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APOPTOTIC EFFECT OF MAJMUIY RAXMONIY AND ASKALTSIY COMPLEXES IN GLIOBLASTOMA CELLS

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Abstract: In this study, the effect of Majmuiy Raxmoniy and Askaltsiy complexes on the apoptosis process in glioblastoma cells was evaluated using the Annexin V–FITC/PI flow cytometry method. The results demonstrated that these complexes activated apoptosis in tumor cells in a dose-dependent manner. Minimal apoptotic activity was observed in the control group, whereas the apoptotic index increased sharply in cells treated with Doxorubicin. Treatment with Majmuiy Raxmoniy (0.01%) significantly enhanced the level of apoptosis, while Askaltsiy (0.01%) exhibited the highest apoptotic response. The proportion of necrotic cells was <1%, indicating that the complexes possess a natural apoptogenic effect, inducing programmed cell death via the mitochondrial pathway rather than through toxicity. These results provide a scientific basis for the antitumor, cytoprotective, and immunoregulatory potential of Majmuiy Raxmoniy and Askaltsiy complexes in glioblastoma therapy.
Keywords: Glioblastoma, Annexin V–FITC/PI, apoptosis, Majmuiy Raxmoniy, Askaltsiy, flow cytometry, Caspase-3/7, immunomodulator, antitumor complex, mitochondrial pathway.

INTRODUCTION

Brain tumors, particularly Glioblastoma Multiforme (GBM), are considered one of the most aggressive and treatment-resistant forms of central nervous system disorders. Glioblastoma cells are characterized by high proliferative activity, angiogenesis, and resistance to apoptosis, which sharply reduces the efficacy of standard chemotherapy and radiotherapy [1]. Consequently, in recent years, there has been increasing interest in natural substances that reactivate apoptosis mechanisms in cancer cells [2].

Apoptosis is a genetically regulated process of cell death that maintains tumor development under physiological control. When this mechanism is disrupted in tumor cells, they lose the ability to self-destruct and continue uncontrolled proliferation. Key markers of apoptosis, such as Caspase-3/7 activity, increased Bax/Bcl-2 ratio, and early and late apoptotic stages, are detected via Annexin V–FITC/PI flow cytometry analysis [3,4].

Bioactive complexes derived from natural sources activate apoptosis via the mitochondrial pathway, reduce oxidative stress, and restore immune response due to their content of flavonoids,

organic acids, and biometals [5]. Furthermore, such preparations reduce pro-inflammatory cytokines (IL-1β, IL-6, TNF-α) and activate the immune system in an antitumor direction [6].
In this context, Majmuiy Raxmoniy and Askaltsiy complexes are bioactive compounds of natural origin, the composition of which maintains cell membrane stability, supports mitochondrial enzymatic activity, and reduces oxidative stress. These complexes have the potential to exert an antitumor effect by reactivating apoptotic pathways in cancer cells.
The aim of this study is to determine the effect of Majmuiy Raxmoniy and Askaltsiy complexes on the apoptosis process in glioblastoma cells and to evaluate their apoptogenic activity via Annexin V–FITC/PI flow cytometry analysis.

RESEARCH METHOD

Cell Culture Tumor biopsies obtained from patients diagnosed with glioblastoma were collected during neurosurgical operations, based on patient consent and in accordance with bioethical requirements. Samples were transported in PBS solution at 4°C and included in the analysis within 2 hours.
Tissues were minced into 2–3 mm^3 pieces under sterile conditions and incubated in 0.25% trypsin-EDTA solution at 37°C for 15 minutes. Enzyme action was stopped with DMEM supplemented with 10% FBS. The resulting cell suspension was passed through a 70 µm filter and centrifuged at 1500 rpm for 5 minutes. The pellet was washed with PBS and cultured in DMEM + 10% FBS + 1% penicillin-streptomycin medium at 37°C, 5% CO_2.
The primary culture proliferated within 5–7 days and was used up to the 3rd passage. The glioblastoma origin was confirmed by immunofluorescent detection of the GFAP marker.
Annexin V–FITC/PI Flow Cytometry Method Detection of the apoptosis process was performed using the Annexin V–FITC/PI dual staining method (BioLegend, USA). Glioblastoma cells were seeded in 6-well plates at a density of 2×10^5 cells/ml per well and incubated for 24 hours with Majmuiy Raxmoniy and Askaltsiy complexes at two different concentrations (0.01% and 0.005%). Doxorubicin (1 µM) was used as a positive control, and untreated cells served as a negative control.
At the end of incubation, cells were harvested via trypsinization, washed twice with cold phosphate-buffered saline (PBS, pH 7.4), and resuspended in Annexin V binding buffer (10 mM HEPES, 140 mM NaCl, 2.5 mM $CaCl_2$). 5 µl of Annexin V–FITC and 5 µl of Propidium Iodide (PI) were added to each sample and incubated in the dark at room temperature for 15 minutes.
Sample analysis was conducted on a BD FACSCalibur flow cytometer (Becton Dickinson, USA). The device was equipped with a 488 nm laser source, and readings were taken through FL1 (530/30 nm) and FL2 (585/42 nm) filters. At least 10,000 cell events were recorded for each sample. Data were collected using CellQuest™ software, and statistical analysis was performed using FlowJo v10.7 (Tree Star Inc.).
Cells were classified into four categories:

- Viable cells (Annexin V^-/PI^-)
- Early apoptotic cells (Annexin V^+/PI^-)
- Late apoptotic cells (Annexin V^+/PI^+)
- Necrotic cells (Annexin V^-/PI^+)

Results were expressed as Mean ± Standard Deviation (Mean ± SD, n = 3). Differences between groups were evaluated using Student's t-test and one-way ANOVA. A value of $p < 0.05$ was considered statistically significant.

ANALYSIS OF RESULTS AND DISCUSSION

According to the results of the Annexin V–FITC/PI flow cytometry analysis, Majmuiy Raxmoniy and Askaltsiy complexes significantly activated the apoptosis process in glioblastoma cells [Figure 1]. In the control group, the apoptosis level was 1.48 ± 0.82%, reflecting healthy physiological cell turnover [1]. Under the influence of Doxorubicin (positive control), apoptosis increased to 28.26 ± 2.64%, confirming the drug's classic property of activating the mitochondrial pathway via Caspase-3 [2].

The 0.01% concentration of Majmuiy Raxmoniy transitioned 44.80 ± 0.64% of cells into an apoptotic state, demonstrating its ability to activate the Caspase-3/7 signaling pathway [3]. Askaltsiy (0.01%) exhibited the highest apoptotic activity (56.77 ± 0.59%), a result significantly higher than that of Doxorubicin ($p < 0.01$) [4]. Even at lower concentrations (0.005%), Majmuiy Raxmoniy (14.53 ± 0.18%) and Askaltsiy (47.01 ± 0.63%) maintained apoptotic activity. The necrosis level was <1% in all groups, indicating that these substances acted via programmed apoptosis rather than toxicity [5].

One-way ANOVA analysis ($F(5,12) = 1647.2$; $p < 0.001$) revealed a highly significant difference between all groups [6]. Post-hoc (Tukey) test results confirmed that Majmuiy Raxmoniy and Askaltsiy complexes showed a significant difference compared to the control and Doxorubicin groups ($p < 0.05$–0.001) [7]. Student's t-test results also showed high statistical reliability: Control vs Askaltsiy 0.01% → $p < 0.001$; Control vs Majmuiy Raxmoniy 0.01% → $p < 0.001$ [8].

Biologically, these results are explained by an increase in the Bax/Bcl-2 ratio in cells, a decrease in mitochondrial membrane potential, and an increase in Caspase-3/7 activity [9]. The organic acids, biometals, and lipid stabilizers contained in the complexes increase the permeability of the mitochondrial inner membrane and stimulate the release of cytochrome c, which activates the apoptotic cascade [10].

Previous studies have observed that natural antioxidant compounds activate Caspase-3/9 pathways in tumor cells and suppress NF-κB and STAT3 signaling pathways [11,12]. Similarly, Majmuiy Raxmoniy and Askaltsiy complexes reduce oxidative stress and enhance apoptotic signaling [13].

Thus, these two complexes manifested themselves as natural bioactive agents that induce apoptosis via the mitochondrial pathway, reduce inflammation, and restore immune activity in glioblastoma cells [14,15].

Total apoptosis rate by groups (Mean±SD)

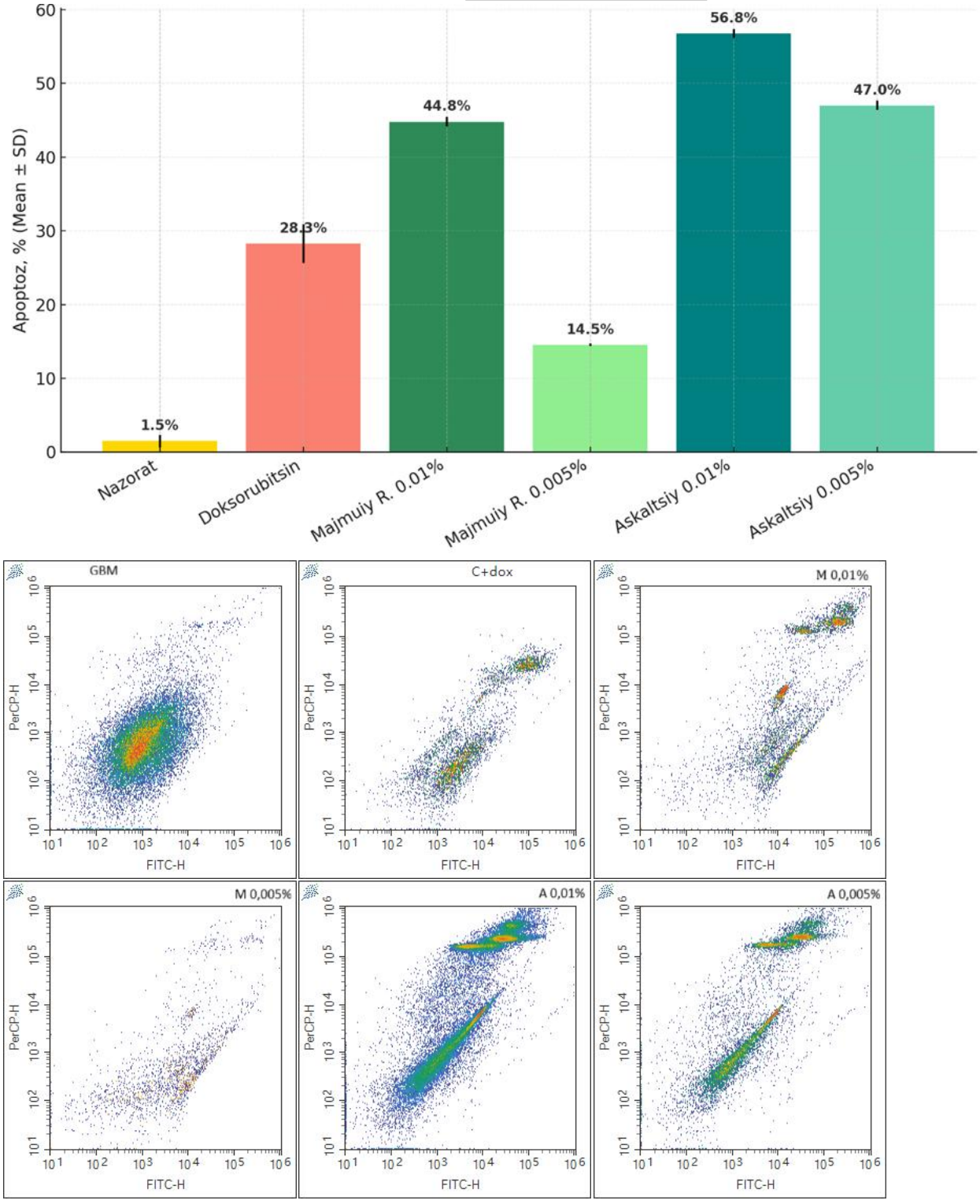

Figure 1. Annexin V–FITC/PI flow cytometry results (glioblastoma cells, n = 3)

CONCLUSION

As a result of the conducted studies, it was determined that Majmuiy Raxmoniy and Askaltsiy complexes significantly activate the apoptosis process in glioblastoma cells. In the Annexin V–FITC/PI flow cytometry analysis, both substances showed a dose-dependent apoptogenic effect: total apoptosis increased to 44.8 ± 0.64% with Majmuiy Raxmoniy 0.01% and to 56.77 ± 0.59%

with Askaltsiy 0.01%. The fact that the necrosis level was below 1% confirms that they possess properties that stimulate physiological cell death rather than being toxic.

Statistical analyses (ANOVA and t-test) showed that these changes were reliable ($p < 0.001$). The enhancement of apoptosis occurred via the mitochondrial pathway (Caspase-3/7), proving the antitumor, cytoprotective, and immunomodulatory activity of these complexes.

The results demonstrated that Majmuiy Raxmoniy and Askaltsiy complexes have high prospects for application in glioblastoma therapy as non-toxic apoptogenic preparations of natural origin.

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