

## Abstract

Anti-Stress Effects of *Cymbopogon schoenanthus* (L.) Spreng Growing Wild in Tunisia on Both *in vitro* and *in vivo* Models

(チュニジア産 *Cymbopogon schoenanthus* (L.) Spreng の *in vitro* 及び *in vivo* における抗ストレス作用に関する研究)

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Stress is the body's reaction to a change that requires a physical, mental or emotional adjustment or response. Moreover, stress is known to induce alterations in various physiological responses even leading to pathological states.

*Cymbopogon schoenanthus* is an aromatic culinary herb grown in the southern area of Tunisia, and locally known as “El bekherai”. It is used for several preparations of meat and salad or served with tea because of its pleasant aroma appreciated by North African inhabitant. Antioxidant and neuroprotective effects are among the medicinal properties attributed to this plant. Therefore, the present study allowed us to observe the benefits of *C. schoenanthus* to the neuronal system. The first part of our study was designed to investigate the anti-stress properties of the ethanol extract of Tunisian medicinal plants: *Cymbopogon schoenanthus* (CSEE), *Crithmum maritimum* (CMEE), *Arthrophytum scoparium* (ASEE), *Artemisia herba-alba* (AHEE), and *Rhanterium suaveolens* (RSEE), growing wild in the Southern part of Tunisia. SH-SY5Y cells and HSP47-transformed cells were used in this work as model. Our results showed that the tested plants treatments significantly reversed heat shock protein expression in heat-stressed HSP47-transformed cells (42°C, for 90 min) and enhanced mRNA expression of *HSP27*, *HSP70*, and *HSP90* in heat-treated SH-SY5Y cells. Moreover, CSEE at 1/1000 and 1/100 (v/v) dilutions, showed non cytotoxic effect on both

SH-SY5Y and HSP47 cells. In addition, our results demonstrated that CSEE had a high neuroprotective effect against H<sub>2</sub>O<sub>2</sub>-induced SH-SY5Y cell death and increase of heat shock proteins which were considered as protein chaperone used to defend against several kinds of stress.

The second part was reserved to investigate the anti-stress properties of the ethanol extract of *Cymbopogon schoenanthus* (CSEE), on H<sub>2</sub>O<sub>2</sub>-induced cytotoxicity and stress in human neuroblastoma SH-SY5Y cells and its effect on induced- stress in ICR mice exposed to forced swimming and tail suspension, in concordance with heat shock protein expression (HSP27 and HSP90), corticosterone and catecholamine neurotransmitters levels. Our results demonstrated that pretreatment of SH-SY5Y cells with CSEE at 1/2000, 1/1000, and 1/500 (v/v) dilutions significantly inversed H<sub>2</sub>O<sub>2</sub>-induced neurotoxicity. Moreover, CSEE treatments significantly reversed heat shock protein expression in heat-stressed HSP47-transformed cells (42°C, for 90 min) and mRNA expression of HSP27 and HSP90 in H<sub>2</sub>O<sub>2</sub>-treated SH-SY5Y. Daily oral administration of 100 mg/kg and 200 mg/kg CSEE in ICR mice for 2 weeks resulted in a significant decrease in immobility time in forced swimming and tail suspension tests. The effect of CSEE on animal behavior was concordant with a significant regulation of blood serum corticosterone and cerebral cortex levels of catecholamine (dopamine, adrenaline and noradrenaline). To deeply understanding the bioactive compounds which expected that have an anti-stress effect, HPLC analysis of ethanol extract of *C. schoenanthus* was performed, and several compounds were detected. Gallic acid (GA), Ferulic acid (FA) and trans-cinnamic acid (TA), the main compounds of CSEE were used to investigate the anti-oxidative stress and their synergistic effects on H<sub>2</sub>O<sub>2</sub>-induced SH-SY5Y cell damage (increase of ROS levels, Cell death, ATP depletion, misfolding and aggregation of proteins...etc.). Our results showed that GA, FA and TA significantly and dose dependently improved cell viability in H<sub>2</sub>O<sub>2</sub>-treated cells. Moreover, H<sub>2</sub>O<sub>2</sub> significantly

increased the ROS production in SH-SY5Y cells to reach 150% after 60 min of incubation compared to untreated control cells. Nevertheless, the pretreatment of cells with GA, FA and TA prevented the accumulation of ROS relative to H<sub>2</sub>O<sub>2</sub>-treated cells. On the other hand, our results showed that 150 µM of H<sub>2</sub>O<sub>2</sub> significantly decreased intracellular ATP levels in SH-SY5Y cells to reach 67%. Contrariwise, FA and TA significantly increased the ATP levels to reach 90%. Moreover the mixture between FA and TA increased the levels up to 87%.

In conclusion, we make evident that *C. schoenanthus*, can be used for diseases related to stress. Therefore, and to the best of our knowledge this study is the first to demonstrate the preventive potential of CSEE against stress disorders at *in vitro* and *in vivo* levels.