Testing modulated electro-hyperthermia using C26 colorectal carcinoma cell line in vitro

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Testing modulated electro-hyperthermia using C26 colorectal carcinoma cell line *in vitro*

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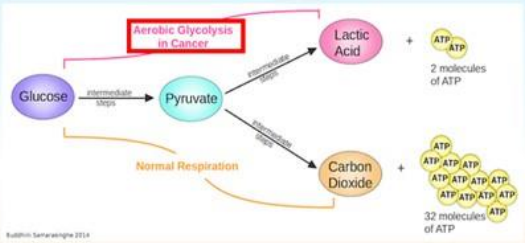
Modulated electro-hyperthermia (mEHT)

- complementary
- 13.56 MHz electromagnetic field
  - safe
  - optimal tissue penetration
- amplitude modulation (AM) of the carrier frequency
- 42 °C
- thermal effect
- high energy absorption
- tumor destruction

Modulated electro-hyperthermia (mEHT)

In a tumor tissue:
enhanced aerobic glycolysis
high water and ion concentration in EC



Modulated electro-hyperthermia (mEHT)

In a tumor tissue:
enhanced aerobic glycolysis
high water and ion concentration in EC

⇩

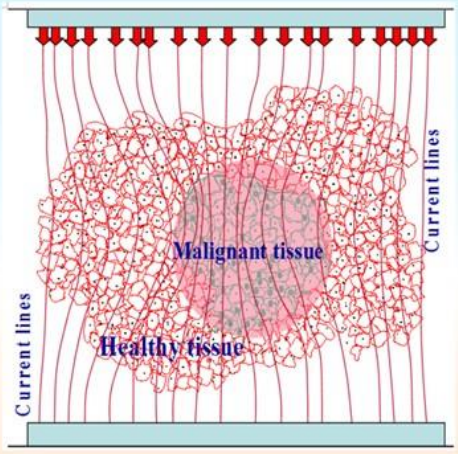
increased conductivity
lower electric resistance

⇩

The electromagnetic field **enriches selectively in the tumor**

Tumor destruction

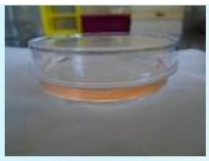
Modulated electro-hyperthermia (mEHT)

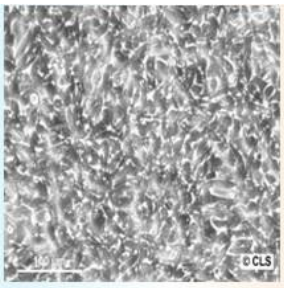


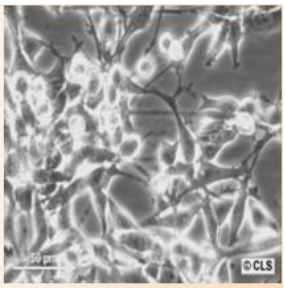
Aims

- ✓ **To set up** optimal and effective **experimental conditions for *in vitro*** studies
- ✓ **To test** mEHT induced
  - **1) morphological changes**
  - **2) cell death**, stress response & growth signaling related marker expression

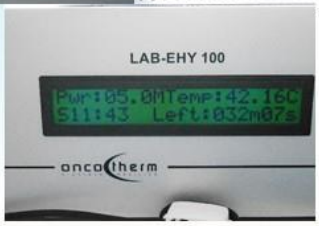
Materials and Methods

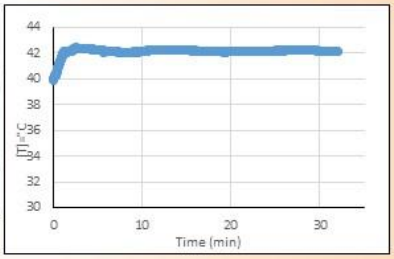


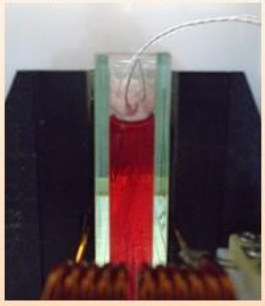


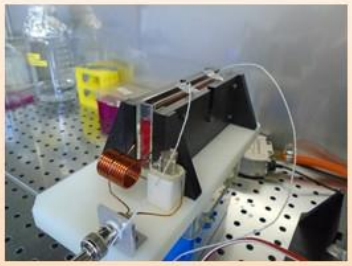


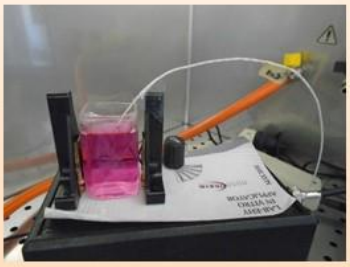
- Mouse colorectal adeno-carcinoma (**C26**)
  - cultured in RPMI 1640 medium
  - on coverslips in Petri dishes (700 000 cell / 4 ml)
- Modulated electro-hyperthermia (**mEHT**)
  - LabEHY 100 (Oncotherm, Paty, Hungary)
  - ~42°C
  - **repeated treatments (2x30 minutes or 2x60 minutes)**
- Experimental groups:
  1. control (kept 37°C in incubator)
  2. modulated electro-hyperthermia (~42 °C)
- Sampling:
  - early (**3h**) and late (**24h**)
  - fixation in ethanol:acetone=3:1
- Morphological changes, stress response, apoptosis and growth signaling related markers were detected











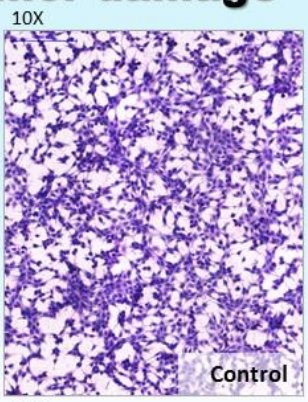
I. 2x30 minutes mEHT treatment

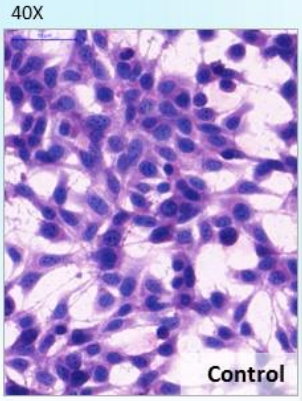
II. 2x60 minutes mEHT treatment

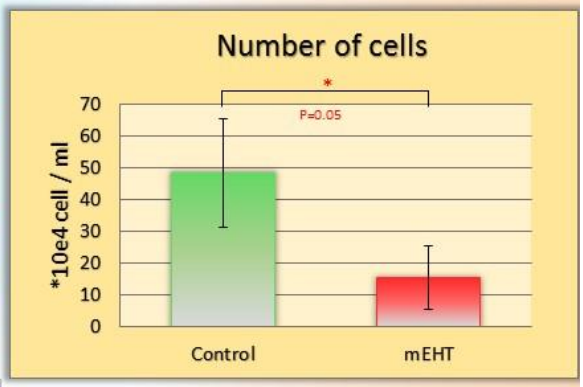
Results –
Tumor damage

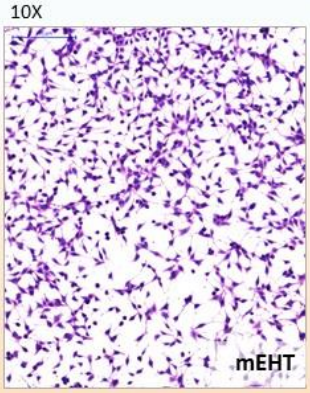
2x30 min mEHT
24 h post-treatment

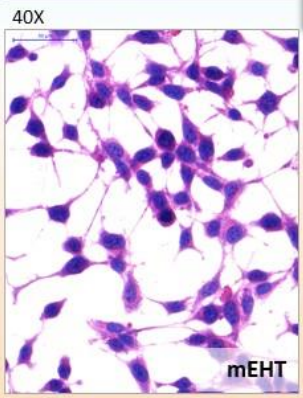
H&E

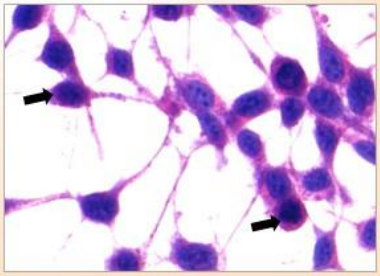












- ✓ Reduced tumor cell number
- ✓ Chromatin condensation → apoptosis

Results –
Immuncytochemistry

2x30 min mEHT
24 h post-treatment

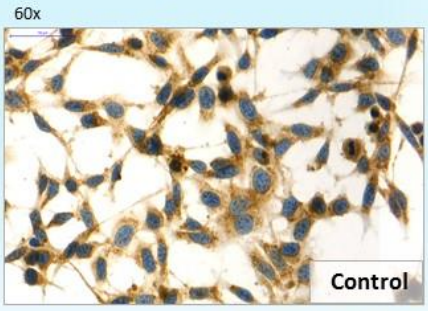
Calreticulin (CRT)

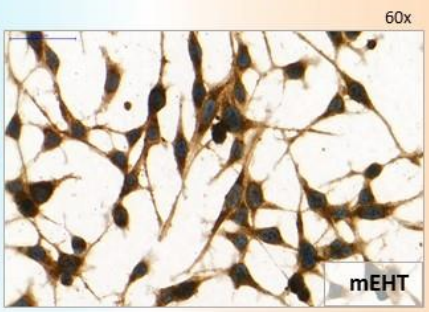
ER chaperone protein

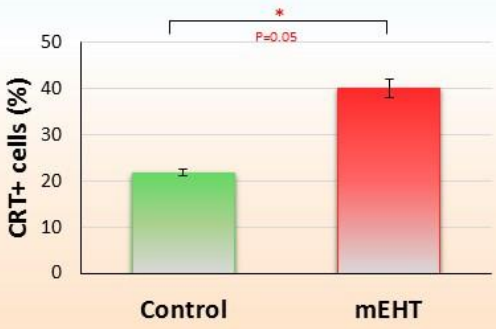
Ecto-CTR is an early marker of apoptosis

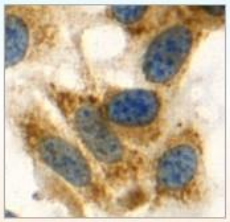
ER → cytoplasm

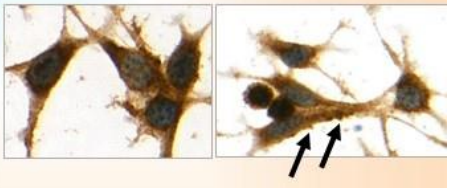
member of DAMP (Damage Associated Molecular Pattern)







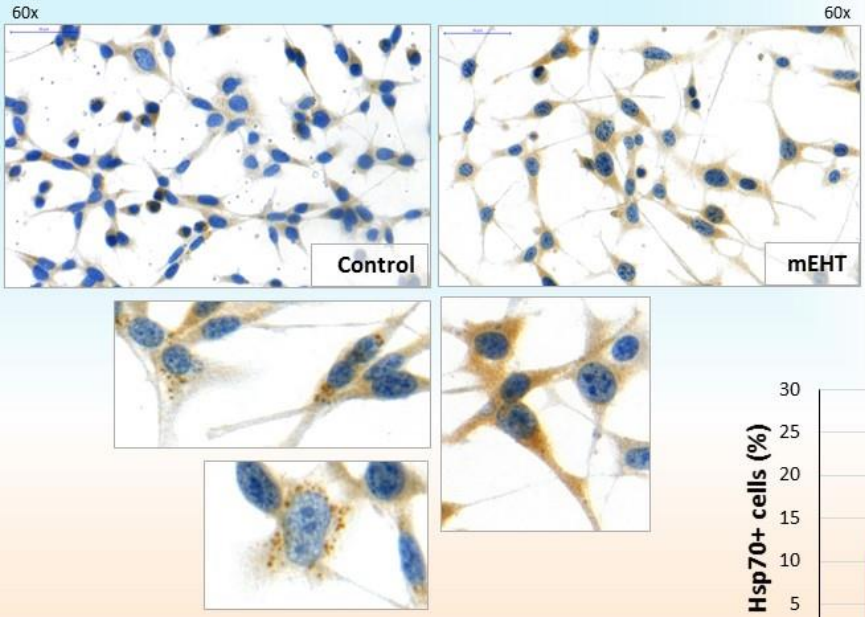




- ✓ Intensive CRT release after 2x30 minutes mEHT treatment 24h post-treatment

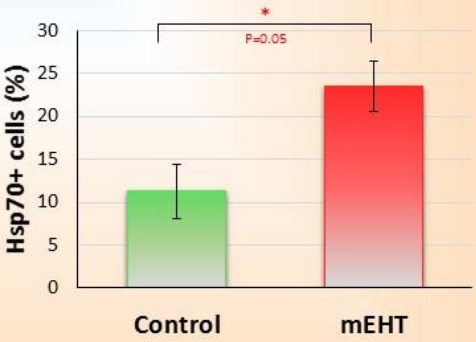
Results –
Immuncytochemistry

2x30 min mEHT
24 h post-treatment



Hsp70

granular → diffuse
heat shock protein
member of DAMP



✓ Visible granular location of Hsp 70 in the control
✓ Became a diffuse pattern due to mEHT treatment

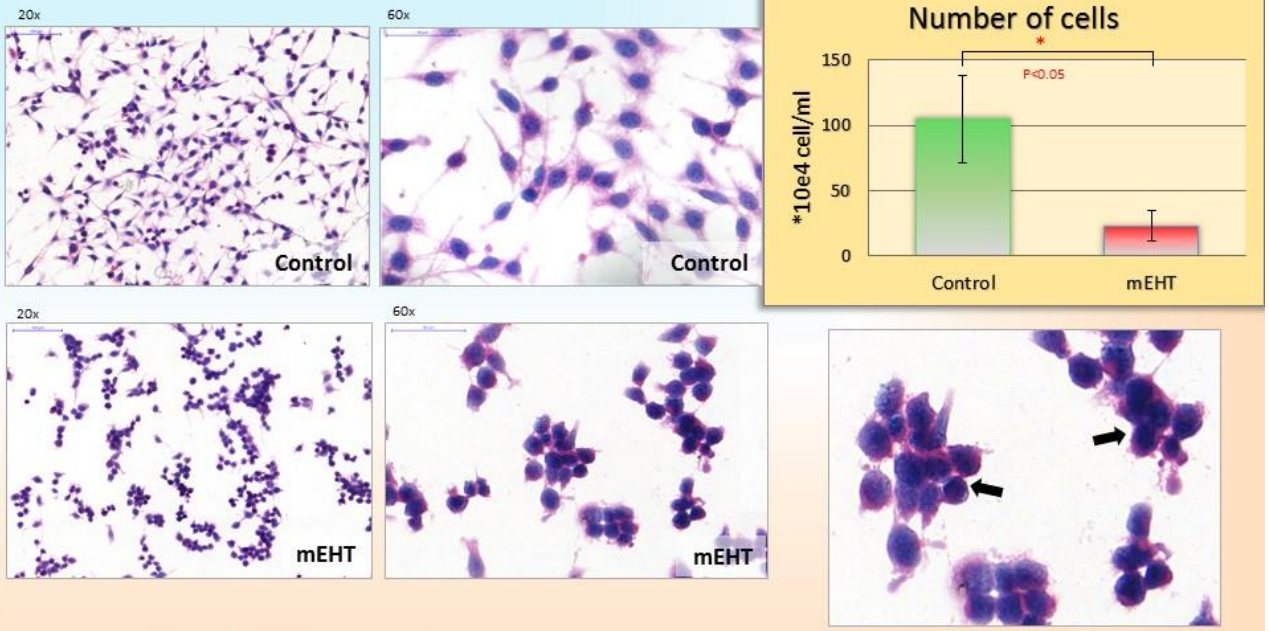
I. 2x30 minutes mEHT treatment

II. 2x60 minutes mEHT treatment

Results –
Tumor damage

2x60 min mEHT
3 h post-treatment

H&E

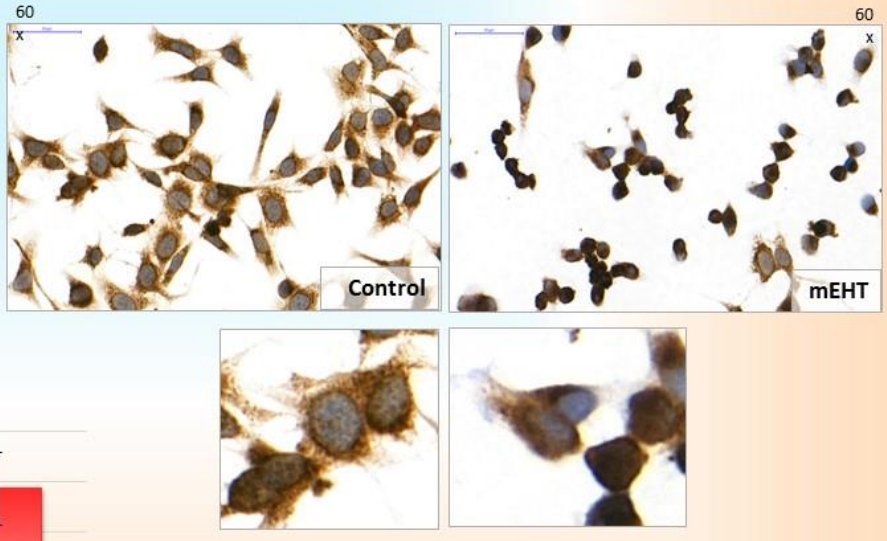


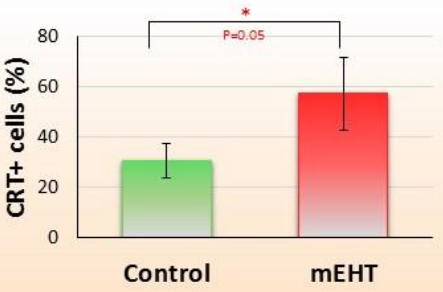
- ✓ Lost of tumor cell processes
- ✓ Reduction of tumor cell number
- ✓ Chromatin condensation → apoptosis

Results –
Immuncytochemistry

2x60 min mEHT
3 h post-treatment

Calreticulin (CRT)

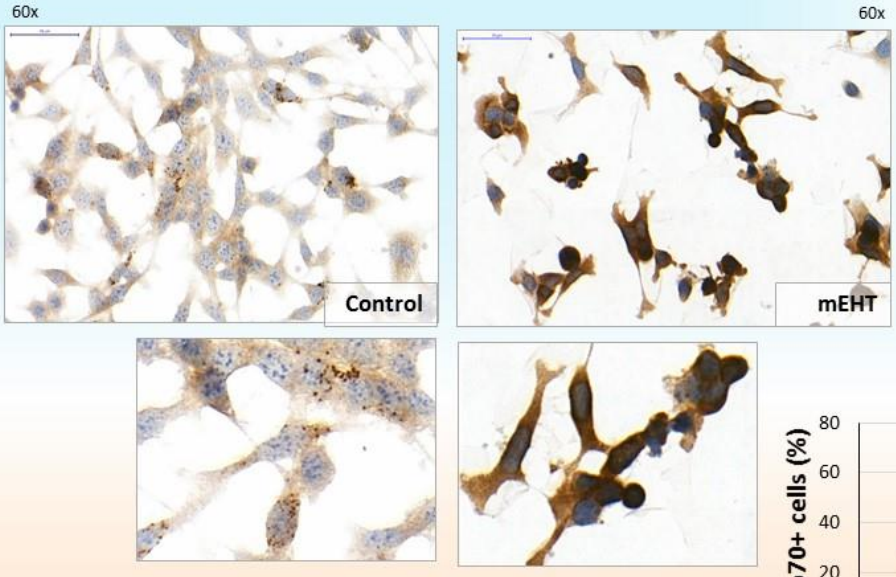




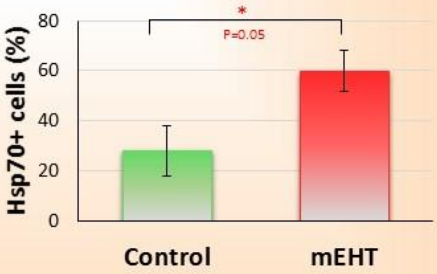
- ✓ mEHT causes more intensive CRT positivity after 2x60 minutes treatment

Results –
Immuncytochemistry

2x60 min mEHT
3 h post-treatment



Hsp70

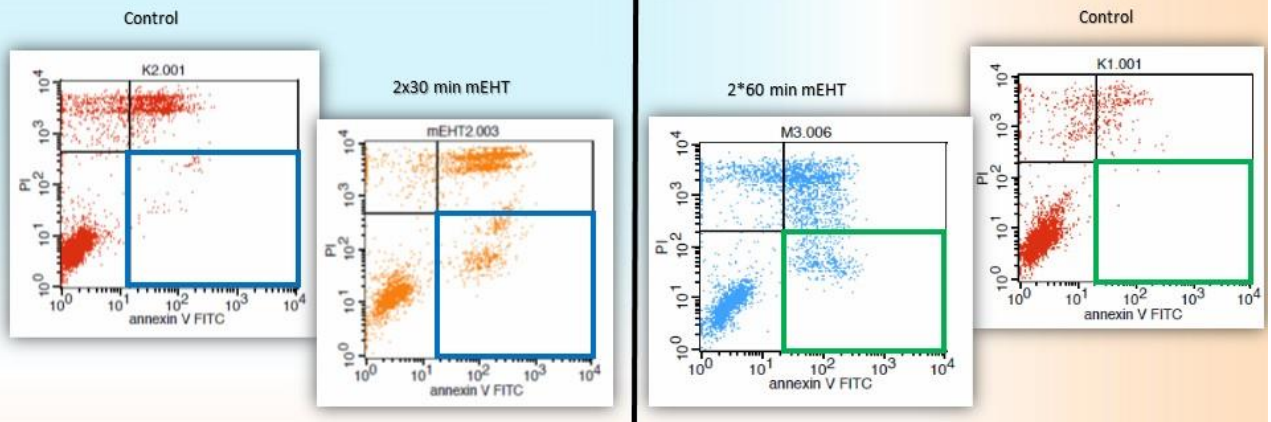


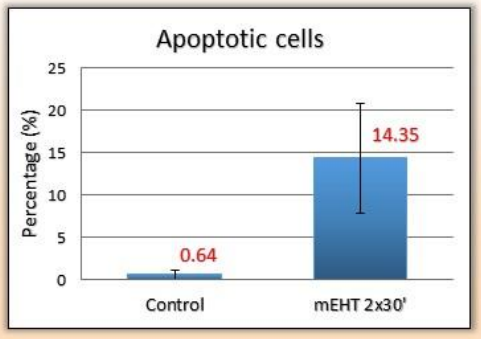
✓ Significant increase of Hsp70 level after 2x60 minutes mEHT treatment compared to control

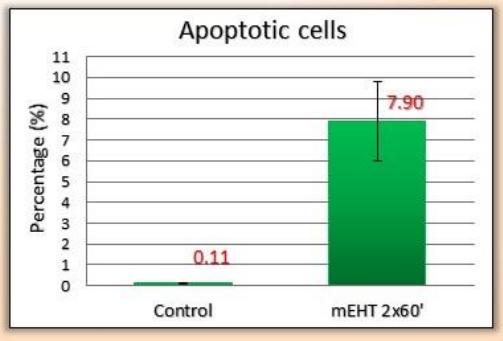
Results –
Flow cytometry

24 h post-treatment

2x30 min | **2x60 min**



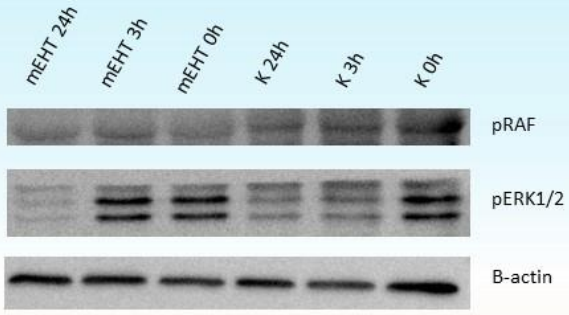


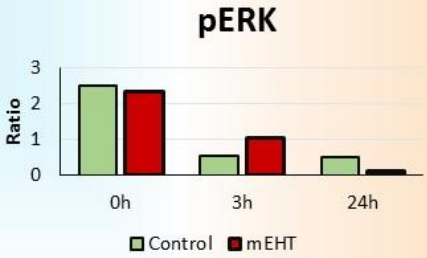


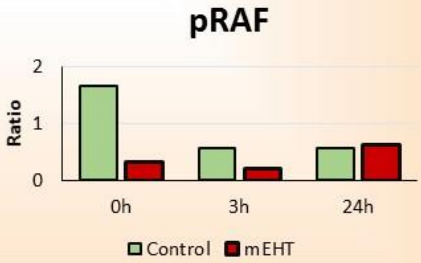
Results –
Protein analysis

2x30 min mEHT

Western blot







✓ Inactivation of pRAF
✓ Activation of pERK 1/2

Conclusion

According to our preliminary results we can summarize that:

- mEHT caused significant **apoptotic cell death** alone besides the release of such damage and stress associated molecular pattern proteins (CRT, Hsp70) which can potentially induce immunogenic response *in vivo.*

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