HORMETIC EFFECTS OF THYMOQUINONE IN LEUKEMIA AND LYMPHOMA CELLS

U. Glamoclija[1,2], L. Mahmutovic[2], B. Babic[2], M. Suljagic[2]
[1]School of medicine, University of Mostar, Bijeli Brijeg bb, 88 000 Mostar, Bosnia and Herzegovina, una.glamoclija@gmail.com
[2]International University of Sarajevo, Hrasnička cesta 15, 71 000 Sarajevo, Bosnia and Herzegovina

INTRODUCTION

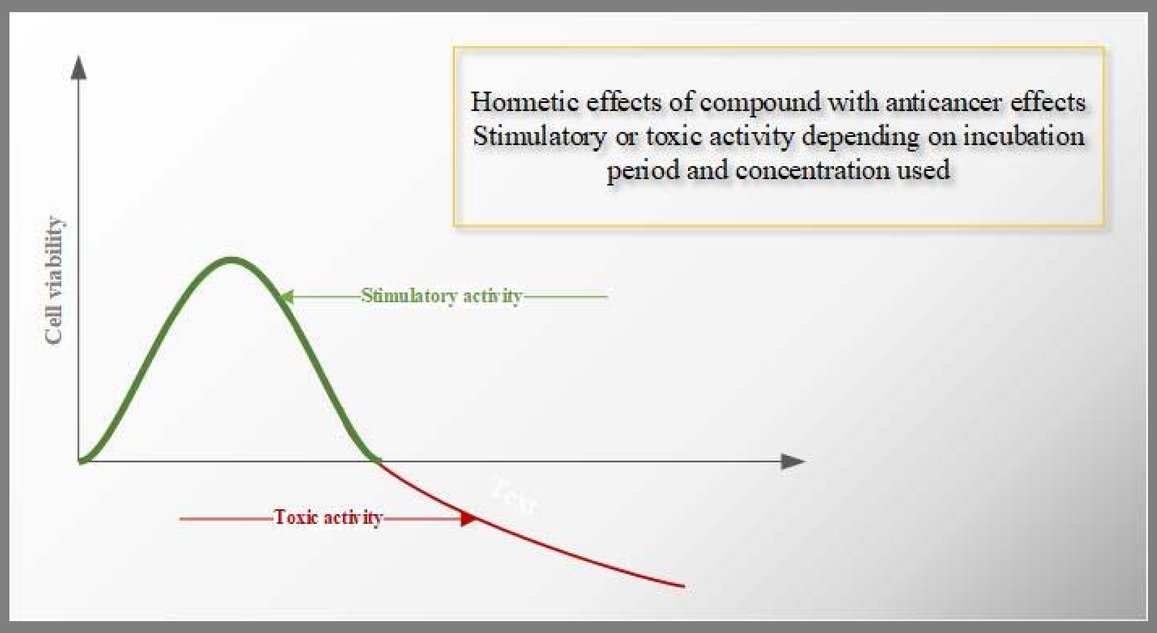
Hormesis is a phenomenon which can be found in many processes of living organisms and represents adaptive mechanism against stressors [1].

In the area of cancer therapeutics, hormesis can be defined as bimodal response to therapy, where low doses stimulate, while high doses inhibit cells [2].

This phenomenon is often overlooked in scientific research, but should gain more attention since it has profound effects on cancer response [3]. Various phytochemicals were reported to have hormetic effects [1,2].

Thymoquinone (TQ) is an active compound isolated from oil of plant *Nigella sativa* L. It inhibits signaling pathways important for cancer development and survival [4].

Several studies have shown concentration dependent inhibition of leukemia and lymphoma cells treated with μM concentrations of TQ [4]. There are no reports about hormetic activity of TQ in cancer cells.



MATERIALS AND METHODS

Viability of cells was determined by the WST-8 assay: CCK-8 Cell Proliferation Assay Kit (Biotool, USA).

Proliferation of cells was determined by using Cell Proliferation BrdU ELISA kit (Roche Applied Science, Germany).

Western blot was performed to evaluate effects of TQ on Phospho-Akt (Ser473) and Phospho-NF-κB p65 (Ser536). Antibodies were purchased from Cell Signaling Technology, Netherlands.

CONCLUSIONS

This is the first report to show thymoquinone hormetic effects in lymphoma and leukemia cell lines.

TQ in low concentrations stimulated phosphorylation of Akt (protein kinase B), while higher concentrations or longer incubation periods resulted in an opposite effect.

In HBL1 cells, NF-kB activation was seen and no stimulation of pAkt was observed.

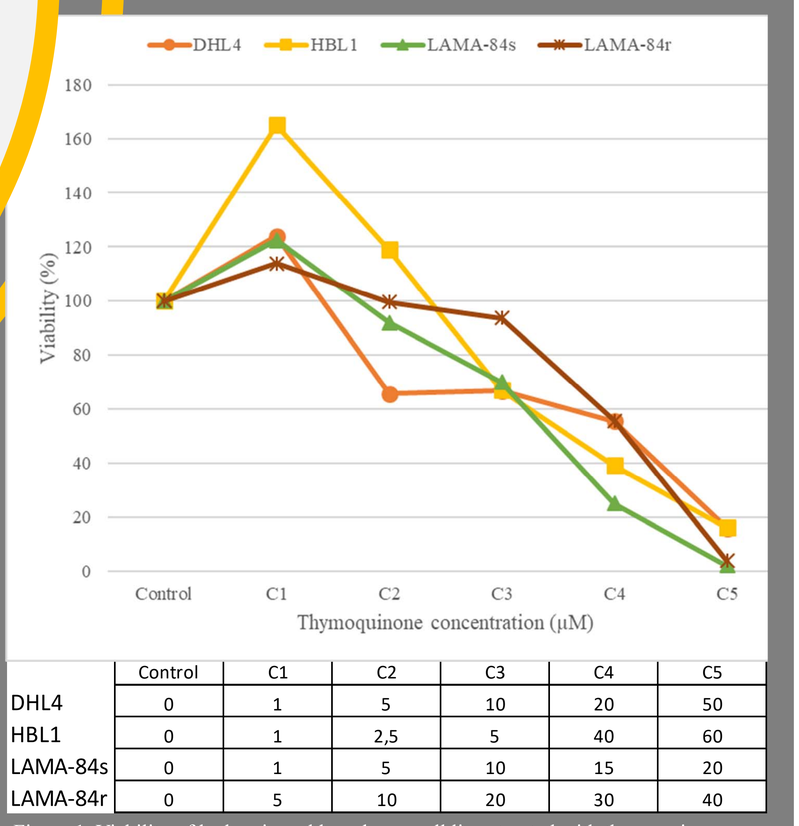
RESULTS AND DISCUSSION

Low doses of TQ stimulated viability of all tested cell lines (Figure 1).

In lymphoma cell lines, low concentrations of TQ increased proliferation for more than 40%. Hormetic effects were more pronounced in high seeding density experiments.

Western blot analysis revealed that TQ in low concentrations or short incubation periods stimulated phosphorylation of Akt, while higher concentrations or longer incubation resulted in an opposite effect (Figure 2). Akt is directly involved in prolonged survival of leukemic cells and represents one of the most important signaling molecules involved in hormetic effects [2].

The only cell line in which no induction of pAkt was seen was HBL1 (Figure 2). In this cell line, 10 and 15 μM thymoquinone caused strong induction of PTEN and inhibition of Akt phosphorylation already after 2 hours of treatment. This cell line belongs to ABC DLBCL dependent on NF-kB activity. Anticipated compensatory mechanism leading to hormetic effects of TQ in this cell line is NF-kB activation, which was observed after 24 and 48 hours' treatment with 15 μM TQ (Figure 3).



	Control	C1	C2	C3	C4	C5
DHL4	0	1	5	10	20	50
HBL1	0	1	2,5	5	40	60
LAMA-84s	0	1	5	10	15	20
LAMA-84r	0	5	10	20	30	40

Figure 1. Viability of leukemia and lymphoma cell lines treated with thymoquinone

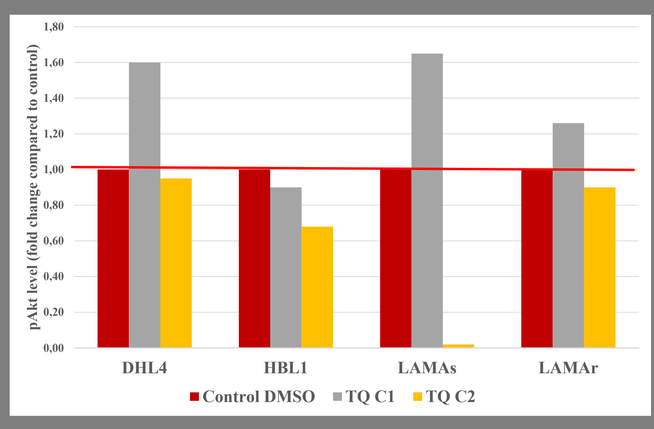


Figure 2. pAkt levels (relative to control) in lymphoma and leukemia cell lines treated with thymoquinone
Red line= value of control DMSO (1,00). All values above red line indicate stimulation of Akt phosphorylation.

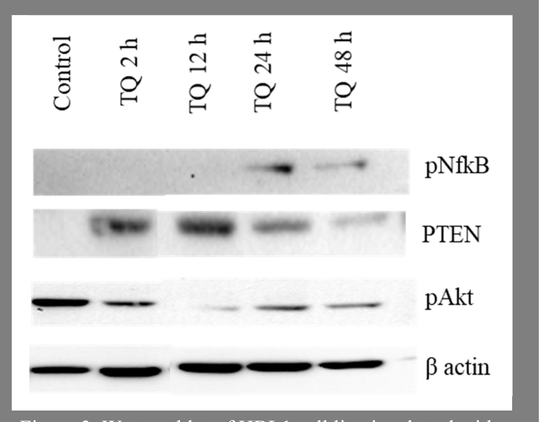


Figure 3. Western blot of HBL1 cell line incubated with thymoquinone for different times

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