

A Field Detector for Genotoxicity from CBRN and Explosive Devices
SPS MYP G5266



*This project
is supported by:*

The NATO Science for Peace
and Security Programme



Medicinal plants and their effects against free radical induced changes in the cell

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Main interest: pharmacognosy, medicinal plants



везена долга



сиврија



феферона



бомбона



цинки



пиран



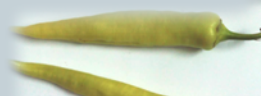
везена куса



златен медал



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хибрид 14530



хибрид 13514



хибрид 13515



фортенсе



куртовска капија



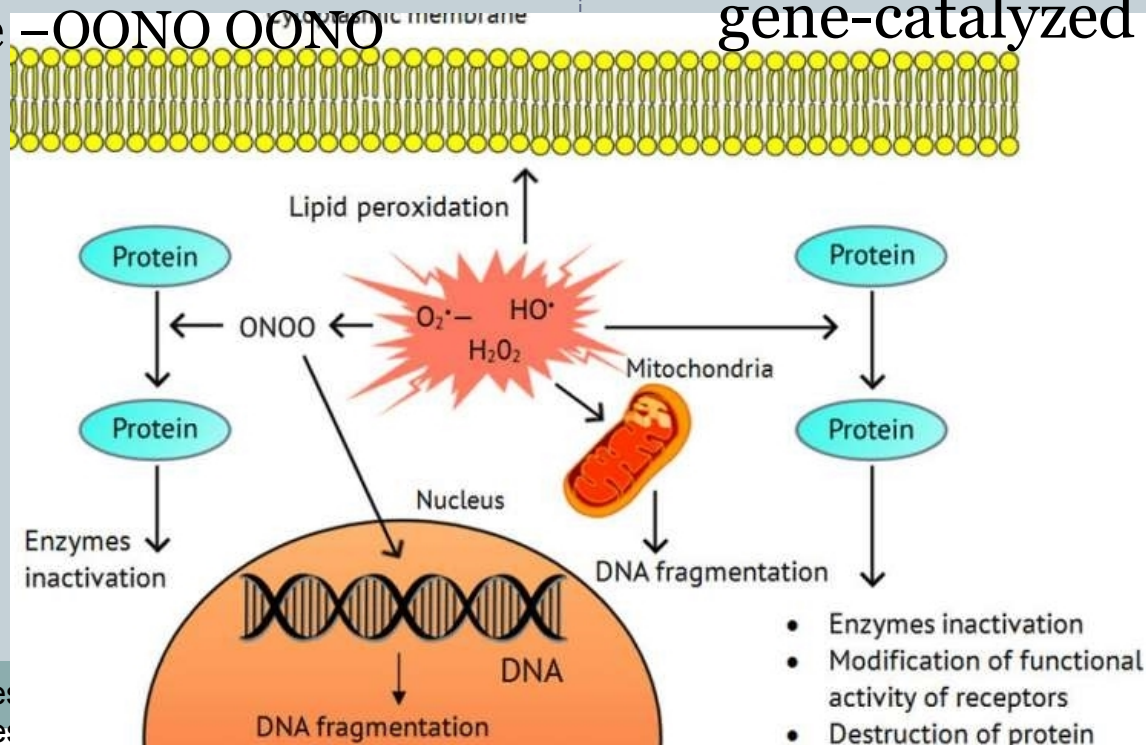
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Pharmacokinetic and pharmacodynamic variability on the effects of certain plant extract v.s pure phytochemical constituent is more than possible.

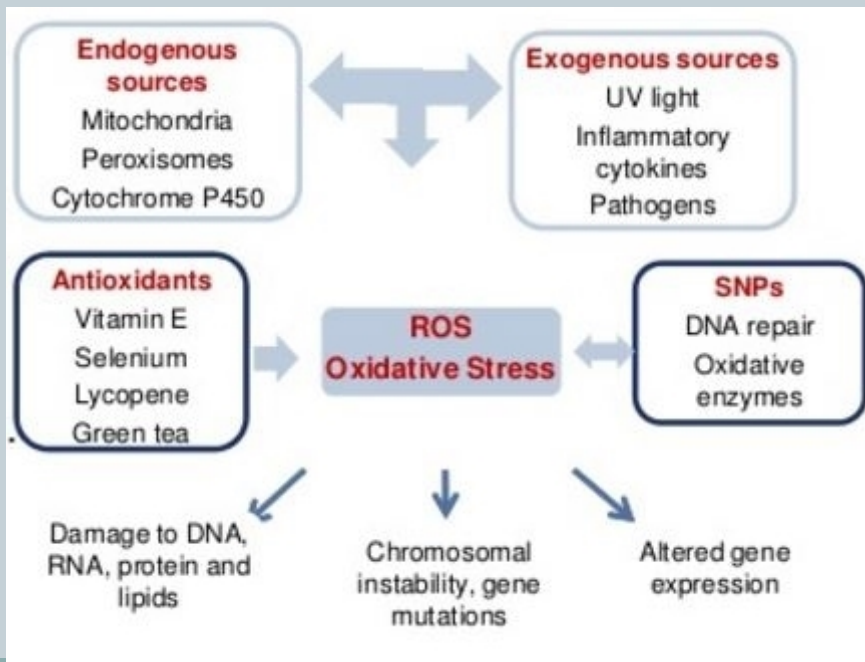
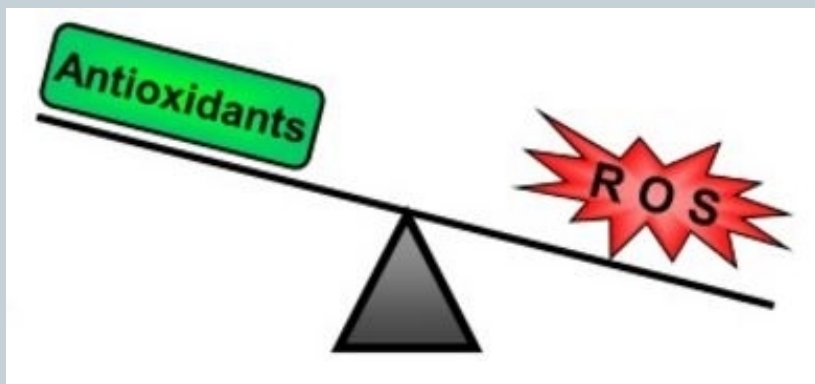
Reactive oxygen species

- **Reactive oxygen species** or **ROS** (superoxide, hydroxyl, and peroxy radicals, and hydrogen peroxide) and
- **Reactive nitrogen species** or **RNS** (nitric oxide and peroxynitrite). (Hydrogen peroxide H_2O_2 and peroxynitrite $-OONO$ $OONO$)

- However, a more important activity of physiological ROS and RNS is their participation in many enzymatic and gene-catalyzed processes.



Oxidative stress: imbalance between ROS and antioxidants



- Maintenance of optimal ROS and RNS levels is a critical condition for normal functioning of physiological processes.
- Disturbance of regulation of ROS and RNS signaling might lead to the activation of dangerous enzymatic cascades and stimulation of numerous pathological states including cardiovascular diseases, hypertension, diabetes mellitus, cancer and carcinogenesis, inflammation, and aging.
- Thus toxic effects of ROS and RNS signaling can depend not only on direct attack of these species on biomolecules (although it might be true for reactive hydroxyl and peroxy free radicals) but to be a consequence of disruption of their physiological levels due to enhancement or reduction of reactive species

Electrochemistry as a tool for estimation of antioxidant capacity

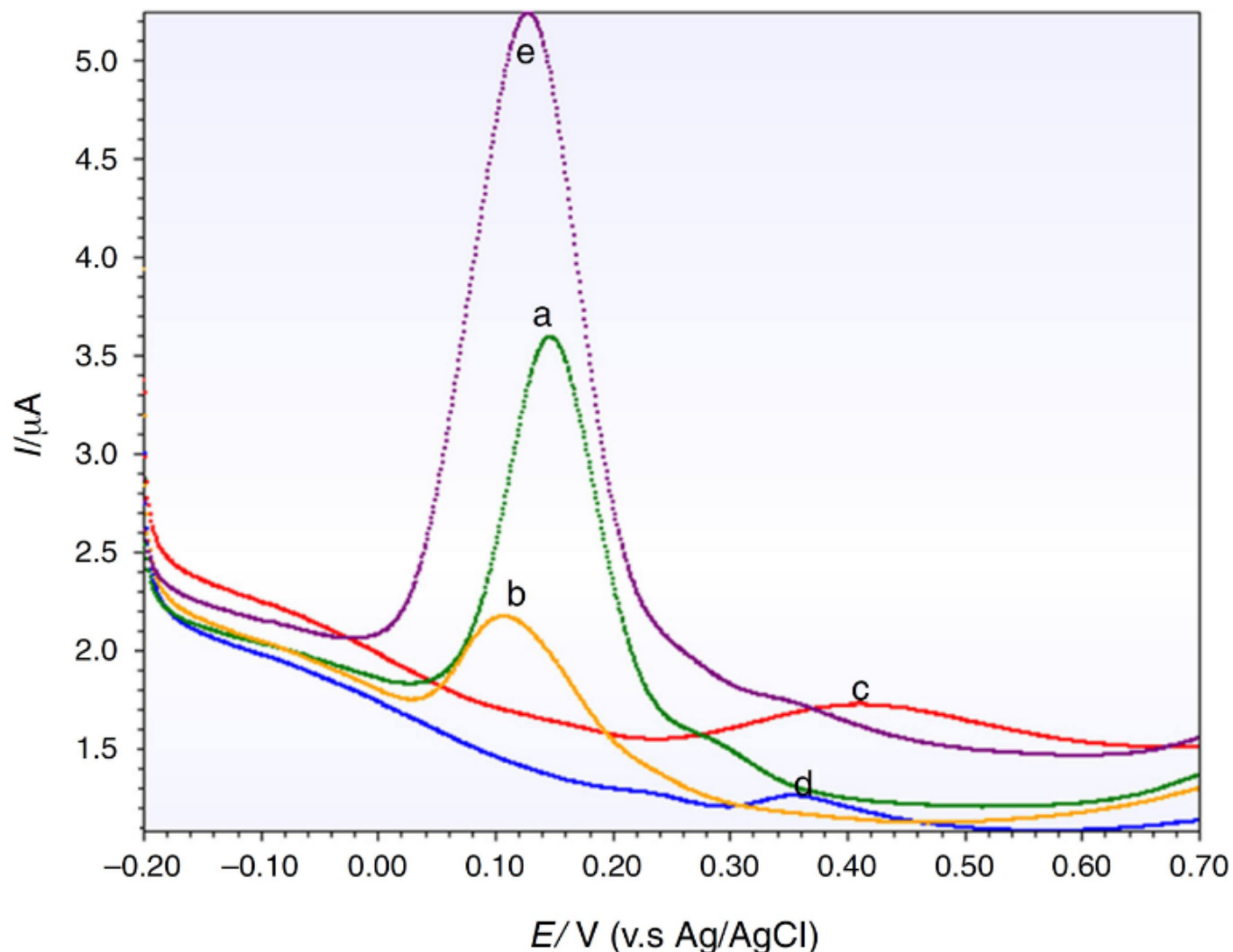


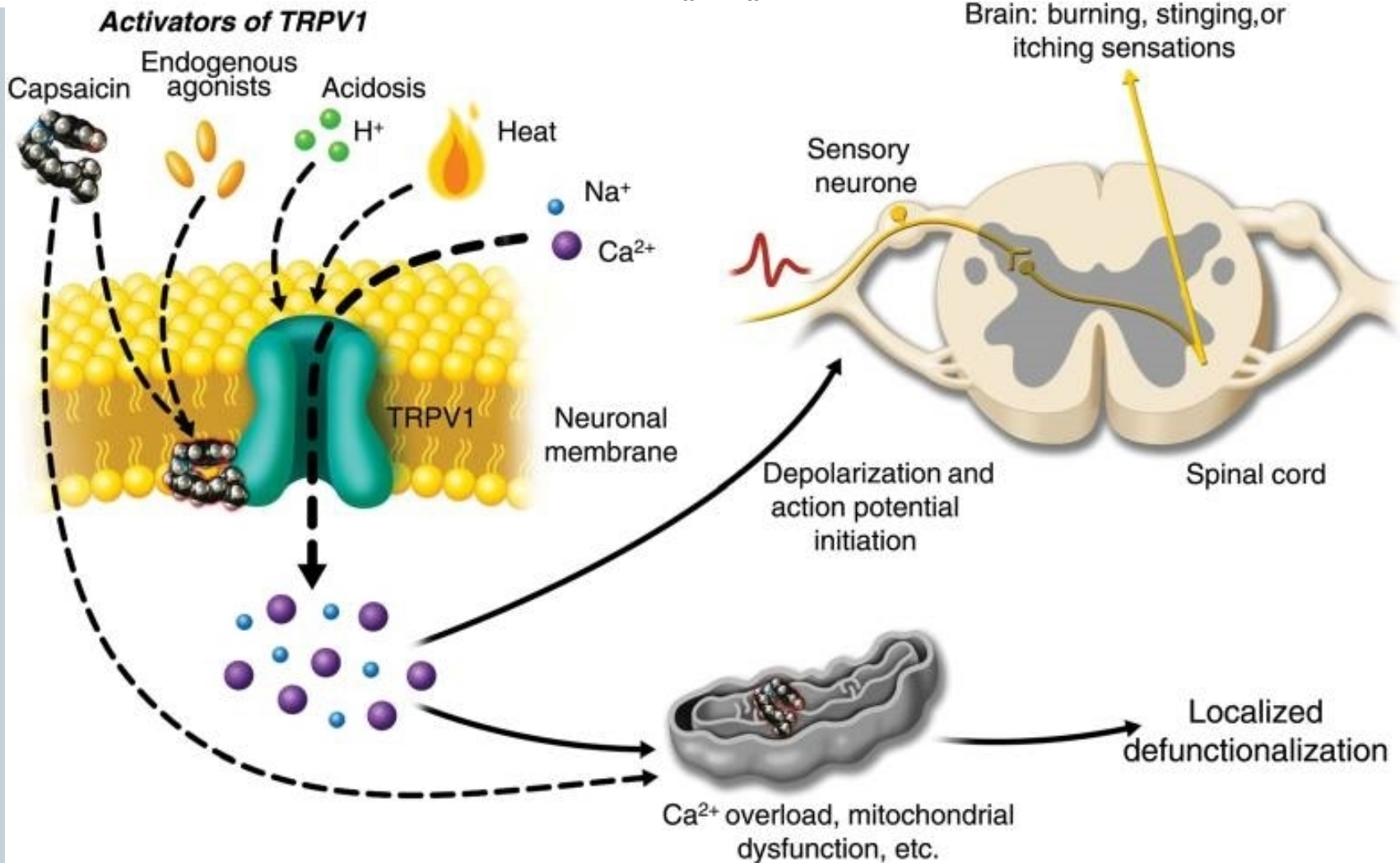
Fig. 7 Square-wave voltammogram of: (A) vitamin E; (B) quercetin, (C) ascorbic acid and (D) capsaicin (10 μM), (E) equimolar mixture containing vitamin E, quercetin, ascorbic acid and capsaicin at concentration of 10 μM , recorded at a glassy carbon electrode in a buffer solution at pH = 7.1. Instrumental parameters were: step potential $dE = 0.001$ V, square-wave amplitude $E_{\text{sw}} = 0.05$ V and frequency of 10 Hz.

ROS and Ca^{2+} two cross-talking messengers

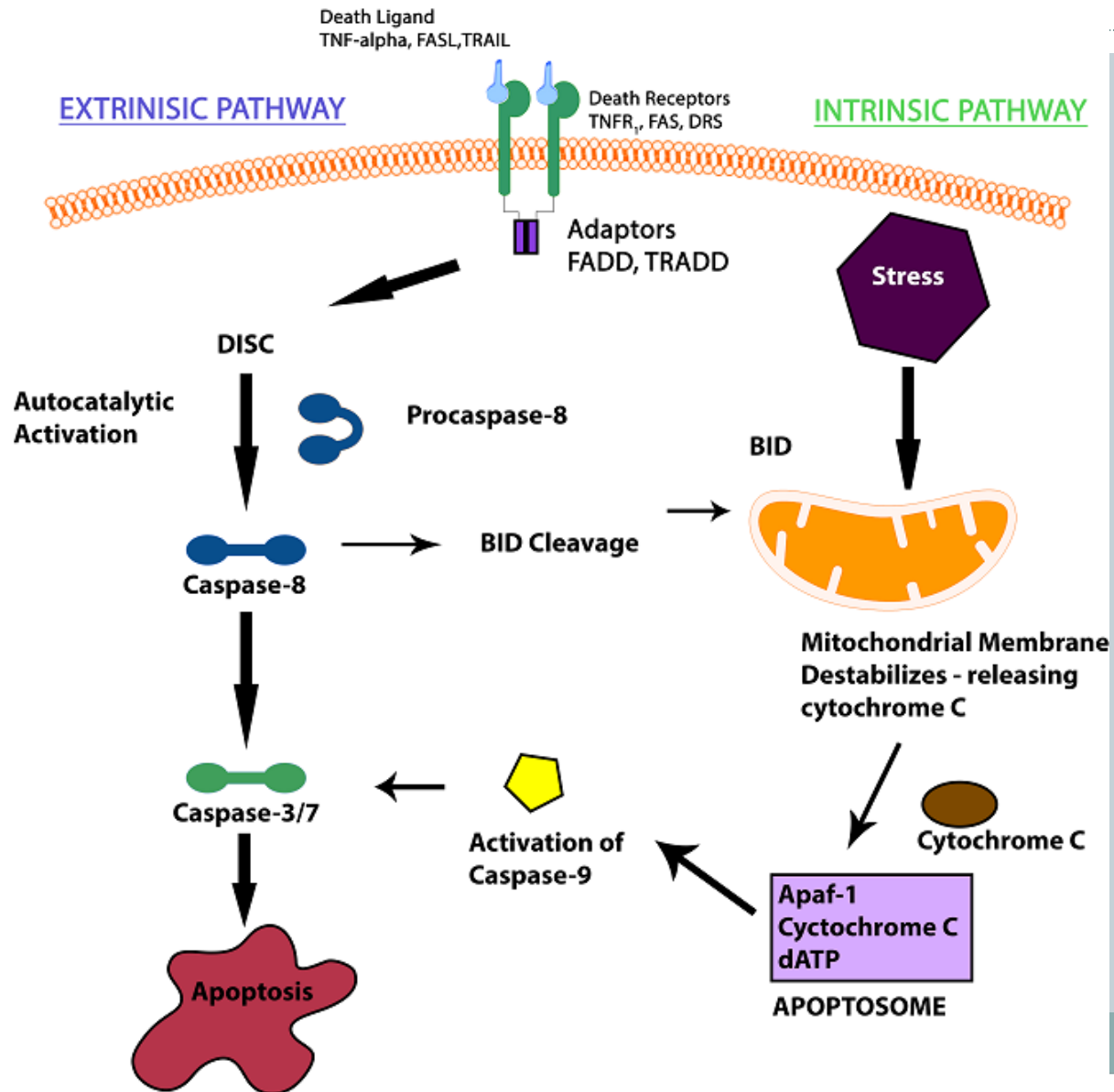


- ROS and Ca^{2+} are two cross-talking secondary messengers in numerous cellular processes.
- Interactions between Ca^{2+} and ROS signaling systems can be both stimulatory and inhibitory.
- There is a delicate balance between the beneficial and detrimental consequences of Ca^{2+} and ROS regarding the mitochondrial function.
- Capsaicin is TRPV1 receptor (Ca^{2+} –channel) agonist.

Activation of TRPV1 receptors



Molecular mechanisms of apoptosis=two ways leading to cell death

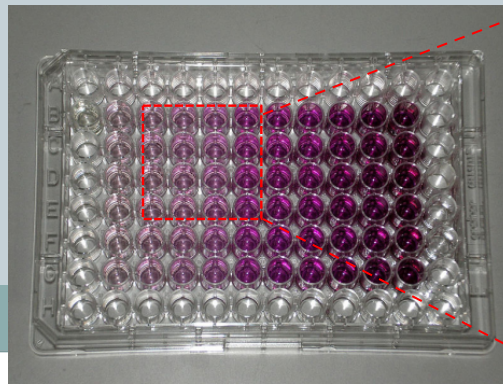
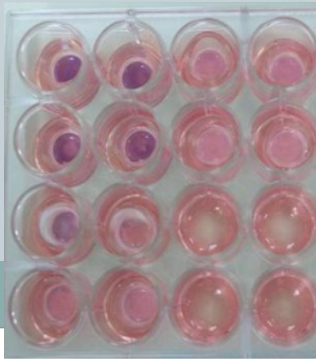
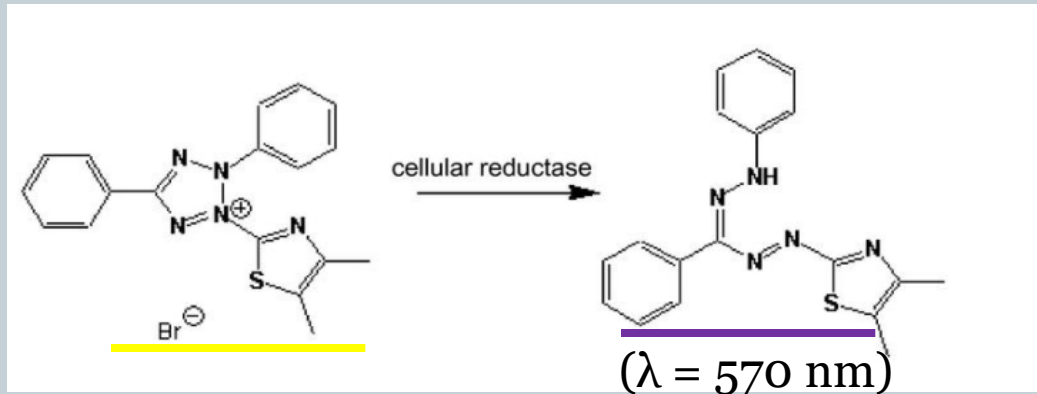


Methods for determination of cytotoxicity of capsaicin MTT and LDH assays

- **MTT assay**

- Colorimetric analysis for measuring the metabolic activity of living cells
- MTT ((3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) –yellow is reduced to violet-blue formazan product

- Method principle :

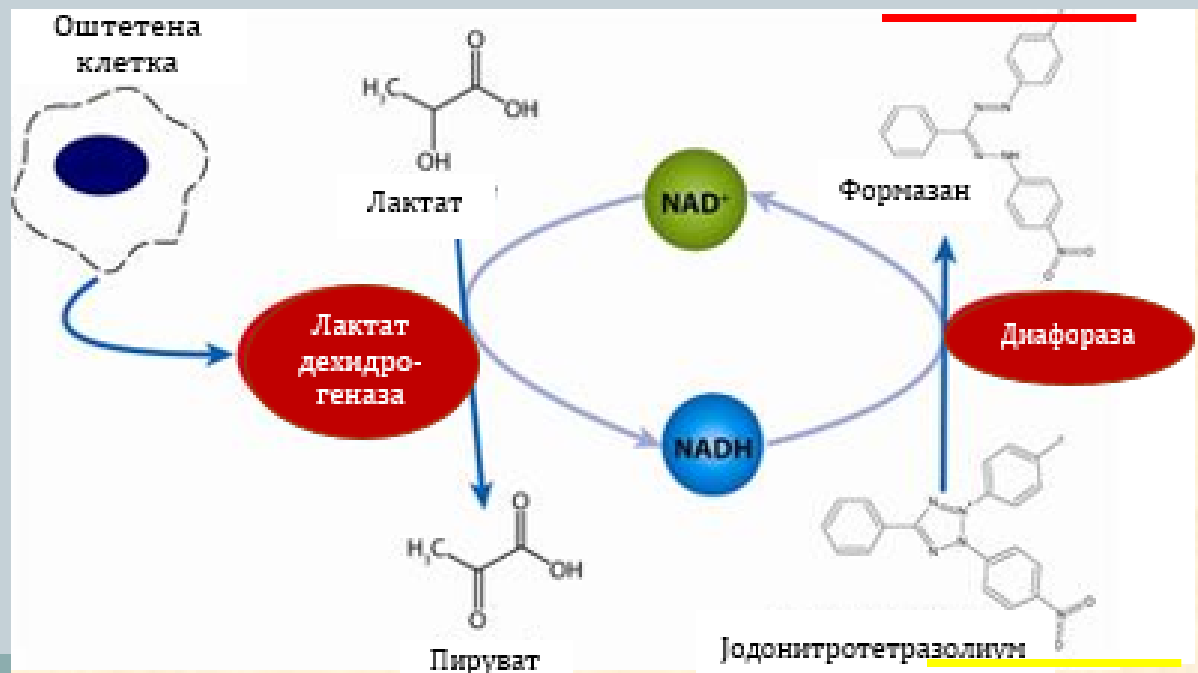


Methods for determination of cytotoxicity of capsiaicn: MTT and LDH assays

- **LDH assay**

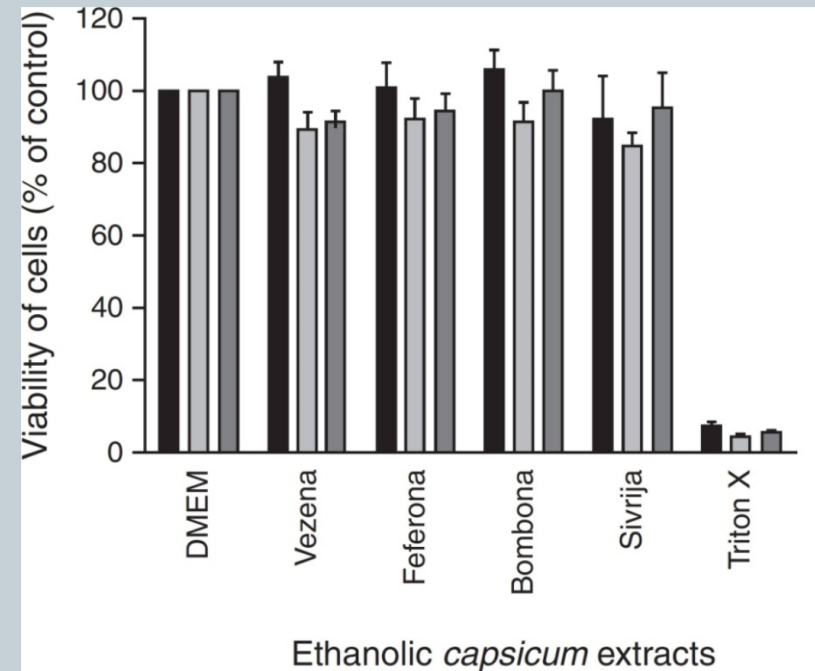
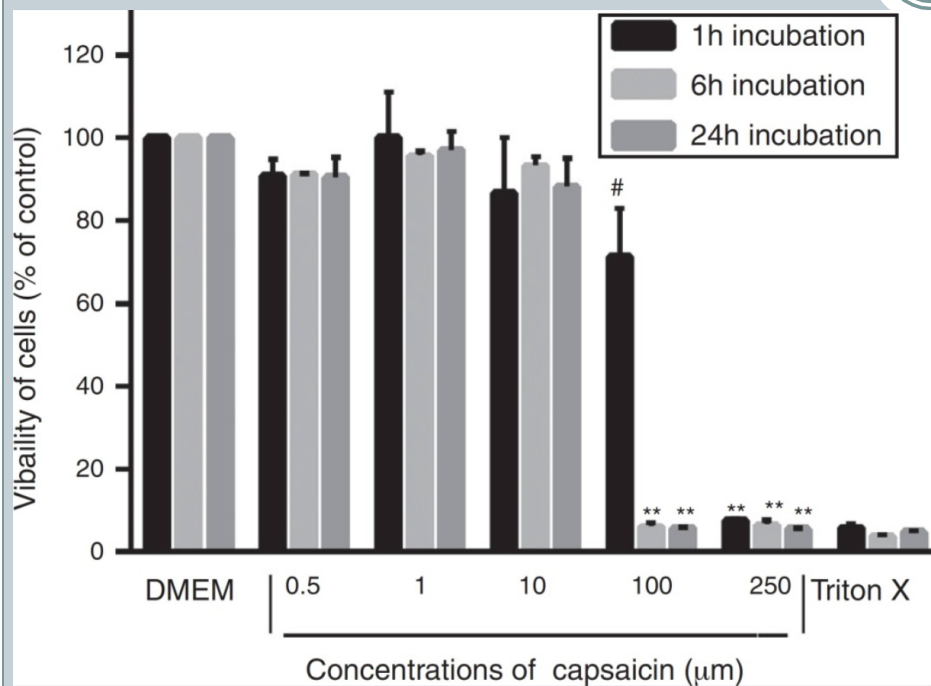
- Method for estimation of cell membrane integrity
- LDH (lactate dehydrogenase) is an intracellular enzyme and it leaks at extracellular compartment in the case of cell necrosis (cell death)

- Method principle



($\lambda = 492 \text{ nm}$)

Capsaicin induces apoptosis through its TRPV1 receptor (Ca²⁺ –channel)



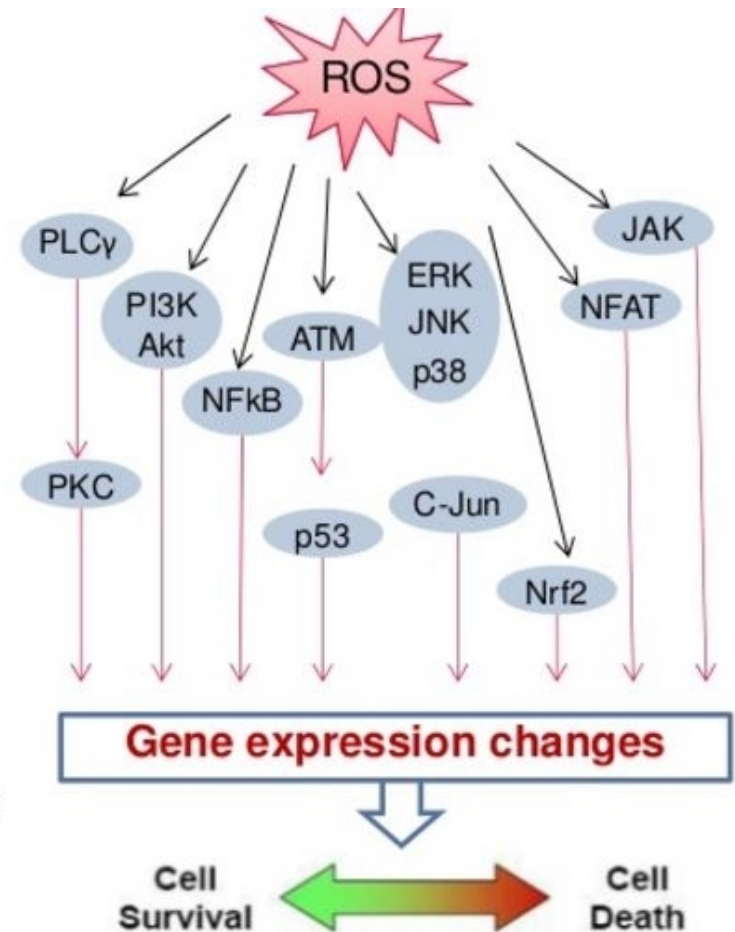
- Effect of different capsaicin concentrations on cell viability (MTT assay) on B104 neuroblastoma cells, incubated for 1, 6 or 24 h. There is a significant difference in the results for the effect of 100 μM capsaicin for 1 h compared to the effect for 6 h. Significance: # ($p < 0.05$), ** ($p < 0.01$) vs. DMEM.

- Effect of ethanolic *Capsicum* extracts (obtained from *Capsicum* genotypes: Vezena, Feferona, Bombona, Sivrija) on cell viability (MTT assay) treated for 1, 6 or 24 h. Viability of the cells was compared to negative control (DMEM).

ROS and genotoxicity ...for further studies

Physiological positive effects of ROS

- ❑ Kill microorganism
 - ❑ Second messenger (H_2O_2)
 - ❑ Cellular differentiation & proliferation
 - ❑ Regulate signal transduction & transcription
-
- Cytokines and growth factor receptors (EGFR, PDGFR, VEGFR, receptors of $TNF\alpha$, $IFN\gamma$ and $IL-1\beta$)
 - Non-receptor tyrosine kinases (JAK, Src, MAPK and PI3K)
 - Protein tyrosine phosphatases
 - Serine/Threonine kinases (Akt, PKC and MAPK)
 - Nuclear transcription factors (NF κ B, AP-1, NFAT, HIF-1 and Nrf2)





**KEEP
PEACE
AND
THANK YOU FOR
YOUR ATTENTION**

- Gratitude to NATO SPS project for supporting young researchers!