

CYTOPROTECTIVE AND CYTOPROLIFERATIVE EFFECTS OF ETHANOL INFUSIONS FROM *MORUS NIGRA* AND *COTINUS COGGYGRIA* HEARTWOOD IN 3T3-L1 PREADIPOCYTES

Milena Pasheva, Danail Pavlov, Oskan Tasinov, Milka Nashar, Yoana Kiselova-Kaneva, Diana Ivanova

Department of Biochemistry, Molecular Medicine and Nutrigenomics, Medical University of Varna

ABSTRACT

PURPOSE: The purpose of the present study was to examine the effect of two ethanol infusions from the heartwood of mulberry tree (*Morus nigra*) and smoke tree (*Cotinus coggygia*) on viability of preadipocyte cells.

MATERIAL AND METHODS: Preadipocyte 3T3-L1 cells were treated with 40% ethanol infusions from the heartwood of both species, as well as with 40% ethanol as a control. MTT-test was applied to assess cell viability.

RESULTS: Ethanol infusions increased cell viability as compared to ethanol-treated controls. Along with the cytoprotective effect, also cytoproliferative effect was established for both infusions.

CONCLUSION: Estimated cytoprotective percentage values of infusion in the nutrient medium could be used in further investigations aimed to explore biological effects of ethanol heartwood infusions from *M. nigra* and *C. coggygia*.

Key words: *Morus nigra*, *Cotinus coggygia*, heartwood infusions, MTT-test, 3T3-L1 preadipocytes, cell viability

INTRODUCTION

Medicinal plants have a long history of use in prophylaxis and treatment of many diseases, therefore the elucidation of their biological effects has been the aim of many scientific studies. *Morus nigra* L. (mulberry tree) and *Cotinus coggygia* Scop. (smoke tree) are traditionally applied in folk medicine for

various complaints. According to some authors the extracts from different parts of mulberry tree (fruits, leaves and bark of the root) exhibit various beneficial effects, such as antiviral, antihyperglycemic, antiatherogenic and hypotensive action (12,5,1). The smoke tree is predominantly used for external administration as an antiseptic, antihemorrhagic and wound-healing remedy (7). However, several authors report that aqueous infusions from leaves can be applied internally for treatment of throat and stomach inflammations, gastric ulcer, diarrhea and even diabetes mellitus (6,9,2).

Although mulberry and smoke tree are popular medicinal plants, there is a lack of data about the biological activity of the extracts from their heartwood. Traditionally in Bulgaria the stems, branches or

Address for correspondence:

Milena Pasheva
Department of Biochemistry, Molecular Medicine and Nutrigenomics,
Medical University Varna, 55 Marin Drinov Str.,
9002 Varna, Bulgaria
e-mail: pasheva19@gmail.com

Received: December 17, 2013

Accepted: January 17, 2013

heartwood from these species are used for coloring of high alcoholic beverages and it is believed that colored drinks in small quantities might possess certain health beneficial effects (personally collected data). Moreover, our recent study estimated a high antioxidant activity of the ethanol extracts from heartwood of both plants, strongly correlating with their high polyphenol content (10). It was found that the ethanol infusion from *C. coggygia* wood had a gastro-protective effect against indomethacin-induced ulcerogenesis in rats (11).

The very first step in the investigation of biological effects of extracts of plant origin is to define the appropriate non-toxic concentrations for further application in different models. The purpose of the present study is to evaluate the effect of 40% aqueous-ethanol infusions from *M. nigra* and *C. coggygia* heartwood on cell viability.

MATERIAL AND METHODS

Infusion preparation

Two ethanol infusions from *Morus nigra* heartwood (EIMNW) and *Cotinus coggygia* heartwood (EICCW) were prepared following the traditional recipe for coloring high alcoholic beverages: 2g dried material from heartwood were placed in 1L 40% Ethanol for 40 days. Heartwood samples were subjected to fumigation following the popular technology for aging of beverages: the wooden chips were boiled for 10 minutes and then saturated with cold water for 24 hours. Finally, the material was dried for 15 minutes at 150-190°C.

Cell culture

3T3-L1 cells were obtained from American Type Culture Collection (ATCC). Preadipocytes were cultured in 75 cm² flasks at 37°C in a humidified chamber containing 5% CO₂. Nutrient medium comprised of phenol red-containing Dulbecco's modified Eagle's medium (DMEM, Lonza) with 4,5g/L glucose, L-glutamine and supplemented with fetal bovine serum (FBS) to final concentration of 10% and penicillin/streptomycin mixture to final concentration of 100U/ml each. Cells were grown in cell culture flasks up to 80% confluence.

MTT assay

Cell viability was assessed using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium

bromide (MTT) assay following the standard procedure (8). This assay is based on the reduction of MTT into purple formazan pigment by the succinate-tetrazolium reductase system in the respiratory chain of mitochondria. This system is active only in metabolically active cells and changes in MTT reductase activity are detectable even before membrane lysis, making the MTT assay a refined marker of cellular viability (3). The absorbance correlates with viable cell number and metabolic activity of the cells. 100µL of MTT solution in PBS (pH=7,4) at concentration of 2mg/mL was added to each well. After 4h of incubation of the plates, the medium was removed and 1 mL of DMSO was added to each well to lyse the cells and dissolve the reduced MTT.

After thorough mixing, 200µL were transferred to 96 plates and the absorbance at λ_{\max} =550 nm was determined using Synergy 2 plate reader (BioTek). Viability of treated cells was presented as percentage of the viability of the non-treated cells (negative control), which was considered as 100%. All treatments were performed in triplicate. Finally, the percentage viability of cells was calculated according to the formula:

$$\text{Cell viability, \%} = \left(\frac{\text{Absorbance}_{\text{sample}}}{\text{Absorbance}_{\text{negative control}}} \right) \times 100, \%$$

Experimental procedure

The cells were collected and seeded in 6 well flasks at density 2x10⁵ cells/well. Then they were treated with EIMNW or with EICCW.

Both infusions were dissolved as follows: 3,125µl, 6,25µl, 12,5µl, 25µl, 50µl, 100µl, 150µl, 200µl, 250µl and 300µl of EIMNW or EICCW were dissolved to 2 ml in phenol red- free DMEM to a final content of the infusions in the nutrient medium of 0,15625%, 0,3125%, 0,625%, 1,25%, 2,5%, 5%, 7,5%, 10%, 12,5%, and 15%, respectively. For MTT test the cells were incubated with the extracts for 20 h. Cells treated with 40% ethanol were used as a control.

Statistical analysis

Statistical analyses were performed using Microsoft Excel Office 2007 software. Differences between the two groups were analyzed by unpaired two-tailed Student's *t*-tests. Data were presented as mean±SD.

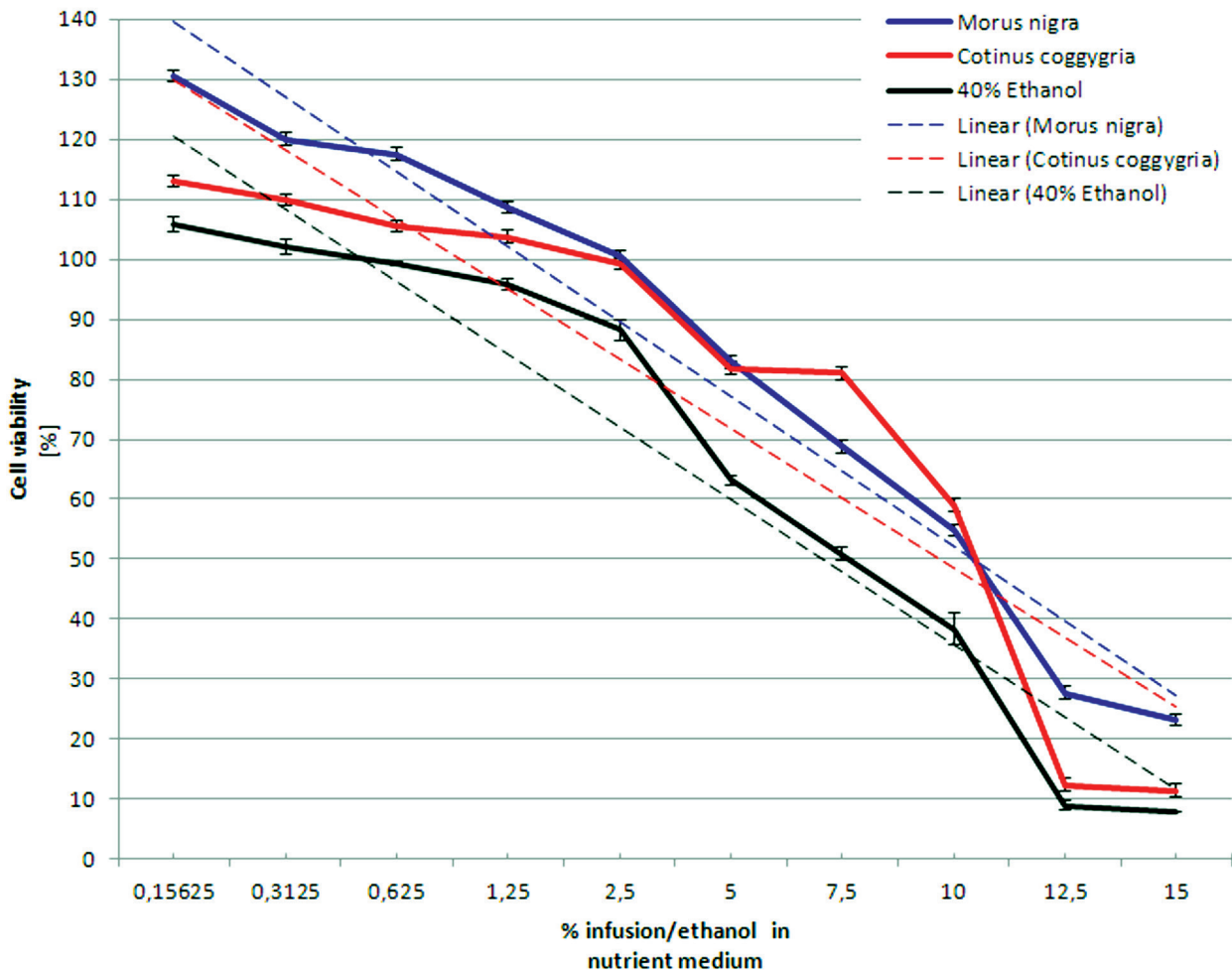


Fig. 1. Viability of 3T3-L1 preadipocytes after treatment with ethanol infusion from *Morus nigra* heartwood (EIMNW) and ethanol infusion from *Cotinus coggygria* heartwood (EICCW); data are presented as mean \pm SD

RESULTS

The effect of EIMNW and of EICCW on 3T3-L1 preadipocytes viability is presented in figure 1. Both infusions exhibited cytoprotective and cytoproliferative effect in percentage values of infusion in the nutrient medium ranging between 0,15% and 2,5%. As presented on the graph, the viability of the cells treated with 40% ethanol (controls) decreased beyond the 0,3% ethanol content in the medium. The cells viability markedly decreased after treatment with higher than 2,5% of each of the two infusions in the culture medium. This tendency was more pronounced in EIMNW treated cells. Viability of cells treated with EIMNW or EICCW increased significantly, demonstrating a cell proliferation inducing activity of the two infusions.

DISCUSSION

Mulberry tree and smoke tree are widely used in folk medicine for different pathological and undesirable conditions. However, leaves and stems from both species, as well as fruits and root bark from mulberry, and not heartwood, are the most popular parts of the plants that are used as sources for preparations. There is a lack of scientific data about biological effects and therapeutic potential of heartwoods infusions of these plants.

The results of the present study estimated the non-cytotoxic effect of 40% ethanol infusions from mulberry and smoke tree heartwood. Viability of 3T3-L1-preadipocytes treated with 0,15%, 0,3%, 0,6%, 1,25% and 2,5% EIMNW and EICCW was above the 100% negative control viability, demon-

strating a cytoprotective and cytoproliferative activity of both infusions (Fig. 1).

The stimulation of the cell proliferation after treatment with both infusions was most probably due to the extracted active compounds from the plant heartwood material. As presented on figure 1, the ethanol controls exhibited reduced cell viability in comparison to the plant infusions. These results are in accordance with a recent study that demonstrated the cytotoxic effect of ethanol. Exposure of 3T3-L1 cells to 40% ethanol resulted in gradual cell viability diminution (4).

On the other hand, it could be suggested that polyphenols contribute to proliferative potential of the infusions. Our recent study revealed high polyphenol content correlating with strong antioxidant activity of EIMNW and EICCW (10).

Reduced viability after application of higher percentages of infusions (above 2.5%) might be due to a toxic effect of some unidentified components extracted in the ethanol heartwood infusion.

The present study is the first report about the effect of *M. nigra* and *C. coggygia* heartwood ethanol infusions on 3T3-L1 cells viability.

CONCLUSIONS

The estimated non-cytotoxic percentage values of the infusions in the nutrient medium may be used in further investigations aimed to reveal biological effects of ethanol heartwood infusions from *Morus nigra* and *Cotinus coggygia* in various *in vitro* and *in vivo* models.

Acknowledgments

The financial support from the European Social Fund within the Project BG051PO001-3.3.06-0028 "Enhancing of the research potential and opportunities for career development in the fields of medicine, healthcare and biotechnology" is greatly acknowledged.

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