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γ- and δ-Tocotrienols interfere with senescence leading to decreased viability of cells

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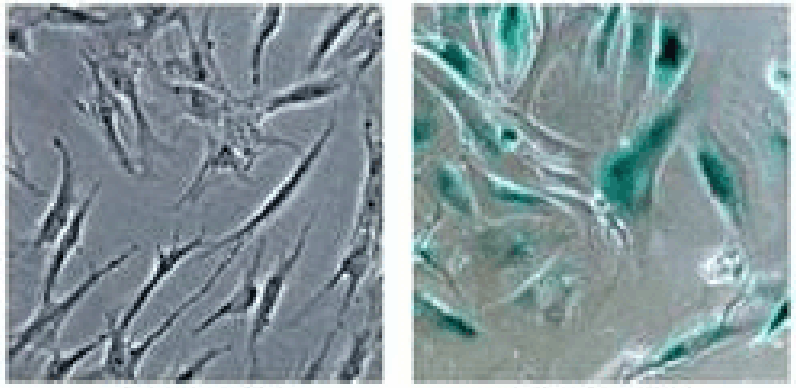
Cell senescence

- a permanent and irreversible cell cycle arrest

Hallmarks

- enlarged and flattened morphology
- a less regular shape
- a larger nucleus
- many vacuoles
- lipofuscin
- ROS
- senescence associated β- galactosidase
- SDF
- SAHF
- p53, p21, p16
- altered metabolism
- SASP



Normal Cell Senescent Cell

Causes

- shortening of telomeres

or

- *DNA damage*
- *oxidative stress*
- *mitochondrial dysfunction*
- *overexpression of activated oncogenes*
- *nucleolar stress*
- *disruption of epigenetic regulation*

The role of senescent cells in organism

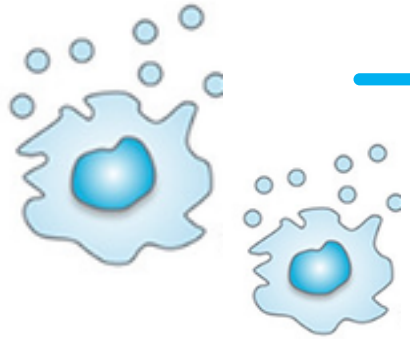
- Development
- Wound healing
- Protection against excessive fibrosis
- Tumor suppression

senescent cells

Removal by immune cells

Accumulation

SASP components



senescent cells

Deleterious efects

- Tumor promotion
- Chronic inflammation
- Age-associated diseases
- Inducing of senescence
- Altering stem cell niche

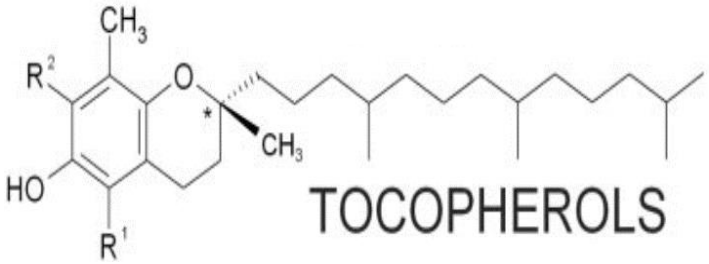
Disturbance of tissue and organ function

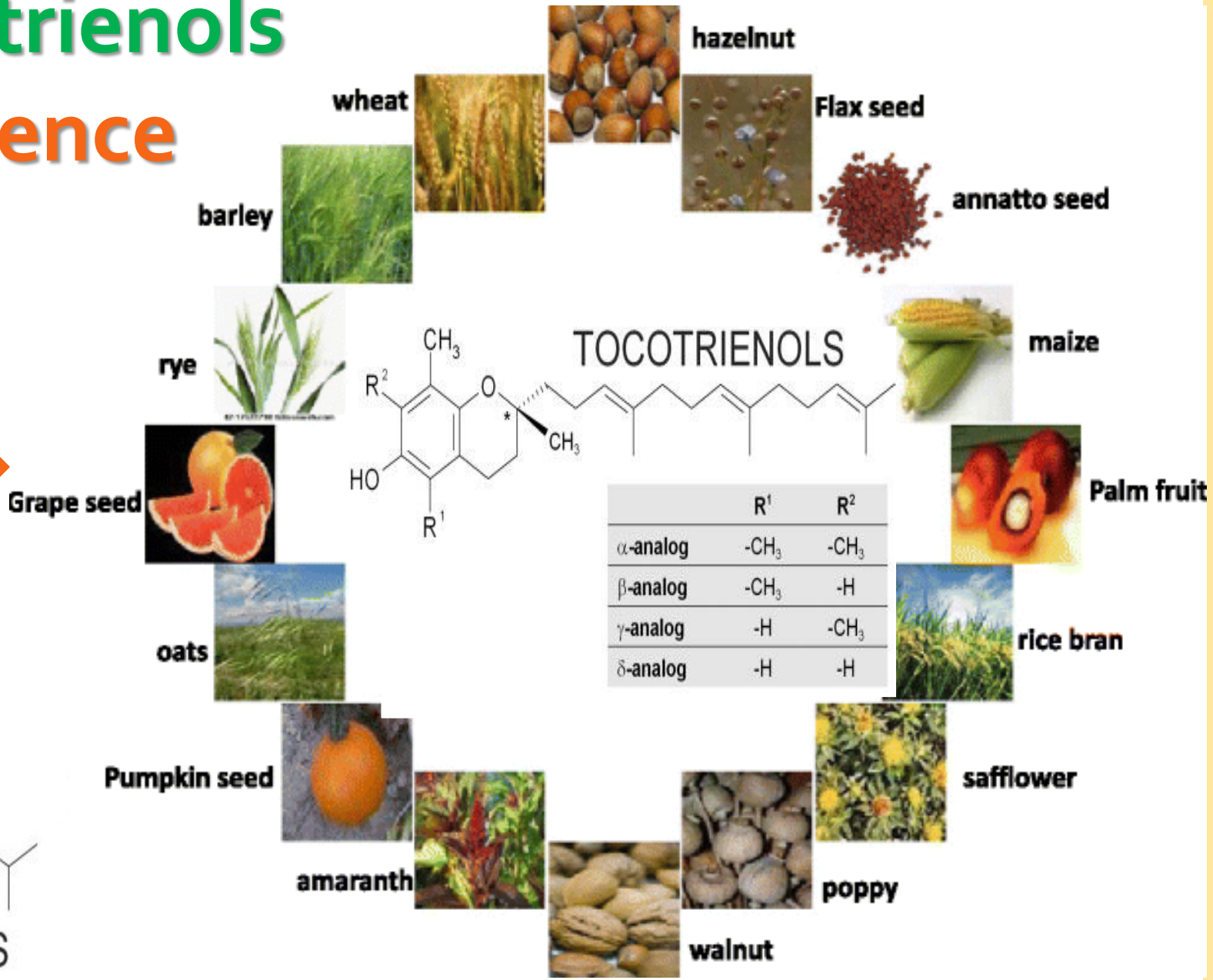
SASP components

Effect of tocotrienols on cell senescence

Vitamin E

- *α-, β-, γ-, δ- tocotrienols*
- *α-, β-, γ-, δ- tocoferols*





Effect of tocotrienols on cell senescence

- *γ-, δ-tocotrienols (T3)*

Pretreatment
- **T3 added before inducing of cell senescence for 24 h**

Cotreatment
- **T3 added along with inducers of cell senescence**

Postreatment
- **T3 added after inducing of cell senescence for 24 h**

- **Cell model**

Human fibroblasts *(MRC-5 cell line)*

- **Induction of senescence**

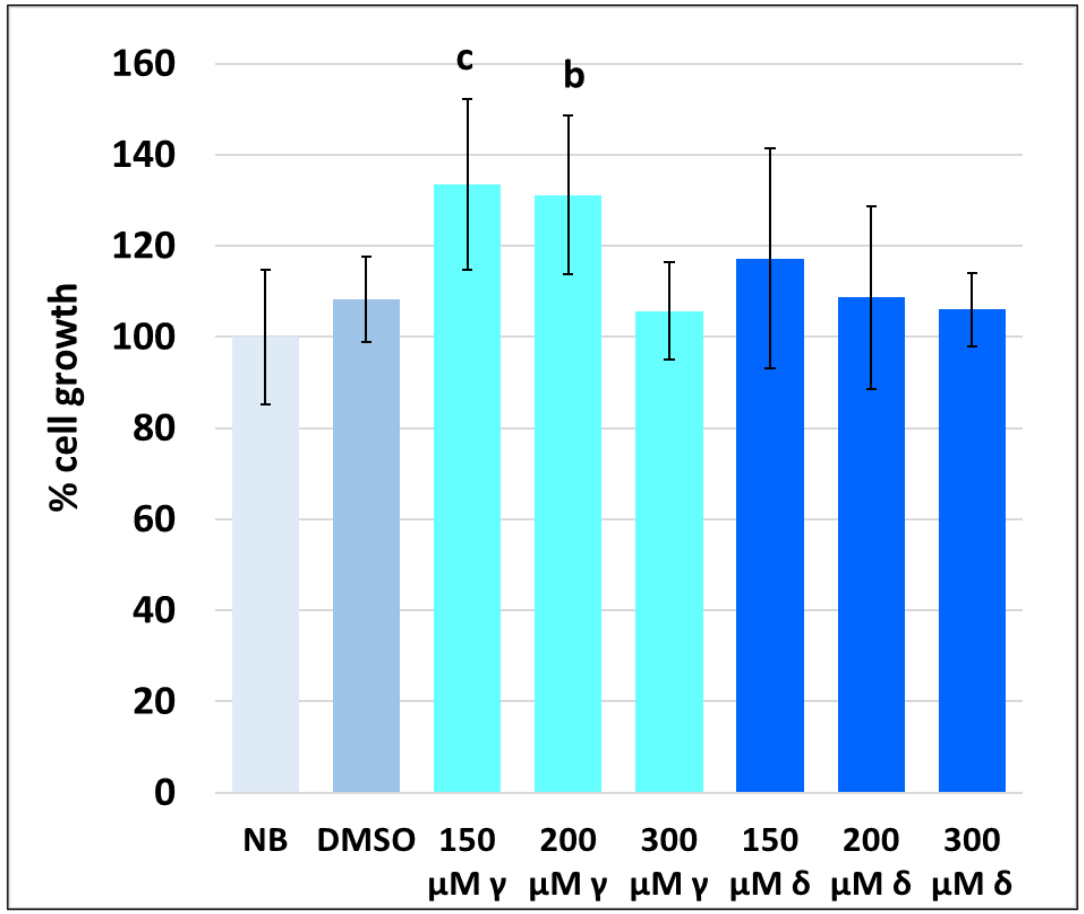
100 µM hydrogen peroxide/0.5h

or

80 µM etoposide/1h

Effect of tocotrienols on cell growth

Normal non-induced cells (0.5 h)



NC – normal cells *(without tocotrienols or dimethyl sulfoxide)*
DMSO – cells treated with DMSO /0.5 h

γ- tocotrienol

or

δ-tocotrienol

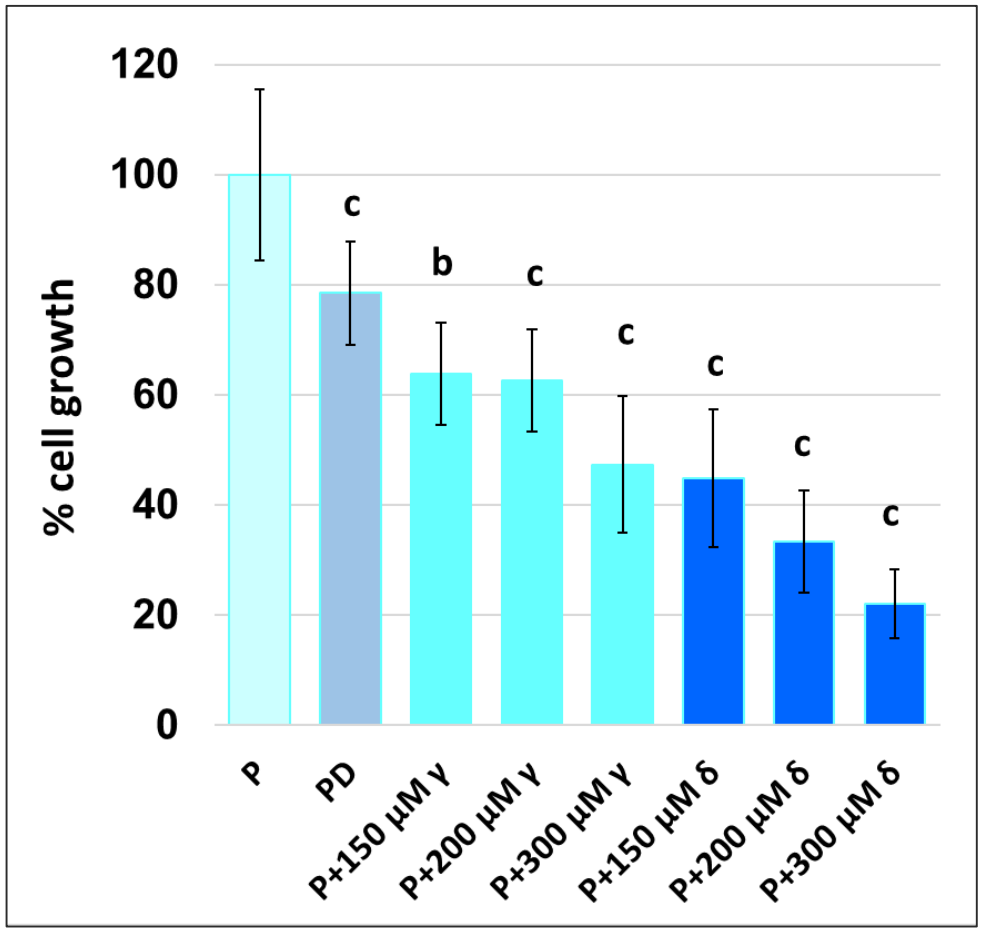
did not lead to a decrease in the cell growth

- *150 and 200 µM γ- tocotrienol*

***significantly* increased cell growth**

Effect of tocotrienols on cell growth

Cotreatment



- **Induction of senescence**
  - ***100 µM hydrogen peroxide / <u>0.5h</u>***

150, 200 and 300 µM γ- tocotrienol

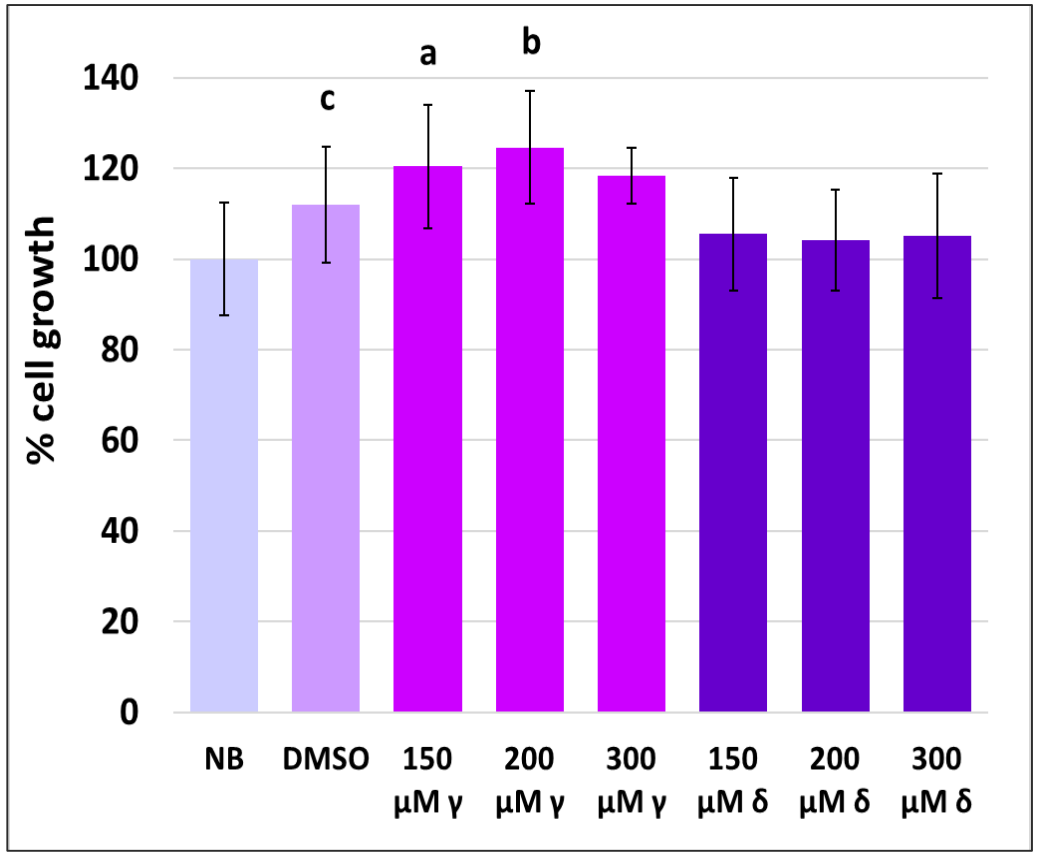
***significantly* reduced cell growth**

150, 200 and 300 µM δ-tocotrienol

***significantly* reduced cell growth**

Effect of tocotrienols on cell growth

Normal non-induced cells (1h)



γ- tocotrienol

or

δ-tocotrienol

did not lead to a decrease in the cell growth

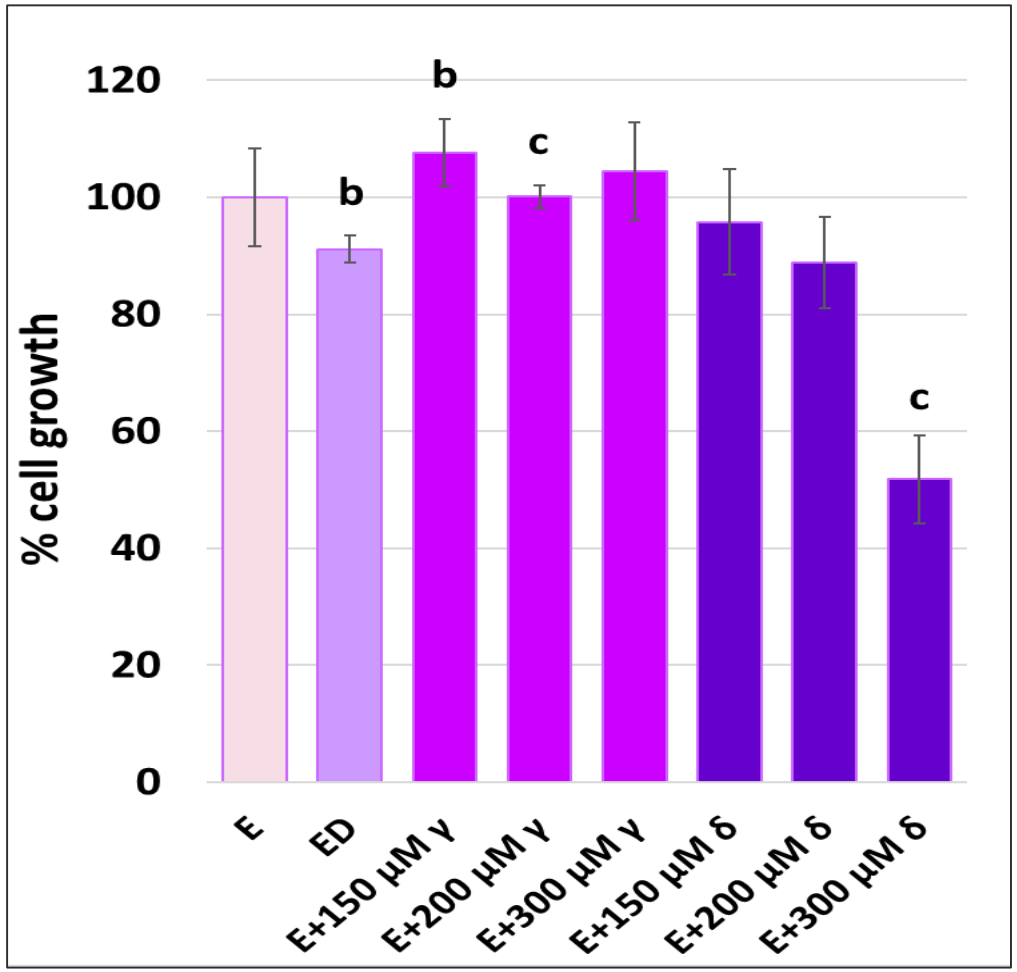
- ***150 and 200 µM γ- tocotrienol***

***significantly* increased cell growth**

NC – normal cells *(without tocotrienols or dimethyl sulfoxide)*
DMSO – cells treated with DMSO /0.5 h

Effect of tocotrienols on cell growth

Cotreatment



- **Induction of senescence**
  - ***80 µM etoposide / 1h***

150 and 200 µM γ- tocotrienol

***significantly* increased cell growth**

300 µM δ-tocotrienol

***significantly* reduced cell growth**

Effect of tocotrienols on cell senescence

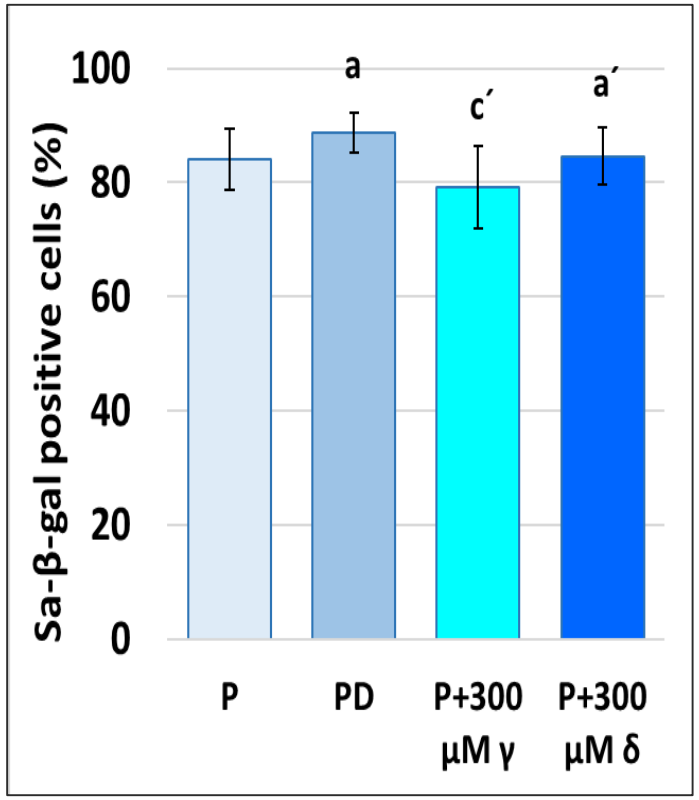
Cotreatment

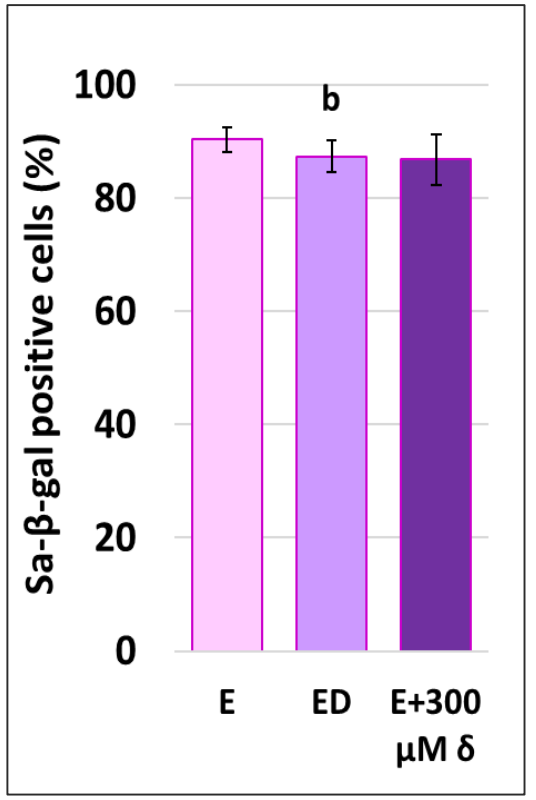
- **Effect of 300 µM γ-tocotrienol and 300 µM δ- tocotrienol on *peroxide induced senescence***

- **Effect of 300 µM δ- tocotrienol on *etoposide induced senescence***

Effect of tocotrienols on SA-β-gal activity

Cotreatment





300 µM γ-tocotrienol / *300 µM δ-tocotrienol*

led to *decreased* SA-β-gal activity

in peroxide induced senescence

300 µM δ-tocotrienol

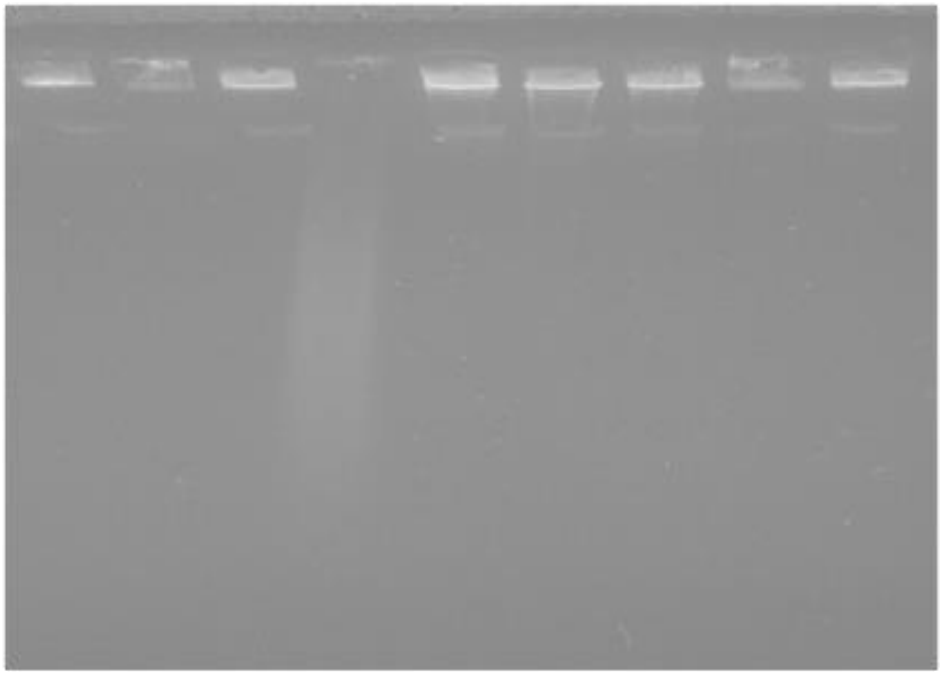
did not lead to decreased SA-β-gal activity

in etoposide induced senescence

Effect of tocotrienols on apoptosis

Cotreatment

E E300δ ED PC NC P P300γ P300δ PD



300 µM γ-tocotrienol

did not lead to formation of an apoptotic ladder

in peroxide induced senescence

300 µM δ-tocotrienol

did not lead to formation of an apoptotic ladder

in peroxide or etoposide induced senescence

Effect of tocotrienols on apoptosis

Cotreatment

300 µM γ-tocotrienol

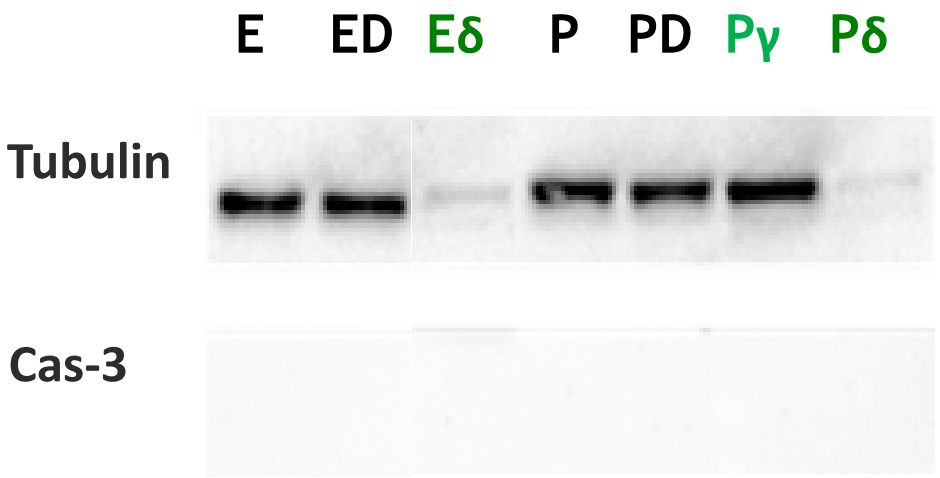
did not lead to caspase-3 protein expression

in peroxide induced senescence

300 µM δ-tocotrienol

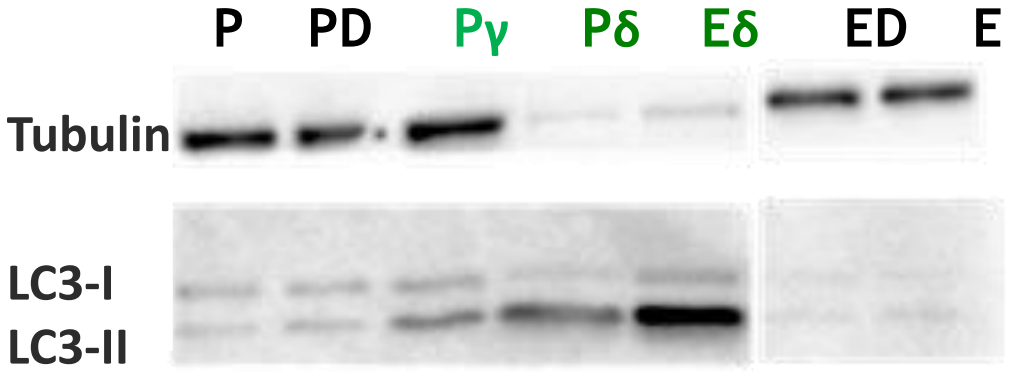
did not lead to caspase-3 protein expression

in peroxide or etoposide induced senescence



Effect of tocotrienols on autophagy

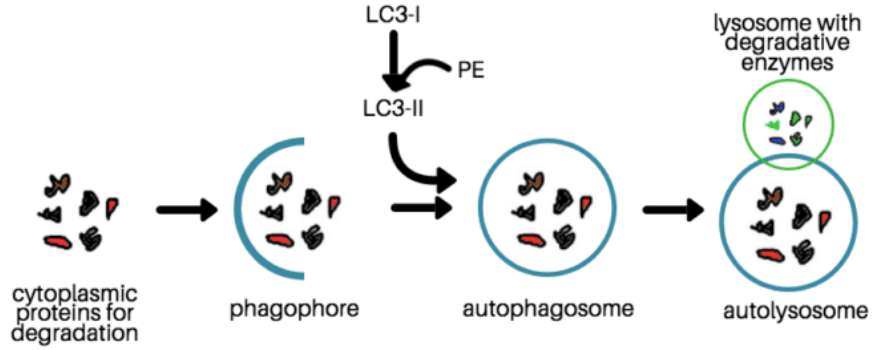
Cotreatment



LC3-II =

LC3-I protein + fosfatidylethanolamine

→ **autophagosomes**



300 µM γ-tocotrienol

led to increased LC3-II protein expression

in peroxide induced senescence

300 µM δ-tocotrienol

led to increased LC3-II protein expression

in peroxide or etoposide induced senescence

Effect of tocotrienols on cell senescence

SUMMARY

- cotreatment with 300 µM γ-tocotrienol

  Peroxide induced senescence

- cotreatment with 300 µM δ-tocotrienol

  Peroxide induced senescence

  or

  Etoposide induced senescence

→ Decreased growth of cells

→ *No apoptosis, no increase of senescence*

→ *LC3-II autophagy protein increased* !

→ Decreased SA-β-gal activity

→ Decreased SA-β-gal activity

(Peroxide induced senescence)

Effect of tocotrienols on cell senescence

CONCLUSION

- cotreatment with 300 µM γ-tocotrienol
- cotreatment with 300 µM δ-tocotrienol

probably induction of cell death *(autophagy?)*

→ *instead of senescence*

in cells growing in stress conditions

Growth of cells in standard condition

was not significantly affected by 300 γ-tocotrienol or 300 µM δ-tocotrienol