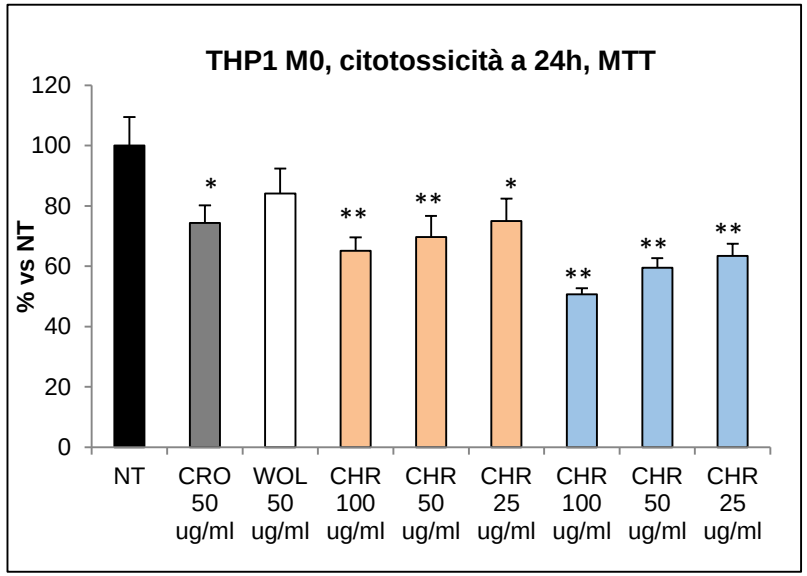
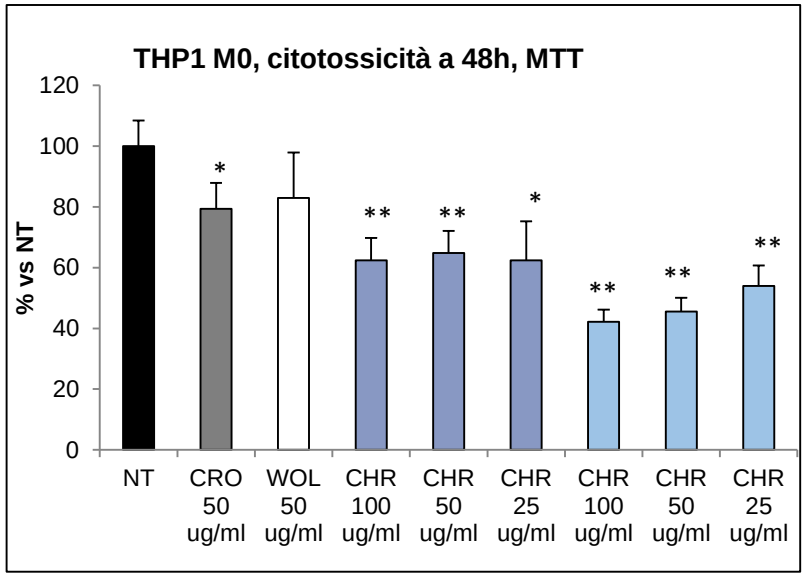
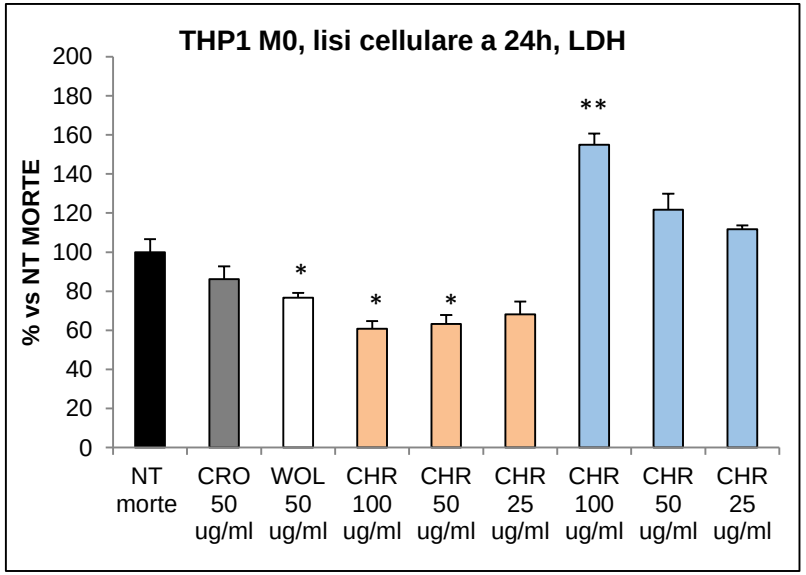
tossicità cellulare THP1-M0 a 24h e a 48h con MTT/LDH



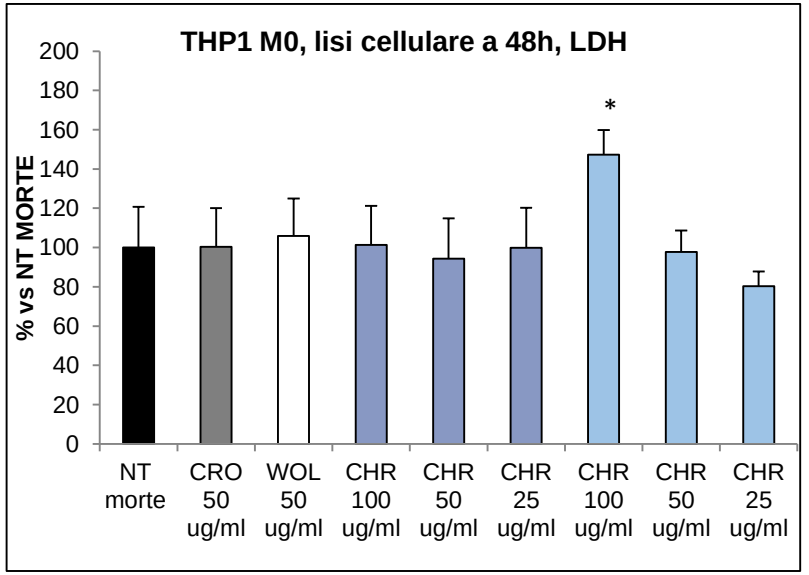
* p<0.05 vs C ** p<0.005 vs C



* p<0.05 vs C ** p<0.005 vs C

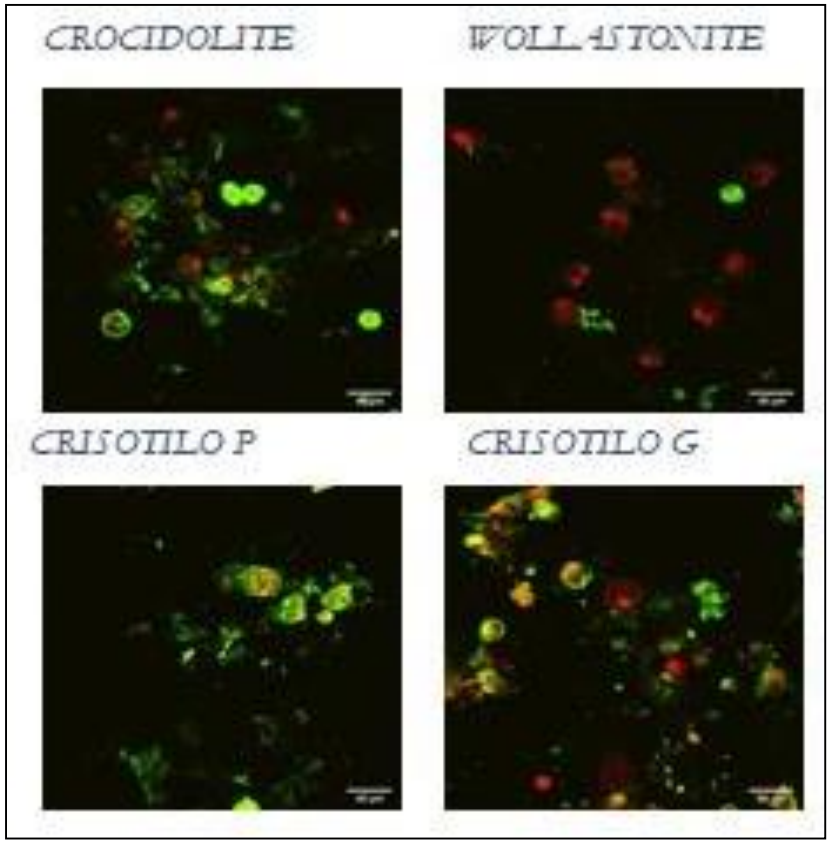


* p<0.05 vs C ** p<0.01 vs C



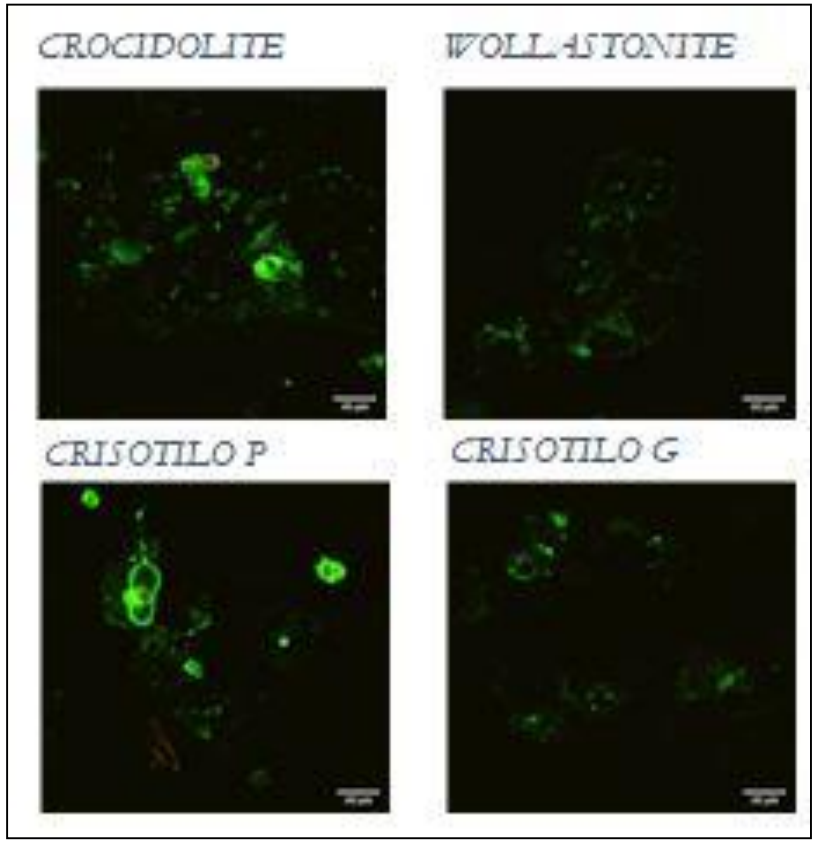
* p<0.05 vs C

Valutazione APOPTOSI a 24h con fibre 50 ug/ml con annessina e ioduro di propidio

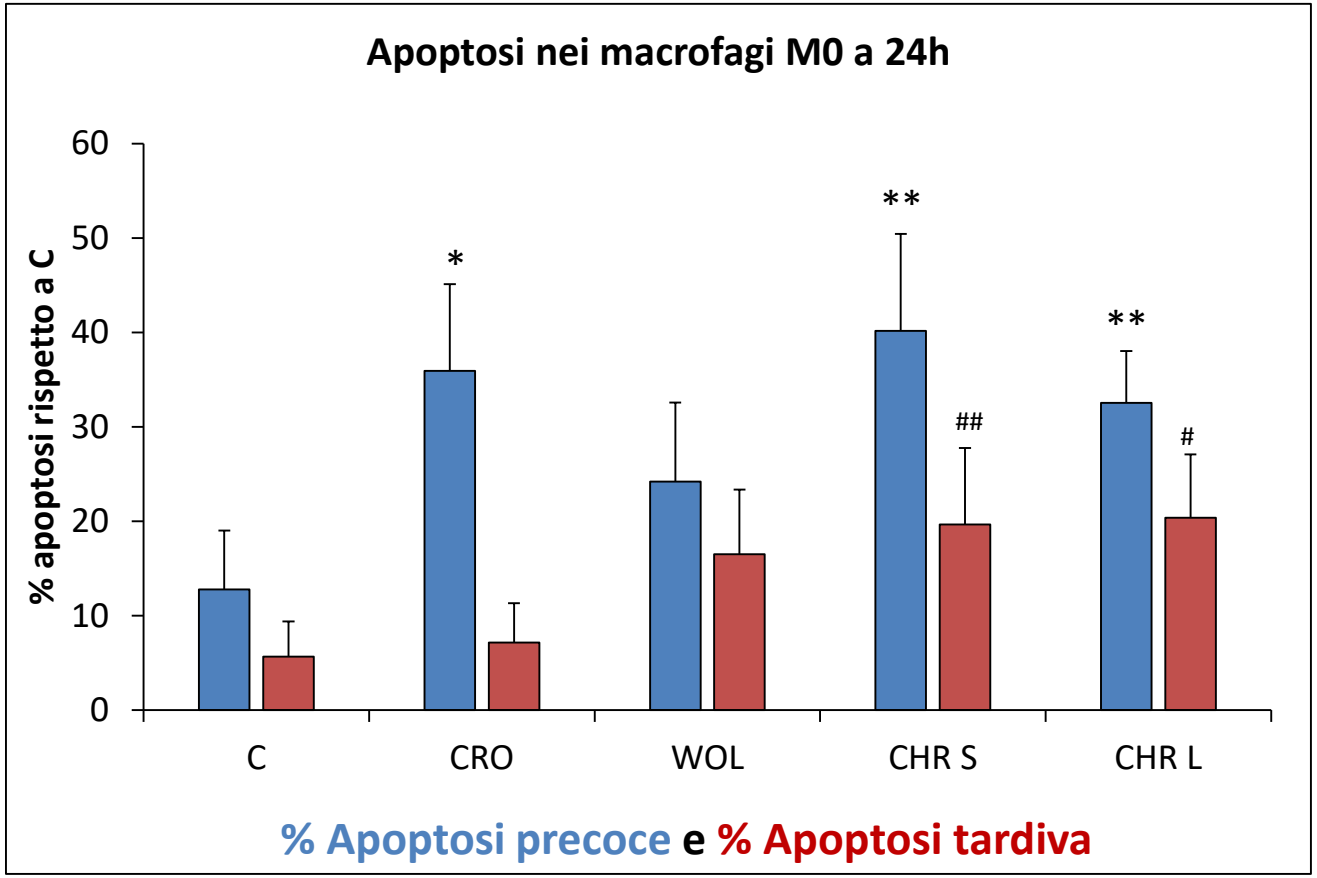


MACROFAGI M0

HECV

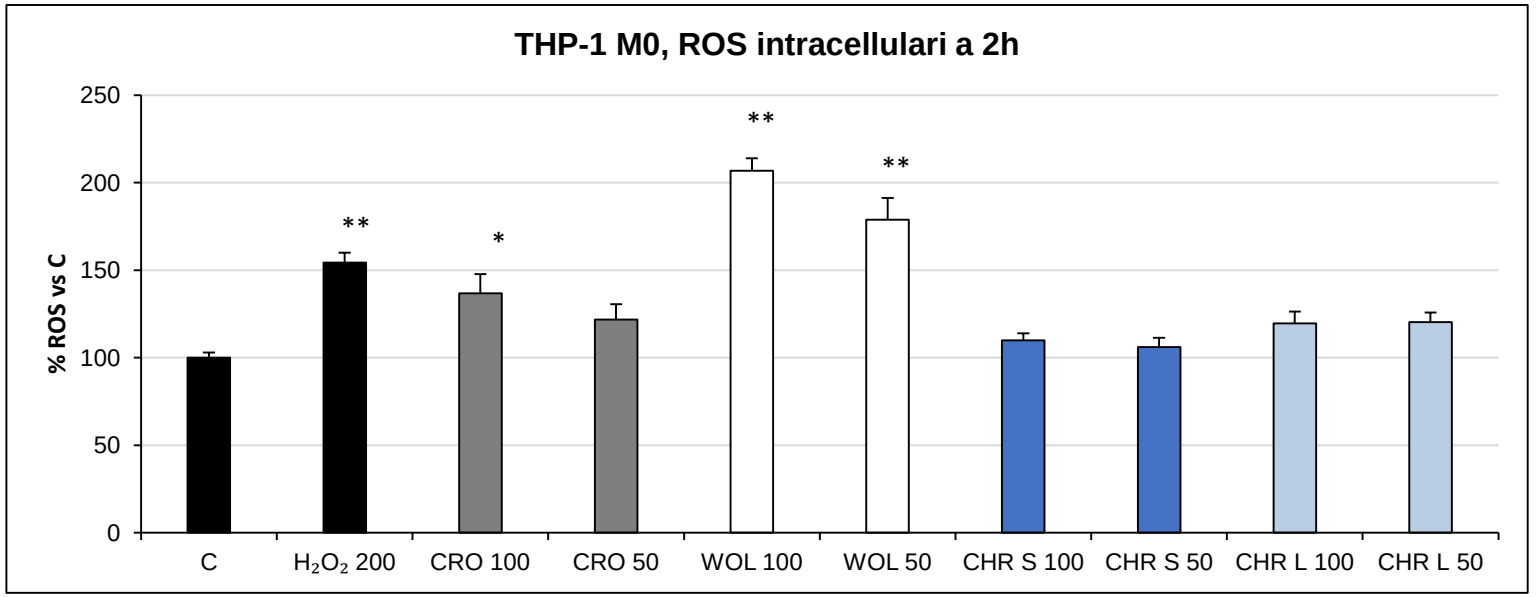


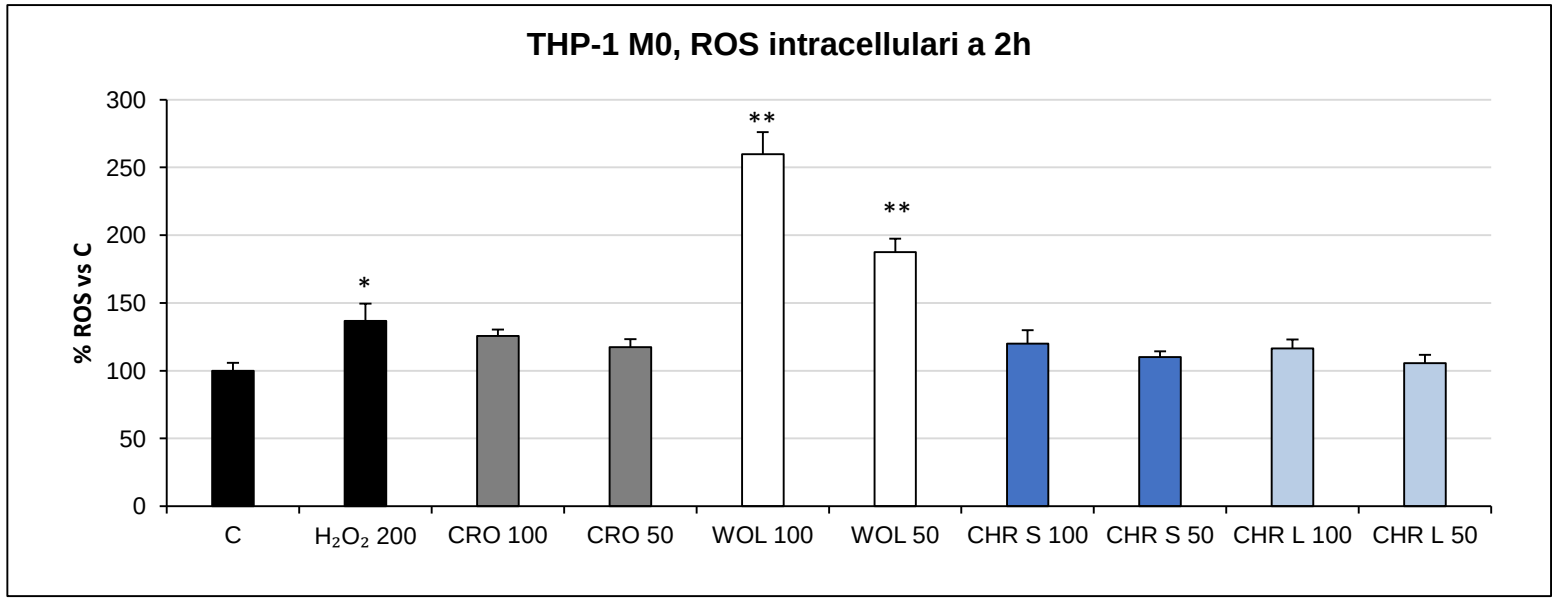
Quantificazione APOPTOSI in M0 a 24h con fibre 50 ug/ml



Early Apoptosis ** p<0,0005 vs C * p<0,005 vs C
Late Apoptosis ## p<0,005 vs C # p<0,01 vs C

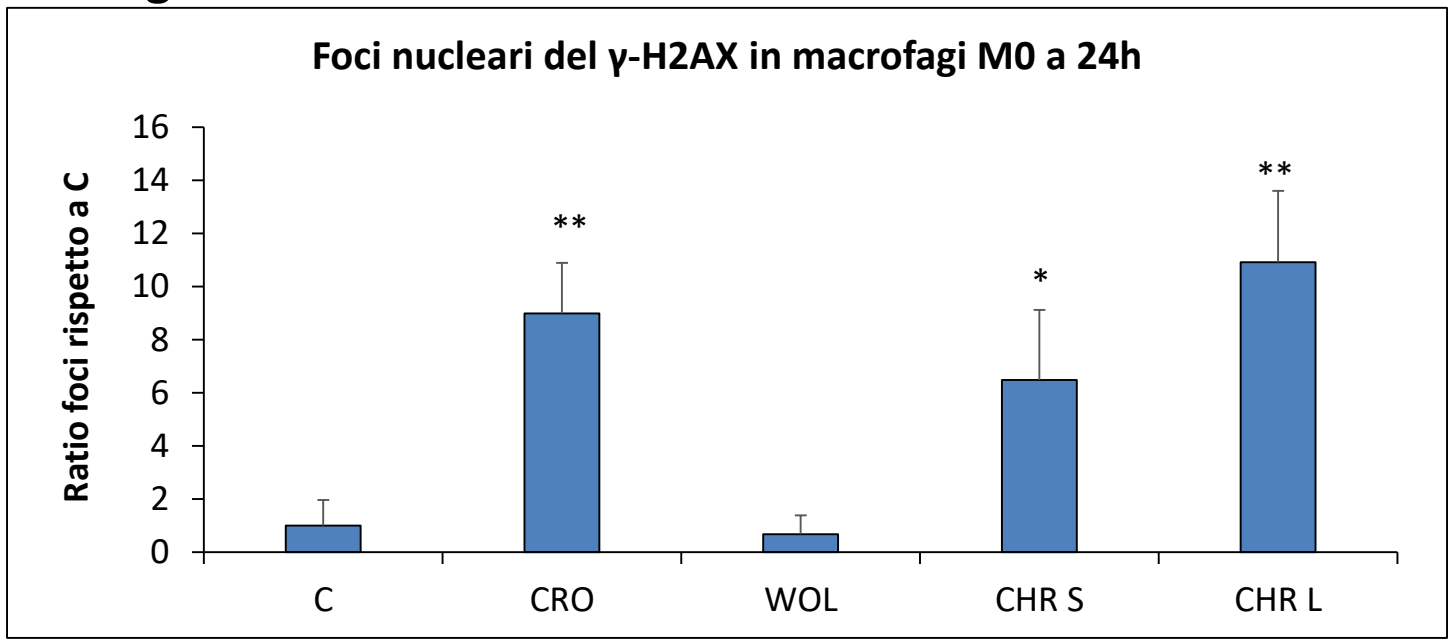
Produzione intracellulare di ROS dopo 2h di esposizione alle fibre con DCF



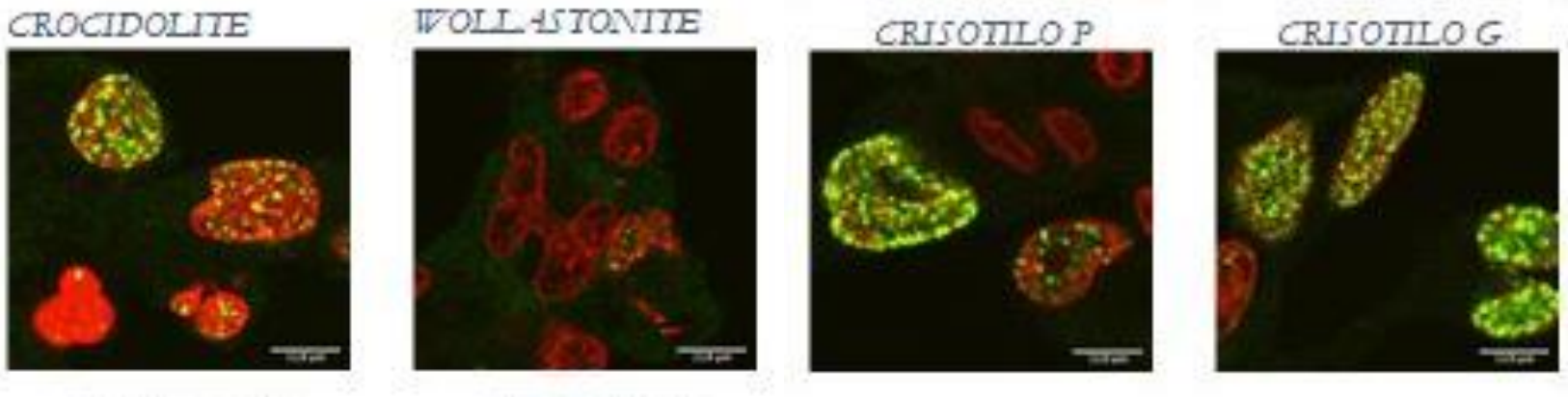


** p<0,0005 vs C * p<0,001 vs C

Valutazione del danno al DNA mediante marcatura del γ-H2AX a 24h nei macrofagi M0

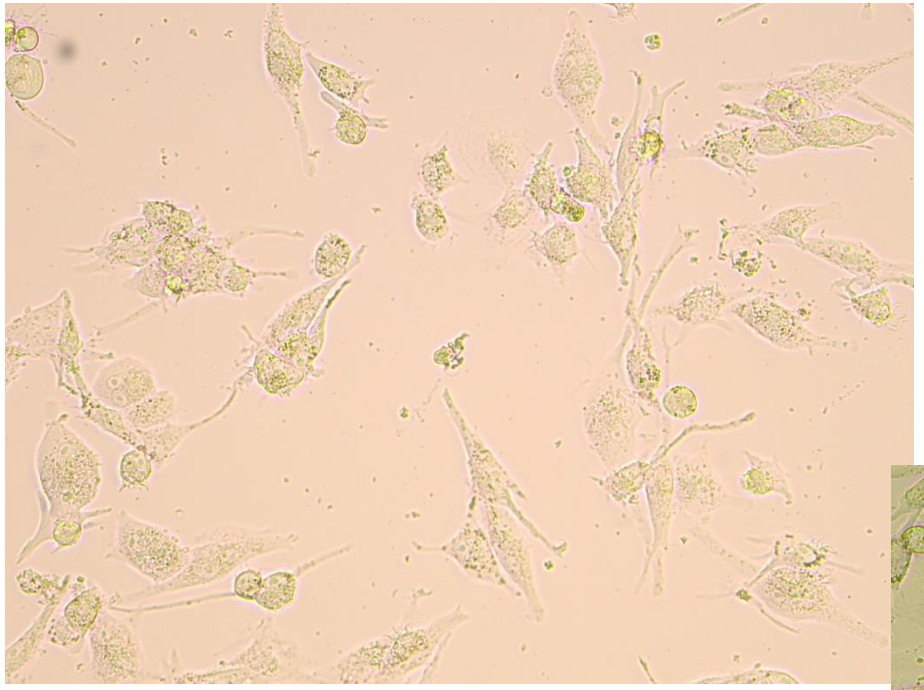


** p<0,0005 vs C * p<0,005 vs C

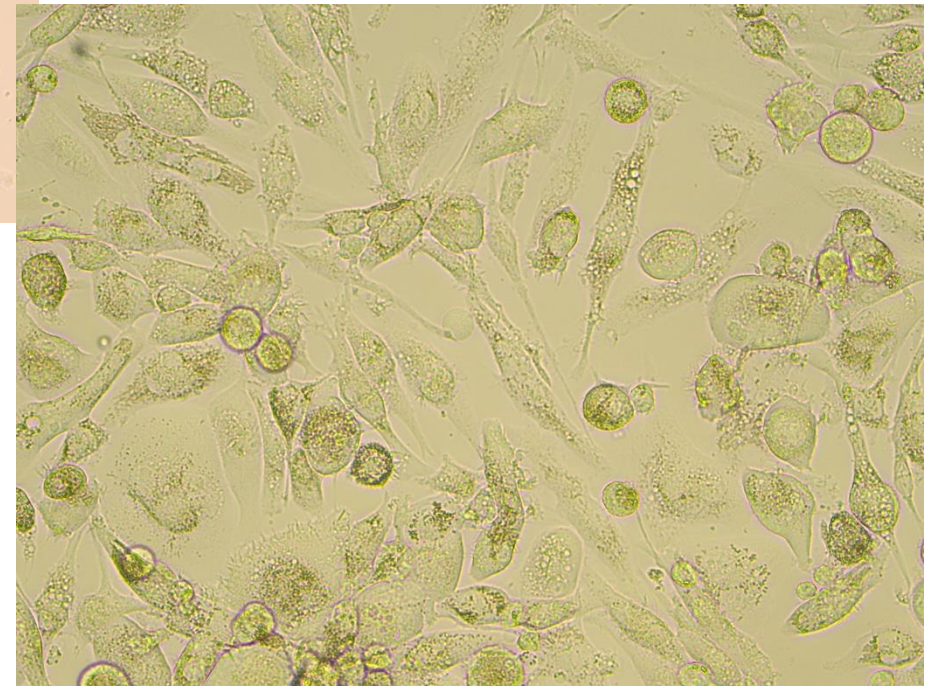


Valutazione fagocitosi in M0 con fibre 10 ug/ml in contrasto di fase

NT a 24h

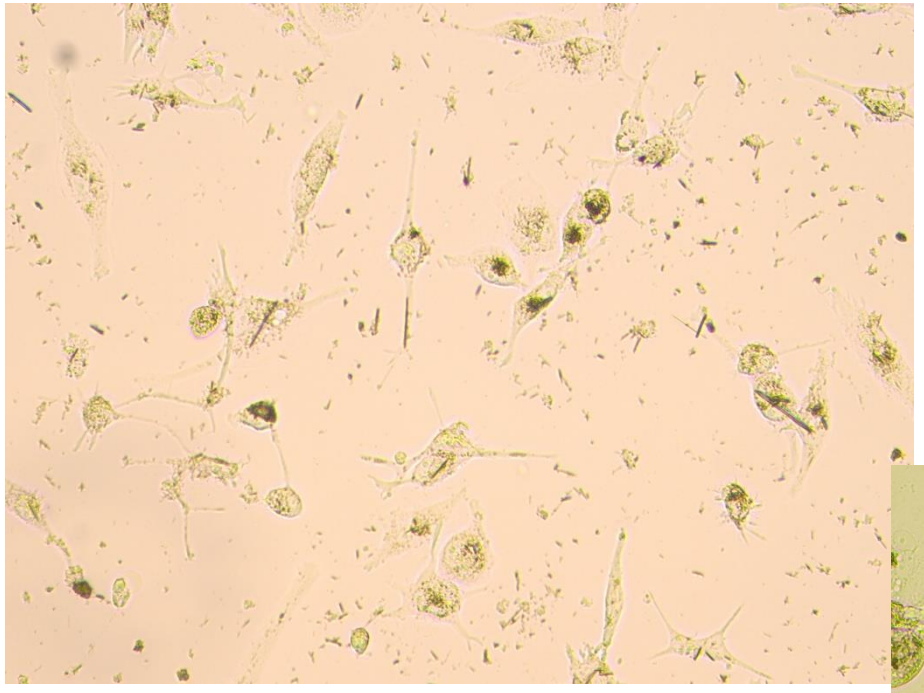


NT a 7gg

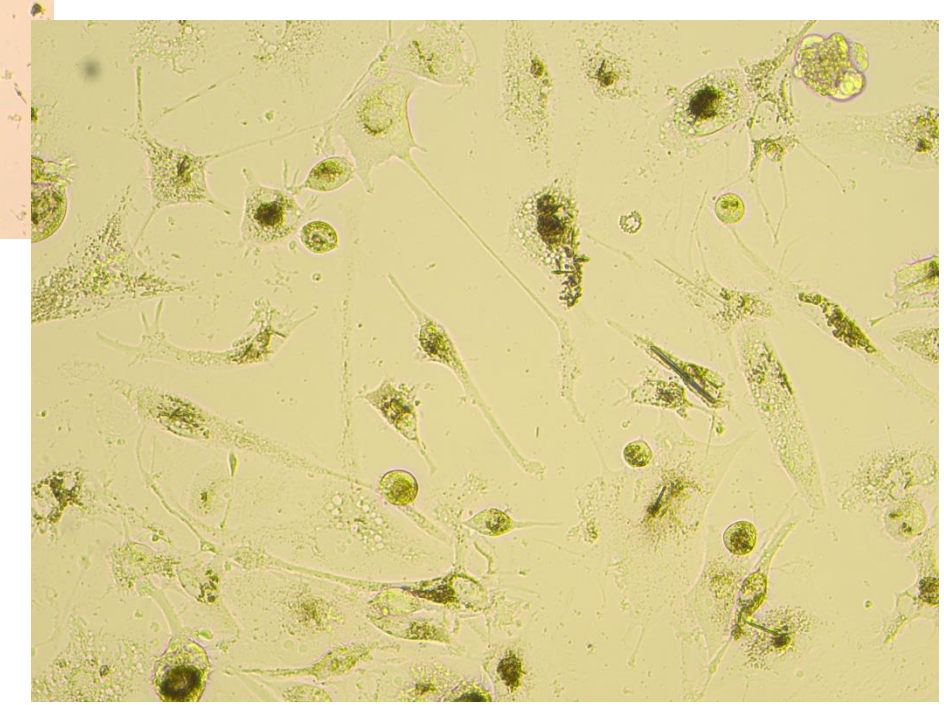


Valutazione fagocitosi in M0 con fibre 10 ug/ml in contrasto di fase

CRO a 24h

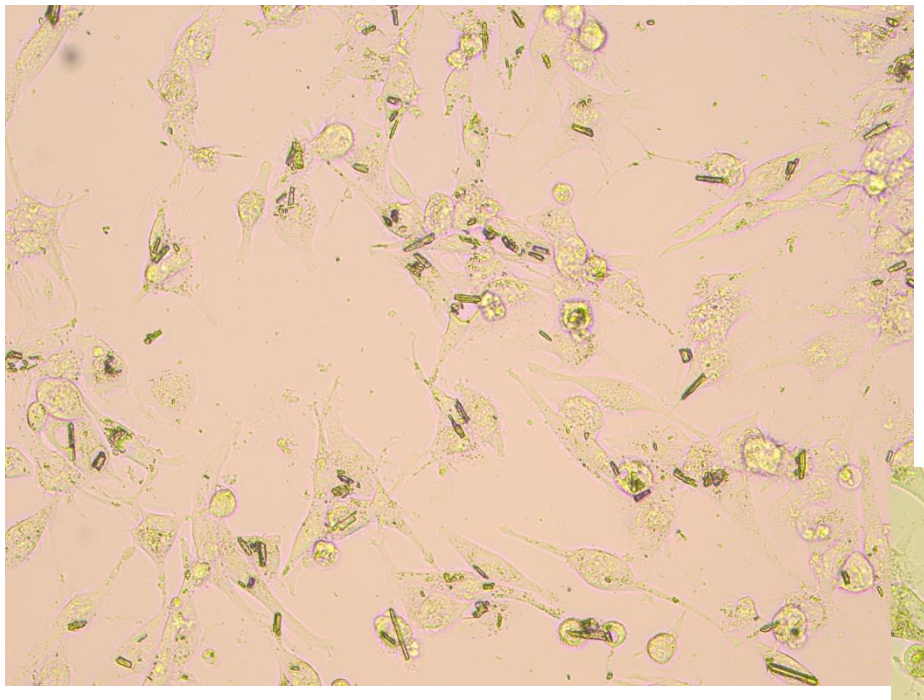


CRO a 7gg

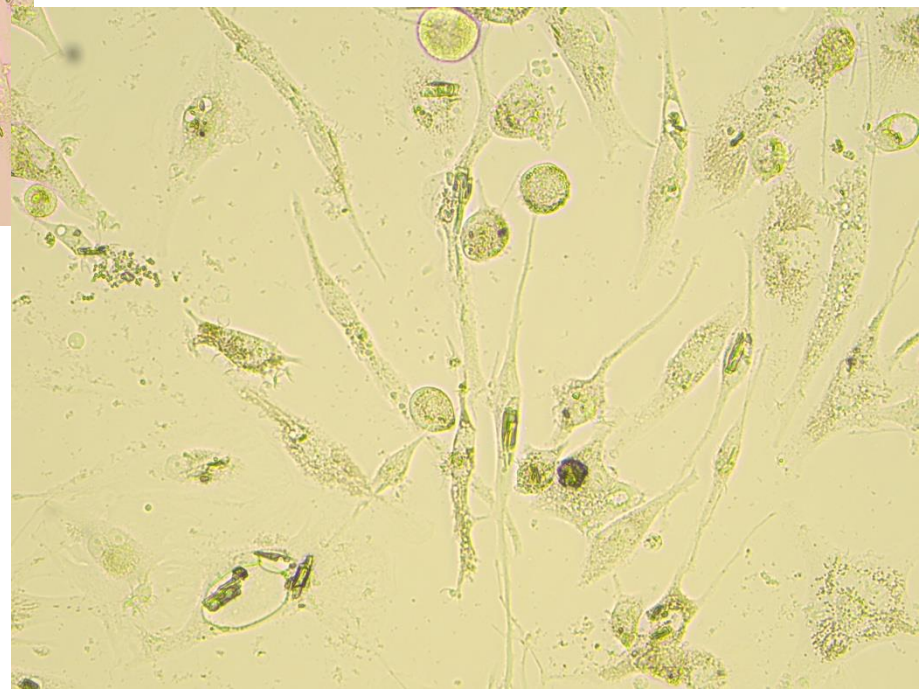


Valutazione fagocitosi in M0 con fibre 10 ug/ml in contrasto di fase

WOL a 24h

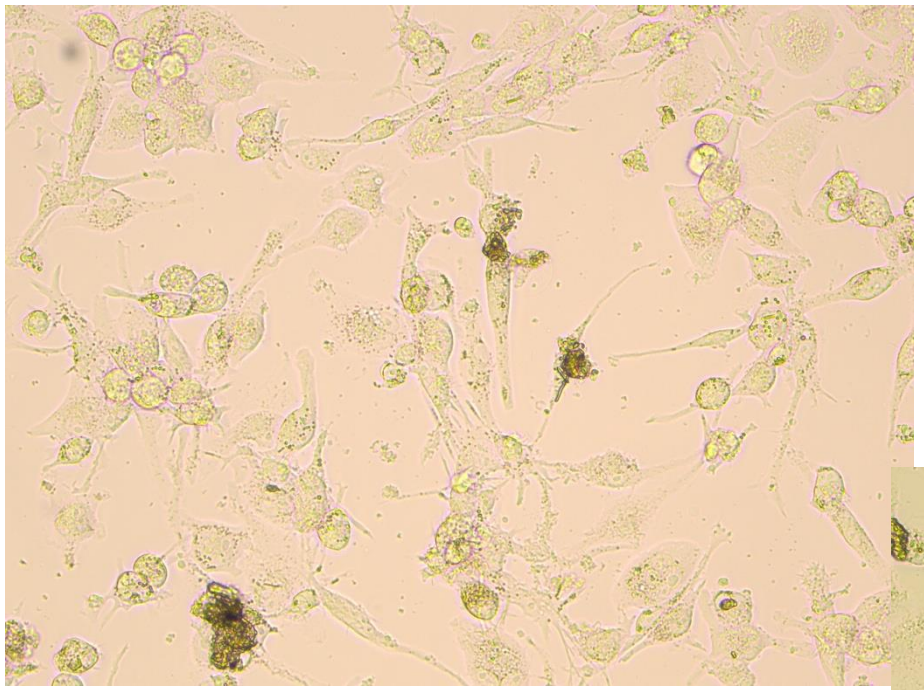


WOL a 7gg

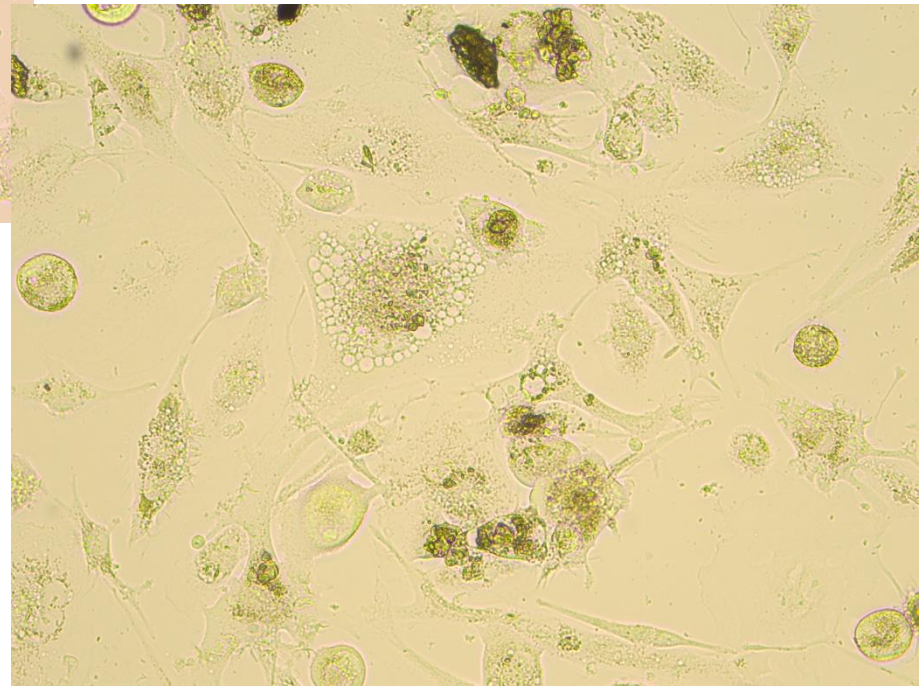


Valutazione fagocitosi in M0 con fibre 10 ug/ml in contrasto di fase

CRI P a 24h

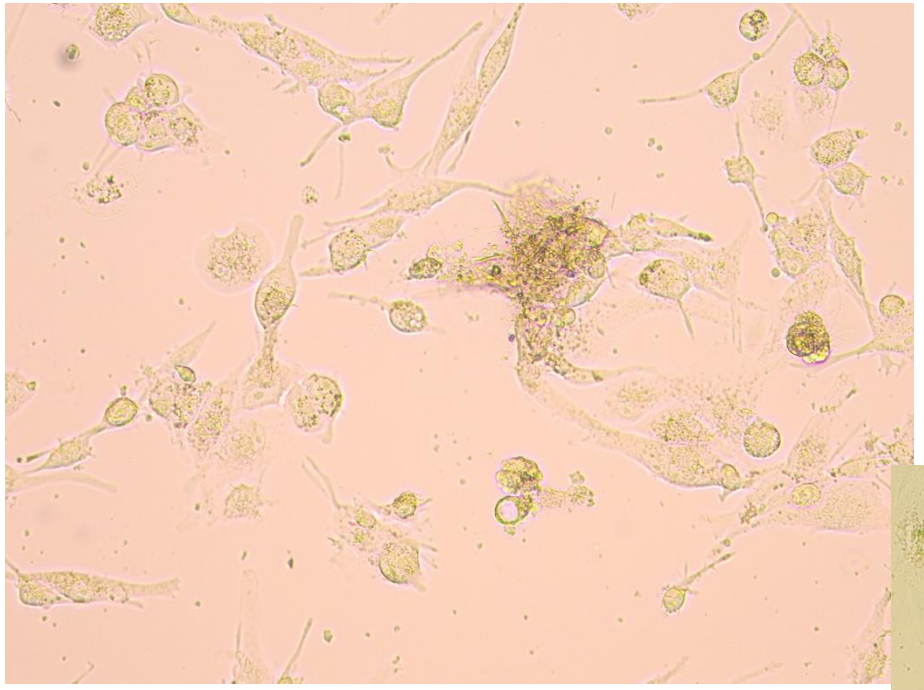


CRI P a 7gg

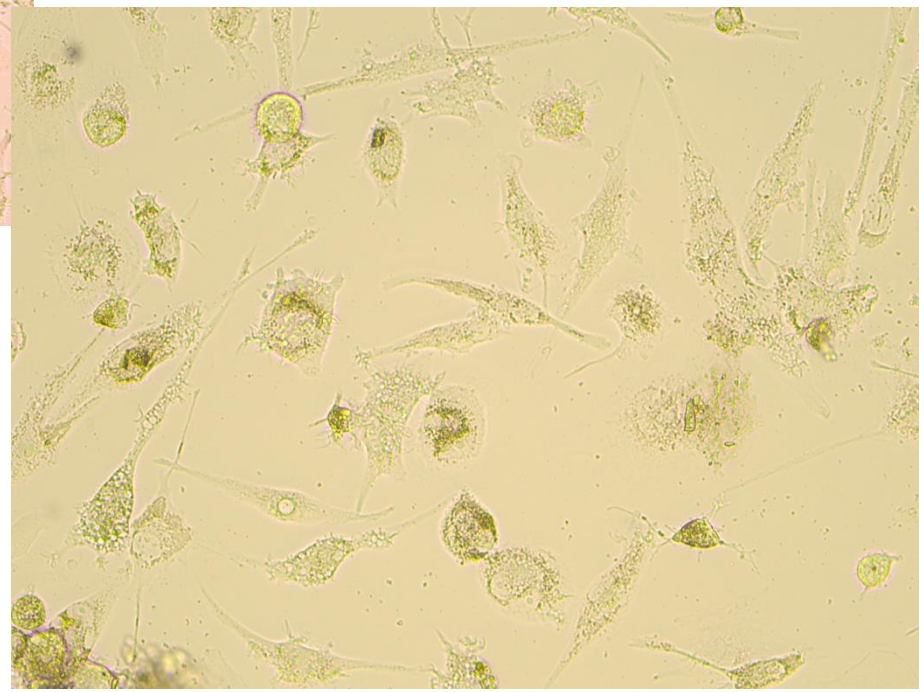


Valutazione fagocitosi in M0 con fibre 10 ug/ml in contrasto di fase

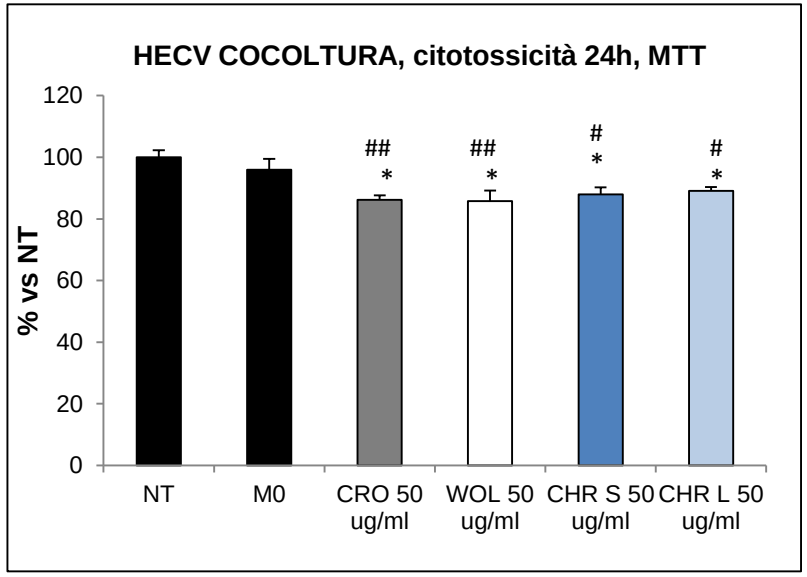
CRI G a 24h



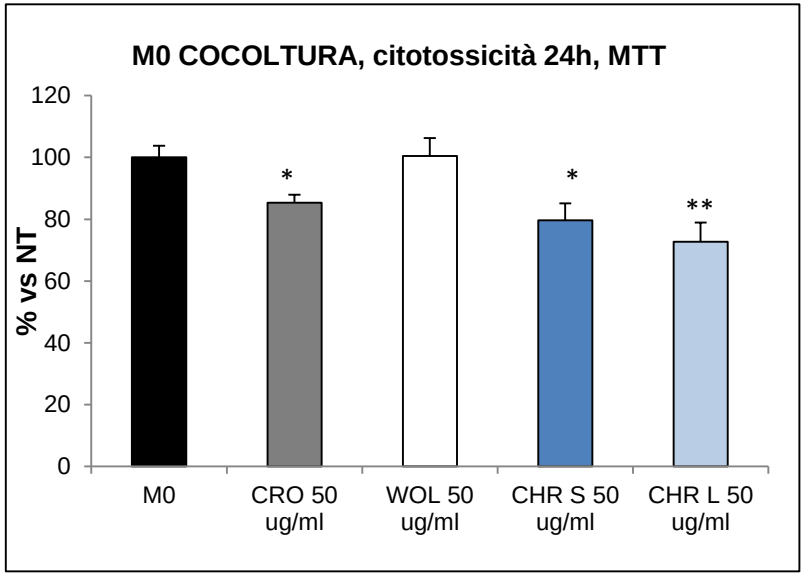
CRI G a 7gg



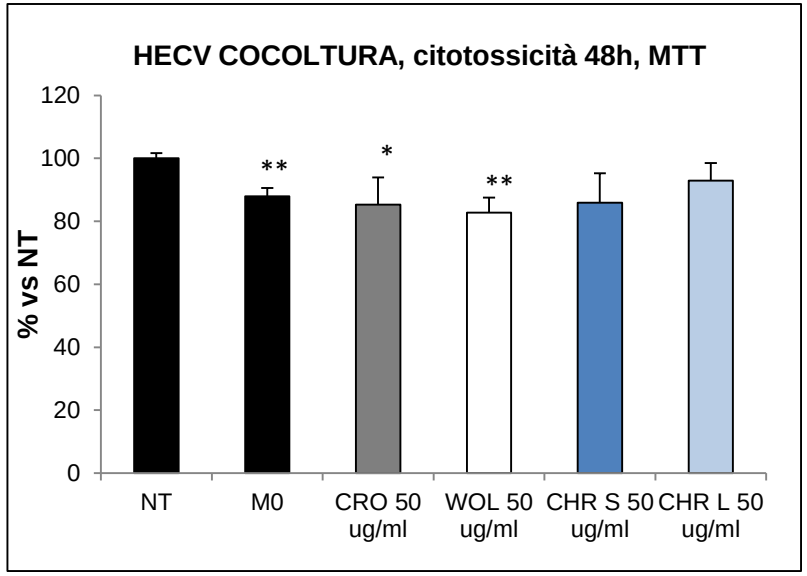
tossicità cellulare cocoltura HECV+THP1-M0 MTT a 24h e a 48h



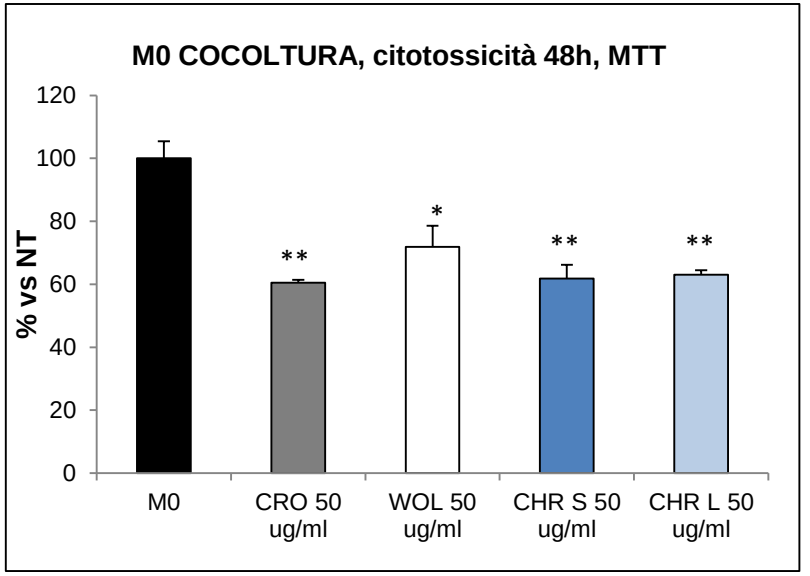
* p<0,0001 vs C ## p<0,001 vs M0 # p<0,005 vs M0



** p<0,01 vs C * p<0,05 vs C



** p<0,0001 vs C * p<0,005 vs C



** p<0,001 vs C * p<0,01 vs C

PCR riassunto

modello	Espressione valutata dopo
THP1 UNDIFF (POLVERI A CONTATTO)	24h e 48h
THP1 M0 (POLVERI A CONTATTO)	6h, 24h (DA FARE 48h e 7gg)
THP1 M0 IN COCOLTURA CON HECV (CON POLVERI SOLO SU THP1)	48h (DA FARE 24h e 7gg)
HECV (POLVERI A CONTATTO)	24h, 48h e 7gg
HECV IN COCOLTURA CON THP1 M0 (CON POLVERI SOLO SU THP1)	24h, 48h e 7gg

Geni visti in THP1	Geni visti in HECV
INFIAMMAZIONE	**INFIAMMAZIONE (IL-1β, IL-6, IL-8, MCP1)**
IL-1β, IL-6, IL-8, MCP, TNFα	**ATTIVAZIONE ENDOTELIO (ICAM-1, VEGF)**
DIFFERENZIAMENTO	**FIBROSI (TGFβ, FIBRONECTINA)**
CD163, CXCL10	**TRANSIZIONE ENDOTELIO-MESENCHIMA (α-SMA, COLLAGENE-1A, MMP-9)**

Remarks.2

In M0-THP-1 cells, both chrysotile fractions induced a remarkable dose-dependent cell death and the exposure to CHR L resulted in an overall higher mortality rate, even compared to CRO. Moreover CHR L induced the highest rates of cell necrosis, while no necrosis was found with CHR S. CHR L e CHR S induced similar high levels of both early and late apoptosis, while with WOL we observed mainly dead cells, likely due to a necrotic effect.
The highest ROS production was observed with WOL in both cell lines and only CHR L caused a slight ROS increase.
Both CHR fractions induced a significant amount of double-strand breaks in the DNA (~CRO)

The same pattern of cell mortality was observed in a co-culture set-up where M0 macrophages were exposed to the mineral fibers, while the HECV monolayer below was not influenced in terms of viability. Regarding the gene expression profile in HECV, the inflammatory genes are up-regulated at 24h and there is no fibrosis. At 48h this expression increase is significant with CRO and CHR L, while they are down-regulated with CHR S, although in a co-culture set-up the down-regulation is present also in CRO and CHR L (not WOL). At 7 days EMT and inflammatory genes are down-regulated.