UNIVERSIDAD COMPLUTENSE MADRID

AFUSAN INVESTIGATION GROUP

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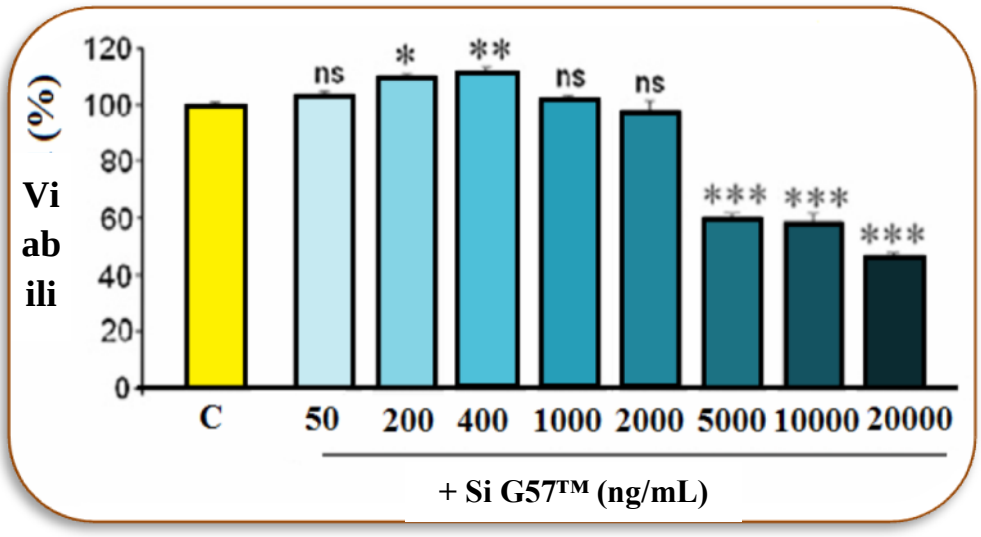
NEUROPROTECTIVE EFFECTS OF SILICON ON SH-SY5Y NEUROBLASTOMA CELL LINE

Silicon effects:

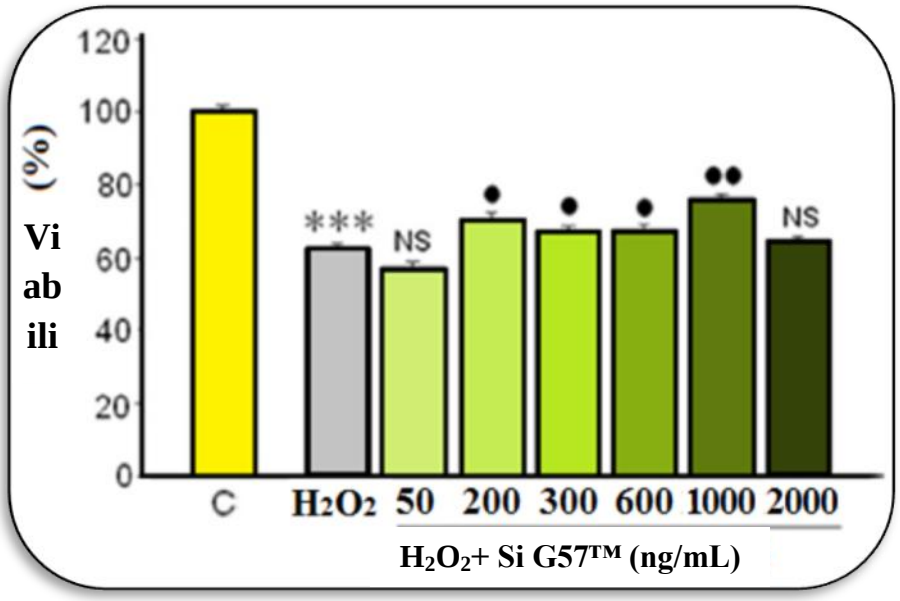
1. On cell viability (tested by MTT method)
   a) Basal conditions (without stimuli)



Significantly increase of cell viability with silicon G57™ at 200 and 400ng/mL is observed. Potential toxic effect from dose of 5000ng/mL, due to the loss detected in cell viability.

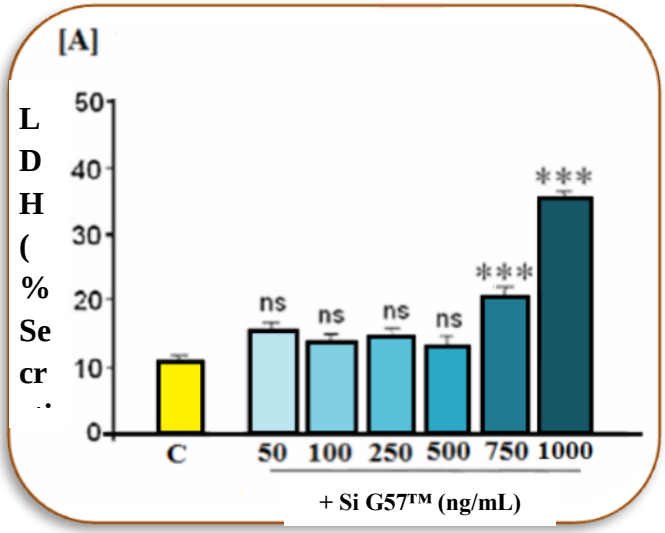
This positive effect of silicon G57™ on cell viability is not because of an increase in cell proliferation. There isn't higher cell division rate, as the analysis of cyclin E and PCNA levels by Western Blot, both cell cycle markers, don`t reveal any significantly differences between silicon treated cells and control ones (Garcimartín et al., 2015, Chemosphere).

b) In presence of oxidative agent (hydrogen peroxide)

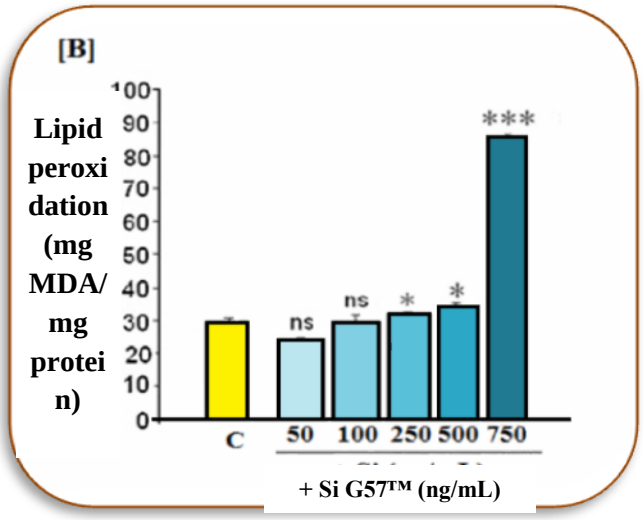


Significantly increase of cell viability respect to hydrogen peroxide from 50ng/mL to 1000ng/mL of silicon G57™.

2. On necrosis cell death (evaluated by lactate dehydrogenase activity)
    a) Basal conditions (without stimuli)

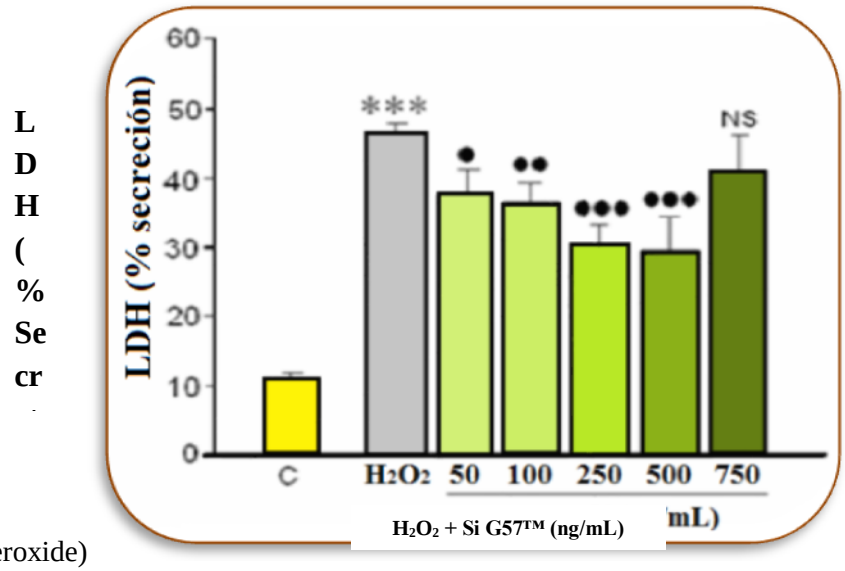


Significantly increase of LDH secretion, linked to cell death through necrosis mechanism, is detected with silicon G57™ concentration higher than 500ng/mL in a dose-dependent manner. Hence, potential toxicity of silicon from 750ng/mL must be considered. Between the causes of LDH secretion, it was found lipid peroxidation.



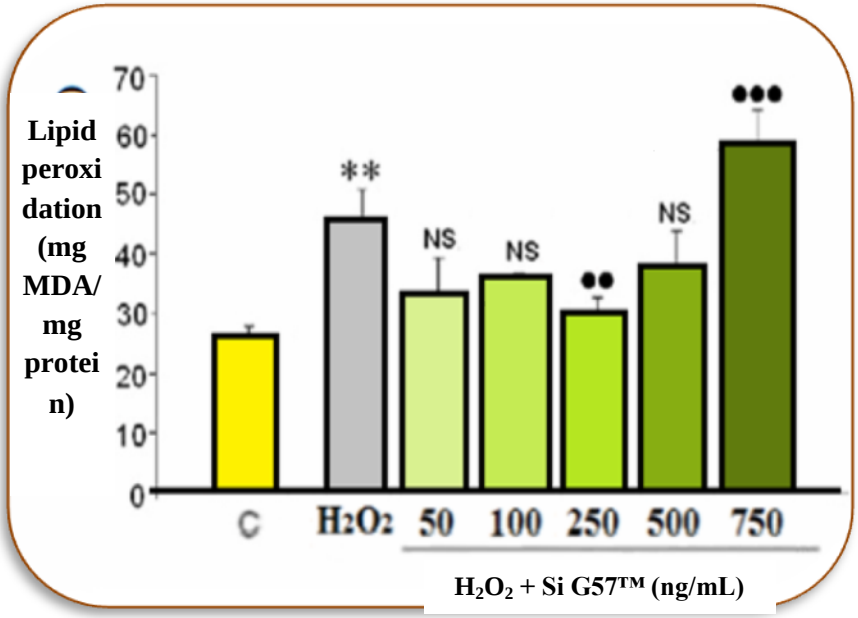
An important increase in lipid peroxidation is detected with 750ng/mL of silicon G57™, confirming that this is the mechanism of necrosis.

b) In presence of oxidative agent (hydrogen peroxide)



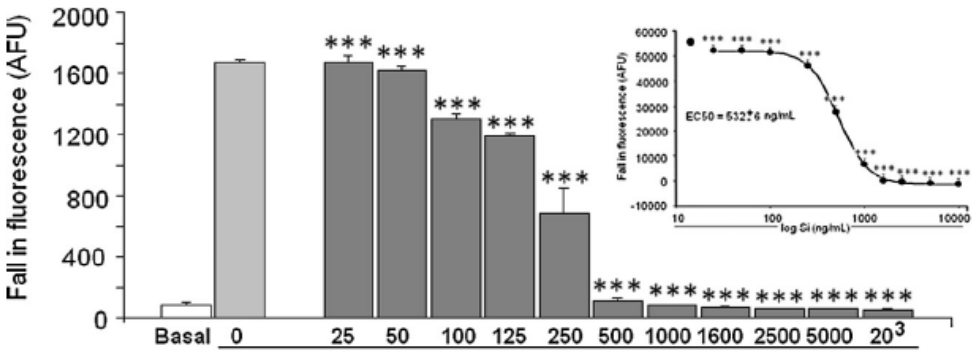
A relevant neuroprotective effect is observed with silicon G57™ treatment in the presence of hydrogen peroxide. On the other hand, a significantly fall in cell death by necrosis caused by hydrogen peroxide is detected with silicon from 50ng/mL to 500ng/mL.

Also, lipid peroxidation levels were tested, verifying that necrosis is caused by this process. The differences between silicon and hydrogen peroxide are significant at the dose of 250ng/mL.



3. On free radicals

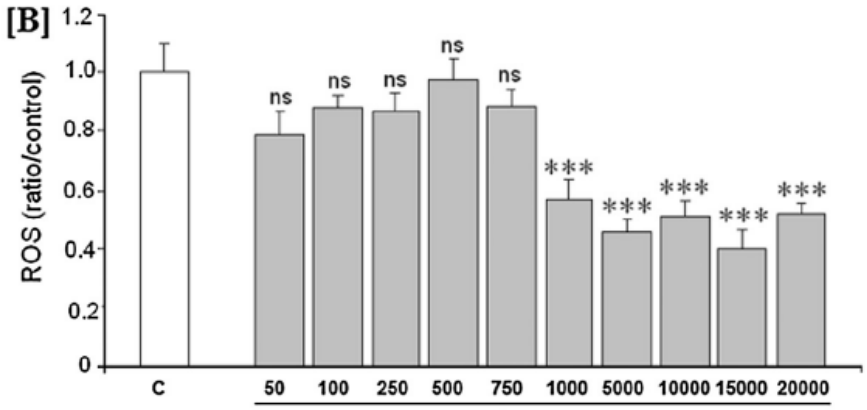
a) Antioxidant capacity of silicon measured by ORAC in vitro method



AAPH + Si G57™ (ng/mL)

The fall in fluorescence in ORAC method is inversely proportional to the antioxidant capacity of the assayed compound. In the figure it can be seen how silicon has significantly differences with AAPH (corresponding to concentration 0 of silicon). The fall of fluorescence with silicon is significantly lower than without it, from the dose of 250ng/mL to the highest evaluated, 20µg/mL. Silicon G57™ is a potent antioxidant agent.

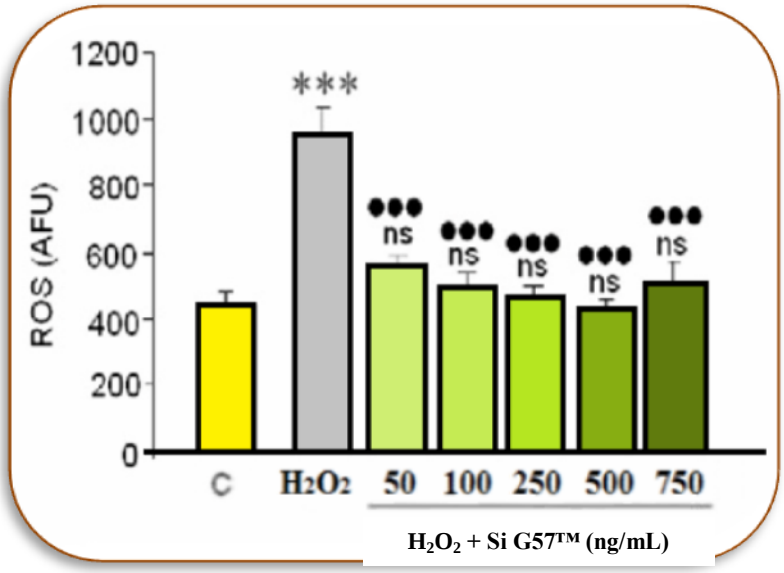
b) Basal conditions (without stimuli)



+ Si G57™ (ng/mL)

In cell experiments, with normal conditions, silicon significantly decreases free radical production from a concentration of 1000ng/mL.

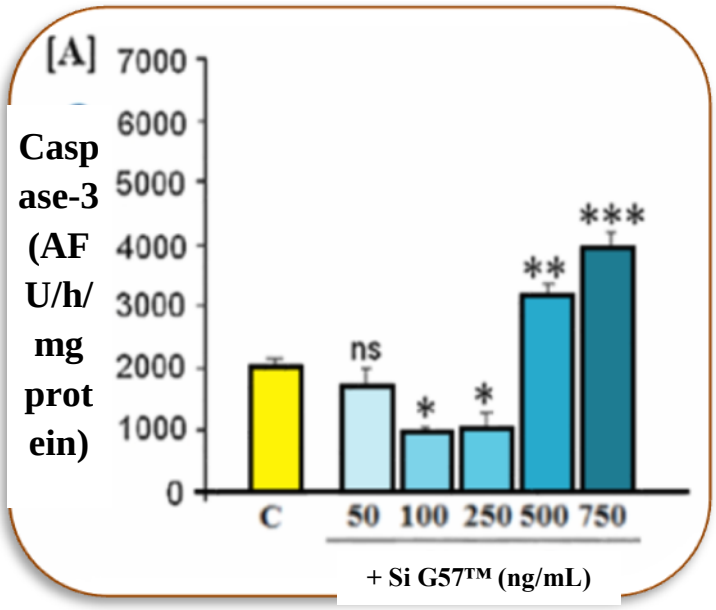
c) In presence of oxidative agent (hydrogen peroxide)



When hydrogen peroxide is present, which produces high amount of ROS, silicon is able to significantly reduce ROS generation from 50ng/mL to 750ng/mL, the highest dose tested. In fact, silicon can keep the ROS at the same levels that control cells without hydrogen peroxide.

4. On cell death by apoptosis (measured by caspase-3 activity)

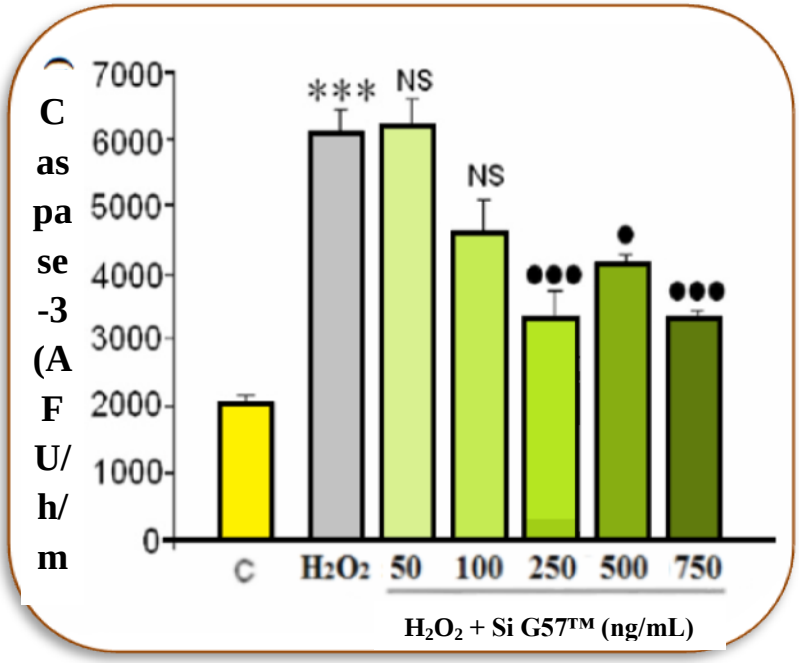
   a) Basal conditions (without stimuli)



Silicon G57™ treatment at 100 and 250ng/mL significantly decreases caspase-3 activity in comparison with control cells without treatment, suggesting a basal antiapoptotic effect which can explain the higher viability commented above.

On the other hand, at 500ng/mL and 750ng/mL of silicon, it is found an increase in caspase-3 activity respect to control cells, indicating a toxic effect from these doses.

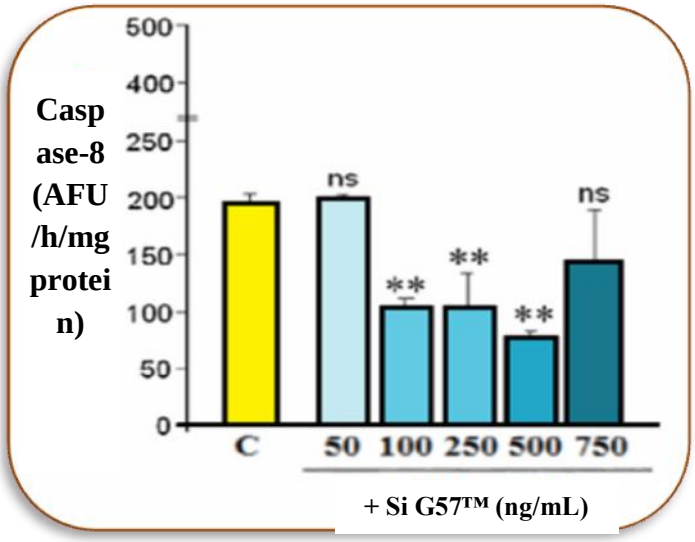
b) In presence of oxidative agent (hydrogen peroxide)



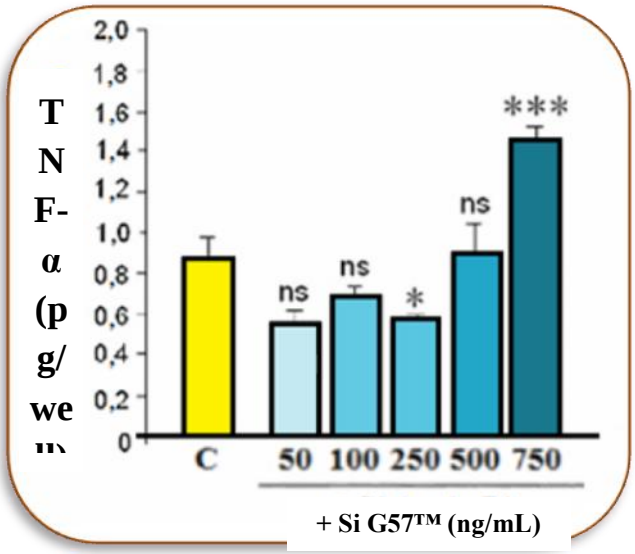
With hydrogen peroxide, silicon G57™ at 250, 500 and 750ng/mL concentrations significantly reduces apoptosis respect to the toxic, even nearly 50% of decrease.

5. Apoptosis pathway (extrinsic and intrinsic pathways)

a) Basal conditions (without stimuli)



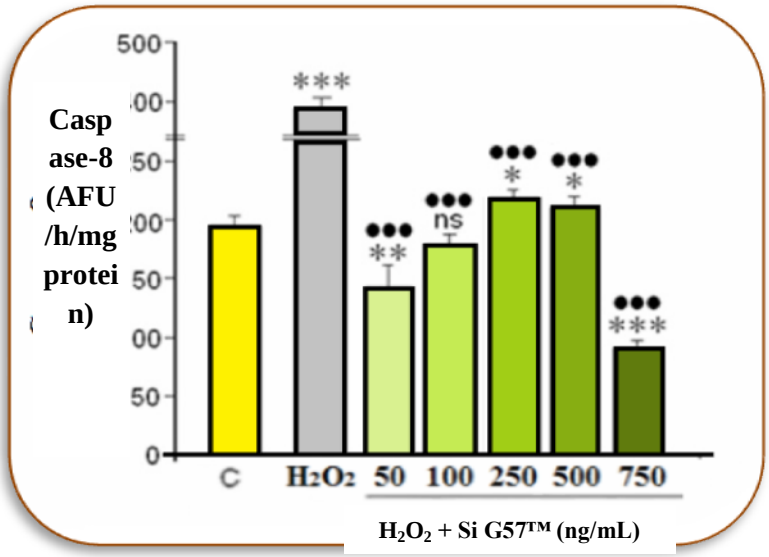
Finally, in order to know the pathway involved in the mechanism of silicon, it was measured activities of both activating enzymes, caspase-8 and caspase-9. Caspase-9 did not show differences between silicon and control cells; while activity of caspase-8 displayed a strong decrease with silicon treatment, at doses 100, 250 and 500ng/mL. Accordingly, antiapoptotic effect of silicon is linked to extrinsic pathway of apoptosis. Between the molecules that activates caspase-8, TNF-α is one of the more important.



TNF-α levels in the cells supernatant are significantly lower in cells treated with 250ng/mL of silicon. Therefore, the descend in TNF-α levels can be one of the mechanisms participating in antiapoptotic effect of silicon.

Los niveles de TNF-alfa liberado al sobrenadante fue significativamente menor en las células tratadas con silicio 250ng/mL. Por ello, el descenso de los niveles de TNF-alfa puede ser uno de los mecanismos que participan en el efecto antiapoptótico del silicio.

b) In presence of oxidative agent (hydrogen peroxide)



Hydrogen peroxide significantly increase caspase-8 activity respect to control cells. All concentrations tested of silicon G57™ promoted a significantly fall in caspase-8 activity in comparison with hydrogen peroxide. Even with silicon at 100ng/mL, caspase-8 shows similar activity than control cells.

CONCLUSIONS

Our studies in SH-SY5Y cell line under basal conditions and treated with hydrogen peroxide, and oxidant agent, demonstrate that silicon can act as a potent antioxidant compound, and also displays an antinecrotic and antiapoptotic effect, especially at the concentrations of 100 and 250ng/mL.