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# Exploring the Therapeutic Effects of *Radix astragalus* on Glioblastoma Multiforme Cell Culture

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## ABSTRACT

**Objective:** This study investigated the effectiveness of *Radix astragalus*, a traditional herbal remedy, in combating Glioblastoma Multiforme (GBM), a highly aggressive brain tumor. Current therapies for GBM are limited in their efficacy, highlighting the urgent need for innovative treatment strategies.

**Methods:** Our research employed advanced methods to assess the anti-tumor properties of *Radix astragalus* extracts on GBM cell lines in vitro. We evaluated cell viability and proliferation using MTT and LDH assays. Furthermore, we analyzed the oxidative stress levels within GBM cells by measuring Total Antioxidant Capacity (TAC), Total Oxidant Status (TOS), and malondialdehyde (MDA) values. These comprehensive techniques provided valuable insights into the intricate interactions between GBM cells and the extracts.

**Results:** The results were highly promising. *Radix astragalus* extracts significantly reduced GBM cell viability, demonstrating a potent antitumorigenic effect. Additionally, the extracts effectively countered the oxidative stress within GBM cells, a key factor promoting tumor growth and progression. Moreover, antioxidant assays revealed enhanced antioxidant activity in GBM cells treated with *Radix astragalus* compared to untreated controls.

**Conclusion:** These findings unveil the remarkable potential of *Radix astragalus* as a novel therapeutic approach for GBM treatment. The extract's ability to target both GBM cell viability and oxidative stress offers a promising avenue for future cancer research. Further investigations are warranted to explore the full potential of *Radix astragalus* in developing effective therapies for this challenging form of brain cancer.

**Keywords:** GBM, *Radix astragalus*, Anticancer

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## Introduction

Glioblastoma multiforme (GBM), often referred to as glioblastoma, is the most common primary malignant brain tumor with an incidence of approximately 3.2 per 100,000 people per year in the United States. It is thought to have its genesis in neuroglial stem or progenitor cells (Le Rhun et al., 2019). Despite the utilization of a range of sophisticated therapeutic modalities, including surgical intervention, radiotherapy, chemotherapy, targeted therapy and supportive care, the median survival time of the majority of patients diagnosed with glioblastoma multiforme (GBM) remains below 15 months, with a 5-year survival rate of less than 3% (Fisher & Adamson, 2021). Despite the availability of numerous treatment options, GBM remains a formidable challenge due to its highly invasive nature and the limited efficacy of current therapies. Although surgical intervention followed by adjuvant radiation and chemotherapy can prolong survival, the overall prognosis for patients with GBM remains dismal, with most patients experiencing recurrence and disease progression. Consequently, there is an immediate requirement to identify novel therapeutic targets and develop more effective treatment strategies in order to enhance patient outcomes. The use of temozolomide, an orally available alkylating agent, has demonstrated some benefits in the treatment of GBM (Wang et al., 2023). However, the emergence of chemoresistance has limited its effectiveness.

The development of new drugs with alternative mechanisms of action is critical to overcome chemoresistance and improve survival outcomes. Additionally, targeted therapies that specifically target GBM cells while sparing healthy brain tissue may provide a more effective and less toxic alternative to traditional chemotherapy. It is therefore imperative to identify and develop innovative treatment options for GBM. Herbs have been utilized by humans for at least 60,000 years to treat various diseases, indicating their diverse pharmacological properties, including anti-cancer activity (Wang et al., 2020). Herbal formulations can act on multiple targets through their various constituents, thereby playing a crucial role in important biological processes and exhibiting therapeutic effects during the progression of diseases (Yang et al., 2018). One such traditional Chinese medicine is *Radix astragalus* (RA), several studies have reported the antitumor activity of RA. It has been demonstrated that RA extracts are capable of inhibiting the growth of lung adenocarcinoma cells (Wei et al., 2020). Zhang et al. demonstrated that the laryngeal carcinoma SCC15 cell line exhibited concentration-dependent antitumor activity following treatment with total RA glucosides (Wang et al., 2020). The multi-targeted action and diverse constituents of RA make it an attractive option for treating various diseases, including cancer (Guo et al., 2019). The antitumor activity of RA may be attributed to its ability to regulate key signaling pathways involved in cancer development and progression. Thus, further investigation of the mechanisms underlying RA's antitumor activity is warranted to fully understand its therapeutic potential in cancer treatment.

Considering the potential therapeutic benefits of RA in cancer treatment, we aimed to investigate its effect on GBM and elucidate its underlying molecular mechanism. The objective of this study was to provide a scientific foundation for future research on the potential therapeutic use of RA in the treatment of GBM.

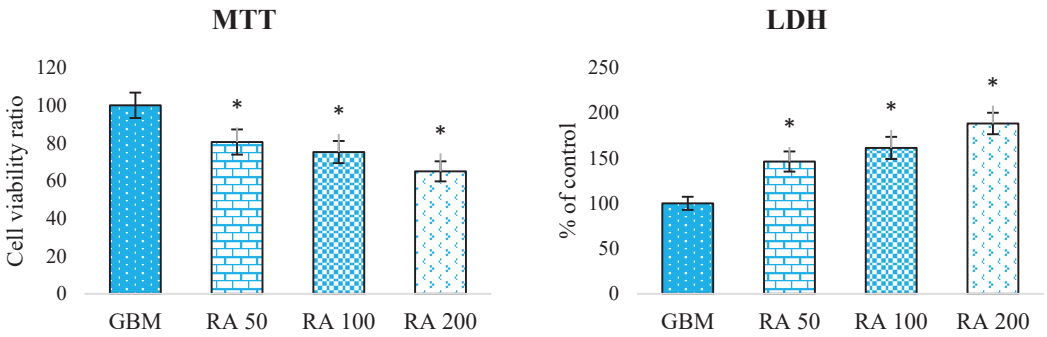


**Figure 1.** Cell viability test results.

## Methods

### Cell culture

GBM cells were used in the studies for anticancer activity. The cells were incubated in RPMI 1640 medium containing penicillin (100 units/mL), streptomycin (100

µg/mL), fetal bovine serum (10%), and L-glutamine (2.5 mM) at 37 °C with a humidified atmosphere of 5% CO2 and 95% air. The culture medium was replaced on three occasions each week.

**MTT assay**

In order to assess the cytotoxicity of RA, the MTT assay was conducted in triplicate on GBM cells. The cells were cultivated in 96-well plates with a capacity of 5 × 103 cells per well. They were subjected to a 24-hour treatment with RA at doses of 50 µg/mL, 100 µg/mL, and 200 µg/mL. After that, each well received 20 µL of MTT solution, and the plate was covered with foil and incubated at 37°C for two hours. After that, the MTT solution was discarded, and 150 µL of dimethylsulfoxide (DMSO) was used to dissolve the blue-violet formazan that had developed in the wells. The plate was incubated under light-deprived conditions for a duration of 30 minutes, following which the optical density values were measured at a wavelength of 570 nm employing an Elisa Reader (Ferah Okkay et al., 2021). Untreated cells were employed as a negative control for assessing cytotoxicity, indicative of 100% mitochondrial function.

**Lactic dehydrogenase release**

To evaluate cell death resulting from membrane disruption, the release of lactic dehydrogenase (LDH) was assessed. The LDH activity was determined through spectrophotometry analysis in the culture medium at a wavelength of 340 nm, focusing on the reduction of nicotinamide adenine dinucleotide (NAD) (Birdal et al., 2024). The percentage of LDH release was computed as the proportion of the total quantity found in both the cellular lysate and the culture medium. The findings were presented in terms of the percentage of LDH that was released.

**Measurement of oxidative burden**

The assessment of antioxidative capacity within cell culture supernatants was conducted through the employment of automated methodologies measuring total antioxidant capacity (TAC) and total oxidant status (TOS) utilizing commercially available kits provided by Rel Assay Diagnostics®. In order to ascertain the TAC levels, the specimens underwent treatment with Reagent 1 followed by the measurement of absorbance at 660 nm (Okkay et al., 2021). After the addition of Reagent 2 and incubation, the absorbance was read again to measure the reduction of ABTS radicals. The TOS levels were assessed through the treatment of the samples with Reagent 1 followed by the measurement of absorbance at 530 nm. The introduction of Reagent 2 resulted in a chromogenic reaction within the solution, and the intensity of the color was quantified using spectrophotometric method. Both TAC and TOS analyses were performed in 48-well plates using kit standards

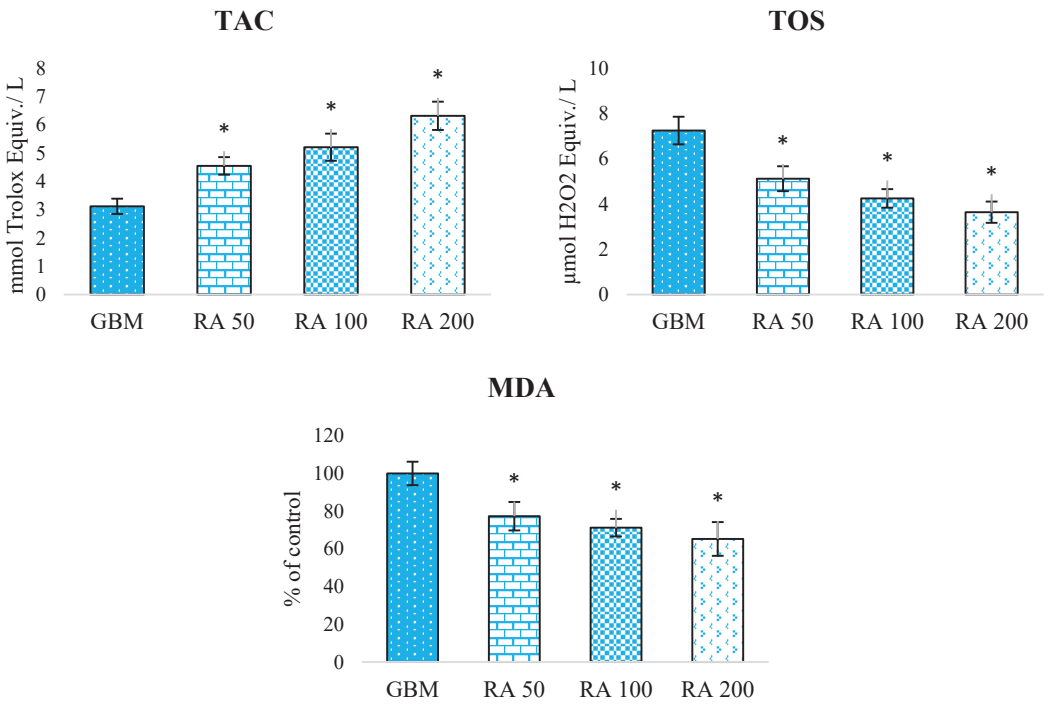


**Figure 2.** Oxidative stress analysis results.

(Trolox 1 mmol/L and H202 10 µmol/L, respectively) and dH2O as controls. The results were read in a plate reader and interpreted according to the manufacturer's instructions.

### Statistical analysis

Using IBM SPSS 22.0, one-way analysis of variance (ANOVA) with post hoc Tukey's test ($p < 0.05$) was used for all analyses. The data was shown as mean ± SD.

## Results

### Cell viability

The colorimetric MTT test was employed to determine the percentage of GBM cell line viability. To assess the effectiveness of RA, GBM cells were treated with various concentrations of RA for 24 hours. Results are presented in Fig. 1. The optimal concentration and duration of RA treatments were found to be 200 µg/ml, which significantly inhibited cell growth in a dose- and time-dependent manner compared to the control group ($P < 0.05$).

### Measurement of oxidative burden

The study found that the TAC levels in the RA group were significantly higher ($p < 0.05$) compared to the GBM group. This means that the RA group had a higher overall antioxidant capacity, which may indicate a lower oxidative burden. On the other hand, the TOS and MDA values in the RA group were significantly lower ($p < 0.05$) compared to the GBM group. This suggests that the RA group had a lower overall level of oxidative stress.

To visually represent these findings, the study included a figure (Fig. 2) which presumably shows the TAC, TOS and MDA levels for both groups. Based on the results, we can infer that the TAC levels for the RA group are higher than the GBM group, while the TOS and MDA levels for the RA group are lower than the GBM group.

## Discussion

GBM is a remarkably aggressive and fatal primary neoplasm of the brain, demonstrating resistance towards traditional therapeutic modalities including chemotherapy and radiotherapy. (Davis, 2016). Hence, it is imperative to discern novel and efficacious therapeutic strategies for GBM. This investigation delves into the plausible therapeutic impacts of RA on GBM cell populations. The outcomes of our research indicate that RA significantly diminishes the survival rate of GBM cells, as assessed through MTT and LDH analyses. This suggests that RA may have cytotoxic effects on GBM cells, which could be explored further in future studies.

Moreover, it was noted that RA resulted in an elevation of the overall antioxidant potential while concurrently reducing the overall oxidative status in GBM cells. These findings suggest that RA may have anti-oxidant properties that could potentially counteract the oxidative stress that is often observed in cancer cells. Our findings align with prior research studies that have documented the anti-cancer properties of RA across a range of cancer types such as breast, gastric, and lung cancer (Li et al., 2020; Wu et al., 2018). Previous literature indicates that the mechanisms responsible for the anti-cancer effects of RA are intricate, involving the regulation of diverse signaling cascades, including the PI3K/Akt/mTOR pathway, the NF-κB pathway, and the JAK/STAT pathway (Zhou et al., 2018).

Despite the encouraging findings of our research, it is important to acknowledge certain constraints. Initially, the scope of our investigation was limited to examining the impact of RA on GBM cells in a controlled laboratory setting, necessitating additional research to validate its therapeutic efficacy in live subjects. Second, the mechanisms underlying the cytotoxic and anti-oxidant effects of RA on GBM cells are still unclear and require further investigation.

## Conclusion

In conclusion, our study provides evidence that RA has potential therapeutic effects on GBM cells. The cytotoxic and anti-oxidant effects of RA on GBM cells suggest that it may have a role as an adjunctive therapy for GBM. Future research endeavors should focus on elucidating the mechanisms that underlie the anti-tumor properties of RA, as well as exploring its potential as a therapeutic intervention for GBM.

A part of this study was presented as an oral presentation at the **3rd International Black Sea Modern Scientific Research Congress**.

Ethics Committee Approval: Since the cell line was studied in vitro, an ethics committee decision is not required.
Informed Consent: Since it is an in vitro study, participant consent is not required.
Peer-review: Externally peer-reviewed.
Author Contributions: Concept - UO, IFO; Design- UO, IFO; Supervision- UO, IFO; Resources- UO, IFO; Data Collection and/or Processing- UO, IFO; Analysis and/or Interpretation- UO, IFO; Literature Search- UO, IFO; Writing Manuscript- UO, IFO; Critical

Review- UO, IFO.

**Conflict of Interest:** The authors have no conflicts of interest to declare.

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