different effects on the viability of SH-SY5Y cells. Furthermore, an escalation in the concentration of MGO does not induce toxicity in SH-SY5Y cells until a certain threshold is exceeded.

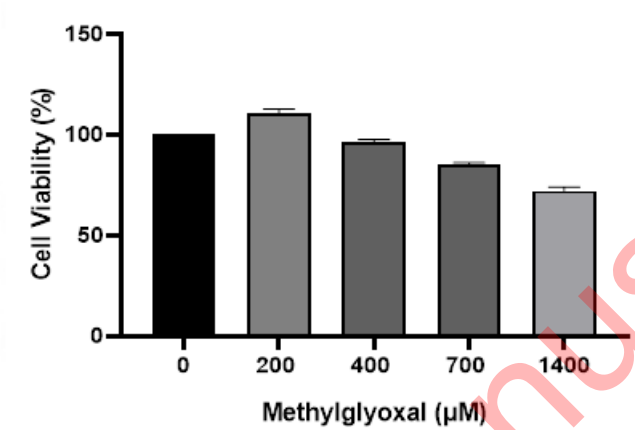

Figure 1. Effects of MGO on SH-SY5Y cell viability. MTT assay was used to determine the viability of SH-SY5Y cells after 24 h of stimulation with various concentrations of MGO. Data are presented as the mean±SD of triplicate experiments.

miR-125b and miR-107 gene expressions

miRNAs may be involved in the pathogenesis of AD by affecting different signaling pathways. Therefore, we investigated the effects of MGO on the expression of miR-125b, miR-107 and genes related to oxidative stress signaling in SH-SY5Y cells. Based on our results, MGO increased and decreased the expression levels of miR-125b and mir-107 genes, respectively, in SH-SY5Y cells ($p<0.05$) (Figure 2). It has been demonstrated that the expression of miR-107 is significantly decreased in patients with AD .[22] The results of our study suggest that MGO may decrease the expression of miR-107. Several miRNAs, such as miR-9, miR-124, miR-125b, and miR-132, are specifically expressed in the central nervous system.[23] Moreover, their dysregulation has been correlated with neurodegenerative diseases, including AD. Through SphK1, miR-125b regulates inflammatory factors and oxidative stress, thereby controlling neuronal growth and apoptosis.[24] miR-125b is highly expressed in AD and causes cognitive deficits[12] is associated with high levels of miR-125b expression and cognitive deficits .[13] This may increase the expression of miR-125b. It is known that the miR-125b gene plays a role in AD and can be stimulated by MGO. Therefore, analysis of miRNAs and genes associated with oxidative stress signaling pathways may contribute to a better understanding of AD pathogenesis.

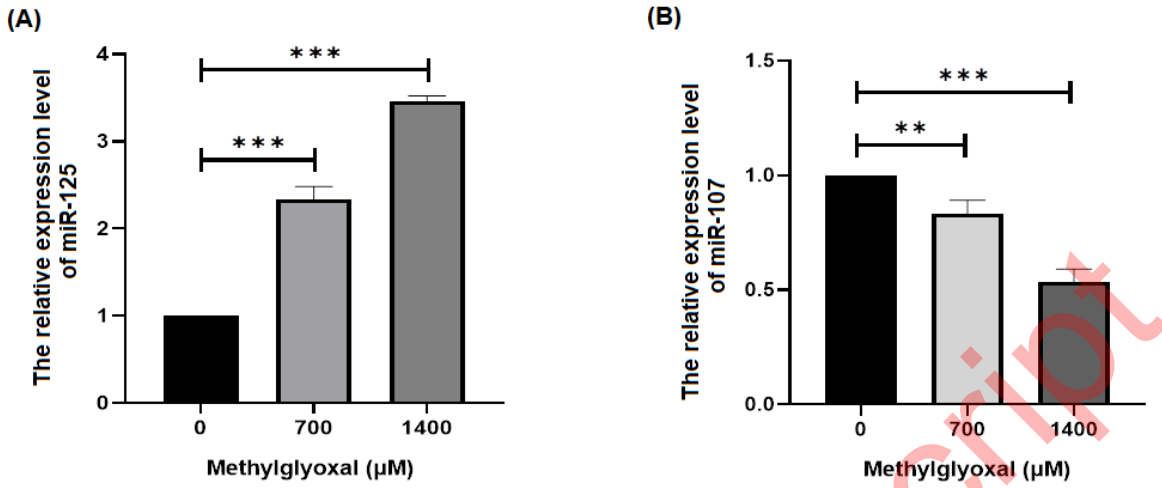

Figure 2. Differences in miR-125b and miR-107 expression between different doses of MGO in SH-SY5Y cells. This figure shows the expression of miR-125b and miR-107 in the SH-SY5Y cell line. (A) Rate of change in miR-125b expression between the groups (B). Rate of differences in miR-107 expression between the groups. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$.

MGO changed the expression of genes related to the Oxidative Stress pathway

The progression of AD has been linked to oxidative stress. ROS can modify lipids, DNA, RNA, and proteins in the brain.[25] The generation of ROS and reactive nitrogen species (RNS) can be attributed to both exogenous and endogenous sources.[26] Due to their high oxygen consumption, lipid content, and lack of antioxidant enzymes, neuronal cells are susceptible to oxidative stress.[27] Several studies have shown that oxidative damage to macromolecules and the accumulation of their products increase with time and that the relationship between ROS production and antioxidant activities (the enzymes superoxide dismutase, catalase, and glutathione peroxidase) is disturbed with age.[28-30] Unsaturated fatty acids and iron are abundant in the nervous system. The nervous system is susceptible to oxidative damage due to its high lipid and iron content. Oxidative stress is thought to be a major cause of the pathophysiology of AD.[31, 32] Therefore, we investigated the changes in the expression of genes involved in oxidative stress, which may be important in AD. A PCR array was performed using SH–the SY5Y cells to investigate the effect of MGO on the expression of genes related to oxidative stress signaling. In addition, fold changes expression was determined using web-based RT2-based PCR array analysis (Figure 3). Differences in expression greater than twofold were considered acceptable limits (Table 2).

Our study showed that the expression of genes associated with the oxidative stress signaling pathway, such as CCS, CYBB, CYGB, DHCR24, PRDX3, AKR1C2, and SPINK1, was increased when SH-SY5Y cells were treated with MGO (700 μm). The expression of genes associated with oxidative stress signaling pathway such as AOX1, CCS, CYBB, DUSP1, EPHX2, EPX, FOXM1, GPX3, HSPA1A, MT3, PRDX3, PRDX6, SLC7A11, and SPINK1 increased when target cells were treated with MGO (1400 μm). In our study, MGO increased the expression of oxidative stress pathway genes in SH-SY5Y cells. The results of our study showed that the level of MGO concentration has a different effect on the expression of genes related to the oxidative stress signaling pathway. An increase in the level of MGO may have a greater effect on the expression of genes related to the oxidative stress signaling pathway. Different physiological functions are expressed by miRNAs in different brain regions, which

influence the pathogenesis of AD. Whether miR-107 and miR-125b are gene regulators of oxidative stress metabolism in AD needs to be investigated. The role of other miRNAs may also be investigated. Furthermore, it is understandable that the limitations of cell lines in mimicking AD and the events that occur in AD strengthen the field for detailed investigations in animal models.

Table 2. PCR array analysis of Oxidative Stress pathway-associated genes exposed to different concentrations of MGO compared with the control group.

Gene	MGO (700 µM)		MGO (1400 µM)	
	Fold change*	P-Value	Fold change*	P-Value
AOX1	0.08	0.07	**2.1**	**0.001**
CCS	**2.51**	**0.002**	**3.31**	**0.003**
CYBB	**15.14**	**0.0013**	**93.39**	**0.0001**
CYGB	**4.92**	**0.0027**	0.80	**0.012**
DHCR24	**2.13**	**0.003**	**0.84**	**0.01**
DUSP1	1.40	**0.003**	**5.45**	**0.003**
EPHX2	1.91	**0.01**	**2.69**	**0.001**
EPX	1.44	**0.02**	**2.34**	**0.0003**
FOXM1	1.51	**0.023**	**4.77**	**0.0001**
GPX3	1.92	**0.002**	**5.68**	**0.0001**
HSPA1A	1.29	0.05	**2.24**	**0.001**
MT3	0.97	0.91	**3.62**	**0.001**
PRDX3	**2.55**	**0.003**	**14.98**	**0.0002**
PRDX6	1.04	0.64	**7.54**	**0.0003**
AKR1C2	**2.66**	**0.001**	1.28	**0.02**
SLC7A11	1.85	**0.004**	**2.76**	**0.003**
SPINK1	**2.22**	**0.03**	**10.82**	**0.003**

* A fold change of more than two was considered an acceptable value. Statistical significance was set at $P < 0.05$.

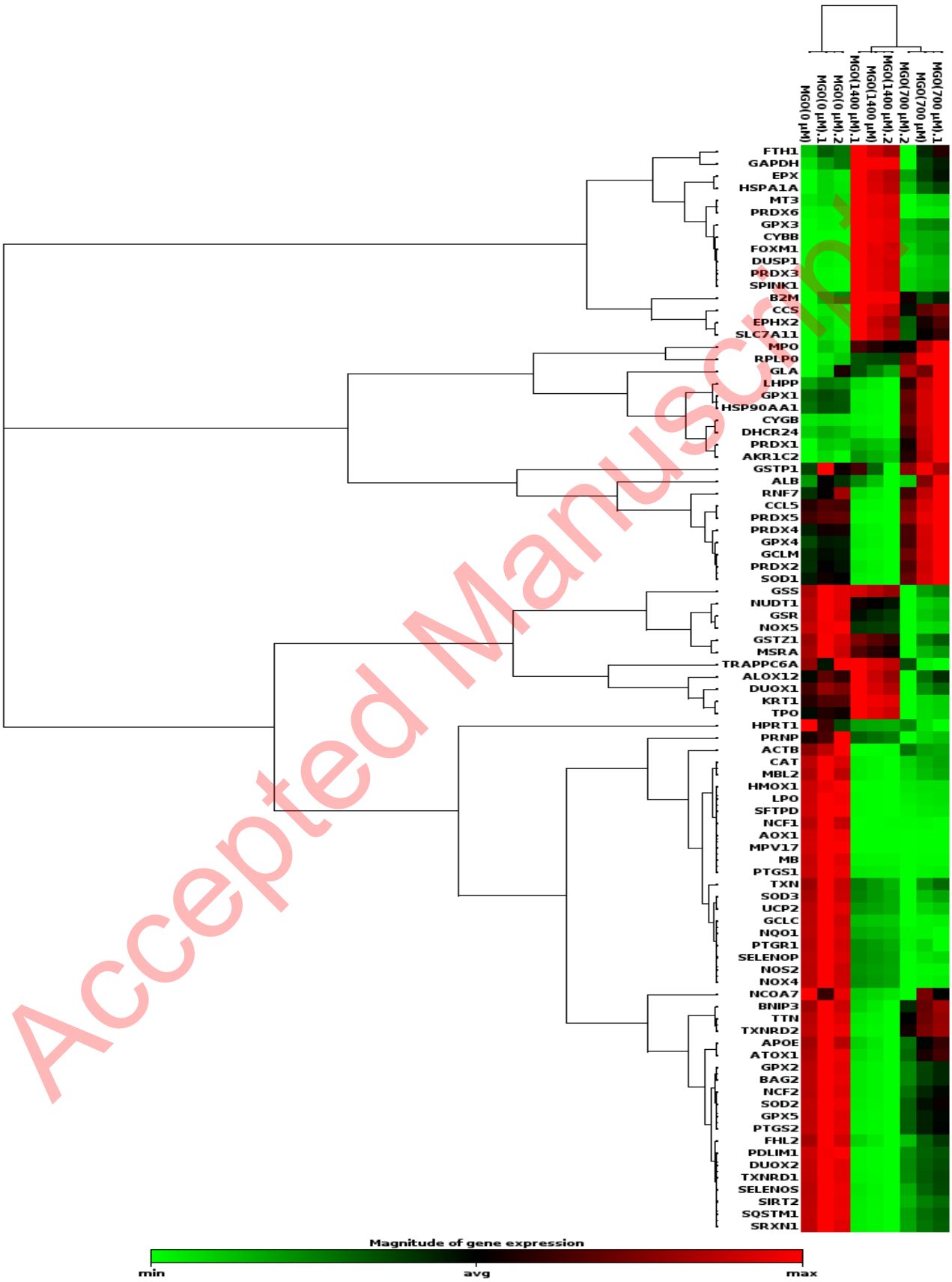

Figure 3. Clustergram analysis of oxidative stress pathway-associated genes after incubation with different concentrations of MGO in SH-SY5Y cells.

4 CONCLUSIONS

Our research examined the effects of MGO on SH-SY5Y neuronal cells by assessing the levels of miR-125b, miR-107 and related genes in the oxidative stress pathway. We found that MGO concentrations up to 700 µM did not adversely affect cell survival. The changes in miR-125b and miR-107 expression in the presence of MGO suggest their involvement in the cellular response to MGO. Furthermore, the expression of certain genes related to oxidative stress was modified by MGO at concentrations of 700 µM and 1400 µM, suggesting a dose-response relationship. These results highlight the importance of exploring the targeting of MGO, miR-125b, and miR-107 as a potential therapeutic avenue for treating AD or alleviating its severe symptoms. Further research is needed to clarify the exact molecular interactions responsible for these observed effects and to confirm the viability of targeting MGO and miRNA regulation as a therapeutic intervention. Future research may lead to breakthroughs in the development of targeted treatments to combat oxidative stress and its role in AD.

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Author contributions

Behrouz Shademan and **Alireza Nourazarian** were involved in the study design; **Behrouz Shademan** and **Hadi Yousefi** were involved in manuscript writing; **Hadi Yousefi** contributed to drawing the manuscript tables; and **Alireza Nourazarian** gave consent for the final version of the manuscript. **Behrouz Shademan, Hadi Yousefi,** and **Alireza Nourazarian** contributed to data analysis.

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Availability of data and materials

The data and materials used in this study are available.

Ethical Approval

The study protocol was approved by the Ethics Committee of Khoy University of Medical Sciences (IR.KHOY.REC.1400.011).

Conflict of interest statement

The authors declare no conflict of interest.

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