Electronic Supplementary Material (ESI) for Toxicology Research.

SUPPLEMENTARY MATERIAL

Oxidative Stress and Mitochondrial Impairment Mediated Apoptotic Cell Death Induced by Terpinolene in *Schizosaccharomyces pombe*

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mRNA expression assay

After washing with PBS, total RNA was isolated as reported previously (Bähler and Wise, 2017) and DNase I treatment was performed. RNA quantity was evaluated spectrophotometrically at 260 nm and 280 nm; 260/280 ratios were 1.8-2. RNA integrity was determined by 1% agarose gel electrophoresis. cDNA synthesis was performed using Sensifast cDNA synthesis kit (Bioline, Turkey). 1 µg total RNA, 1 µM oligo(dT) primer, 1 mM dNTP mix, 1x RT-reaction buffer, 10 U Reverse Transcriptase were used for each reaction and reaction volume was completed to a total volume of 20 µl with molecular grade water. RT reaction was performed at 42 ^{0}C for 25 minutes. At the end, RT enzyme was inactivated at 85 ^{0}C for 5 minutes. cDNA samples were stored at -20 ^{0}C. Quantitative PCR was performed using SYBR Green Master mix (Roche Applied Science) and QuantStudio5 Thermal Cycler (Applied Biosystems). Gene specific primers were designed using Primer3Plus (Version: 2.3.6) software and ordered from Sentromer Inc. (Turkey). Primers were used at 0.3 µM quantitation and presented in Table S1. The fold changes for the isoforms were normalized against β-actin reference gene (primer set is given in Table S1). PCR reactions for each sample were set as three technical replicates and performed in 10 µl total reaction volume. Reaction mix was prepared following the manufacturer's instruction (Roche, Turkey). Reaction conditions: Incubation at 94 ^{0}C for 10 min, 40 cycles of 94 ^{0}C for 15 s, 56 ^{0}C for 30 s and 72 ^{0}C for 30 s. Melting curve analysis of PCR products was performed at the end of each polymerase reaction to confirm a single PCR product was detected. The reaction efficiency corrected ΔΔCq formula was used to determine relative quantification.

Table S1: Gene specific primers used in RT-PCR reactions in this study.

SOD1	Forward: 5' AACAGCATCATTGGCCGTAC 3' Reverse: 5' ATGACACCACAAGCGTTACG 3'	Tm: 58.9 ^{0}C Tm: 58.9 ^{0}C	Product size: 119 bp
GPx1	Forward: 5' AGAAGCAACTTGGCCTTGAG 3' Reverse: 5' TTCGAGATGTTCAGGCTTGC 3'	Tm: 58.7 ^{0}C Tm: 59.3 ^{0}C	Product size: 113 bp
Pca1	Forward: 5' TTATGACAGACACGGCAAGC 3' Reverse: 5' GCATCATTAGGTTGGGCATC 3'	Tm: 59.9 ^{0}C Tm: 60.3 ^{0}C	Product size: 100 bp
Sprad9	Forward: 5' TACGCAAGTCAAGCCCATAC 3' Reverse: 5' GCAGATTCACTGCCATAACC 3'	Tm: 58.8 ^{0}C Tm: 58.2 ^{0}C	Product size: 105 bp
Act1	Forward: 5' TGTATTCCCCTCGATTGTCGG 3' Reverse: 5' CACGCTTGCTTTGAGCTTCAT 3'	Tm: 59.59 ^{0}C Tm: 60.07 ^{0}C	Product size: 101 bp

References

Bähler J. and Wise J. A., Preparation of Total RNA from Fission Yeast, *Cold Spring Harbor Protocols*, 2017, 4, pdb.prot091629. doi: 10.1101/pdb.prot091629.