

Anti-stress Mechanism of Action of PHYTOCEE®: Effect on CRHR1 and 11 β -HSD1

OBJECTIVE

To elucidate the anti-stress mechanism of action of PHYTOCEE®.

MATERIALS AND METHODS

Corticotropin releasing hormone receptor 1 (CRHR1) assay

CHO-K1 cells (10000 cells/well) were added to 384 well plate and incubated for 20 h at 37°C and 5% CO₂. The cells were treated with PHYTOCEE® at noncytotoxic concentration (0.16 to 100 μ g/mL or 50 μ M Antalarmin hydrochloride, an antagonist for 30 min, followed by incubation for 4 h with 50pM of corticotropin releasing factor (CRF) (agonist). 8 μ L of the LiveBLAzer–FRET B/G substrate mixture was then added and β -lactamase expression was estimated to quantify CRHR1 activation.

11 β -hydroxysteroid dehydrogenase type 1 (11 β -HSD1) gene expression

The trypsinized monolayer of H295R cells were seeded to 6 well plates at a density of 1 X 10⁶ cells under regular growth conditions. The cells were incubated with PHYTOCEE® at various concentrations (20-40 μ g/ml) along with 10 μ M forskolin for 24 h at 37°C with 5% CO₂. 11 β HSD1 gene expression was assessed. 11 β HSD1 is critical for conversion of active form of cortisol / corticosterone.

RESULTS

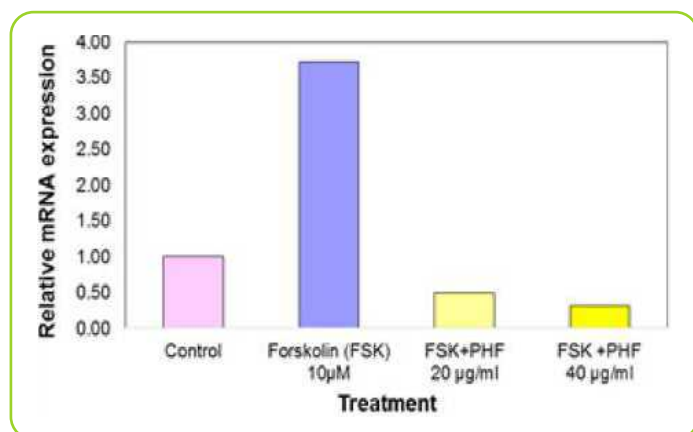


Figure 1: Effect of PHYTOCEE® on mRNA expression of 11 β -HSD1 in H295R

11 β -HSD1, 11 β -hydroxysteroid dehydrogenase type 1

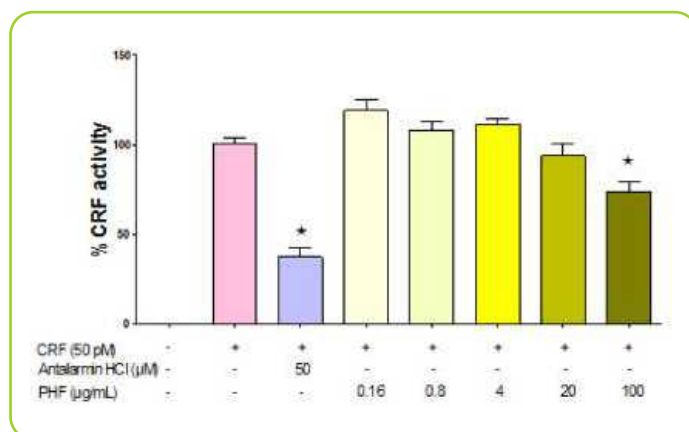


Figure 2: Effect of PHYTOCEE® on CRF induced CRHR1 activity in CHO-K1 DA cells

Values are expressed as Mean; n= 3; *p<0.05, Treatment Vs. CRF stimulated control
CRF, Corticotropin releasing factor; PHF, PHYTOCEE®, CRHR1, Corticotropin releasing hormone receptor 1

CONCLUSIONS

PHYTOCEE® administration acts as CRHR1 antagonist, hindering ACTH-mediated effects, and downregulated 11 β -HSD1 expression.

OUTCOME

Thus, PHYTOCEE® inhibits cortisol release in chronic stress could prevent major stress-related pathological and behavioural changes.