procedures of ONSM include the use of magnetic resonance imaging

(MRI), particularly with gadolinium-enhanced fat-suppression sequences. This is regarded as the most efficient method of diagnosis as it has removed the need for tissue biopsy (Parker et al., 2018; Patel et al., 2017). Several other methods of diagnosis are also in practice which include Contrast-enhanced CT scans, Ultrasound, Biopsy, and Multifocal visual-evoked potential. In addition, Horowitz et al. (2023) examined the efficacy of ^{68}Ga-DOTATOC PET as a non-invasive, sensitive, and extremely specific technique to overcome the challenges associated with the diagnosis of ONSM. The study reported that ^{68}Ga-DOTATOC PET helped in delineating the volume of tumor before performing the radiation procedure which led to a reduced exposure to doses of radiations. The article suggested that ^{68}Ga-DOTATOC PET has to be conducted before proceeding with radiotherapy for meningiomas, which have not been histologically proven. The utilizing of this technique would be particularly helpful in cases left undiagnosed after MRI. The treatment options for patients diagnosed with ONSM include general observation, radiotherapy, surgical procedures, local chemotherapy, hydroxyurea, systemic chemotherapy and hormonal therapy (Parker et al., 2018). Sasano et al. (2019) conducted a study to examine the efficacy of radiation therapy that involved intensity modulation for treating patients suffering from ONSM. The essay reported a significant improvement in the treatment through IMRT and its achievement in preserving visual functions of patients, and it particularly highlighted the efficacy of early treatment with IMRT for improving visual functions in patients. IMRT relies on a device, such as a multi-leaf collimator to adjust in the intensity of spatial and temporal radiations and for irradiating a precise focal target from various directions. As a result, it helps achieve a balanced dose distribution on a 3D plane. An advanced version of traditional stereotactic radiation therapy has proven more efficient and non-invasive as it reduced the exposure of adjacent tissues to radiations. Therefore, IMRT is regarded as an effective treatment of ONSM.

3. Method

CCK-8 Cell viability assay.

The HBL-52 optic nerve sheath meningioma cell line serve as a genetically transmitted model of meningioma which was acquired and used in the CCK-8 Cell viability assay. The cells were cultured in a 5% CO2 incubator at 37°C in MDEM medium that contained 10% fetal bovine serum (FBS). After subjecting the cells to different concentrations of diosgenin, 30 µl of CCK-8 was added to the cell culture plates. After 12 hours of incubation at 37°C, the absorbance was measured at 450 nm using a microplate reader (Bio-Rad, Hercules, USA) from Bio-Rad. The absorbance values were used to determine the level of cell cytotoxicity.

To conduct an in vitro wound healing assay for cell migration, 6-well plates were seeded with 2×105 cells/ml of HBL-52 cells and left to incubate until they formed an 85% monolayer of confluent cells. We treated HBL-52 optic nerve sheath meningioma cells with different doses of diosgenin. After that, we removed the cells from the DMEM medium and washed them twice with PBS.

A sterile pipette with a 100 ml capacity was utilized to create a straight cell-free incision within the wells. After that, the cells were left to fix for 40 minutes before being stained with a solution

of 3.5% ethanol and 1.55% crystal violet dye. The cells were rinsed once more, and a photograph was captured. After 24 hours of culture, the plates were photographed again using an inverted microscope. Cell migration into the scratched area was determined by photographing randomly designated fields.

Assay for cell invasion

Using Matrigel-coated trans well chambers with a polycarbonate filter free of polyvinylpyrrolidone and a 6-mm pore size, we assessed diosgenin's impact on the HBL-52 cells' propensity to invade. The upper chambers were filled with 150 ml of cell cultures, while the bottom chambers contained only medium. The cells were taken out of the upper chamber after 24 hours of incubation, and any invaded cells were fixed with methanol. The cell cultures were then stained with 1.55% crystal violet dye for 40 minutes. At a magnification of 200×, the number of invaded cells was counted using an inverted microscope. Electron microscopy assessment of autophagy

To determine whether diosgenin inhibited autophagy in HBL-52 optic nerve sheath meningioma cells, this experiment employed electron microscopy. To summarize, HBL-52 cells were exposed to varying concentrations of diosgenin for a duration of 24 hours. In the following steps, the cells were isolated by trypsinization, followed by two washes with PBS, and finally, fixed in a solution of 2% glutaraldehyde in 0.1 M phosphate buffer.

Fluorescent microscopy for the assessment of cell death

Fluorescence microscopy with DAPI staining was utilized to examine the apoptotic effects of diosgenin on HBL52 optic nerve sheath meningioma cells (Stelzer et al., 2021). To summarize, HBL-52 cells were cultured in 6-well plates with a cell density of 1×105 cells per ml and exposed to diosgenin at different doses for 24 hours at 37°C. Following that, 30 µl of cell culture was seeded onto glass slides and then stained using DAPI. A fluorescence microscope was used to examine the slides after they were covered with cover slips.

Analysis of the cell cycle and western blotting

Diosgenin molecule was administered to HBL-52 optic nerve sheath meningioma cells (cultivated in DMEM medium at a cell density of 1×106 cells/ml) in multiple concentrations ranging from 0 to 30 µM. Afterward, western blotting was done for protein analysis (Pillai-Kastoori et al., 2020).

Data analysis

Three separate experiments were applied to obtain the results, which are shown as the mean values with standard deviations. SPSS 17.0 software (SPSS, Inc., Chicago, IL, USA), The student's t-test was worked to compare the groups. A statistically significant difference was indicated by a p-value less than 0.05.

4. Results

Etiology of "Optic Nerve Sheath Meningiomas (ONSM)" is not clearly known, and they are typically idiopathic. However, exposure of the individuals to ionizing radiation is often related to meningiomas. ONSM is found to be related to "neurofibromatosis (NF) type 2." One of the most commonly observed cytogenetic abnormality within the context of meningiomas contain the absence of chromosome 22 long arm, incorporating the region, associated with NF2 gene. However, in the past research, the surgical treatment and the radiation therapy were considered to be most commonly used for managing ONSM, whereas limited focus has been given on the role of disogenin in this regard which can be used for managing ONSM. Thus, the present study has been effective in overcoming this limitation by evaluating the impact of disogenin within the context ONSM.

4.1 Antiproliferative effects exerted by disogenin within HBL-52 ONSM cells

"CCK-8 (Cell Counting Kit-8) assay" was utilized for evaluating cytotoxicity incorporated by diosgenin within HBL 52 ONSM cells at different doses of the associated molecule. Figure 1(A) shows diosgenin's chemical structure, whereas the cytotoxicity results are shown in figure 1(B). Therefore, the results obtained from CCK-8 assay clearly showed that diosgenin resulted in antoproliferative effects within the HBL 52 ONSM cells. Within this context, the value of IC50 was found to be 8.6 Mm. Thus, this molecule was found to have a low value of IC50 which shows that it has high cytotoxicity causing potency within the oncogenic cells.

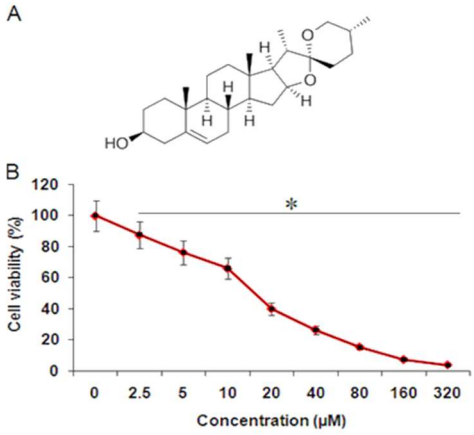

Figure 1. Diosgenin's chemical structure (A) showing CCK-8 assay, (B) shows diosgenin's cytotoxic impacts on HBL-52 ONSM cells viability ($p < 0.05$)

4.2 Inhibition of invasion as well as cell migration within HBL-52 ONSM cells via Diosgenin

In order to determine effectiveness of diosgenin in decreasing the invasion and migration of tumorous cell, "transwell assay" as well as "wound healing assay" were conducted. Therefore, figures 2 and 3 show that Diosgenin results in inhibitory impacts on both cell invasion and migration within a dose-dependent manner. It was clearly observed from the width of the wound at higher molecule doses. Therefore, these results show that Diosgenin might be effective in

reducing the spreading of cancer cells, by acting as an effective anti-oncogene agent. This can also be effective in reducing the spreading of genetically transmitted ONSM.

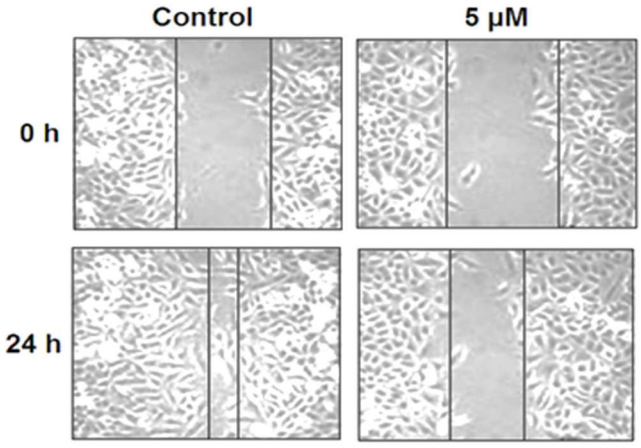

Figure 2. Inhibition of invasion as well as cell migration within HBL-52 ONSM cells via Diosgenin

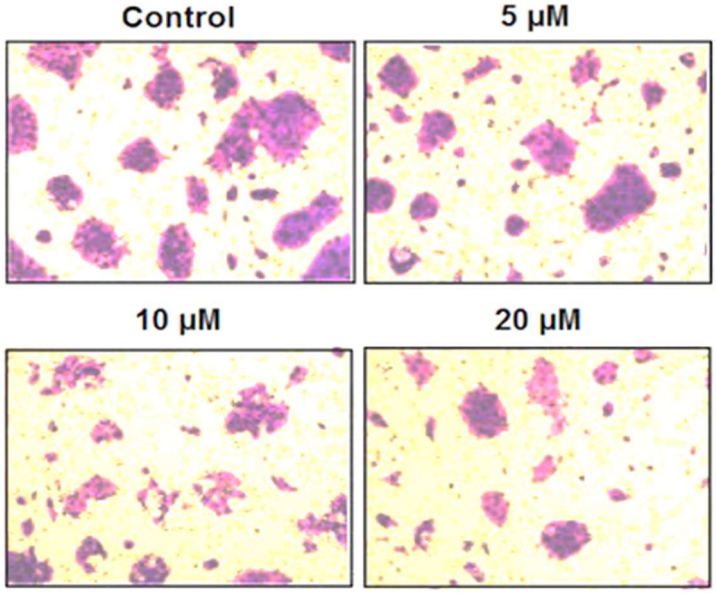

Figure 3. Inhibition of invasion as well as cell migration within HBL-52 ONSM cells via Diosgenin

4.3 Autophagic cell death induced by Diosgenin within HBL-52 ONSM cells

In order to further investigate diosgenin's mode of action within the context of its anti-neoplastic properties in HBL-52 ONSM cells, an electron microscopy was considered. Figure 4 shows that a dose of 5 μM of diosgenin, autophagic and autophagosomes vacuoles can be observed more clearly within treated cells than control cells. Later on, "western blot assay" was conducted to ensure

autophagy. Figure 5 shows important results in this regard, which clearly shows that molecule has a positive impact on "autophagy-associated protein expressions," which include LC3-II, p62 and LC3-I. Therefore, disogenin has resulted in a dose-dependent increase within the context of LC3-I and LC3-II expression, whereas p62 expression was inhibited.

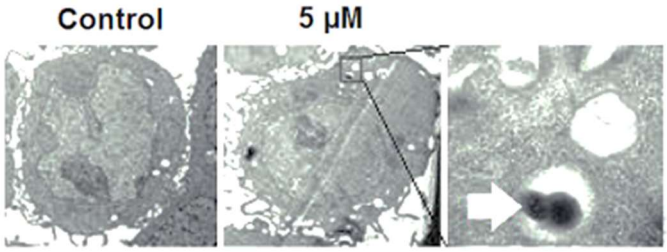

Figure 4. Formation of vacuoles and autophagosomes within HBL-25 ONSM cells due to induction of diosgenin

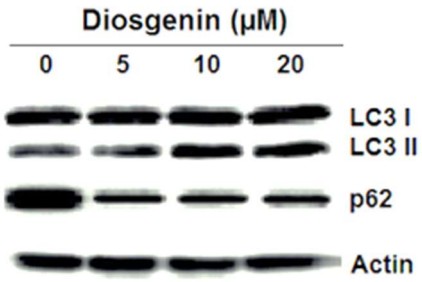

Figure 5. Diosgenin effects on different ""autophagy-associated protein expressions," which include LC3-II, p62 and LC3-I

4.4 "Apoptotic" cell death induced by Diosgenin within HBL-52 ONSM cells

Diosgenin was not only able to develop autophagy within the HBL-52 ONSM cells, but it also led to the induction of apoptotic effects within these cells. For this purpose, fluorescence microscopy was used and DAPI was taken as the staining agent. Afterwards, it was also ensured by western blot, after checking Bcl-2 and Bax protein expressions. These proteins are found to be associated with apoptosis. Therefore, Diosgenin also resulted in significant chromatin condensation, nucleus splitting, apoptotic cascade representatives and nuclear fragmentation as shown in figure 6. For that reason, with the increased concentration of diosgenin, apoptotic cascade was also found to be increased. It has also been observed that diosgenin resulted in the increased expression of Bax, however Bcl-2 expression was found to be decreased, dependent on the dose as shown in figure 7.

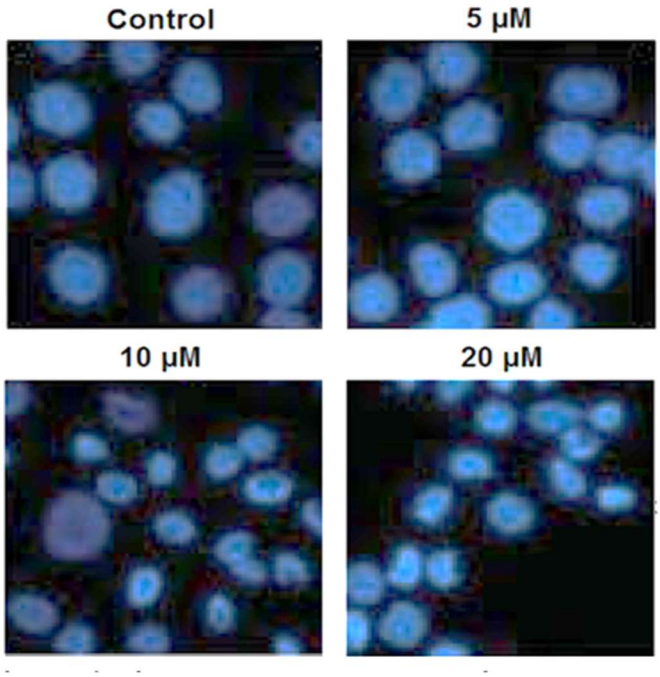

Figure 6. Fluorescence microscopy, utilizing DAPI staining which shows that diosgenin could result in the induction of programmed cell death as stated by increased nuclear fragmentation and chromatin condensation with the increasing dose of diosgenin

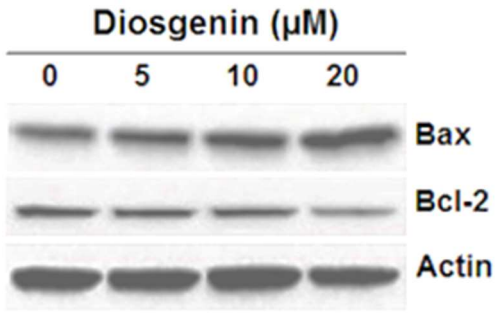

Figure 7. Diosgenin effects on different protein expressions which are related to autophagy, including p62, LC3-I and LC3-II

4.5 "G0/G1 cell cycle arrest" induced by Diosgenin

Diosgenin's cytotoxic effects were found to be mediated via phase distribution of cell cycle arrest. The findings attained from the measurements of flowcytometric showed that the molecule resulted in the arrest of G0/G1 cell cycle, depending on the concentration. Therefore, with the increase dosage of diosgenin, upsurge was also observed within the HBL-52 ONSM cells within the G0/G1 phase. Figure 8 shows that the introduction of a dose of 30 μM of diosgenin can increase the G0/G1 cells percentage by 70%.

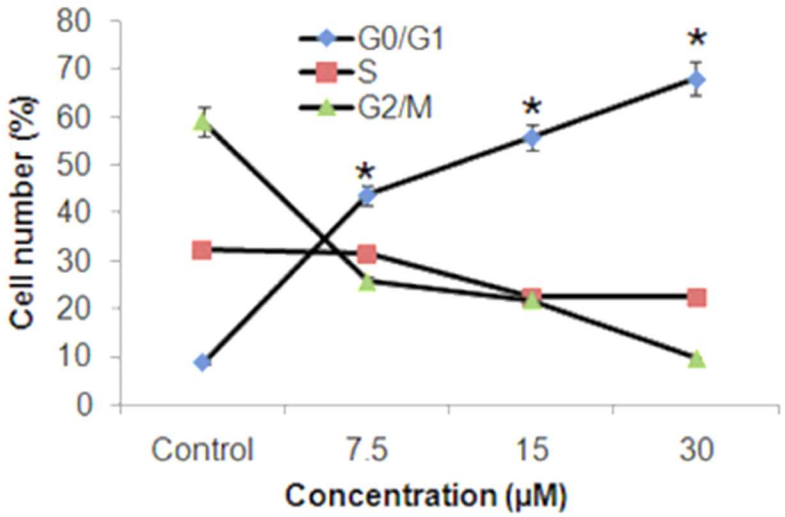

Figure 8. Arrest of G0/G1 cell cycle is triggered by diosgenin within the context of HBL-52 ONSM cells as stated by "flow cytometry"

5. Discussion

The present study focused on examining the impact of diosgenin for managing ONSM. The current essay documents the anti-proliferative activity of diosgenin, which is a plant extract. The paper reports the antitumor effects of diosgenin through a wide variety of molecular mechanisms, including apoptosis, generating reactive oxygen species, and inhibiting cell migration. This article aimed at an evaluation of the anticancer effects of diosgenin against HBL-52 ONSM cells and also examined the way this molecule acted on cancerous cells by analyzing its effects on call autophagy, cell cycle, apoptosis, and cell migration and cell invasion. A previous study conducted by Zhu et al. (2020) has also reported the significant anticancer potential of diosgenin by focusing on its chemotherapeutic activity for eliminating benign proliferative cells. Thus, diosgenin has emerged as a beneficial molecule to be used in treating cancer. It has been reported to induce ROS-mediated autophagy, inhibit oncological cells, and initiate cytotoxicity on selective tumorous cells (Bhardwaj et al., 2021). However, not much focus has been put on the anticancer role of diosgenin in HBL-52 ONSM cells. Therefore, the present study has reported its role on HBL-52 ONSM cells and deciphered its impact on cell cycle, apoptotic activities, autophagy, and call migration. The results of the present study reported that diosgenin led to anti-proliferative activities in cells growing in IC50 values of 8.6 μM. This low value of IC50 indicated a high potential of diosgenin for inducing cytotoxicity in oncological cells. Thus, the present study indicates that diosgenin can be used as a potential treatment to inhibit the proliferation or the growth of ONSM tumors. As diosgenin induces cytotoxicity in selective cells, particularly in neoplastic cells, it indicates its potential to inhibit the growth of ONSM tumors. The present study also reported the effectiveness of diosgenin for inhibiting migration and invasion of oncogenic cells. The results reported that diosgenin had the potential to act as an anti-metastatic agent for decreasing the migration of cancerous cells to invade healthy cells. The width of wound at the areas with high concentration of diosgenin, thus, indicated the effectiveness of this molecule for inhibiting the migration of

cancerous cells. Hence, diosgenin can be used as an anti-metastatic agent to inhibit the migration of ONSM tumors and prevent the invasion of healthy cells by the cancerous cells. Furthermore, the present study also reported that diosgenin could trigger the formation of autophagosomes, thus leading to autophagy. The results reported a dose-dependent increase in LC3-I and LC3-II expression and decrease in p62 expression caused by diosgenin. Consequently, diosgenin has the potential to eliminate malfunctioning organelles and cells from the body, leading to the destruction of tumors. In addition, the present study reported that diosgenin was not only effective in developing autophagy within the HBL-52 ONSM cells, but also resulted in the induction of cellular apoptosis within the cancerous cells. The fluorescence microscopy and western blot helped reveal the impact of diosgenin on cellular apoptosis. Finally, the present study also reported that diosgenin was effective in triggering G0/G1 cell cycle arrest. Thus, the present study's findings provide valuable evidence regarding the effectiveness of diosgenin for treating ONSM.

6. Conclusion

The present study has focused on the anti-neoplastic activity induced by the use of disogenin molecule. The results extracted from the current paper highlight the significant anti-tumoral role of this molecule in HBL-52 optic nerve. Diosgenin emerged as a potential anti-metastatic agent because of its significant role in inhibiting the migration of tumor cells and invasion of healthy cells. Furthermore, this molecule has been associated with inducing autophagy and apoptosis. Thus, diosgenin can be practiced as a potential treatment for destroying neoplasm cells without harming the healthy cells. This has been evidenced by the selective cellular apoptosis induced by diosgenin. Moreover, diosgenin was also effective in triggering the G0/G1 cell cycle arrest of the HBL-52 optic nerve sheath meningioma cells, which was indicated by flow cytometry. This is a potential benefit of diosgenin, which can be useful in arresting the tumoral cells and preventing the invasion of healthy cells.

7. Implications

The present study holds significant implications for suggesting potential treatment for curing ONSM. The essay has focused on the potential of a rarely explored molecule, diosgenin, for treating ONSM. Thus, the findings of this study are significant because they suggest potential treatment of tumor process. The role of diosgenin as an anti-proliferative agent holds significant implications in inhibiting the growth of tumoral cells. Thus, the oncologists should focus on the potential of diosgenin for inhibiting the proliferation of tumors in the body. Moreover, the significant role of this molecule for inhibiting cell invasion and migration holds immensely significant implications in the field of ONSM. The present paper highlights the role of this molecule for preventing neoplastic cells from invading healthy cells in the body. In consequence, the role of diosgenin as an anti-metastatic agent should be further explored to the cure of this illness. In addition, the selective cytotoxicity of diosgenin also holds significant implications as the greatest concerns associated with ONSM treatment include the potential harm to the healthy cells. This selective cytotoxicity of diosgenin has the potential to revolutionize the treatment,

which should be explored at a bigger level. Thus, the current dissertation illuminates a variety of implications for enhancing the effectiveness of ONSM.

8. Limitations and Future Directions

The essay has presented valuable findings to examine the potential of diosgenin for inhibiting the proliferation and movement of neoplastic cells, however the study has several limitations. One of the goals of this dissertation is to demonstrate the effects of diosgenin on HBL-52 cells, although the anticancer effects of this molecule may not be uniform and may exhibit a variation based on the cell type. Therefore, future studies can examine the impact of diosgenin on a different cell type to present a more comprehensive conclusion regarding its effectiveness in treating ONSM. Moreover, its effects on a different cell type can be compared with those on HBL-52 cells. In addition, more studies should be performed for exploring the efficacy of diosgenin to treat of ONSM through successive trials.

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