Original Article

Biphasic Neuroprotection from Depression-induced Oxidative Stress

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ABSTRACT

Objective: To create a two-hit cellular model of oxidative stress in depression and to assess the neuroprotective effects of fluoxetine and 808 nm photobiomodulation on cell survival, interleukin-6, glutathione and major depressive disorder depending on their doses.

Study Design: Experimental study

Place and Duration of Study: This study was conducted at the University of Babylon, Iraq from 15th January 2026 to 30th April 2026.

Methods: The SH-SY5Y cells were subjected to corticosterone (100 μM) and H_2O_2 (100 μM) treatment for 24 hours, after which we used fluoxetine (0.1-50 μM) and photobiomodulation (2.5-60 J/cm^2).

Results: Exposure to dual-hits resulted in decreased viability up to 59.6% ($p<0.0001$). The fluoxetine demonstrated hormesis effect: cell viability reached 102.4% when fluoxetine was used at a concentration of 1 μM. Moreover, there was statistically significant decrease in interleukin-6 (1.42 vs. 3.97 pg/mL), major depressive disorder (8.3 vs. 12.0 mg/dL), and increased levels of glutathione (44.1 vs. 29.2 μM). The photobiomodulation applied at 10 J/cm^2 restored viability to 107.1% ($p<0.0001$) and had similar effect.

Conclusion: Both fluoxetine and 808 nm photobiomodulation confer biphasic neuroprotection in a clinically relevant dual-hit depression model, establishing dose thresholds for future synergy investigations.

Key Words: Major depressive disorder, Corticosterone, Oxidative stress, Fluoxetine, Photobiomodulation, Hormesis, Glutathione, Malondialdehyde, Interleukin-6

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INTRODUCTION

A global prevalence of ~280 million suffers from major depressive disorder (MDD); however, less than 50% succeed in achieving a state of remission under conventional treatment, indicating the failure of the monoamine theory.[1,2] Major depressive disorder is currently viewed as a complex disease associated with HPA axis dysfunction, neuroinflammation, oxidative stress, and mitochondrial malfunctioning.[3]

Chronic stress causes high cortisol levels, induces glucocorticoid resistance, triggers inflammation by activating NLRP3 and releasing pro-inflammatory cytokines (IL-6, IL-1β, TNF-α), whereas GR translocation reduces mitochondrial activity, resulting in superoxide production and perpetuation of the oxidative-inflammatory process.[4,5]

The SH-SY5Y neuroblastoma line featuring GR expression, as well as the expression of SERTs and catecholaminergic phenotypes, was selected for modeling those processes.[6,7] The double-hit approach involving corticosterone and H_2O_2 (100 μM concentration) simulating excessive glucocorticoid secretion and oxidative damage causing 40% reduction in cell viability was used. FLX effects on several signaling pathways (BDNF/TrkB/CREB pathway stimulation and NF-κB inhibition) apart from SERTs[8] and 808 nm laser-induced up-regulation of NRF2 were tested.[9] This study establishes optimal dose thresholds for both interventions as a basis for future synergy testing.[10]

METHODS

This experimental study was conducted at University of Babylon, Iraq from 15th January 2026 to 30th April 2026 vide letter No. 4982 dated January 1, 2026. SH-SY5Y cells were maintained in RPMI-1640 supplemented with 10% FBS and 1% penicillin-streptomycin (Gibco) at 37°C, 5% CO_2 Passages below 15 were used throughout.

Dual-hit stress model: Cells were simultaneously exposed to corticosterone (100 μM; Sigma-Aldrich) and freshly prepared H_2O_2 (100 μM; Sigma-Aldrich) in

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2% FBS medium for 24 h. Vehicle controls received 0.1% DMSO.

Fluoxetine treatment, stress induction, cells were treated with fluoxetine hydrochloride (0.1-50 µM; Macklin) for 24 h (0.1% DMSO). Three biological replicates with 4-5 technical replicates (MTT) or 3 (biochemical assays) were performed.

Photobiomodulation, an 808 nm diode laser (Dragon Lasers, China; 50 mW, 0.25 W/cm^2) was applied transcutaneously from below at fluences of 2.5-60 J/cm^2 (10-240 s). A single session was delivered immediately post-stress; thermal effect was negligible ($\Delta T < 0.5°C$).

Cell viability (MTT assay): MTT (0.5 mg/mL; Carl Roth) was incubated for 4 h; formazanwas dissolved in DMSO and absorbance read at 570/630 nm (BioTek ELx800). Viability was expressed as percentage of control.

Lipid peroxidation, MDA was quantified by the TBARS method (absorbance at 535 nm; $\varepsilon = 1.56 \times 10^5$ $M^{-1}cm^{-1}$) and normalized to protein concentration (Bradford assay).

Reduced glutathione was measured colorimetrically using DTNB (412 nm) against a standard curve and normalized to protein content.

Interleukin-6 was quantified in cell supernatants by sandwich ELISA (Elabscience, E-EL-H6156; 450/570 nm) per manufacturer's instructions and normalized to protein concentration.

The data was analyzed through SPSS-25. One-way ANOVA with Dunnett's post hoc test (viability vs control) or Šidák's test (biochemical markers vs. stressed group) A P<0.05 was considered as significant.

RESULTS

H_2O_2 treatment at 50-300 µM for 24 h decreased viability in a dose-dependent manner (IC_{50} = 150-200 µM; Table 1, Fig. 1). CORT treatment from 100 to 500 µM reduced viability in a similar fashion (Table 2, Fig. 2).

Co-treatment with CORT 100 µM and H_2O_2 100 µM led to a reduction in viability to 59.6% (p<0.0001), significantly higher compared to both conditions tested separately (85.6% and 82.9% respectively (Table 3, Fig. 3).

FLX at 0.1-50 µM demonstrated hormetic protective effects. Doses lower than micromolar were ineffective; the first protective effect was achieved only by FLX concentrations higher than 0.7 µM, where viability reached 55.6% (p=0.0416). The maximal protective effect occurred at a concentration of 1 µM with viability restoration to 102.4% (p<0.0001). Protective effect decreased with 5 µM and 10 µM concentrations, while it became ineffective at 25 µM and higher concentrations (Table 4, Fig 4).

Table No. 1: Dunnett's multiple comparisons test for H_2O_2 cytotoxicity

H_2O_2 (µM)	Mean difference vs control (%)	95% CI (%)	P-value
50	-4.46	-24.29 – 15.37	0.9714 (NS)
100	21.74	1.91 – 41.57	0.0282 (*)
150	31.05	11.22 – 50.88	0.0014 (**)
200	53.82	33.99 – 73.65	<0.0001 (****)
250	76.17	56.34 – 96.00	<0.0001 (****)
300	85.88	66.05 – 105.70	<0.0001 (****)

NS=Not significant, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 vs. control (one-way ANOVA, Dunnett's test)

Table No. 2: Dunnett's multiple comparisons test for corticosetrone cytotoxicity

CORT (µM)	Mean difference vs control (%)	95% CI (%)	P-value
100	20.69	3.78 – 37.60	0.0139 (**)
200	26.00	9.09 – 42.91	0.0021 (**)
300	64.13	47.21 – 81.04	<0.0001 (****)
400	69.63	52.71 – 86.54	<0.0001 (****)
500	61.75	44.84 – 78.66	<0.0001 (****)

*p<0.05, **p<0.01, ****p<0.0001 vs. control (one-way ANOVA, Dunnett's test)

Table No. 3: Dunnett's multiple comparisons test for H_2O_2 and CORT combination model

Comparison	Mean 1 (Control %)	Mean 2 (Treated %)	Mean Difference vs Control (%)	95.00% CI of Difference	P Value
Control vs. CORT 100 µM	100.0	85.59	14.41	1.075 to 27.74	0.0340*
Control vs. H2O2 100 µM	100.0	82.94	17.06	3.731 to 30.39	0.0129*
Control vs. CORT 100 µM + H2O2 100 µM	100.0	59.59	40.41	27.07 to 53.74	<0.0001****

*p<0.05, ****p<0.0001 vs. control (one-way ANOVA, Dunnett's test)

Table No. 4: Šidák's multiple comparisons test for fluoxetine rescue effects vs. stress

Comparison	Mean Difference vs Stress (%)	95.00% CI of Difference	P Value
Fluoxetine (0.1 μM) vs. stress	9.221	-12.86 to 31.30	0.9083 (ns)
Fluoxetine (0.3 μM) vs. stress	6.982	-15.10 to 29.07	0.9833 (ns)
Fluoxetine (0.7 μM) vs. stress	22.61	0.5266 to 44.69	0.0416 (*)
Fluoxetine (1 μM) vs. stress	69.42	47.33 to 91.50	<0.0001 (****)
Fluoxetine (5 μM) vs. stress	36.86	14.77 to 58.94	0.0001 (***)
Fluoxetine (10 μM) vs. stress	22.14	0.05416 to 44.22	0.0491 (*)
Fluoxetine (25 μM) vs. stress	-12.00	-34.08 to 10.08	0.6919 (ns)
Fluoxetine (50 μM) vs. stress	-18.50	-40.58 to 3.583	0.1597 (ns)
Stress vs. control	-66.99	-89.07 to -44.90	<0.0001 (****)

ns = not significant; *p<0.05, ***p<0.001, ****p<0.0001 vs. stress group (one-way ANOVA, Šidák's test)

Table No. 5: Šidák's multiple comparisons test for laser rescue effects vs stress

Comparison	Mean Difference vs Stress (%)	95.00% CI of Difference	P Value
Laser (10s) vs. stress	-1.516	-28.89 to 25.86	>0.9999 (ns)
Laser (20s) vs. stress	30.68	3.308 to 58.05	0.0208 (*)
Laser (40s) vs. stress	58.35	30.98 to 85.72	<0.0001 (****)
Laser (60s) vs. stress	41.23	13.86 to 68.60	0.0010 (**)
Laser (120s) vs. stress	-13.30	-40.67 to 14.07	0.7366 (ns)
Laser (240s) vs. stress	-34.74	-62.12 to -7.373	0.0067 (**)
Stress vs. control	-51.33	-78.70 to -23.96	<0.0001 (****)

ns = not significant; *p < 0.05, **p < 0.01, ****p < 0.0001 (one-way ANOVA with Šidák's test)

Table No. 6: Šidák's multiple comparisons test for IL-6 levels vs. induction control

Comparison	Mean Difference vs Induction (pg/mL)	95.00% CI of Difference	P Value
Induction vs. control	1.505	0.4229 to 2.586	0.0035 (**)
Fluoxetine (1 μM) vs. induction	-2.545	-3.627 to -1.464	<0.0001 (****)
Fluoxetine (5 μM) vs. induction	-2.939	-4.020 to -1.857	<0.0001 (****)
Fluoxetine (10 μM) vs. induction	-1.435	-2.516 to -0.3529	0.0055 (**)
Laser (10s) vs. induction	-0.9427	-2.024 to 0.1391	0.1141 (ns)
Laser (20s) vs. induction	-0.9505	-2.032 to 0.1313	0.1091 (ns)
Laser (40s) vs. induction	-1.738	-2.819 to -0.6559	0.0008 (***)
Laser (60s) vs. induction	-1.470	-2.552 to -0.3882	0.0044 (**)

ns = not significant; *p < 0.05, **p < 0.01, ****p < 0.0001 (one-way ANOVA with Šidák's test)

Table No. 7: Šidák's multiple comparisons test for GSH levels vs. induction

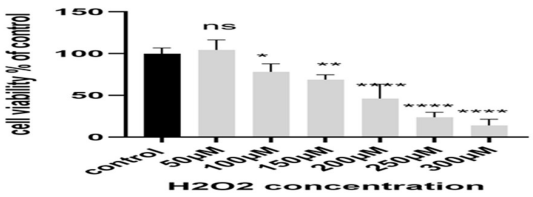
Comparison	Mean Difference vs Induction (pg/mL)	95.00% CI of Difference	P Value
Induction vs. control	-15.83	-23.06 to -8.606	<0.0001 (****)
Fluoxetine (1 μM) vs. induction	14.93	7.706 to 22.16	<0.0001 (****)
Fluoxetine (5 μM) vs. induction	14.77	7.539 to 21.99	<0.0001 (****)
Fluoxetine (10 μM) vs. induction	8.633	1.406 to 15.86	0.0135 (*)
Laser (10s) vs. induction	0.1333	-7.094 to 7.361	>0.9999 (ns)
Laser (20s) vs. induction	2.433	-4.794 to 9.661	0.9503 (ns)
Laser (40s) vs. induction	9.900	2.672 to 17.13	0.0041 (**)
Laser (60s) vs. induction	4.700	-2.528 to 11.93	0.3912 (ns)

ns = not significant; *p < 0.05, **p < 0.01, ****p < 0.0001 (one-way ANOVA with Šidák's test)

Table No. 8: Šidák's multiple comparisons test for MDA levels vs. induction

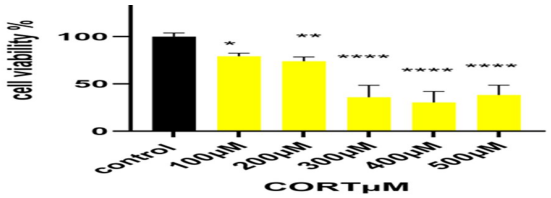
Comparison	Mean Difference vs Induction (pg/mL)	95.00% CI of Difference	P Value
Induction vs. control	4.804	1.759 to 7.848	0.0010 (**)
Fluoxetine (1 µM) vs. induction	-8.450	-11.49 to -5.406	<0.0001 (****)
Fluoxetine (5 µM) vs. induction	-6.264	-9.308 to -3.220	<0.0001 (****)
Fluoxetine (10 µM) vs. induction	-3.675	-6.719 to -0.6307	0.0124 (*)
Laser (10s) vs. induction	-3.110	-6.154 to -0.06533	0.0434 (*)
Laser (20s) vs. induction	-4.424	-7.469 to -1.380	0.0023 (**)
Laser (40s) vs. induction	-6.325	-9.369 to -3.281	<0.0001 (****)
Laser (60s) vs. induction	-5.667	-8.711 to -2.623	0.0002 (***)

ns = not significant; *p<0.05, **p<0.01, ****p<0.0001 (one-way ANOVA with Šidák's test)



ns = Not significant, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 vs. control

Figure No. 1: Concentration-dependent effect of H_2O_2 (0–300 µM, 24 h) on SH-SY5Y viability (MTT assay)



*p<0.05, **p<0.01, ****p<0.0001 vs. control

Figure No. 2: Concentration-dependent effect of corticosterone (100–500 µM, 24 h) on SH-SY5Y viability (MTT assay)

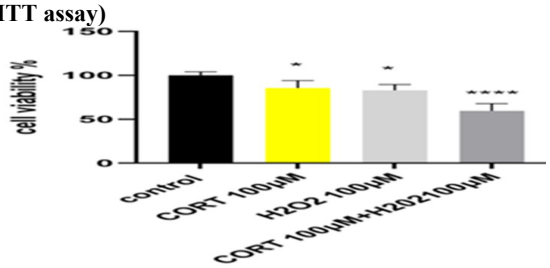


Figure No. 3: Effect of individual and combined corticosterone (100 µM) and H_2O_2 (100 µM) exposure on SH-SY5Y viability (MTT assay, 24 h)
[*p<0.05, ****p<0.0001 vs. control]

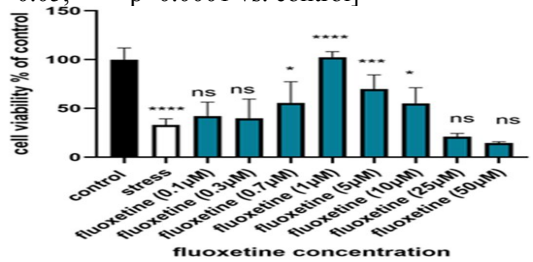


Figure No. 4: Dose-dependent protective effect of fluoxetine (0.1–50 µM, 24 h) against corticosterone/H_2O_2-induced stress in SH-SY5Y cells (MTT assay) [ns = not significant; *p<0.05, ***p<0.001, ****p<0.0001 vs. stress group]

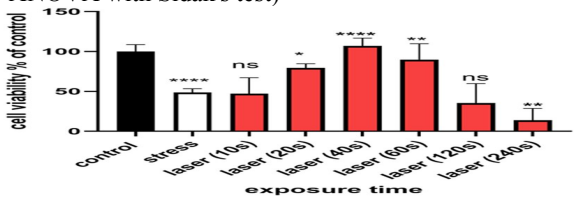


Figure No. 5: Dose-dependent protective effect of 808 nm PBM (10–240 s; 2.5–60 J/cm²) against corticosterone/H_2O_2-induced stress in SH-SY5Y cells (MTT assay, 24 h) [ns = not significant; *p<0.05, **p<0.01, ****p<0.0001 vs. stress group]

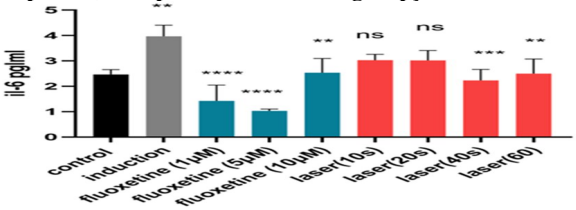


Figure No. 6: Effects of fluoxetine (1–10 µM) and 808 nm PBM (10–60 s) on IL-6 levels in corticosterone/H_2O_2-stressed SH-SY5Y cells (ns = not significant; **p<0.01, ***p<0.001, ****p<0.0001 vs. stress group)

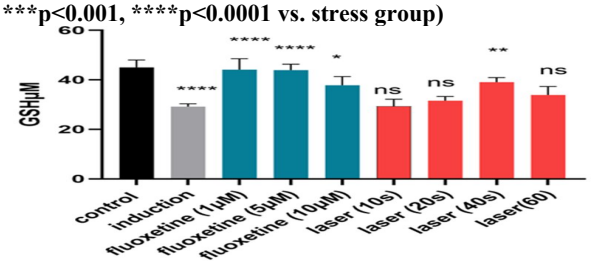


Figure No. 7: Effects of fluoxetine (1–10 µM) and 808 nm PBM (10–60 s) on GSH levels in corticosterone/H_2O_2-stressed SH-SY5Y cells. ns = not significant; *p<0.05, **p<0.01, ****p<0.0001 vs. stress group

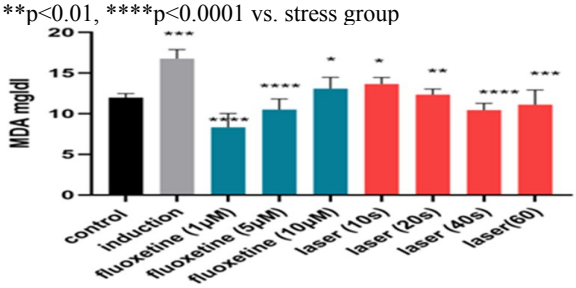


Figure No. 8: Effects of fluoxetine (1–10 µM) and 808 nm PBM (10–60 s) on MDA levels in corticosterone/H_2O_2-stressed SH-SY5Y cells [*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 vs. stress group]

Biphasic viability recovery by PBM follows Arndt-Schulz curve - PBM (10-240 s): 10 s ineffective; 20 s rescued to 79.4% (p=0.0208); 40 s maximum effect (107.1%, p<0.0001); 60 s (89.9%, p=0.0010); 120 s ineffective; 240 s toxic (13.97%, p=0.0067) [Table 5, Fig. 5].

FLX and PBM suppress IL-6: Stress elevated IL-6 to 3.97 pg/mL (control 2.47 pg/mL, p=0.0035). FLX 1 µM reduced IL-6 to 1.42 pg/mL (p<0.0001); 5 µM to 1.03 pg/mL (p<0.0001). PBM 40 s reduced IL-6 to 1.74 pg/mL (p=0.0008); 60 s to 2.50 pg/mL (p=0.0044) (Table 6, Fig. 6).

DISCUSSION

This study presented a double hit CORT/H_2O_2 SH-SY5Y cell model exhibiting features specific for MDD, such as reduced viability, decreased GSH content, increased MDA content, and increased IL-6 production. Effects of the combination of CORT and H_2O_2 are synergistic, however, the precise nature of this phenomenon requires validation since it might differ from the classic glucocorticoid sensitization based on the recent data about neuroprotective effects of CORT in SH-SY5Y cells.[11,12] Effects of both FLX and 808 nm PBM treatment are consistent with biphasic (hormetic) behavior in accordance with Arndt-Schulz law. FLX at 1 µM resulted in close-to-completeness biochemical normalization, including decreased sub-basal level of MDA, indicating its antioxidant effects.[13,14]

Application of PBM with duration of 40 seconds (10 J/cm²) normalized all tested parameters, except for incomplete restoration of GSH level, which can be related to different nature of effects. Toxicity at 240 seconds (60 J/cm²) represents a destructive dose of light, and the possible presence of photothermal effects cannot be excluded.[15] Noteworthy, even sub-therapeutic doses of PBM treatment decreased MDA content, pointing to independent membrane protection.[16]

Overall, both interventions address the oxidative-inflammatory axis through orthogonal mechanisms, justifying future combinatorial studies.[17]

CONCLUSION

The corticosterone/H_2O_2 dual-hit paradigm provides a reliable in vitro model of major depressive disorder-related oxidative and neuroinflammatorystress. Fluoxetine (1 µM) and 808 nm photobiomodulation (10 J/cm^2) each demonstrated hormetic neuroprotection, improving viability, glutathione, and interleukin-6 while reducing major depressive disorder. Their mechanistically distinct profiles support future combinatorial synergy investigations.

Author's Contribution:

Concept & Design or acquisition of analysis or interpretation of data:	Zahraa Qusay Muslim, Salman Mohammad Salman
Drafting or Revising Critically:	Zahraa Qusay Muslim, Kaiser N. Madlum
Final Approval of version:	All the above authors
Agreement to accountable for all aspects of work:	All the above authors

Conflict of Interest: The study has no conflict of interest to declare by any author.

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