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Cytotoxic Effects of Hypericum Perforatum on Glioblastoma Cells by Inducing Oxidative Stress, Autophagy and Apoptosis

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ABSTRACT

AIM: To identify the autophagy mechanism T98 glioma cells.

MATERIAL and METHODS: Three groups were created with T98 human glioblastoma cells; Group 1: T98 glioma cells without treatment (Control group). Group 2: T98 glioma cells treated with 3 µl/ml JWO. Group 3: T98 glioma cells treated with 6 µl/ml JWO. The cell proliferation, oxidative stress, types of cell death were studied at IC50 dose of JWO.

RESULTS: The proliferation of glioma cells was inhibited in 5.296 µl/ml dose. JWO induced apoptosis in T98 glioma cells in comparison with the control and there was statistically significant difference ($p<0.001$). Apoptosis was analyzed via TUNEL method and results were checked by flow cytometry. We also investigated the effects of JWO on autophagy in T98 glioma cells by immunostaining LC3-II and MDC fluorescent stainings. The differences between JWO treated and control group were notably significant ($p<0.001$). The immunofluorescence staining resultsof LC3-II was confirmed by Western blotting analysis.

CONCLUSION: JWO seems to be an effective treatment agent for glioblastoma. Not only does it induce apoptosis via oxidative stress but also affects the autophagy. The use of JWO in combination with other treatment options may increase the efficacy of treatment.

KEYWORDS: Autophagy, Glioblastoma, St John's Wort Oil

INTRODUCTION

Glioblastoma (GBM) or WHO grade IV neoplasm with astrocytic differentiation is the most aggressive common malignant brain tumor in adults (32,37). The annual incidence of GBM is 3–4 cases per 100,000 people (7). The standard treatment includes gross total surgical resection with minimal neurological complications, radiotherapy, and chemotherapy, mostly with temozolomide. Although the survival time is <1 year from the date of diagnosis, most patients die within 2 years (7,25,32,37). Despite advances in early diagnostic methods and treatment approaches, there is still no effective treatment for GBM.

St. John's wort, also known as *Hypericum perforatum* (HP), is a plant whose extract is widely used in Europe, Asia, and the USA (1). St. John's wort oil (JWO) has sedative and vasoconstrictor properties. Furthermore, it is used for the treatment of neuralgia, fibrositis, menopausal neurosis, depression, excitability, anxiety, bacterial/viral infections, and wounds (topical preparation) (1,8,11,16,17).

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The effects of JWO on tumor cells such as metastatic melanoma, breast cancer, pancreatic cancer and GBM have been investigated recently (2,10,13,22,42). Preclinical studies using GBM cells have reported that JWO acts via the mechanism of apoptosis (2,10,29). However, data regarding JWO's effect on the autophagy mechanism in GBM remains unknown. Thus, herein, we aimed to evaluate the effects of JWO on cell viability, oxidative stress, apoptosis, and autophagy in a T98 human GBM cell line.

MATERIAL and METHODS

The study was approved by the Ethics Committee of Manisa Celal Bayar University (No: 20.478.486/1237). The T98 human GBM cell line (CRL-1690) was used, and it was acquired from the American Type Culture Collection (USA). The effects of JWO on cell viability, oxidative stress, autophagy, and apoptosis were studied via 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyltetrazolium bromide (MTT) analysis, TUNEL assay, immunofluorescence staining of inducible nitric oxide (NO) synthase (iNOS), endothelial nitric oxide synthase (eNOS), and microtubule-associated protein 1 light chain 3-II (LC3-II) respectively. In addition, oxidative stress, autophagy, and apoptosis were assessed via monodansylcadaverine (MDC) staining, flow cytometry (annexin-V FITC/propidium iodide [PI]), and western blotting assay (iNOS, eNOS, LC3-I and LC3-II), respectively.

Preparation of Oil Extracts

HP was collected from the rural fields of the Manisa province in July. After being dried, 50 g of HP was immersed in olive oil (500 cc) in glass jars and left in the sun daily for 12 hours (h) for 4 weeks (33). This extract was used in the study experiments without any further processing.

Cell Line

The T98 cell line was seeded in Eagle's minimal essential medium (M4526, Merck), which contained 10% fetal bovine serum (S1810, Biowest) and 100 IU penicillin/streptomycin (PS-B; Capricorn Scientific). The cells were cultured in 95% air and 5% CO_2 at 37°C until approximately 70–80% confluency was achieved. The cell media was discharged every third day.

MTT Assay

To detect the IC_{50} dose of JWO, an MTT (20,395.02, Serva Electrophoresis) assay was performed. The cells were cultured in 96-well plates (1×10^4 cells/per well) for 24 h. The different concentrations of JWO (0, 2, 5, 10, 15, 20, 25, and 30 µl/ml) and 0.2% (v/v) dimethyl sulphoxide (DMSO; A3672, AppliChem), as a negative control, were applied to the cells. After being incubated for 24 h, the media was replaced with fresh media (100 µl). In addition, 10 µl of MTT reagent (5 mg/ml in distilled water) was added to each well. The plates were incubated for 4 h. Subsequently, the media with MTT was discarded, and 100 mL of DMSO was added to each well. The cells were incubated for 30 min. Finally, the absorbance was measured using an ELISA plate reader at a wavelength of 490 nm (20).

Immunofluorescence

Cells were stained using the immunofluorescence technique to detect eNOS and iNOS for oxidative stress and LC3-II for autophagy. After the application of 0 (control), 3, and 6 µl/ml of JWO for 24 h, the cells were fixed in 4% paraformaldehyde for 30 minutes (min) and subsequently, washed with phosphate buffer saline (PBS; 00-3002, Invitrogen, Camarillo, CA, USA) for 5 min. Thereafter, the cells were incubated with 0.1% Triton X-100 (A4975, AppliChem) at 4°C for 15 min to achieve permeabilization and washed with PBS. To quench the endogenous peroxidase activity, 3% hydrogen peroxide solution (108,600, Merck) was used for 5 min. After washing the cells in PBS, they were incubated with monoclonal anti-eNOS (sc-376751, Santa Cruz), anti-iNOS (sc-7271, Santa Cruz), and anti-LC3-II (ab51520, Abcam) for 18 h at 4°C. The control samples were not incubated with any primary antibody. The cells were washed in PBS one last time and treated with a secondary antibody (ab150084, Abcam) for 1 h. For counterstaining, the cells were dyed using 4',6-diamidino-2-phenylindole dihydrochloride (DAPI; AR1177, Boster Bio). The cells were covered with mounting medium (AML060, Scytek, Logan, Utah, USA) and examined under an inverted-florescence-phase microscope (IX71, Olympus, Japan). The immunoreactivity was evaluated in 10 randomly chosen areas according to the H-score method. The intensity of staining was graded as follows: 0 = no staining, 1 = pale, 2 = moderate, and 3 = intense. Thereafter, the percentage of each intensity was calculated, and the H-score was determined using the following formula: (1+staining intensity/3) ´ percentage of staining. Pi varies from 0% to 100%. The procedure was performed independently by at least two investigators (38).

TUNEL Assay

To determine apoptosis, the ApopTagÒ Plus Peroxidase *In Situ* Apoptosis kit (S7101, Merck) was used according to the manufacturer's instructions. After fixation in 4% (w/v) paraformaldehyde for 30 min, the cells were incubated with 0.1% Triton X-100 for 15 min. After being rinsed with PBS, the cells were treated with an equilibration buffer and subsequently, incubated with the Tdt-enzyme at 37°C for 60 min. Tdt was omitted prior to the end of the reaction as the TUNEL-negative controls. After the stage of "Stop Wash Buffer," the apoptotic cells were labeled with diaminobenzidine (DAB; 00-2020, Zymed, CA, USA), and the cells were dyed with Mayer's hematoxylin (72804E; Microm, Walldorf, Germany) for counterstaining. The cells were washed in distilled water and covered using aqueous mounting medium. The TUNEL-positive cells had characteristic brown nuclei and were quantified in 10 randomly selected fields using an apoptotic index, which was determined as follows: (number of apoptotic cells/total number of cells) ´ 100 (20).

Annexin-V FITC/PI flow cytometry

The apoptosis rate was determined via the annexin-V FITC-PI (K101, BioVision) assay. The cells were cultured in a 6-well plate until a confluency of 70–80 % was achieved. Thereafter, the cells were treated with 0 (control), 3, 5 (IC50 dose), and 6 µl/ml of JWO for 24 h. Subsequently, the cells were

harvested and centrifuged at 1,000 rpm. After the cells were resuspended in a binding buffer, 5 µl of annexin-V FITC and 5 µl of PI were added. The cells were analyzed using a flow cytometry device (Ex = 488 nm, Em = 530 nm, and FL1 and FL2 detectors [BD Accuri™ C2 plus]) (20). Flow cytometric analysis was performed three times.

MDC Staining

The autophagic vacuoles were defined via MDC fluorescent staining (D4008, Sigma). After the application of 0, 3, and 6 µl/ml of JWO for 24 h, the cells were washed with PBS. Thereafter, the cells were incubated with MDC (70 µmol) at 5% CO_2 and 37°C. After the cells were washed with PBS again, the samples were evaluated under a florescence microscope (BX43, Olympus, Japan) using a DAPI filter attachment. The staining was evaluated using the H-score method, as described earlier, in 10 randomly chosen areas.

Western Blotting

After JWO was added, the cells were lysed using mammalian protein extraction reagent (M-PER). The protein concentrations were ascertained via the Pierce™ BCA assay kit (Thermo). Electrophoresis was carried out in 4% tris-glycine SDS buffer at a constant voltage of 90 V. Thereafter, the proteins were transferred from the gel to the membranes for western blotting analysis. The membranes were treated with the primary antibodies of eNOS (sc-376751, Santa Cruz), iNOS (sc-7271, Santa Cruz), and LC3A/B (ab58610, Abcam) and incubated overnight at 4°C. The samples were washed with TBST, and the membranes were treated with secondary antibodies for 1 h. Subsequently, the membranes were scanned at 600 dpi resolution (20).

Statistical Analysis

Each experiment and evaluation was performed three times. The results were analyzed using GraphPad (San Diego, CA, USA). One-way ANOVA was performed, and the results are presented as means and standard deviations. Statistical significance was set at $p \leq 0.05$. (40).

RESULTS

The T98 GBM cells were replicated and confluent. JWO was applied at increasing concentrations to obtain the IC50 dose. The toxic effect of JWO on the T98 GBM cell line at 2, 5, 10, 15, 20, 25, and 30 µl/ml doses for 24 h was evaluated. The IC50 dose was 5.296 ± 0.950 µl/ml. To determine the efficacy of the 6 µl/ml dose on the T98 glioma cells, a lower dose (3 µl/ml) was chosen and a double combination was applied (Figure 1).

Compared to the control group, the number of viable cells decreased and the spindle-shaped cells disappeared in the 6 µl/ml JWO group. In addition, there was a decrease in intercellular connections and degeneration after JWO treatment (Figure 2).

JWO Increased the Oxidative Stress in T98 GBM Cells

Oxidative stress is a condition that results from an imbalance between reactive oxygen species (ROS) and free radicals. ROS can trigger DNA damage, leading to genetic changes and ultimately tumor formation.

In this study, we stained for eNOS and iNOS to evaluate oxidative stress.

In the control group, eNOS was detected around the Golgi apparatus. The regular Golgi apparatus observed in the control group began to disintegrate in the 3 µl/ml JWO group.

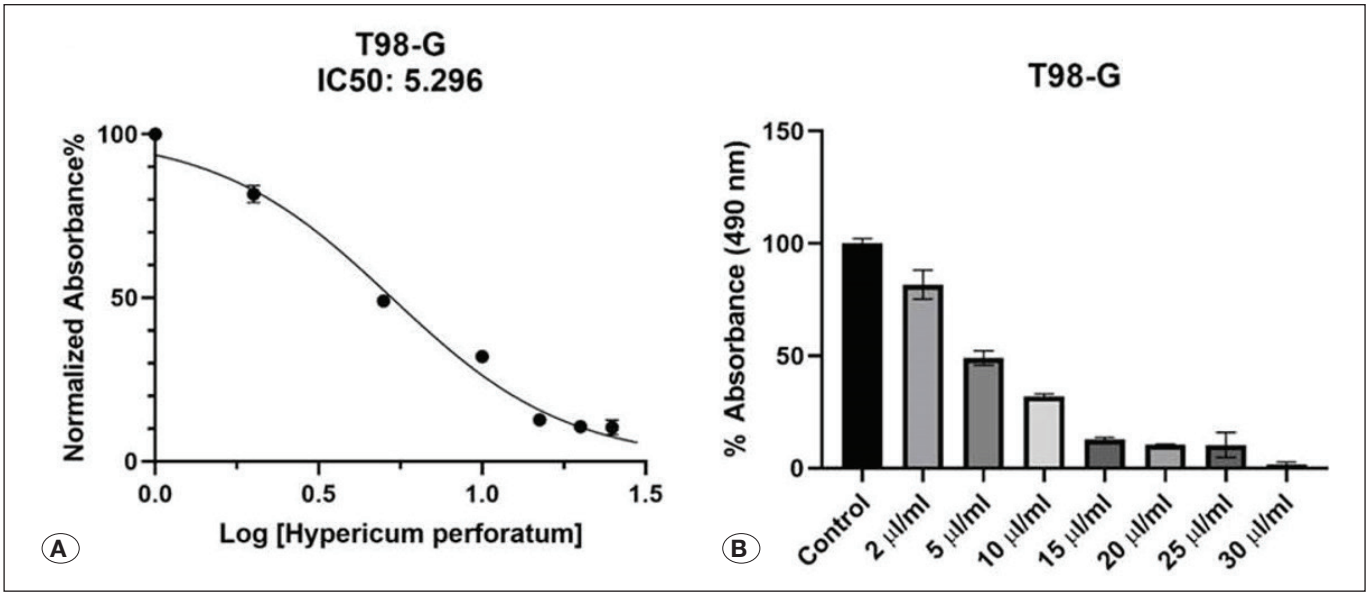

Figure 1: The absorbance logarithmic changes **(A)**, and percent vitality absorbance values **(B)** of JWO at different concentrations (***p<0,001).

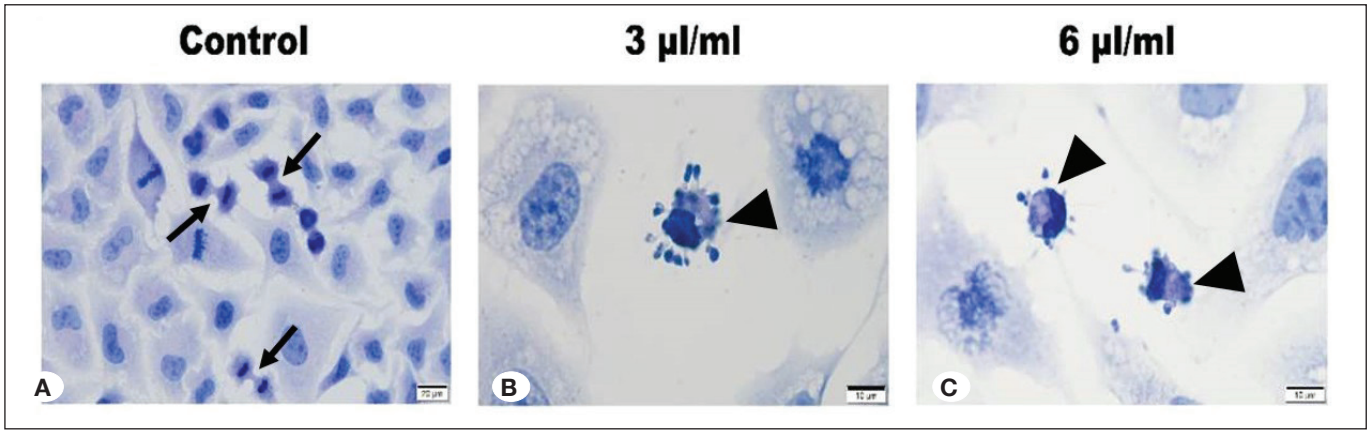

Figure 2: T98 glioma cells were fixed and stained with hematoxylin-eosin. While high mitotic activity was observed in the control group (arrows) **(A)**, apoptotic and degenerative changes (arrow heads) occurred after JWO treatment **(B and C)**. Scale bars: 10 µm.

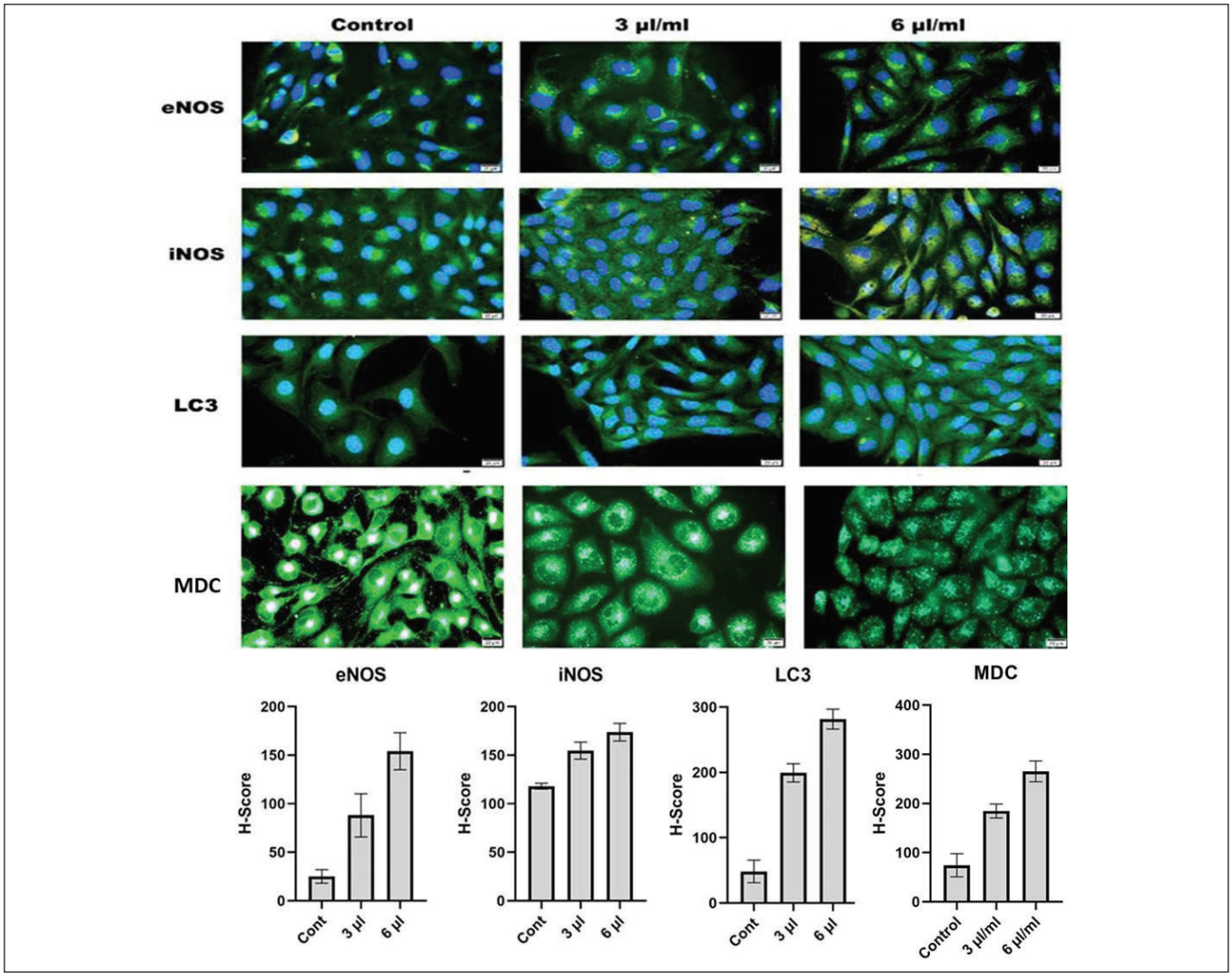

Figure 3: Dose-dependent changings for eNOS, iNOS, LC3-II and MDC immunofluorescence stainings in T98 glioma cells. The increase was observed for eNOS, iNOS, LC3-II and MDC immunofluorescent stainings after JWO treatment in T98 glioma cell. These changings were found to be significant (***p<0,001)with dose dependent manner in the analysis performed with the H-score (mean ± SD). Nuclei: Blue, Immunopositivity: Green. Scale bars: 20 µm.

At 6 µl/ml of JWO, the Golgi apparatus ceased to be linear and became large vesicles. There was a significant difference between the groups (p<0.001) (Figure 3).

In the control group, iNOS was detected in vesicular areas close to the nucleus. In the 3 µl/ml JWO group, iNOS was proximal to the nucleus, separated from the vesicular structure, and homogeneously distributed in the cytoplasm. However, these changes became more pronounced at 6 **µl/ml** of JWO. The differences between the groups were statistically significant (p<0.001) (Figure 3).

JWO Induced Autophagy in T98 GBM Cells

Autophagy is a natural conserved degradation of macromolecules and organelles by the cell via a lysosome-dependent mechanism. We evaluated the effects of JWO on autophagy in T98 glioma cells by immunostaining for LC3-II and performing MDC fluorescent staining (Figure 3).

Immunostaining for LC3-II revealed small amounts of autophagic vesicles that were scattered in the cytoplasm in the control group. In the 3 µl/ml JWO group, the amount of autophagic vesicles began to increase in the cytoplasm. However, at 6 µl/ml of JWO, the autophagic vacuoles were small and dispersed in the cytoplasm. The differences in LC3-II staining between the groups were statistically significant (p<0.001) (Figure 3).

MDC fluorescence staining revealed autophagic vesicles that were densely packed near the nucleus and in the Golgi apparatus in the control group. In addition, they were distributed as small vesicles in the cytoplasm. The vesicle size increased in the 3 µl/ml JWO group, and it was generally widespread in the cytoplasm. In the 6 µl/ml JWO group, the vesicle size was similar to that in the 3 µl/ml JWO group. In addition, lipid droplets were detected in the cytoplasm. The differences in MDC staining between the groups were statistically significant (p<0.001) (Figure 3).

The immunofluorescence staining results of eNOS, iNOS and LC3-II were confirmed via western blotting analysis (Figure 4). Beta-actin was used as a loading control for western blotting samples. The eNOS, iNOS and LC3-I protein expressions increased in a dose-dependent manner after JWO treatment, and the increases were statistically significant (p<0.001). However, LC3-II protein expression decreased in a dose-dependent manner after JWO treatment, and these decreases were statistically significant (p<0.001). Quantitative evaluation of the western blotting analysis, revealed that the increases and decreases in folds were more pronounced (Figure 4).

JWO Has an Apoptotic Effect on T98 GBM Cells

TUNEL staining was used to assess apoptosis and determine the link between oxidative stress and programmed cell death (Figure 5). Compared to the control group, there was a dose-dependent increase in the apoptotic cells labeled with TUNEL staining. The number of cells determined by the apoptotic index increased significantly in a dose-dependent manner (p<0.001) (Figure 5). The TUNEL immunohistochemistry staining results were confirmed by flow cytometric analysis (Figure 6). We determined that JWO induced apoptosis up to concentrations of 6 µl/ml and induced necrosis at higher doses. Flow cytometry was performed using Annexin-V FITC/PI

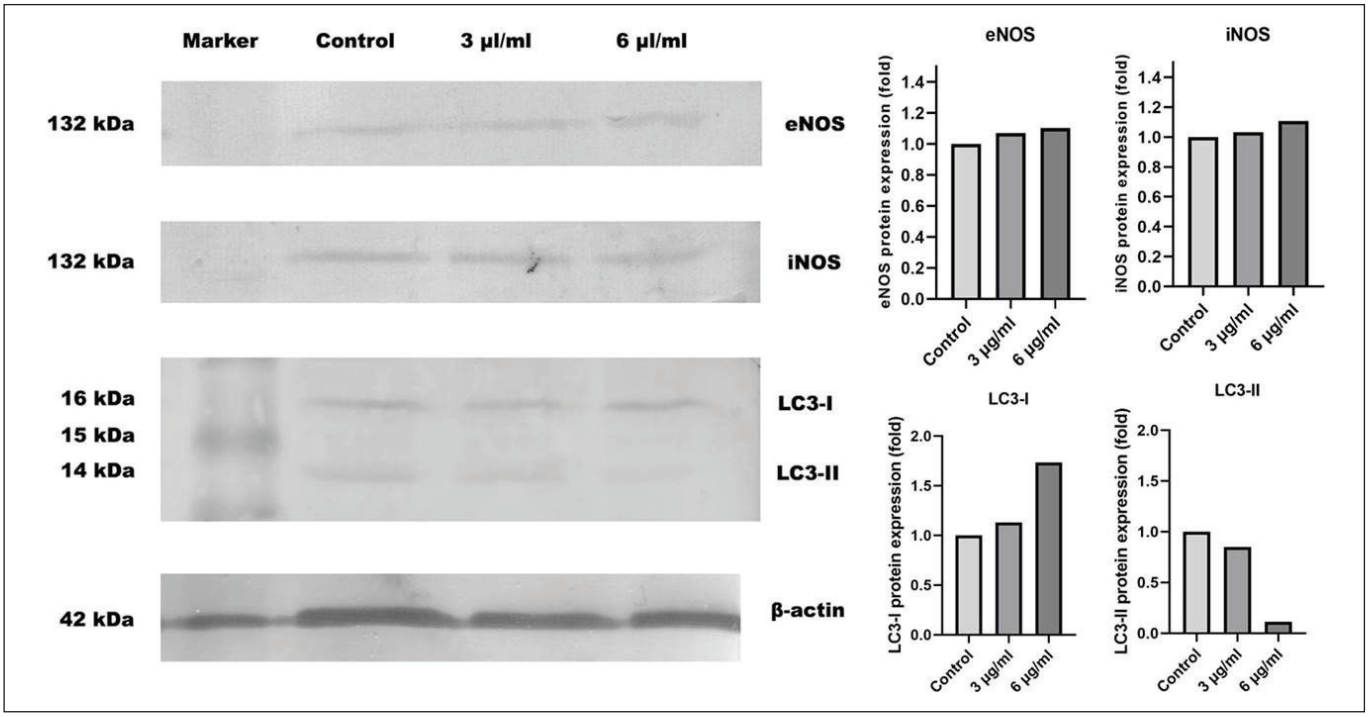

Figure 4: The Western blotting analysis of eNOS, iNOS, LC3-I and LC3-II protein expression levels after the application of 0, 3, and 6 µl/ml JWO (***p<0,001).

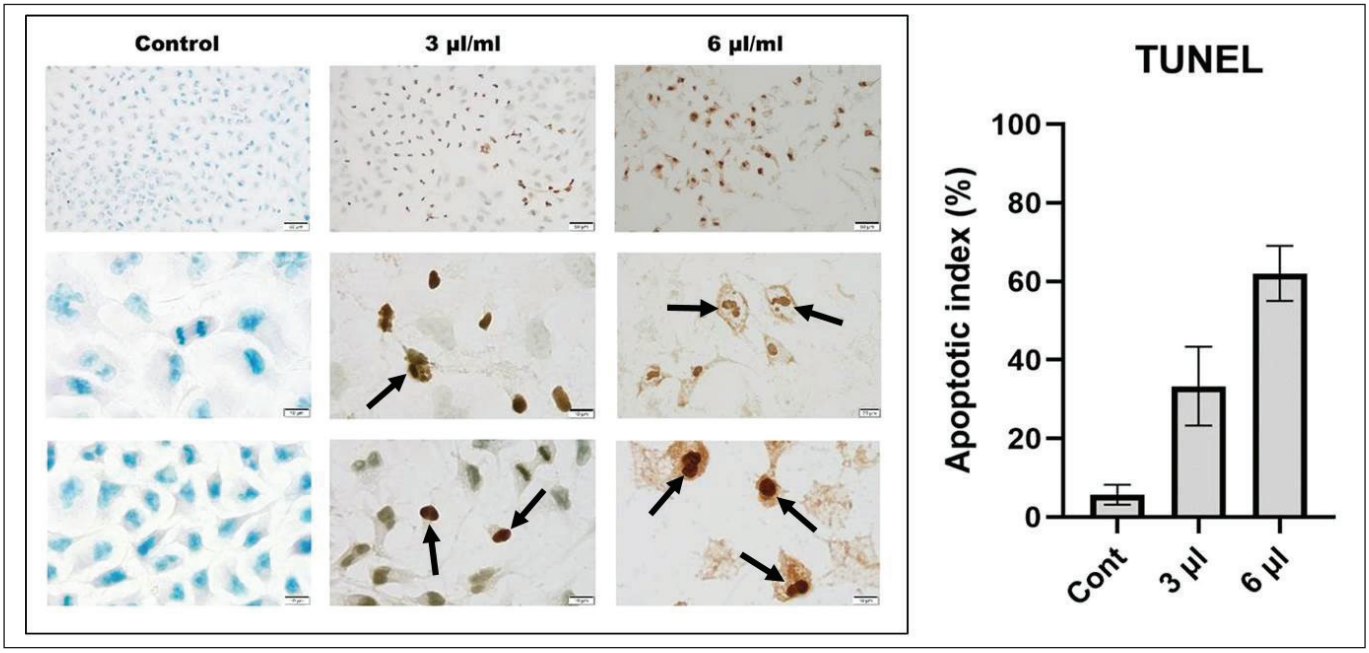

Figure 5: Representation of dose-dependent apoptotic deaths in T98 glioma cells by TUNEL method. Apoptotic index observed in cells as a result of TUNEL staining (% ± SD) (***p<0,001). Scale bars: 20 µm.

staining to examine early/late apoptosis and necrotic rates. In the 3 µl/ml JWO group, the early apoptosis, late apoptosis, and necrosis rates, were 21.8%, 6.5%, and 2.4%, respectively. In the 5 µl/ml JWO group, the early apoptosis, late apoptosis, and necrosis rates were 20.9%, 21.9%, and 8.9%, respectively. In the 6 µl/ml JWO group, the early apoptosis, late apoptosis, and necrosis rates were 17.6%, 8.8% and 5.8%, respectively (Figure 6).

DISCUSSION

Although new treatment options for GBM have been reported, the successful treatment rates remain low. A growing percentage of the patients with GBM often use herbal supplements to support the treatment (2,18). JWO is reportedly effective in the treatment of mild-to-moderate depressive disorders. Most studies report that its effect is related to reuptake of several neurotransmitters such as dopamine, serotonin, glutamate, noradrenaline, and gamma-aminobutyric acid (1,11,28)

JWO extracts have been studied in detail and many secondary metabolites such as hyperoxide, hyperforin, hypericin, isoquercitrin, quercitrin, quercetin, and chlorogenic acid have been identified. Of these, the two important compounds of the pharmaceutical industry are hyperforin and hypericin (1,14,21,26). Hypericin is as a promising antitumor agent and a potential treatment option against neurodegenerative diseases such as Alzheimer's disease (9). The successful use of hypericin in photodynamic therapy for cancer is due to its photosensitizing property. An apoptotic signal is induced when hypericin is exposed to light. This includes the production of ROS, which cause tumor cell death. Hypericin administration also increases the cytokine levels, which triggers the activation of immune cells and an inflammatory response (26). Moreover, irradiation studies in D54Mg GBM cells have demonstrated that when hypericin enters the cell, it accumulates in the endoplasmic reticulum and Golgi apparatus rather than in the mitochondria. Hypericin causes Ca^{2+} release from these organelles, and the excessive Ca^{2+} in the cytoplasm induces cell death (35). However, other studies have reported that some other compounds found in the JWO extract may interact with individual cell growth mechanisms with more pronounced effects than hypericin (2). Matić et al. examined the mechanisms of the anticancer activity of six different JWO extracts including hypericin in different malignant cell lines (19). They determined that hypericin and two other extracts had strong cytotoxic effects against cervical cancer HeLa cells and chronic myelogenous leukemia (K562) cells. Roscetti et al. showed that HP L. extract had a dose- and time-dependent inhibitory effect on the growth of K562 erythroleukemic cells and induced apoptosis. In contrast, purified hypericin had a mild cytotoxic effect on cell growth and no effect on the induction of apoptotic cell death (27). *Hypericum hircinum*, from the same genus as HP, is a plant with antiproliferative and antioxidant effects on tumor cells such as A431 human squamous carcinoma, PC human prostatic adenocarcinoma, T98G human GBM, and B16-F1 mouse melanoma cells. However, its oil components are different from those of HP. The essential oil of *Hypericum hircinum* consists of cis-b-guaiene (29.3%), d-selinene (11.3%), isolongifolan-7-a-ol (9.8%), and (E)-caryophyllene (7.2%). Thus, the causative agent that has a toxic effect on tumor cells differs (24). Therefore, we used JWO instead of its extract in this study. Our study results demonstrate that JWO has a cytotoxic effect on GBM cells in a dose-dependent manner, and it exhibits a significant in-

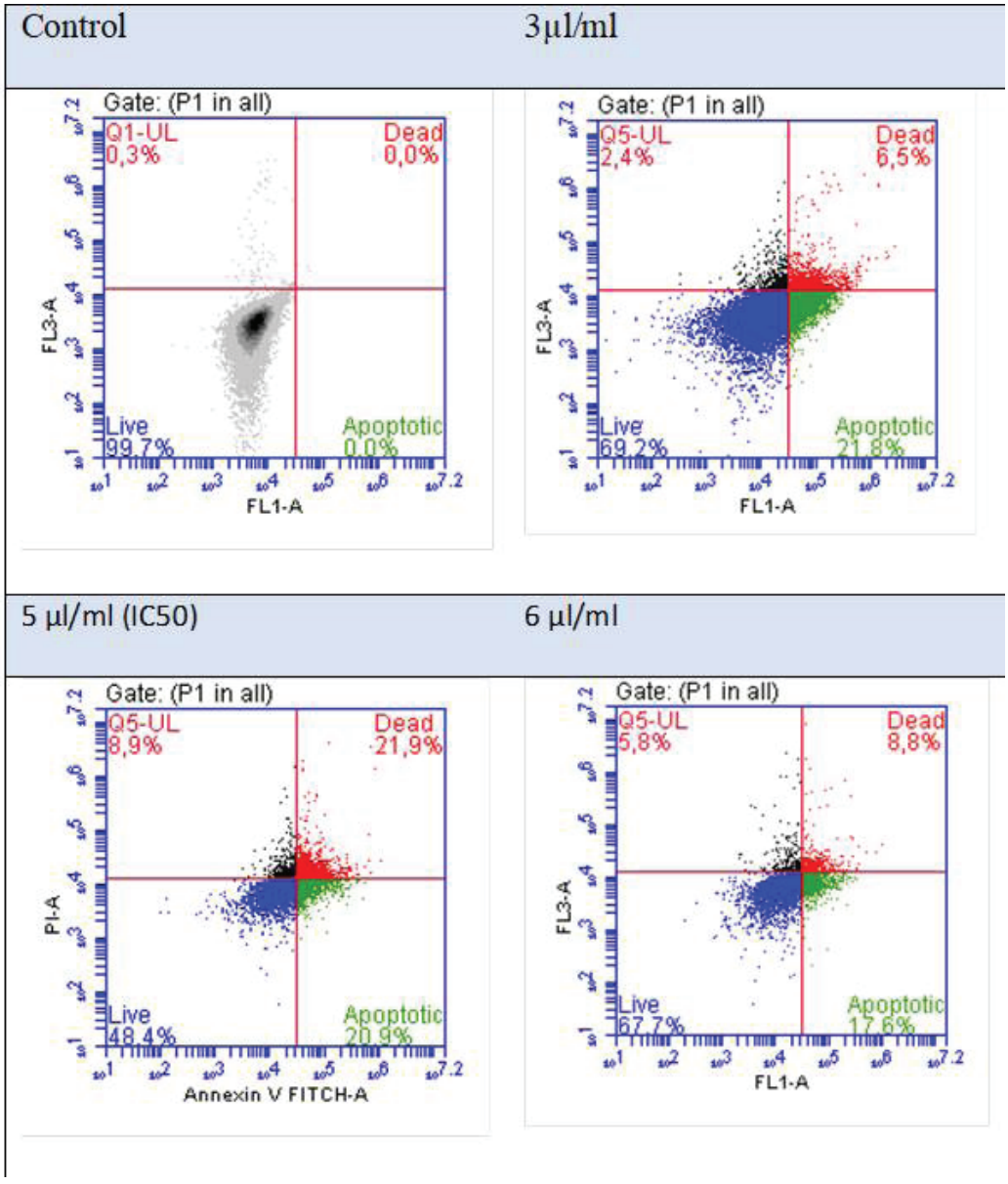

Figure 6: Graphics were demonstrated live-apoptotic /late-apoptotic and necrotic cell death in T98 glioma cells different JWO dose treatments, via Annexin-V/FITC flow cytometry analysis.

hibitory effect on proliferation at a dose of 5.296 ± 0.950 µl/ml after 24 h of incubation.

The metabolic functions of oxidative stress and its potential role in tumor development and progression have been explored for decades. Free radicals, particularly ROS, are considered common mediators of apoptosis. The degree of oxidative stress in a cell determines the balance between the rate of ROS production and the activity of detoxifying scavengers. (30). If the antioxidant defense is insufficient, the damage caused by oxidative stress can lead to the activation of genes responsible for apoptosis. (23). The presence of ROS such as NO can be detected by the enzyme NO synthase. Low NO levels trigger the onset of cancer, and high NO levels cause tumor cell death. (20). Several studies have demonstrated that numerous natural compounds have anticancer properties and cause cell death by increasing oxidative stress in GBM cells (15,20). In our study, JWO was added to T98 GBM cells. We detected increased immunoreactivities of eNOS and iNOS, which was statistically significant, and the result was confirmed by western blot analysis. This increase indicates that JWO mediates cell death through oxidative stress. Detection the amount of antioxidant enzymes, such as glutathione peroxidase-1, catalase, and superoxide dismutase, which play a role in minimizing the effects of oxidative stress, in future studies will support our findings.

In multicellular organisms, there is a homeostatic balance between the number of new cells produced via mitosis and the number of damaged or unnecessary cells that are excreted from the body. Numerous genes are involved in regulating mitosis, detecting cellular abnormalities, and regulating the programmed cell death known as apoptosis. While some of these regulatory genes act to induce mitosis, others inhibit mitosis-induced apoptosis or other forms of programmed cell death, such as pyroptosis or autophagy. (3). Therapeutic resistance of GBM may result from the down-regulation of proapoptotic proteins and up-regulation of antiapoptotic proteins. This in turn leads to genetic instability and activation of oncogenes that promote cell survival and resistance to chemotherapy, radiotherapy and immunotherapy (34). The intrinsic or mitochondrial pathway of apoptosis occurs via the induction of Bcl-2-associated X protein (Bax), a proapoptotic molecule, and the decrease in mitochondrial membrane potential. However, the antiapoptotic proteins, such as Bcl-2, are inhibited (43). Hsu FT et al. investigated the mechanism by which hyperforin, an extract of JWO, activates apoptosis in GBM (10). They identified that hyperforin induces apoptotic cascades and prevents antiapoptotic-related protein expression in GBM. They also stated that the hyperforin-mediated antiapoptotic potential in GBM is associated with the inactivation of EGFR/extracellular signal-regulated kinases/nuclear factor kappa-light chain-enhancer activated B cell signaling (10). Another study demonstrated the proapoptotic effect of HP-derived hypericin in the U87 GBM cell line. Annexin V/PI analysis revealed apoptosis in GBM cells after the administration of hypericin. Furthermore, the signaling pathways, including phosphatidylinositol-3-kinase/Akt, mitogen-activated protein kinase, tumor necrosis factor, and hypoxia-inducible factor-1 pathways, which play an important role in cell survival and proliferation, are down-regulated after hypericin administration (6). We used TUNEL staining and annexin V/PI assay to determine the apoptosis rate. Our study demonstrated that JWO produces an apoptotic effect in a dose-dependent manner on GBM cells. The differences between JWO-treated and control groups were statistically significant. Additionally, the annexin V/PI analysis results supported the existence of apoptosis. These methods demonstrate cells that have reached the final stage of apoptosis. It would be clearer if we can identify whether apoptosis occurs via the extrinsic or intrinsic pathway.

Autophagy is considered a typical type of programmed cell death and depends on lysosomal degradation of cellular organelles and protein recycling. Autophagic cells often differ from the typical apoptotic cells. Autophagic cells almost always contains multilayered or bilayered intracellular membrane structures enclosing cytoplasmic material or subcellular organelles (4,12). These structures, called autophagosomes, form in response to nutrient starvation or oxidative stress. Autophagosomes cause the formation of fatty acids, amino acids and ATP, which ensures cell survival and homeostasis (4,12). Similar to apoptosis, autophagy can be triggered by several factors. High levels of ROS within the cell can also cause autophagy. In cancer cells, the mitochondrial structure changes and causes abnormal molecular mechanisms. ROS are produced during aerobic glycolysis, and the excessive production and accumulation of ROS causes cellular death. Wen et al. demonstrated autophagy via mitochondria in GBM cells using isoaaptamine. Isoaaptamine causes the overproduction of ROS in GBM and a decrease in the levels of superoxide dismutase-1 and catalase, which play a role in oxidative stress control, in the cytoplasm. The expression of LC3B (LC3-II) and p62 increase during autophagy and are markers of autophagy. Agents such as isoaaptamine increase ROS production, causing cancer cell death via apoptosis or autophagy under the effect of oxidative stress (36). Activation of the autophagy process can be achieved by mTORC1 inhibition. The inhibition of mTORC1 is based on the inhibition of receptor tyrosine kinase or rapamycin. This activates ATG protein complexes cascade that begins with the activation of PI3KC3-C1 and ULK1 complexes as well as phagophore formation (31). One of the most commonly used markers for monitoring autophagy is membrane-bound lipidated LC3 (LC3-II), which is formed by the lipidation of cytosolic LC3 (LC3-I). Increased LC3 level is indicative of high autophagy levels (4,31). In addition to LC3 levels, MDC staining of autophagic vesicles can be used to detect autophagy (39,40).

Although there are several studies that have demonstrated that JWO induces apoptosis in breast cancer and non-small cell lung cancer via autophagy (5,41), we did not find any study reporting its autophagic effect in GBM. In this study, compared to in the control group, the autophagy markers such as LC3 level and MDC staining were significantly positive within the JWO-treated groups. The increase in LC3-I expression was confirmed by western blotting analysis. The LC3-II level was lower. The higher concentrations of JWO could have suppressed the lipidation of LC3-I, which may explain why only the presence of LC could be detected. The detection of LC3-I indicates that JWO induced apoptosis in GBM cells via autophagy activation.

CONCLUSION

JWO appears to be an effective treatment option for GBM. It induces apoptosis via oxidative stress in addition to affecting autophagy. The use of JWO in combination with other treatment options may increase the efficacy of standard treatments. However, further studies on JWO should be undertaken.

Declarations

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Availability of data and materials: The datasets generated and/or analyzed during the current study are available from the corresponding author by reasonable request.

Disclosure: The authors declare no competing financial interests.

AUTHORSHIP CONTRIBUTION

Study conception and design: MM, UUU
Data collection: MM, MIT
Analysis and interpretation of results: FC, BG, MIT
Draft manuscript preparation: MM,UUU, IA, EK
Critical revision of the article: MM, BG, MIT
Other (study supervision, fundings, materials, etc...): MB, MG, EK
All authors (MM, UUU, FC, IA, EK, MB, BG, MK, MIT) reviewed the results and approved the final version of the manuscript.

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