74th SIF National Congress

Bergamot essential oil (BEO) provides cytoprotection in neuronal cells exposed to heavy metals.

Rosanna Mallamaci[1], D. Meleleo[2], A. Barbarossa[3], M.N. Sgobba[1], A. Carocci[3].

[1] Dpt. of Biosciences, Biotechnologies and Biopharmaceuticals, University "Aldo Moro" of Bari, Italy; [2] Dpt. of Science of Agriculture, Food, Natural Resources and Engineering, University of Foggia, 71122, Foggia, Italy;[3] Dpt. of Pharmacy-Pharmaceutical Sciences, University of Bari "Aldo Moro", 70125 Bari, Italy

AIMS

Bergamot essential oil (BEO) is obtained from the fresh fruit of the bergamot tree, grown primarily in the Italian region of Calabria. Known for its unique fragrance, BEO is used in various products such as perfumes, cosmetics, pharmaceuticals, and food products. BEO has diverse biological activities, including its impact on the cardiovascular system, antimicrobial properties against common pathogens, and neuropharmacological effects. It is also used in aromatherapy to relieve stress, mood disorders, and cancer-related pain. The release of amino acids by BEO, which act as neurotransmitters in the hippocampus, can influence both normal and pathological synaptic plasticity and provide neuroprotection during cerebral ischemia and pain episodes. BEO mainly contains limonene, c-terpinene, β-pinene, linalool, and linalyl acetate, known for their powerful antioxidant effects. All essential oils with a high D-limonene content have a significant free radical scavenging effect, predominantly disabling the production of reactive oxygen species (ROS) resulting in a positive impact on inflammation, immune responses and cellular health. Present epidemiological and experimental investigations have suggested that exposure to heavy metals, such as cadmium (Cd), mercury (Hg), and lead (Pb), could potentially contribute to the onset of neurodevelopmental disorders and/or accelerate the progression of neurodegenerative and neurological diseases. This study aims to investigate whether BEO can mitigate neurotoxicity induced by heavy metals (Cd^{2+}, Hg^{2+} or Pb^{2+}) in SH-SY5Y cells, which serve as a model for neuronal cells.

- ✓ Several reports indicate the possibility of using the medicinal plant extracts comprising polyphenols, flavones, flavonoids, tannins, alkaloids, and terpenes, which are known antioxidants, as alternative to neurotoxicity therapy.
- ✓ Cd^{2+}, Hg^{2+} or Pb^{2+} were used to reproduce an *in vitro* model of heavy metals-induced toxicity in SH-SY5Y cells that could mimic an *in vivo* heavy metals chronic exposure in the brain.

METHODS

The viability of SH-SY5Y cells post-exposure to heavy metals, either individually or in conjunction with BEO, was assessed using MTT, LDH, and Calcein assays to evaluate the effects of necrotic cell death. Additionally, the DCF-DA assay was conducted to ascertain BEO protective capacity against oxidative stress induced by heavy metals in SH-SY5Y cells. The antibacterial activity of BEO was tested against various bacterial strains, with particular attention to Gram-positive and Gram-negative bacteria.

MIC (mg/mL)						
	Microorganisms					
	Gram-positive			Gram-negative		
	E. faecalis ATCC 29212	*S. aureus* ATCC 29213	*S. aureus* ATCC 43300	*E. coli* ATCC 25922	*E. coli* ATCC 35218	*K. pneumoniae* ATCC 13883
BEO	17.6	4.4	8.8	8.8	17.6	35.2

Table 1. Antibacterial activity of BEO expressed as Minimum inhibitory concentration (MIC, mg/mL)

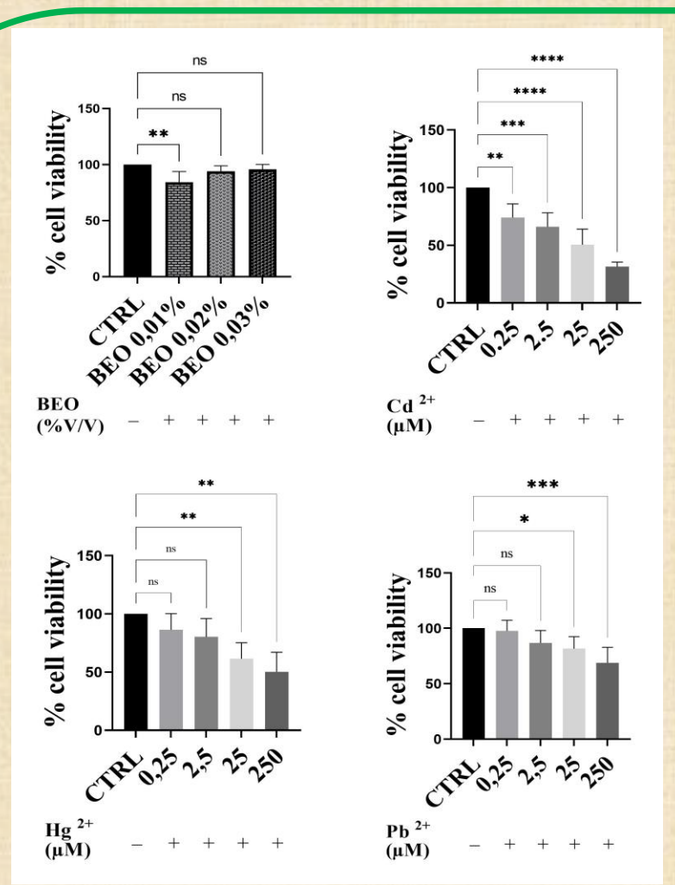

Figure 1. Effects of BEO (0.01, 0.02, 0.03%), Cd^{2+}, Hg^{2+}, or Pb^{2+} (0.25–250 µM) on SH-SY5Y cell viability after 24 h of exposure (MTT assay).

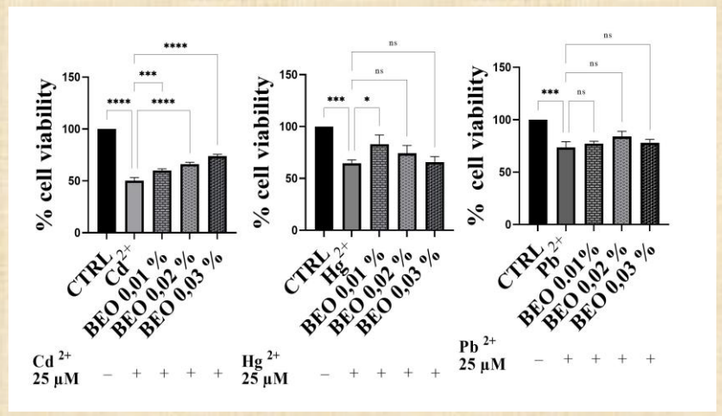

Figure 2. Effect of simultaneous treatment with Cd^{2+}, Hg^{2+}, and Pb^{2+} (25 µM) and BEO (0.01–0.03 %) on SH-SY5Y cell viability after 24 h of exposure (MTT assay).

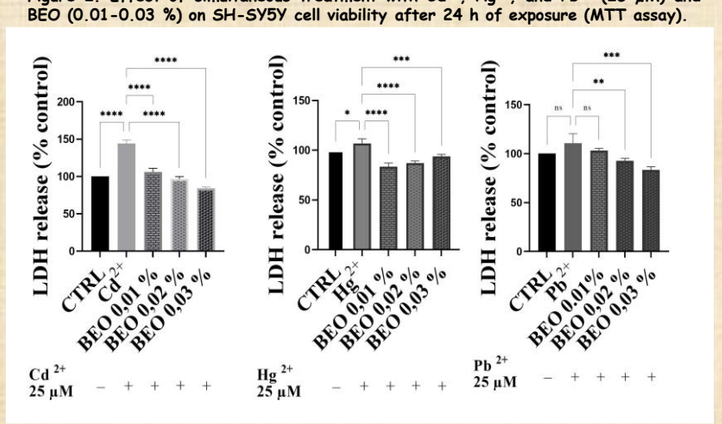

Figure 3. LDH activity of SH-SY5Y cell after of 24 h of exposure with different concentrations of BEO (0.01–0.03 %) and Cd^{2+}, Hg^{2+}, and Pb^{2+} (25 µM).

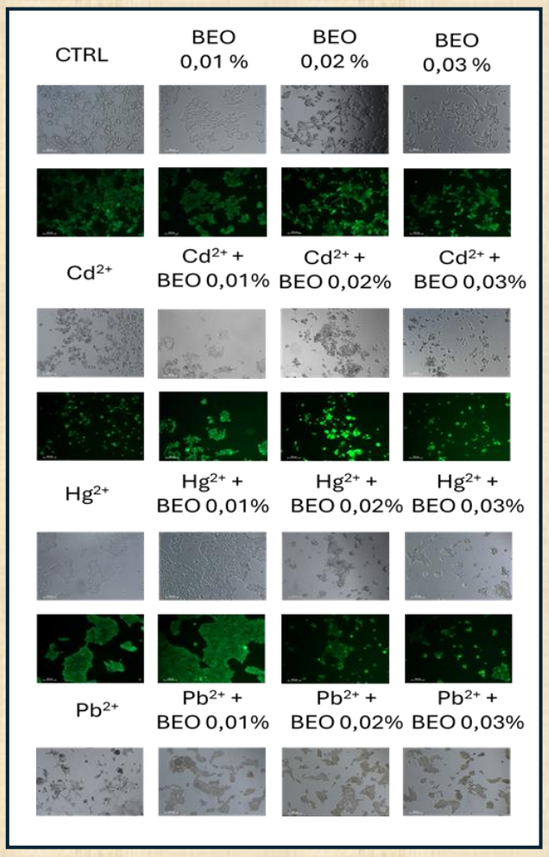

Figure 4. Live/dead viability assay of SH-SY5Y cells cell after 24 h of exposure with different concentrations of BEO (0.01–0.03 %) and Cd 2+, Hg 2+, and Pb 2+ (25 µM). Cells were stained for esterase activity using calcein-AM (green).

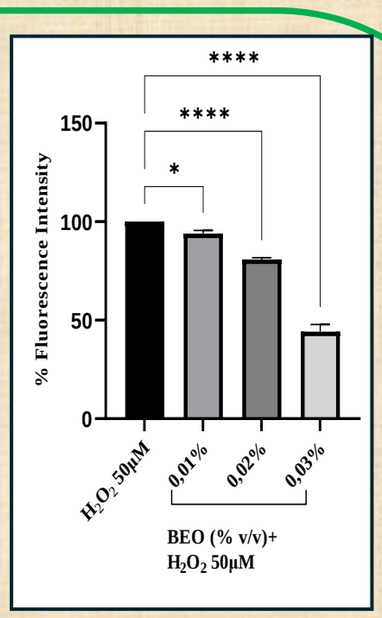

Figure 5. DCFH oxidation in SH-SY5Y cells after 24h incubation with BEO in the presence of hydrogen peroxide (H_2O_2). Bars represent 'medium ± standard 248 deviation' values obtained from three individual experiments. Significant differences versus H_2O_2 : not significant differences (ns, P > 0.05), ** P < 0.01, *** P < 0.001.

RESULTS

Recent research has demonstrated the protective properties of BEO in neuronal cells subjected to heavy metal exposure. Heavy metals such as lead, cadmium, and mercury are known to induce oxidative stress and cytotoxicity in neuronal cells, leading to potential neurodegenerative effects. The study demonstrated BEO ability to increase the survival of SH-SY5Y cells following heavy metal exposure. Pre-treatment with BEO yielded a notable, dose-dependent cytoprotective effect, particularly against cadmium (Cd)-induced toxicity. This protective effect was substantiated by a decrease in lactate dehydrogenase (LDH) release when cells were treated with both Cd and BEO, as opposed to cells treated solely with Cd. Since oxidative stress is one of the mechanisms of Cd^{2+}, Hg^{2+} or Pb^{2+} induced neurodegeneration, we aimed at exploring the potential ability of BEO to reduce oxidative stress triggered by heavy metals in SH-SY5Y cells. Moreover, a significant reduction in reactive oxygen species (ROS) production, prompted by hydrogen peroxide (H_2O_2) or heavy metals, was observed in the same cellular model.

The results indicated that BEO significantly reduced cell death and oxidative stress markers compared to untreated cells. Additionally, microbiological experiments highlighted BEO antibacterial action, further showcasing its potential therapeutic benefits.

CONCLUSIONS

- ✓ These findings suggest that BEO showed to have good protective activity against SH-SY5Y cells exposed to Cd^{2+}, Hg^{2+} or Pb^{2+} toxicity, counteracting the cytotoxic effects of these metals in the proposed cellular model.
- ✓ The toxicity that was identified by the MTT assay and the measurement of LDH release and was reversed by treatment with BEO offers further evidence that BEO can have a positive impact on neuronal cell protection.
- ✓ Our study showed how effective BEO is at reducing oxidative stress provoked by the stimulus with both H_2O_2 and heavy metals, making this effect more pronounced against Cd induced oxidative stress.
- ✓ Future studies will be essential to explore these protective effects further and to uncover new potential applications.

- ✓ Dosoky, N.S.; Setzer, W.N. Biological Activities and Safety of Citrus spp. Essential Oils. Int. J. Mol. Sci. 2018; 19(7):1966.
- ✓ Mallamaci, R.; Conforti, F.; Statti, G.; Avato, P.; Barbarossa, A.; Meleleo, D. Phenolic Compounds from Tropea Red Onion as Dietary Agents for Protection against Heavy Metals Toxicity. *Life* 2024, *14*, 495.
- ✓ Mannino, F.; Pallio, G.; Imbesi, C.; Scarfone, A.; Puzzolo, D.; Micali, A.; Freni, J.; Squadrito, F.; Bitto, A.; Minutoli, L.; et al. Beta-Caryophyllene, a Plant-Derived CB2 Receptor Agonist, Protects SH-SY5Y Cells from Cadmium-Induced Toxicity. *Int. J. Mol.Sci.* 2023, *24*, 15487
- ✓ Mallamaci, R.; Storelli, M.M.; Barbarossa, A.; Messina, G.; Valenzano, A.; Meleleo, D. Potential Protective Effects of Spirulina (*Spirulina platensis*) against In Vitro Toxicity Induced by Heavy Metals (Cadmium, Mercury, and Lead) on SH-SY5Y Neuroblastoma Cells. *Int. J. Mol. Sci.* 2023, *24*, 17076.
- ✓ Yousef, A.O.S.; Fahad, A.A.; Abdel Moneim, A.E.; Metwally, D.M.; El-Khadragy, M.F.; Kassab, R.B. The neuroprotective role of coenzyme Q10 against lead acetate-induced neurotoxicity is mediated by antioxidant, anti-inflammatory and anti-apoptotic activities. *Int. J. Environ. Res. Public Health.* 2019, *16*(16), 2895.