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Vanillin based chalcone analogue induces cell death in HeLa and HCT-116 cell line through mitochondrial apoptotic pathway

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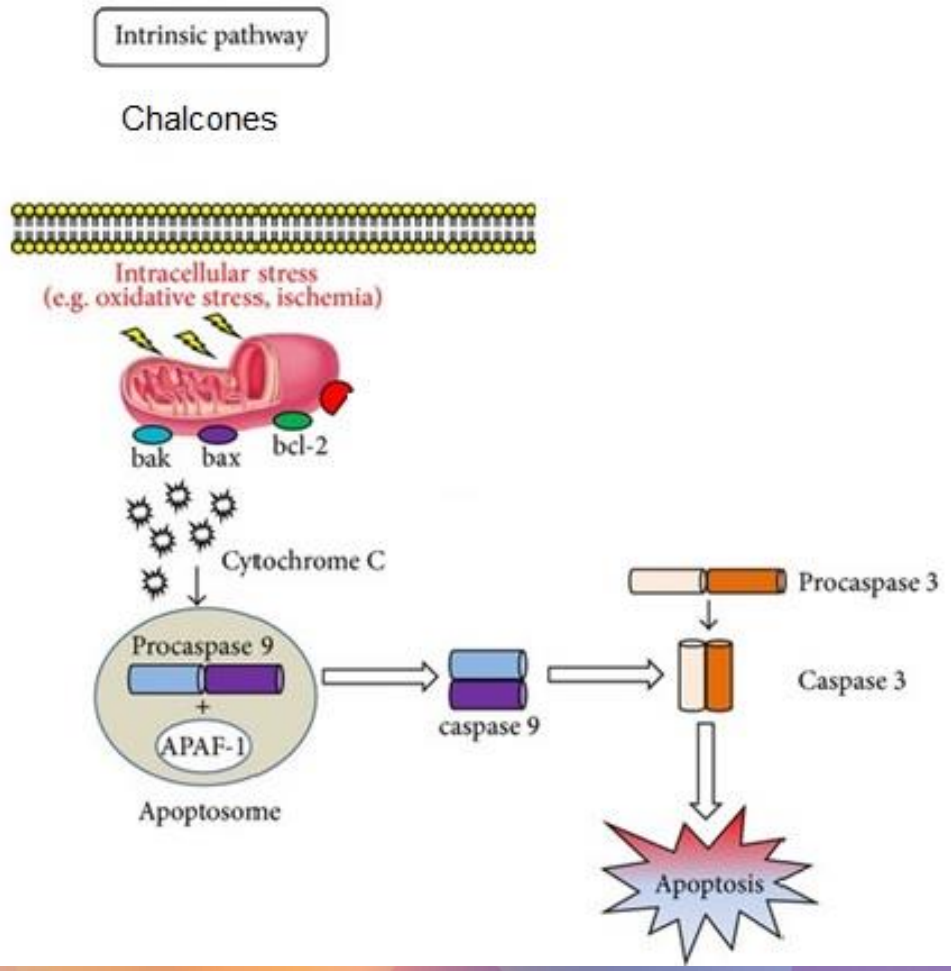
Graphical Abstract

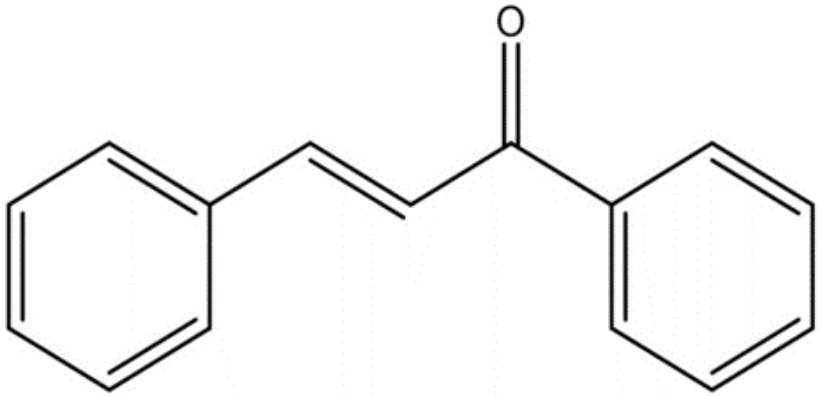


Abstract:
Application of organic heterocyclic compounds as anti-tumor therapeutics are limited due to therapeutic drawbacks including resistance of tumor cells, non-selectivity of the administered drug towards healthy cells and abundance of unwanted side effects.
The aim of our research was to investigate and compare both the antitumor effect and the mechanism of action of newly synthesized, so far untested chalcone analogue on HeLa, HCT-116 cells and healthy human fibroblast lung cell line (MRC-5).
The antitumor efficiency of investigated chalcone analogue was compared to the antitumor effects of dehydrozingerone and cysplatin that were used as referent substances. Cytotoxic and apoptotic effect were evaluated using both MTT test and flow cytometry by Annexin V-FITC/7-AAD staining.
The results of our investigation indicated that newly synthesized chalcone analogue H5 expressed more powerful antitumor effect compared to the effects of both dehydrozingerone and cisplatin. Cell death was mediated via apoptotic pathway.

Keywords: apoptosis, chalcone, cell death

Introduction

Chalcones represent precursor compounds for flavonoids biosynthesis in plants. Chalcones, 1,3-diaryl-2-propen-1-ones, have unique chemical structure with conjugated double bonds and delocalized -electron system on both aromatic rings.



Chalcones structural formula

Various studies have shown that chemical structure of chalcone is responsible for their antitumor effect.

Introduction

In our research we have evaluated antitumor effect of vanillin based chalcone analogue (E)-1-(3-metoxy-4-propoxyphenyl)-5-methylhex-1-en-3-on (marked as H5) on tumorous HeLa, HCT-116 cells and healthy MRC-5 cells.

The concentrations of H5 that were used in order to determine the cytotoxic effect were in the range of 0.3; 1; 3; 10; 30; 100 and 300 µM.

Antitumor effect of our chalcone analogue was compared with the effect of dehydrozingerone and cysplatin after 24 and 48 h treatment.

Methods that were used in our experiments were MTT test for the determination of cytotoxic effect and flow cytometry by Annexin V-FITC/7-AAD staining for the purpose of determination of the type of the cell death and expression and localization of N terminal, active Bax protein using immunofluorescence method.

Results and discussion

The data indicate that H5 showed the concentration-dependent significant cytotoxic effect on both HeLa and HCT-116 cells after 24 and 48 h treatment (slide 7 and 8).

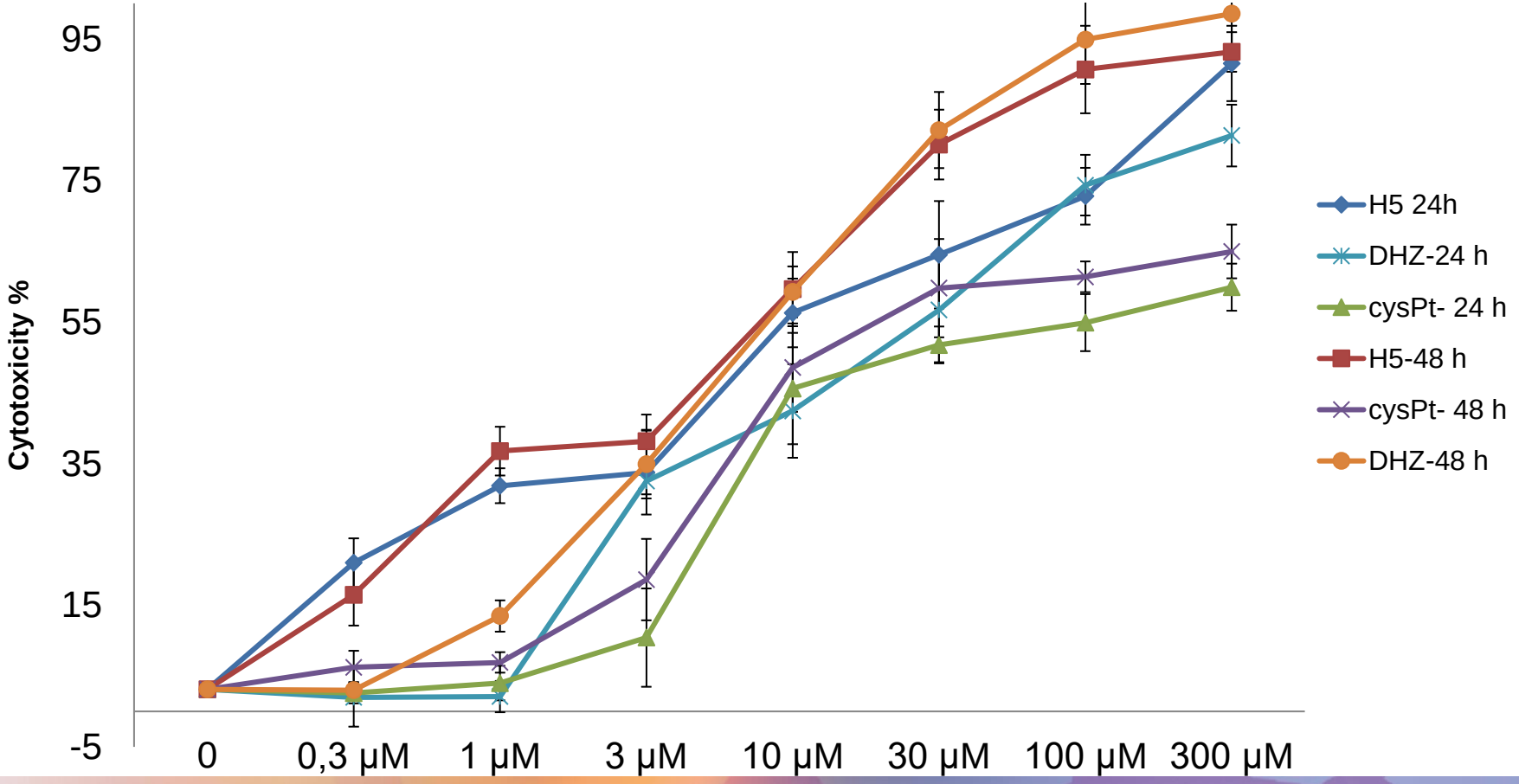
The cytotoxic effect of H5 on healthy MRC-5 cells in evaluated time periods and concentrations was not significant (slide 9).

IC50 values for all tested substances that were tested in both time intervals were calculated and demonstrated on slide 10.
These concentrations were used in order to determine apoptotic mechanism.

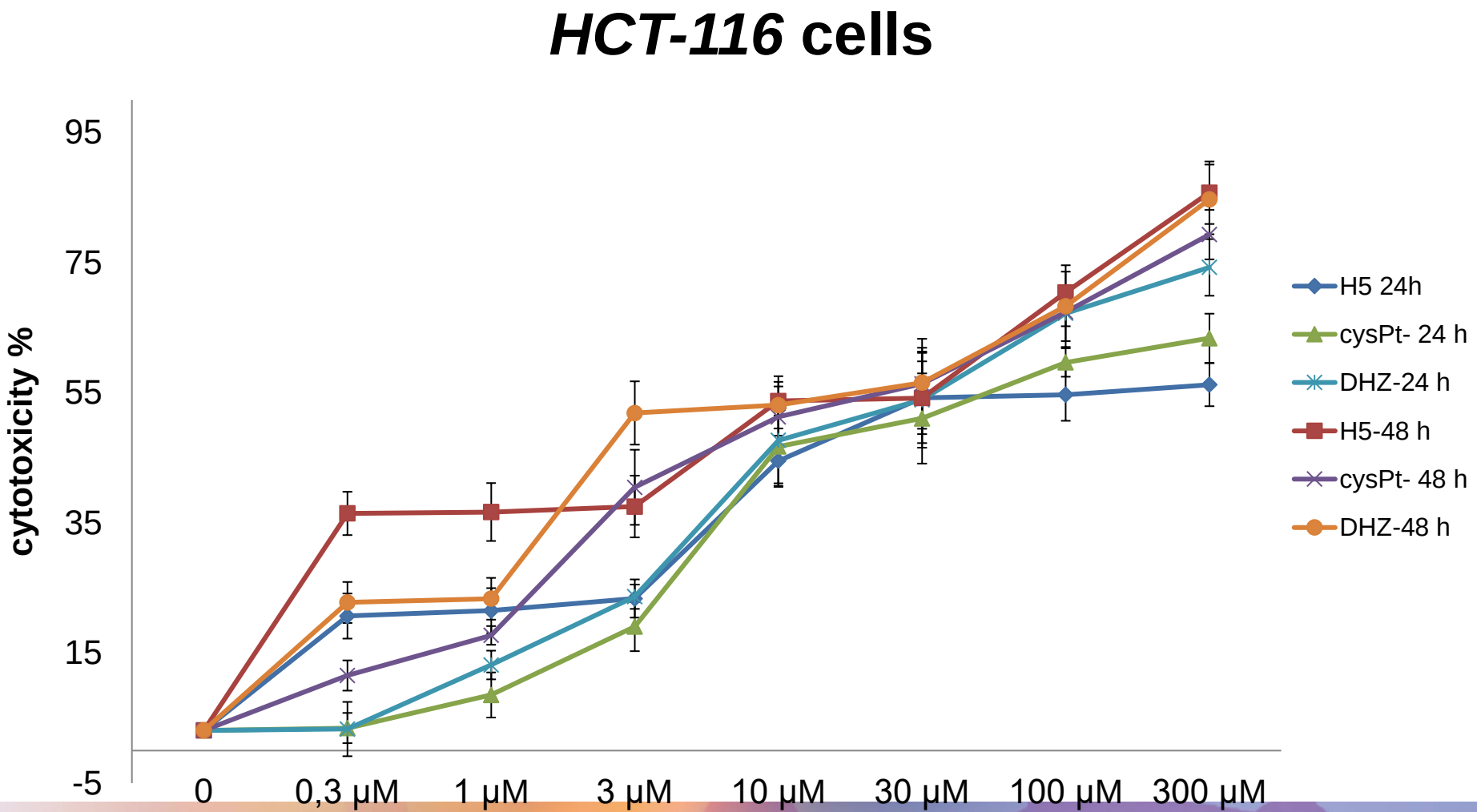
In the next experiment we have evaluated the type of the cell death induced by H5 (slide 11) as well as expression of active Bax protein (slide 12).

Results and discussion

HeLa cells

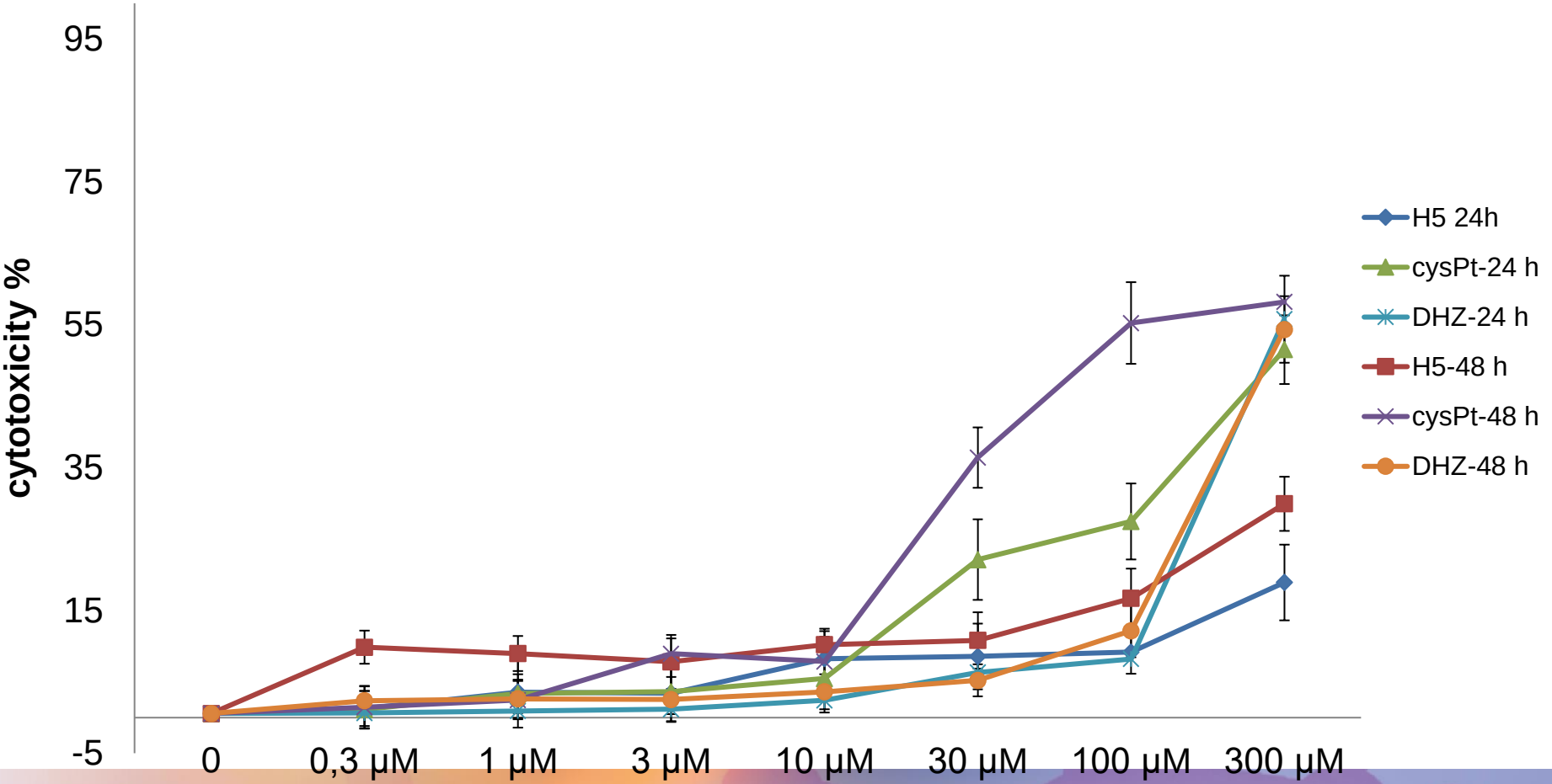


Results and discussion



Results and discussion

MRC-5 cells

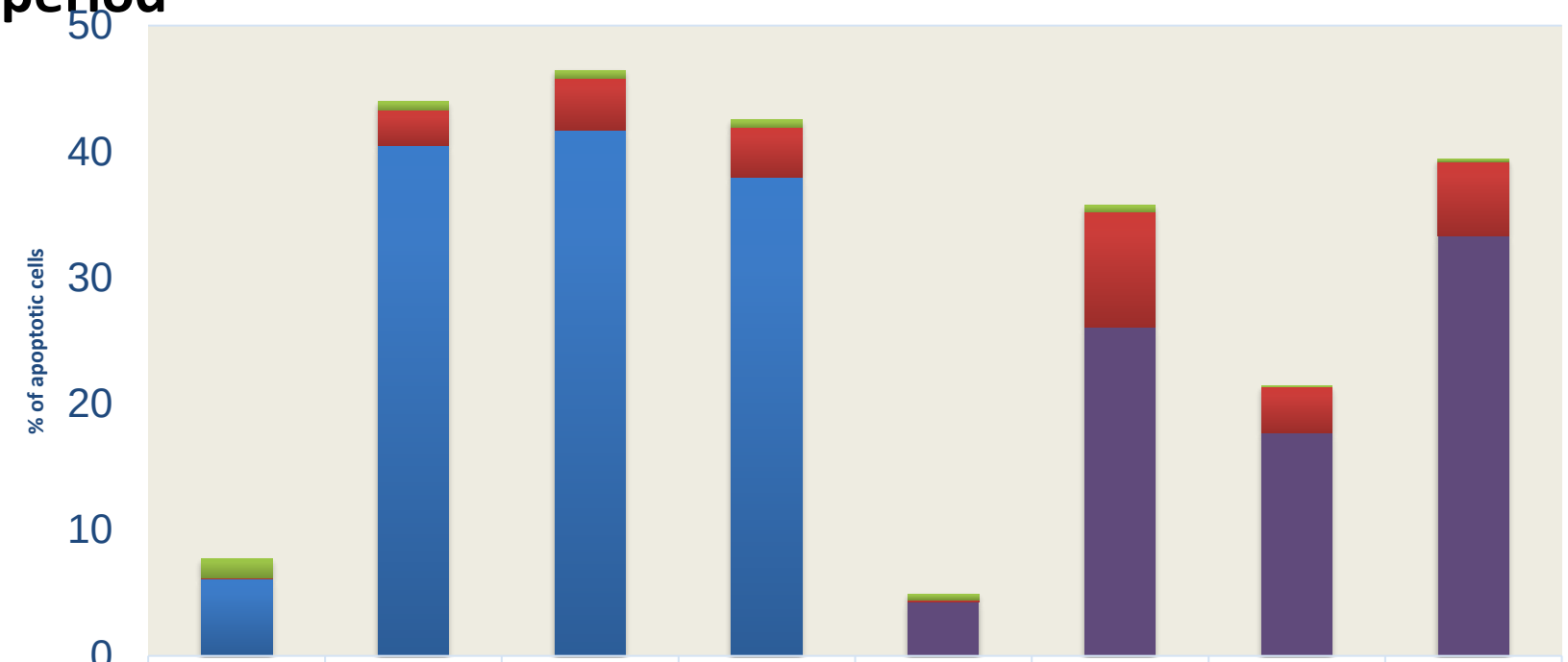


Results and discussion

IC_{50} concentration values for all tested substances during 24 and 48 h period

IC_{50}	*HeLa*		*HCT-116*		*MRC-5*	
	24 h	48 h	24 h	48 h	24 h	48 h
H5	4,11 ± 1,46	3,55 ± 1,10	5,62 ± 1,17	5,09 ± 0,87	> 300	> 200
cysPt	19,60 ± 3,22	9,70 ± 1,40	7,17 ± 1,15	4,82 ± 1,45	>100	44,25 ± 14,72
DHZ	9,61 ± 2,16	5,41 ± 1,57	5,71 ± 0,66	2,85 ± 0,69	> 300	> 200

Apoptotic effects of all tested substances during 24 and 48 h period



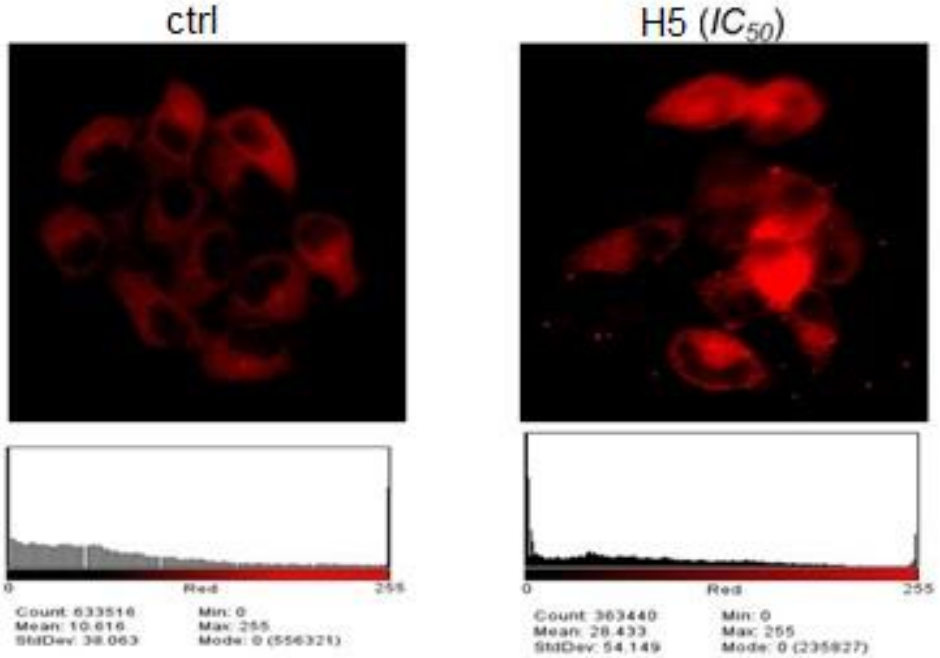
	ctr	H5	DHZ	cisPt	ctr	H5	DHZ	cisPt
N	1.53	0.69	0.66	0.62	0.45	0.55	0.11	0.22
LA	0.07	2.81	4.10	3.92	0.18	9.10	3.60	5.95
EA	6.12	40.48	41.69	37.98	4.29	26.10	17.72	33.28

N- necrosis; LA- late apoptosis; EA- early apoptosis

Results and discussion

In order to determine the apoptotic mechanism involved in H5 induced cell death, HeLa and HCT-116 cells were treated with IC50 concentration of H5, after witch the expression and localization of mitochondrial, active Bax (N-terminal) protein was evaluated using specific antibody and immunofluorescence method. Intensity of immunofluorescence was evaluated using ImageJ software.

Results indicate that treatment of HeLa and HCT-116 cells with H5 caused 2.7 and 2.6 fold increase in the expression of N terminal, active Bax protein compared to control, untreated cells.

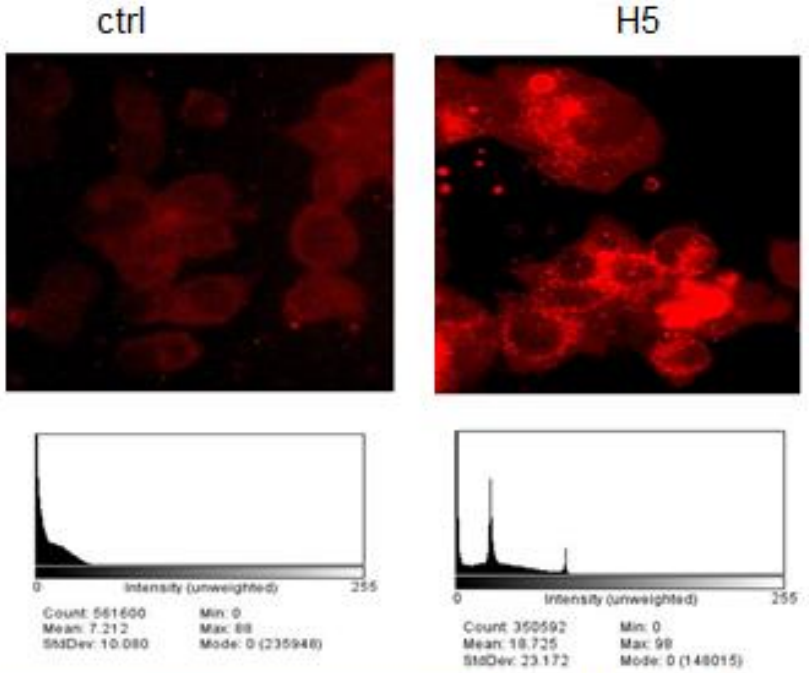


HeLa	MFI for Bax
контрола	10,6
X5 (IC_{50})	28,4

fold increase
1
2,7

Results and discussion

The data indicate that H5 treatment caused delocalization of active, N terminal Bax protein from cytosol to mitochondria, consequently causing the release of cytochrome c into cytosol and formation of APAF-1, thus indicating the involvement of mitochondria in H5 mediated cell death



HCT-116	MFI for Bax
CTRL	7.2
H5 (IC_{50})	18.7

fold increase
1
2.6

Results and discussion

There are many options in cancer therapy, including surgery, radiotherapy and immunotherapy, but nevertheless treatment with anti-cancer drugs due to their non-selective cytotoxicity remains the primary option.
Due to the cytotoxic and numerous adverse effects of currently available anti-cancer drugs on healthy cells, it is necessary to synthesize new compounds with more efficient antitumor effect.
Chalcones are precursor compounds for flavonoids biosynthesis in plants. Changes in their chemical structure have offered a high degree of biological diversity that has proven to be useful for the development and synthesis of new medicinal agents.
These medicinal agents exhibit lesser toxicity on normal cells and greater selectivity towards tumor cells compared to natural agents.
For example, twelve structural Millepachine analogues (especially(3-hydroxy-4-methoxyphenyl)(5-methoxy-2,2-dimethyl-2H-chromen-8-yl)methanone) showed more effective anti-proliferative activity on five human cancer cell lines (A549, HeLa, HCT 116, A2780 and MGC803) compared to natural compound, Millepachine.

Conclusions

We herein reported for the first time anti-tumor effect H5 chalcone analogue and we showed that our synthesized chalcone had more effective antitumor effect compared to other anti-tumor drugs, cysplatin and dehydrozingerone.

Antitumor effect was carried out with induction of inner, mitochondrial apoptotic pathway, the expression of active, N terminal, mitochondrial Bax protein was significantly higher in H5 treated cells compared to the control cells. This finding point out involvement of inner, mitochondrial pathway in apoptosis realization.

The effect of H5 on healthy cells was insignificant compared with the effect on HeLa and HCT-116 cells.

Acknowledgments

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