

EFFECTS OF THE TOLUENE AND METHANOL EXTRACT OF SENNA (*CASSIA ANGUSTIFOLIA VAHL*) ON VIABILITY AND PROLIFERATION HELA CELLS

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SUMMARY

Senna (*Cassia angustifolia* Vahl.) is used in food and pharmaceutical technologies as officinal drugs and natural laxative. The aim of the study was to investigate the effect of toluene and methanol Senna extracts on the viability and proliferation of HeLa cells. The senna leaves were extracted in Soxhlet's extractor and obtained toluene and methanolic extracts were used for determination of effects on viability and proliferation. Cytotoxic effect of different concentrations (0.1%, 0.01%, 0.001% and 0.0001%) extracts was investigated in HeLa cells *in vitro*. MTT test showed significant cytotoxic activity for toluene extract, especially the concentration of 0.1%, while the tested concentrations methanolic extract did not show cytotoxic activity.

Key words: *Cassia angustifolia*, viability, proliferation, HeLa cell, MTT, cytotoxic activity.

SRPSKI

EFEKAT TOLUENSKOG I METANOLSKOG EKSTRAKTA SENE (*CASSIA ANGUSTIFOLIA VAHL*) NA VIJABILNOST I PROLIFERACIJU

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SAŽETAK

List sene (*Cassia angustifolia* Vahl.) se koristi u prehrambenoj i farmaceutskoj tehnologiji kao oficinalna droga i prirodni laksativ. Cilj studije je ispitati efekte toluenskog i metanolskog ekstrakta Sene na vijabilnost i proliferaciju HeLa ćelija. Ekstrakcijom lišća sene sa Soxhlet-ovim ekstraktorom dobijeni su toluenski i metanolski ekstrakti, koji su korišćeni za ispitivanje efekta na vijabilnost i proliferaciju. Citotoksični efekat različitih koncentracija (0,1%, 0,01%, 0,001% i 0,0001%) ekstrakata ispitan je na HeLa ćelijama u *in vitro* uslovima. MTT test pokazuje visoku citotoksičnost toluenskog ekstrakta, naročito onog koncentracije 0,1%, dok ispitivane koncentracije metanolskog ekstrakta ne pokazuju citotoksičnost.

Ključne reči: *Cassia angustifolia*, vijabilnost, proliferacija, HeLa ćelije, MTT, citotoksična aktivnost.

INTRODUCTION

In recent years, interest in studying the different extracts of traditional medicinal plants as a source of potential cytotoxic activity has been increasing [1,2]. *Cassia* genus belongs to the family Fabaceae (also called Legumino-

sae), includes over 700 species which because of therapeutic efficacy are used in medicinal purposes [3,4,5]. *Cassia* L. and *Senna* Mill. are commonly used medicinal plants for a broad range of diseases and conditions including constipation, parasitic skin diseases, hypercholesterolemia, hypertension, inflammation, pain relief, and antiplatelet aggregating activity [4,5,6]. *Cassia angustifolia* is a plant widespread in tropical regions of East Africa and Asia, where it

grows as a bush which reaches height of 300 cm [4]. The drug consists of dried leaves of Senna [7]. *Cassia angustifolia* is used more than the other species of the genus *Cassia* because the drug is purer, more homogeneous and more stable composition. The mechanism of action of sennosides is well studied. Sennosides pass through the stomach and small intestine unchanged. They are activated only in the large intestine under the action of bacterial enzymes that hydrolyze them and reduce their active form [8,9]. Previous studies of active components from Senna leaves were investigate the genotoxic effects on normal cell lines [10]. The sennosides, main active metabolite of Senna, show a very low toxicity in rats and low genotoxicity in bacterial strains [6,9,10]. The laxative property of Senna is based on two glycosides sennoside A and sennoside B [11]. The strongest laxative sennosides A and B, shows the lowest toxicity, while fraction that contains rhein 8-glycoside with a minimum efficiency laxative, has increased toxicity [12,13]. Emodin and aloe-emodin showed toxic effects on *Salmonella typhimurium*, on hepatocytes and fibroblasts from mouse [14]. In other studies, such an effect has not been confirmed. In research carried out by Mori mice were treated with 1% hydroxyanthraquinone 480 days, there were neoplasms in the stomach, liver and intestine [15,16]. Sieger and Abendroth in there works show that there is a specifed risk factor in chronic use of laxatives [17,18]. All other tests which were performed did not provide convincing evidence that chronic use of Senna causes functional and structural effects in the body [19].

STYDY OBJECTIVE

The aim of the study was to investigate the effect of toluene and methanol Senna extracts (*Cassia angustifolia* Vahl) on the viability and proliferation of HeLa cells.

MATERIAL AND METHODS

Cells. The extracts were tested on the human cervical carcinoma cell line - HeLa. Cells were cultured in Dulbecco's Modified Eagle's Minimal Essential Medium (DMEM, PAA Laboratories GmbH) with supplements of 2 mM L-glutamine, 100 µg/ml streptomycin, 100 units/ml penicillin and 10% fetal bovine serum.

Extracts. Toluene and methanol (MeOH) extracts of Senna (*Cassia angustifolia*) were prepared by extracting finely milled plant leaves in a Soxhlet extractor for 3 hours. After that, the extract was evaporated under reduced pressure in a vacuum evaporator. The toluene and methanol extracts, obtained in this way, were diluted with enriched DMEM up to final concentrations of 0.0001%, 0.001%, 0.01% and 0.1% (v / v).

MTT test. The MTT test is a widely accepted method for the rapid and accurate testing of the cytotoxicity of various materials, as well as of cell viability and cell proliferation. This method is based on the capacity of the mitochondrial dehydrogenase of viable cells to reduce tetrazolium salts and create colored formazan products. The number of living cells is directly proportional to the quantity of generated formazan.

Effects on cell viability. HeLa cells were seeded in a 96-well plate for cultivation. In order to determine the effect of Senna extract on the growth of HeLa cells, there

were seeded 10^5 cells in each well. The cells were incubated for 24 hours. After incubation, the medium was replaced with methanol and toluene extracts in different concentration. Saponin (with a concentration of 2 mg/ml) was used as a positive control. The negative control consisted in cells growing in pure DMEM. It was done in triplicate. The cells were incubated for 24 hours in an atmosphere saturated with water vapor, with 5% of CO₂ at 37 °C. (Jouan, EG 110IR)

Effects on cell proliferation. In order to determine the effect of Senna extract on the proliferation of HeLa cells, 10^4 cells were seeded in the wells. After 24 hours, Senna extract was added. Cisplatin (with a concentration of 6×10^{-4} mg/ml) was used as a positive control. The cells were incubated for 72 hours in an atmosphere saturated with water vapor, with 5% of CO₂ at 37 °C. It was done in triplicate.

Upon completion of the incubation, the medium was extracted, the cells were washed with 100 µl of PBS and there was added a quantity of 20 µl of MTT [3-(4,5-dimethylthiazol-2)-2,5-diphenyltetrazolium bromide]. After 4 hours of incubation at 37 °C, the MTT was removed and 100 µl of isopropanol were added, in order to dissolve the formed formazan crystals. The spectrophotometric measurement of MTT reduction was carried out at 540 nm, on multi-channel photometer Multiscan Ascent No354, Thermo Labsystems, Finland.

The viability/proliferation percentage of HeLa cells was obtained on the basis of the following formula:

% Viability (proliferation) = average value of absorbance of each test extracts / average value of control absorbance

RESULTS

Effects of the toluene and methanol extract of Senna after incubation for 24 hours. Figure 1 shows that cell viability ranges between 23.64% and 89.98%, after 24 hours of incubation with toluene extract of Senna. The 0.1% toluene extract of Senna shows the largest decrease in the viability of HeLa cells (23.64%), while the extract with a concentration of 0.01% shows the weakest effect on cell viability (89.98%). The viability of the cells with methanol extracts, after 24 hours of incubation is in the range of 70.7% to 101.94%. The lowest viability of HeLa cells was manifested MeOH extract concentrations of 0.0001%. The concentration of 0.001% and 0.01% were showed approximately the same effect on the viability of HeLa cells and which was around 100%. The viability of the HeLa cells at a concentration of 0.1% of MeOH extracts was 93.9%. The results do not indicate the existence of a dose relationship between the concentration of methanol extracts of *Cassia angustifolia* and cell viability. HeLa cell viability at a concentration of 0.0001% of the extract is 70.70%, which suggested that there was a slight cytotoxic effect. Other concentrations of MeOH extract did not have cytotoxic effect on the cells during the incubation period of 24 hours. The cell viability with the saponin was 17.7%

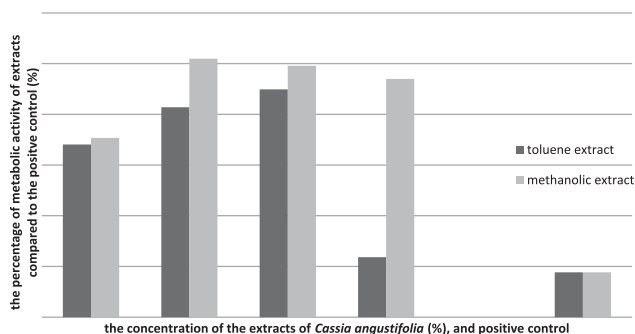


Figure 1. The percentage of the metabolic activity in the HeLa cells compared to the positive control after incubation for 24 hours with different concentrations of toluene and methanol extract of species *Cassia angustifolia*

Effects of the toluene and methanol extract of Senna after incubation for 72 hours. Figure 2 shows the viability of HeLa cells in the presence toluene extract of Senna after 72 hours of incubation. The viability of HeLa cells ranges between 71.10% and 101.51%. The highest reduction of cell proliferation (28.9%) occurs when the concentration of the toluene extract is 0.0001%. All other toluene extracts showed little effect on cell proliferation, while a 0.001% concentration showed an increase in the viability of HeLa cells by 1% in comparison to the negative control. The viability of the cells with methanol extracts, after 72 hours of incubation is in the range of 77.73% to 131.95%. MeOH extract with a concentration of 0.0001% displayed the greatest effect on the reduction of cell proliferation, while cell viability was 77.74%. Concentrations of 0.001% and 0.01% showed an increase in the viability of HeLa cells by about 130%, indicating that the given concentrations of Senna had a pronounced proliferative effect. When the concentration of the MeOH extract of Senna was 0.1%, cell viability amounted to 92.06%, displaying a slight suppression of cell proliferation in comparison to the control group.

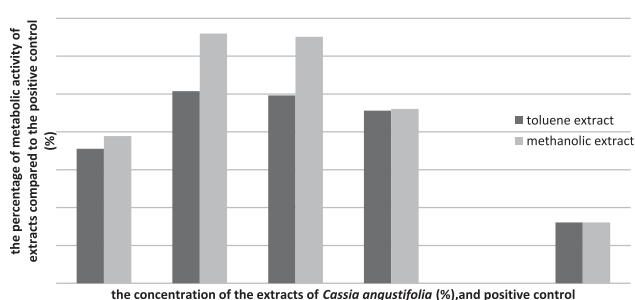


Figure 2. The percentage of the metabolic activity in the HeLa cells compared to the positive control after incubation for 72 hours with different concentrations of toluene and methanol extract of species *Cassia angustifolia*

DISCUSSION

The results, after incubation of 24 hours with a toluene extract showed an inhibitory effect on cell viability. The greatest degree of influence had the highest concentration (0.1%). A concentration of 0.0001% to 0.01% showed a smaller cytotoxic effect than the maximum concentration. Based on the results, which were obtained

after 24 hours of incubation HeLa cells with methanol extracts of *Cassia angustifolia* can be concluded that with increasing concentration of 0.0001% to 0.01% increases cell viability, and then at a concentration of 0.1% fell again [2,6,10].

The results obtained for both extracts indicate that there was no dose dependence between the concentration of Senna extracts and cell viability. By comparing the different concentrations of the tested extracts, it is evident that extracts with middle concentrations had the best effect on the viability and proliferation of HeLa cells. By comparing the results of different types of extracts, we can see that the toluene extract of *Cassia angustifolia* had a higher inhibitory effect on the viability and proliferation of HeLa cells, unlike methanol, which has a stimulating effect in certain concentrations. The results obtained in this study should be considered as the consequence of the effects of various chemical compounds. The presence of chemical compounds in the extract depended on the solvent that was used. In addition to that, the incubation process led to the interaction of the compounds in the extract, as well as to the interaction with their metabolic products, which may have had first an inhibitory effect and later a stimulatory effect on cells at certain extract concentrations [12,13].

CONCLUSION

After analyzing the impact of toluene and methanol extract of *Cassia angustifolia* on the viability of HeLa cells, we can conclude the following: Toluene extract with a concentration of 0.1% displayed a high cytotoxicity after 24 hours of incubation, while extracts with lower concentrations were slightly cytotoxic. After 72 hours of incubation, the tested toluene extracts did not inhibit the growth of cells. The tested methanol extracts displayed no cytotoxicity, except in the case of the lowest concentration, which exhibited low cytotoxicity and cytostaticity. Different (toluene and a methanol) extracts of the same plant species have different effects on the viability and proliferation of HeLa cells. The results could contribute to further research and finding new use of Senna, in order to extend the therapeutic indication and use of the chemotherapy but also the treatment of conditions which may find application.

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