Kragujevac J. Sci. 46 (2024) 39-48
doi: 10.5937/KgJSci2400015N

UDC 606:61:595.784.2:616.34-006
Original scientific paper

SILK OF THE INDIAN MEAL MOTH *Plodia interpunctella* (HÜBNER, 1813) AFFECTS THE HUMAN COLON CANCER CELLS

Danijela Nikodijević[11], Milena Milutinović[1]*, Nikola Radenković[1], Stefan Blagojević[1], Anđela Vasiljević[1], Vladimir Jurišić[2], Dragana Predojević[1], Filip Vukajlović[1], Snežana Pešić[1]

[1]*University of Kragujevac, Faculty of Science, Department of Biology and Ecology, Radoja Domanovića 12, 34000 Kragujevac, Serbia*
[2]*University of Kragujevac, Faculty of Medical Science, P.BOX 124, 34000 Kragujevac, Serbia*
*Corresponding author; E-mail: milena.milutinović@pmf.kg.ac.rs

(Received September 26, 2024; Accepted December 04, 2024)

ABSTRACT. The insect silk protects the larvae that produce it. This implies that it may contain biologically active substances that can be used in medicine. In this paper, the cytotoxic and proapoptotic potential of Indian meal moth (*Plodia interpunctella*) silk extract was examined after 72 h of exposure to the SW-480 human colorectal adenocarcinoma cell line. Apoptosis was examined by monitoring the cell morphological changes by fluorescent staining, acridine orange/ethidium bromide, and DAPI, as well as by flow cytometry. The results showed that silk extract has a very good cytotoxic and proapoptotic activity. The selective effect of silk indicates the presence of a high level of biocompatibility with normal human cells. Based on the results of increased gene expression for caspase 8 and 9 in silk extract-treated cells compared to control, apoptosis was induced by a caspase-dependent pathway.

Keywords: colon cancer, *Plodia interpunctella*, apoptosis, caspases, biocompatibility

INTRODUCTION

The increased incidence of colon carcinoma requires the need to find drugs that will have an inhibitory effect on cancer cells, without or with minimum side effects on normal cells in the human body (PIASEK *et al.*, 2009; PAGANI, 2010; SUNG *et al.*, 2020; ABADI *et al.*, 2021). The appearance of inflammation, resistance, necrosis, and many other unwanted effects lead to doubt even with drugs used according to the protocol, especially after their long-term use (MILLS *et al.*, 2018). Eliminating the possibility that an antitumor agent causes necrosis in cancerous cells and affects healthy human cells is of great importance. Thus, an

ORCID ID:
D. Nikodijević - 0000-0002-6453-0313; M. Milutinović - 0000-0002-5075-5650;
N. Radenković - 0000-0002-8458-2022; S. Blagojević - 0000-0002-5640-6267;
V. Jurišić - 0000-0001-6525-128X; F. Vukajlović - 0000-0003-4879-0171;
D. Predojević - 0000-0002-9374-0563; S. Pešić - 0000-0001-7762-1372.

increasing number of substances are being tested from natural sources, based on their biocompatibility (KUMAR and MANDAL 2019a; MILUTINOVIĆ *et al.,* 2019; 2020; NIKODIJEVIĆ *et al.,* 2019; 2021).

Animal products have found great importance in research related to antitumor therapy. In animal secretions, many substances have great potential for antitumor drug development. Many peptides from the secretions of wasps, bees, and other insects show a big potential for modulation of some signaling pathways in some pathological conditions (LEONARDI *et al.,* 2007; ZHENG *et al.,* 2015; ROY and BHARADVAYA, 2021). Insects, such as the Indian meal moth (IMM), *Plodia interpunctella* (Hübner, 1813), produce silk that protects their larvae. It provides optimal conditions for the development of their next developmental stage (pupa) inside the silk cocoon, protection from parasites and predators, drying of the larvae during metamorphosis, etc. (VOLLRATH and SELDEN, 2007; CAPPELLOZZA *et al.,* 2022; BARVE *et al.,* 2022). This fact led to the conclusion that silk contains a greater number of potentially useful compounds (MONDAL *et al.,* 2007). The composition of silk depends on the type of organism that produces it, due to a difference in the glands for its production. Therefore, the silk of spiders is mainly composed of spidroin, while moth silk predominantly contains fibroin (TAHIR *et al.,* 2019). In addition to fibroin, the silk of moths mainly contains proteins from the sericin group, which are considered good biopolymers with proven therapeutic value (KUNZ *et al.,* 2016; SURYAWANSHI *et al.,* 2020). Sericin consists of several amino acids such as glutamine, glycine, alanine, tryptophan, and others, which play a role in metabolic processes essential for humans (RANGI and JAJPURA, 2015). Freshly produced silk of the IMM, as well as of many other Lepidoptera, can contain secretions of the mandibular salivary glands on its threads. Saliva contains digestive enzymes, antioxidants, protein inhibitors, and many other small molecules with important biological activities. In addition to being necessary for insects' digestion, and protection (DONG *et al.,* 2013; RIVERA-VEGA *et al.,* 2017), it can also be important for humans. In this regard, the silk of the IMM has shown significant antitumor (MILUTINOVIĆ *et al.,* 2019) and antimicrobial activity (STEFANOVIĆ *et al.,* 2020), which indicates a great potential for more detailed studies on the mechanism of its action and the possibility of use.

Based on the assumption that silk may be the source of substances with anticancer properties, this study aimed to investigate the potential of silk extract to inhibit proliferation and induce apoptosis of SW-480 colorectal cells, as well as the triggering mechanism.

MATERIALS AND METHODS

Chemicals

Phosphate buffered saline (PBS) and Dulbecco's Modified Eagle Medium (DMEM) were obtained from GIBCO, Invitrogen, USA. Ethidium bromide (EB), 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT), absolute ethanol and chloroform (molecular biology grade) were obtained from SERVA, Germany. Acridine Orange (AO) was obtained from Acros Organics, New Jersey, USA. The Kit for qPCR AMPLIFYME SG UNIVERSAL Mix was from BLIRT, Poland. Nuclease-free water and TRIzol were from Ambion, USA. Kit for translating RNA into complementary DNA (High-Capacity cDNA Reverse Transcription Kits) from Thermo Scientific, USA. All solvents and chemicals were of analytical grade. Annexin V-FITC was purchased from BD Biosciences.

Silk collection

The silk used in this research was produced by IMM larvae (420 larvae in total) in The Laboratory for General and Applied Entomology, Faculty of Science, University of

Kragujevac. IMM larvae were reared on a standard laboratory diet for *P. interpunctella* (SILHACEK and MILLER, 1972) for ten years, in transparent plastic containers for mass rearing (1.2 L in volume), in a thermostat chamber at 28 ± 1 ºC, relative humidity, 60 ± 10% and 14:10 (light:dark) photoperiod. The 30 specimens of 5th instar IMM larvae were placed into sterile glass Petri dishes (60 mm in diameter and 15 mm height) and left to produce silk at 24 ± 3 ºC, relative humidity, 60 ± 10% and 14:10 (light:dark) photoperiod. This process was repeated in two replicates (two Petri dishes). After 48 hours, silk-producing larvae in two Petri dishes were substituted by 30 new 5th instar IMM larvae, and this process was repeated for 15 days. Visible impurities (mainly larval excrement) between larval shifts were removed from the silk with fine painter's brushes. During the production of silk in Petri dishes, larvae were not fed. After 15 days, all larvae were removed from Petri dishes. The silk produced after 15 days was used in experiments conducted in the Laboratory for Cell and Molecular Biology, Faculty of Science, University of Kragujevac.

Silk extract preparation

The sample of IMM silk was extracted with DMSO for 24 h. After that time, the cell culture medium was added to make a stock solution with a final concentration of 1 mg/mL (conditionally, because the silk was not completely dissolved). The stock solution was sterilized using 0.2 mm membrane filters and diluted in the concentration range (DMSO was not above 0.5% in the highest certain concentration). For all experiments, silk extract was used in one concentration obtained by MTT assay (IC_{50} value in μg/mL).

Cell lines and culturing

The human colorectal carcinoma cells SW-480 (from ATCC – American Type Culture Collection, USA), and normal human keratinocytes HaCaT cells (from CLS – Cell Lines Service, Eppelheim, Germany), were used to experiment. Cell cultivation conditions have been described in detail previously by MILUTINOVIĆ and associates (2015), and experiments were done with 80% of cell confluence.

Cell viability assay (MTT assay)

The 72-hour effects of silk extract on SW-480 colorectal carcinoma and normal HaCaT cell viability were determined using an MTT assay (MOSMAN, 1983). The cells were seeded in a 96-well plate (10^4 cells/well), and after 24 h were treated with 100 μL of different concentrations of silk extract (0.1 – 500 μg/mL). Untreated cells served as a control, and only DMEM was replaced. The absorbances were read on an ELISA reader (at 550 nm), and percentages of cell viability were obtained as the ratio between the absorbance of treated and control cells (multiplied by 100). Cytotoxicity (i.e., IC_{50} values) was determined from the cell viability curves (by CalcuSyn program, Biosoft v2.0).

Fluorescence microscopic analysis of cell death

Double staining assay (Acridine Orange/Ethidium Bromide – AO/EB) was used for cell death type determination on SW-480 cells (BASKIĆ *et al.*, 2006), under the long-term (72 h) influence of silk extract. After seeding (10^4 cells/well in a 96-well plate), the cells were treated with IC_{50} concentration of silk extract. Detection was performed using an inverted fluorescent microscope (Nikon Ti-Eclipse, Tokyo, Japan) at 400x magnification. Results are presented as a percentage of viable cells (V), early apoptosis (EA), late stage of apoptosis (LA), and necrosis (N) concerning the total cell number per sample (a minimum of 300 cells was counted in each sample).

Nucleus staining

For determining the nucleus morphology, DAPI staining was used (FANG *et al.*, 2018). The cells were seeded in 96-well plates (1.2×10^4 cells/well). After 24 h (at 37 °C with 5% CO_2), the cells were treated with 100 µL of silk extract (IC_{50} concentration of silk extract), and in the control cell, the medium was replaced. When the incubation time of 72 h was expired, the cells were washed with PBS, and fixed with 4% paraformaldehyde (10 min), and 5 µL of DAPI was added (1 mg/mL) for 5 min, protected from light. Nuclei were observed using an inverted fluorescence microscope Nikon Ti-Eclipse, at 400x magnification.

Flow Cytometry

In addition to fluorescent staining, the type of cell death was analyzed by flow cytometry using an Annexin V-FITC kit according to the manufacturer's instructions, by BD FACSVerse™ flow cytometer (San Jose, CA, USA). Analysis was performed after the chronic effect of silk extract (after 72 h). After incubation time, the supernatant was removed, cells were washed with PBS, and then trypsinized and collected from the well. All samples were centrifugated at 1200 rpm for 5 min. and the supernatant was removed. The obtained precipitate was resuspended in 1X HEPES buffer in the concentration of $1 – 5 \times 10^6$ cells/mL. Per each sample, 100 µL of the cell suspension was stained with Annexin V-FITC (5 µL), and incubated for 15 min (room temperature, in the dark). After incubation time, in each sample 1 mL of 1X HEPES buffer was added, and samples were centrifuged for 5 min at 1200 rpm. The supernatant was removed, while the precipitate was resuspended in 100 µL 1X HEPES buffer. After that, the cells were stained with 5 µL of propidium iodide (PI) and incubated for 15 min at room temperature in the dark in the final volume of 300 µL of 1X HEPES buffer. For this analysis, a minimum of 10,000 events were analyzed by the original BD FACSuite software to determine and calculate a percentage of viable, apoptotic (early and late), and necrotic cells.

Quantitative mRNA analysis by qPCR method

Total RNA was isolated from control and SW-480 cells treated with silk extract at a concentration of IC_{50}) (CHOMCZYNSKI and SACCHI, 1987). The cells were seeded in a T25 cm^2 (10^6 cells per flask) and after 80% of confluence were treated with 5 mL of silk extract. RNA isolation was performed 72 h after treatment. The RNA concentrations were measured on a biophotometer (Eppendorf BioPhotometer Plus). The samples of isolated RNA were stored at –80 °C until analysis. For transcribing single-stranded RNA in the cDNA (complementary DNA), the High-Capacity cDNA Reverse Transcription Kit (Thermo Scientific, Waltham, Massachusetts, USA), was used.

The quantification of mRNA expression was performed by using an Applied Biosystems, Quantitative Real-Time system (Applied Biosystems 7500/7500 Real-Time PCR Software v2.0). All amplifications were done using an AMPLIFYME SG UNIVERSAL Mix for qPCR. Each sample contained 10 µL of qPCR Mix, 0.6 µL of forward and reverse primer for specific genes (5 pmoL/µL) 1 µL cDNA, and 7.4 µl nuclease-free water. The results are processed by the $2^{\wedge(-\Delta\Delta Ct)}$ method (SCHMITTGEN and LIVAK, 2008). For this experiment, *β-actin* was employed as a reference gene. Gene-specific primers (forward and reverse sequences) are shown in Table 1.

Statistical analysis

The data are expressed as mean ± standard error (SE). Biological activity was examined in three individual experiments, performed in triplicate for each dose. Statistical

significance was determined using the Student's *t*-test or the one-way ANOVA test for multiple comparisons in SPSS software (version 23.0. Armonk, NY, USA). A p-value < 0.05 was considered significant. The IC_{50} values were calculated from the dose curves by CalcuSyn software.

Table 1. Gene-specific primers.

	Forward	Reverse
β-actin	5′-AAGCAGGAGTATGACGAGTCCG-3′	5′-GCCTTCATACATCTCAAGTTGG-3′
Cas8	5′-AGAGTCTGTGCCCAAATCAAC-3′	5′-GCTGCTTCTCTCTTTGCTGAA-3′
Cas9	5′-GAGTCAGGCTCTTCCTTTG-3′	5′-CCTCAAACTCTCAAGAGCAC-3′

RESULTS AND DISCUSSION

Cytotoxicity of IMM silk extract

The MTT assay was used to examine the long-term effect of *Plodia interpunctella* silk extract (in the concentration range of 1 – 500 μg/ml), on SW480 colon cancer cell viability. Our results showed that silk significantly decreased SW-480 cell viability after a longer time of action (chronic effect after 72 h). A significantly reduced percentage of cell viability also indicated a significant IC_{50} value, which represents a measure of cytotoxicity. According to that, the IC_{50} value indicates the presence of strong cytotoxicity after 72 h (IC_{50} = 32.97 ± 1.39). This result is in agreement with the commercial cytostatics currently used in colon cancer treatment, such as 5-fluorouracil (SMITH *et al.*, 2020). In addition, strong selective activity has been shown, due to non-cytotoxicity on normal HaCaT cells after the same incubation period (IC_{50} > 500 μg/ml), and the results showed that the silk has a selective effect, which is in agreement with the literature data (TERADA *et al.*, 2005; TSUBOUCHI *et al.*, 2005). This implies that silk can be considered as an agent with a reduced risk of side effects.

The cytotoxic effect of silk was also demonstrated on HCT-116 cells originating from colorectal cancer, and silk showed a selective effect without affecting the viability of normal lung fibroblasts (MILUTINOVIĆ *et al.*, 2020). This implies that silk is an excellent treatment that has a high level of biocompatibility and requires further research in this field. The dissolution of silk leads to the release of sericin, which is mostly associated with its biological effects in cells (TAHIR *et al.*, 2019).

Influence of *IMM* silk extract on cancer cell morphology

According to cell morphological changes under the influence of silk treatment, apoptosis predominantly occurred in SW-480 cells, suggesting that silk has a pro-apoptotic effect. The micrographs show the cell morphological changes typical for apoptosis. Fluorescent staining with Acridine Orange and Ethidium Bromide dyes, clearly separate viable cells (green color, with preserved integrity of the nucleus and cell membrane), cells in apoptosis (the cells are lighter, have changed and condensed nuclei, and do not have clear cytoplasm outlines), and necrosis (clearly visible red vesicles) (Figure 1). Staining by DAPI dye shows changes only at the nuclear level that also indicate nuclei condensation, and the presence of apoptosis compared to the control sample (Figure 1).

Induction of apoptosis on SW-480 cells by Indian meal moth silk

Based on the micrographs, the percentage of viable cells, cells in early and late apoptosis, as well as necrotic cells, was calculated. The results showed that apoptosis is the

dominant type of cell death. (Table 2). It is known in the literature that the sericin originating from silk leads to an increase in intracellular oxidative stress and consequently to the induction of apoptosis on A431 epidermal carcinoma cells, as well as on MCF-7 cells originating from breast carcinoma (KUMAR and MANDAL, 2019b).

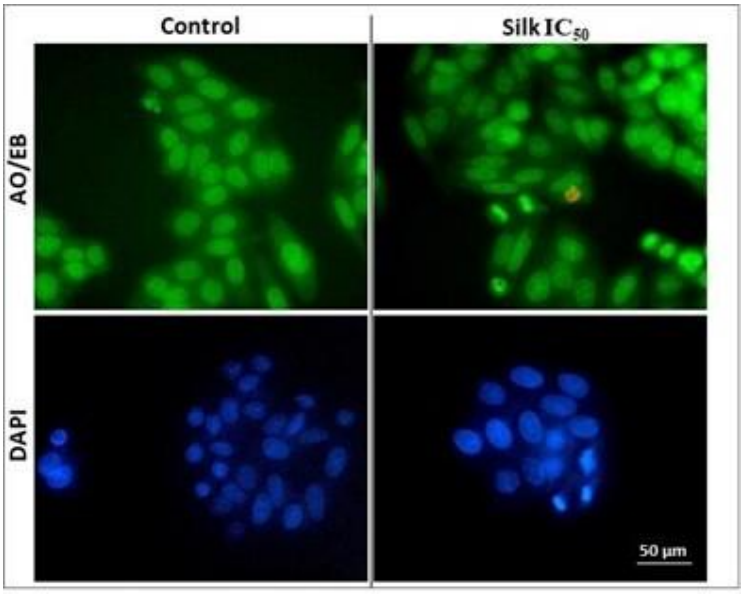

Figure 1. Morphological changes on colorectal cancer SW-480 cells 72 h after IC_{50} silk extract treatment.

Table 2. Percentages of viable and apoptotic cells (early and late stage), and necrosis in control and treated SW-480 cells, were detected by AO/EB staining after 72 h.

SW-480	Control	Silk extract
Viable	93.62 ± 0.06	64.93 ± 4.39*
Early apoptosis	5.66 ± 0.78	19.05 ± 2.82*
Late apoptosis	0.70 ± 0.01	14.62 ± 2.13*
Necrosis	0.02 ± 0.01	1.40 ± 0.41*

The data means ± SE of three independent experiments. *$p < 0.05$ compared to untreated cells.

This result was also confirmed by flow cytometry using annexin-V and propidium iodide staining but high percentages of necrosis were observed in treated cells (Table 3). In this analysis, the increased percentage of necrosis did not represent the percentage of necrotic cells because the staining was non-specific, and cells in the stage of late apoptosis may also be detected as necrotic. With the later stage of apoptosis, an increase in membrane permeability probably occurs, so the cells in late apoptosis may be double-stained and recognized as necrosis. Necrosis is unmistakably detected by staining with ethidium dye since the presence or absence of necrosis is visible. This conclusion is also supported by the fact that the percentage of viable cells and spontaneous apoptosis in control samples obtained using both methods was similar.

According to the literature, the antitumor effect of IMM silk was achieved by inducing apoptosis (MILUTINOVIĆ *et al.*, 2020). In the action of an anticancer drug, the absence of necrosis is of great importance even after a longer period of action, a chronic effect, because this usually leads to the appearance of inflammation and consequently to intoxication, resistance, and many other side effects.

Table 3. Percentages of viable and apoptotic cells (early and late stage), and necrosis in control and treated SW-480 cells, detected by flow cytometry, after 72 h.

SW-480	Control	Silk extract
Viable	93.51 ± 0.31	47.9 ± 0.03*
Early apoptosis	5.93 ± 0.27	1.15 ± 0.04*
Late apoptosis	0.29 ± 0.01	14.36 ± 2.06*
Necrosis	0.27 ± 0.04	36.59 ± 2.05*

The data means ± SE of three independent experiments. *p < 0.05 compared to untreated cells.

Influence of IMM silk extract on expression of mRNA for apoptotic genes

The significant changes in mRNA expression of apoptosis-related genes in silk extract-treated cells compared to control were registered (Figure 2). Namely, the gene expression for both investigated caspases (*Caspase 8* and *9*) was significantly increased, especially *Caspase 9*.

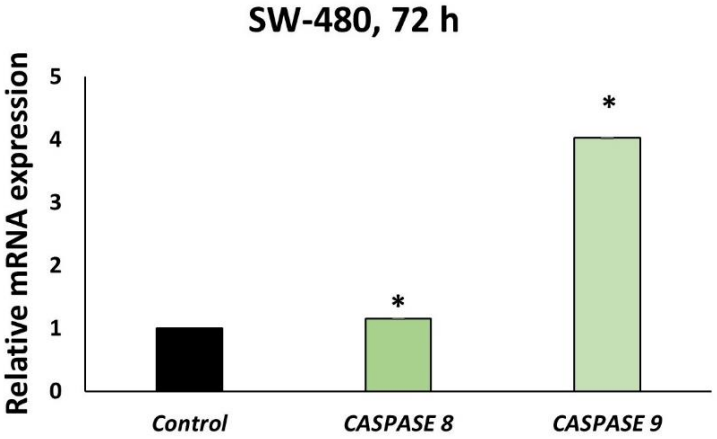

Figure 2. Relative mRNA expression of apoptotic genes under the influence of silk extract in IC_{50} concentration, after 24 h. *p < 0.05 compared to untreated cells.

The increase of both caspases implies that apoptosis occurs through a caspase-dependent mechanism in SW-480 cells exposed to silk extract. The pro-apoptotic effect of silk extract possibly comes from sericin, which has been shown to increase the expression of caspase 3 and decrease the expression of the anti-apoptotic marker *Bcl-2* on SW-480 cells (KAEWKORN *et al.*, 2012). On its fibers, fresh silk contains the components of insect saliva, which can have an anti-inflammatory effect, which can be significant in combined anticancer therapy (WEI *et al.*, 2015). Based on this fact, IMM silk is a good source of many substances that can be tested for antitumor activity.

Insects, with their lifestyle, have contributed to the development of various materials that have gained importance in medical use. The source of bioactive substances such as insect silk, in this case, product of *Plodia interpunctella* moth, represents a good starting point for testing biocompatibility and use in biomaterial production, as well as for testing the mechanisms of its previously demonstrated antitumor effect.

Acknowledgments

This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Agreement no. 451-03-47/2023-01/200122 and 451-03-47/2023-01/200111).

References:

[1] ABADI, A.J., MIRZAEI, S., MAHABADY, M.K., HASHEMI, F., ZABOLIAN, A., HASHEMI, F., RAEE, P., AGHAMIRI, S., ASHRAFIZADEH, M., AREF, A.R., HAMBLIN, M.R., HUSHMANDI, K., ZARRABI, A., SETHI, G. (2021): Curcumin and its derivatives in cancer therapy: Potentiating antitumor activity of cisplatin and reducing side effects. *Phytotherapy Research*. **36**: 189–213. doi: 10.1002/ptr.7305

[2] BARVE, T.V., GODFREY, R.K., STORER, C.G., KAWAHARA, A.K. (2022): Effect of behavioral conditions on silk characteristics in the Indian meal moth (*Plodia interpunctella*). *bioRxiv*; preprint. doi: 10.1101/2022.10.11.511611

[3] BASKIĆ, D., POPOVIĆ, S., RISTIĆ, P., ARSENIJEVIĆ, N.N. (2006): Analysis of cyclohexamideinduced apoptosis in human leukocytes: fluorescence microscopy using annexin V/propidium iodide versus acridin orange/ethidium bromide. *Cell biology international*. **30** (11): 924–932. doi: 10.1016/j.cellbi.2006.06.016

[4] CAPPELLOZZA, S., CASARTELLI, M., SANDRELLI, F., SAVIANE, A., TETTAMANTI, G. (2022): Silkworm and silk: traditional and innovative applications. *Insects* **13**: 1016. doi: 10.3390/insects13111016

[5] CHOMCZYNSKI, P., SACCHI, N. (1987): Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Analitical Biochemistry* **162**: 156–159. doi: 10.1006/abio.1987.9999

[6] DONG, Z., ZHAO, P., WANG, C., YAN, JIANPING, Z., WANG, C. X., LIN, Y., XIA, O. (2013): Comparative proteomics reveal diverse functions and dynamic changes of Bombyx mori silk proteins spun from different development stages. *Journal of Proteome Research*. **1**;12(11): 5213–22. doi: 10.1021/pr4005772

[7] FANG, K., ZHANG, X.H., HAN, Y.T., WU,.G.R., CAI, D.S., XUE, N.N., GUO, W.B., YANG, Y.Q., CHEN, M., ZHANG., X.Y., WANG, H., MA, T., WANG, P.L., LEI, H.M. (2018): Design, synthesis, and cytotoxic analysis of novel hederagenin–pyrazine derivatives based on partial least squares discriminant analysis. *International Journal of Molecular Science* **19**, 2994. doi: 10.3390/ijms19102994

[8] KAEWKORN, W., LIMPEANCHOB, N., TIYABOONCHAI, W., PONGCHAROEN, S. & SUTHEERAWATTANANONDA, M. (2012): Effects of silk sericin on the proliferation and apoptosis of colon cancer cells. *Biological Research* **45**: 45–50. doi: 10.4067/S0716-976 02012000100006

[9] KUMAR, J.P., MANDAL, B.B. (2019a): Silk sericin induced pro-oxidative stress leads to apoptosis in human cancer cells. *Food and Chemical Toxicology* **123**: 275–287. doi: 10. 1016/j.fct.2018.10.063

[10] KUMAR, J.P., MANDAL, B.B. (2019b): Inhibitory role of silk cocoon extract against elastase, hyaluronidase and UV radiation-induced matrix metalloproteinase expression in human dermal fibroblasts and keratinocytes. *Photochemical & Photobiological Sciences* **18**: 1259. doi: 10.1039/C8PP00524A

[11] KUNZ, I.K., BRANCALHAO, R.M.C., CHASKO RIBEIRO, L.F., NATALI, M.R.M. (2016): Silkworm sericin: properties and biomedical applications. *BioMed Research International* **2016**: 1–19. doi: 10.1155/2016/8175701

[12] LEONARDI, A., FOX, J. W., TRAMPUŠ-BAKIJA, A., KRIZAJ, I. (2007): Ammodytase, a metalloprotease from *Vipera ammodytes ammodytes* venom, possesses strong fibrinolytic activity. *Toxicon* **49**(6): 833–842. doi: 10.1016/j.toxicon.2006.12.003

[13] MILLS, E.L., RYAN, D.G., PRAG, H.A., DIKOVSKAYA, D., MENON, D., ZASLONA, Z., JEDRYCHOWSKI M.P., COSTA, A.S.H., HIGGINS, M., HAMS, E., SZPYT, J., RUNTSCH, M.C., KING, M.S., MCGOURAN, J.F., FISCHER, R., KESSLER, B.M., MCGETTRICK, A.F., HUGHES, M.M., CARROLL, R.G., BOOTY, L.M., KNATKO, E.V., MEAKIN, P.J., ASHFORD, M.L.J., MODIS, L.K., BRUNORI, G., SÉVIN, D.C., FALLON, P.G., CALDWELL, S.T., KUNJI, E.R.S., CHOUCHANI, E.T., FREZZA, C., DINKOVA-KOSTOVA, A.T., HARTLEY, R.C., MURPHY, M.P., O'NEILL L. (2018): Itaconate is an anti-inflammatory metabolite that activates Nrf2 via alkylation of KEAP1 Nature 556: 113–117. doi: 10.1038/nature25986

[14] MILUTINOVIĆ, M.G., MAKSIMOVIĆ, V.M., CVETKOVIĆ, D.M., NIKODIJEVIĆ, D.D., STANKOVIĆ, M.S., PEŠIĆ, M., MARKOVIĆ, S.D. (2019): Potential of *Teucrium chamaedrys* L. to modulate apoptosis and biotransformation in colorectal carcinoma cells. *Journal of Ethnopharmacology* **240**: 111951. doi: 10.1016/j.jep.2019.111951

[15] MILUTINOVIĆ, M., STANKOVIĆ, M., CVETKOVIĆ, D., MAKSIMOVIĆ, V., ŠMIT, B., PAVLOVIĆ, R., MARKOVIĆ S. (2015): The molecular mechanisms of apoptosis induced by *Allium flavum* L. and synergistic effects with new-synthesized Pd(II) complex on colon cancer cells. *Journal of Food Biochemitry* **39**(3): 238–250. doi: 10.1111/jfbc.12123

[16] MILUTINOVIĆ, M., ČUROVIĆ, D., NIKODIJEVIĆ, D., VUKAJLOVIĆ, F., PREDOJEVIĆ, D., MARKOVIĆ, S., PEŠIĆ, S. (2020): The silk of *Plodia interpunctella* as a potential biomaterial and its cytotoxic effect on cancer cells. *Animal Biotechnology* **31**(3): 195–202. doi: 10.1080/10495398.2019.1575848

[17] MONDAL, M., TRIVEDY, K., KUMAR, S.N. (2007): The silk proteins, sericin and fibroin in silkworm, *Bombyx mori* Linn., a review. *Caspian Journal of Environmental Sciences* **5**(2): 63–76.

[18] MOSMANN, T. (1983): Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *Journal of Immunological Methods* **65**(1-2): 55–63. doi: 10.1016/0022-1759(83)90303-4

[19] NIKODIJEVIĆ, D., JOVANKIĆ, J., CVETKOVIĆ, D., ANĐELKOVIĆ, M., NIKEZIĆ, A., MILUTINOVIĆ, M. (2021): L-amino acid oxidase from snake venom: Biotransformation and induction of apoptosis in human colon cancer cells. *European Journal of Pharmacology* **910**(2):174466. doi: 10.1016/j.ejphar.2021.174466

[20] NIKODIJEVIĆ, D.D., MILUTINOVIĆ, M.G., CVETKOVIĆ, D.M., ĆUPURDIJA, M., JOVANOVIĆ, M., MRKIĆ, I., JANKULOVIĆ-GAVROVIĆ, M., MARKOVIĆ, S. (2019): Impact of bee venom and melittin on apoptosis and biotransformation in colorectal carcinoma cell lines. *Toxin Reviews* **40**(4): 1272–1279. doi: 10.1080/15569543.2019.1680564

[21] PAGANI, M.D. (2010): The complex clinical picture of presumably allergic side effects to cytostatic drugs: symptoms, pathomechanism, reexposure, and desensitization. *Medical Clinics of North America* **94**(4): 835–852. doi: 10.1016/j.mcna.2010.03.002

[22] PIASEK, A., BARTOSZEK, A., NAMIEŚNIK, J. (2009): Phytochemicals that counteract the cardiotoxic side effects of cancer chemotherapy. *Postepy Higieny i Medycyny Doswiadczalnej (Online)* **63**: 142–158.

[23] RANGI, A., JAJPURA, L. (2015): The Biopolymer Sericin: Extraction and Applications. *Journal of Textile Science & Engineering* **5**: 1. doi: 10.4172/2165-8064.1000188

[24] RIVERA-VEGA, L.J., ACEVEDO, F.E., FELTON, G.W. (2017): Genomics of Lepidoptera saliva reveals function in herbivory. *Current Opinion in Insect Science* **19**: 61–69. doi: 10.1016/j.cois.2017.01.002

[25] ROY, A., BHARADVAJA, N. (2021): Venom-derived bioactive compounds as potential anticancer agents: A review. *International Journal of Peptide Research and Therapeutics* **27**: 129–147. doi: 10.1007/s10989-020-10073-z

[26] SCHMITTGEN, T.D., LIVAK, K.J. (2008): Analyzing real-time PCR data by the comparative C(T) method. *Nature Protocols* **3**: 1101–1108. doi: 10.1038/nprot.2008.73

[27] SILHACEK, D.L., MILLER, G.L. (1972): Growth and development of the Indian meal moth *Plodia interpunctella* (Lepidoptera: Phycitidae) under laboratory massrearing conditions. *Annals of Entomological Society of America* **65**(5): 1084–1087.

[28] SMITH, T., AFRAM, K., NOTTINGHAM, E. L., HAN, B., AMISSAH, F., KRISHNAN, S., TREVINO, H., AGYARE, E. (2020): Application of smart solid lipid nanoparticles to enhance the efcacy of 5-fuorouracil in the treatment of colorectal cancer. *Scientific Reports* **10**: 16989. doi: 10.1038/s41598-020-73218-6

[29] STEFANOVIĆ, O., VUKAJLOVIĆ, F., MLADENOVIĆ, T., PREDOJEVIĆ, D., ČOMIĆ, LJ., PEŠIĆ, S. (2020): Antimicrobial activity of Indian meal moth silk, *Plodia interpunctella*. *Current Science* **118**: 10. doi: 10.18520/cs/v118/i10/1609-1614

[30] SUNG, H., FERLAY, J., SIEGEL, R. L., LAVERSANNE, M., SOERJOMATARAM, I., JEMAL, A., BRAY, F. (2020): Global cancer statistics 2020: Globocane estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer Journal of Clinicians* **71**(3): 209–249. doi: 10.3322/caac.21660

[31] SURYAWANSHI, R., KANOUJIA, J., PARASHAR, P., SAFAR, A. (2020): Sericin: a versatile protein biopolymer with therapeutic significance. *Current Pharmaceuticals Design* **26**(42): 5414–5429. doi: 10.2174/1381612826666200612165253

[32] TAHIR, H. M., JABEEN, P., RAZA, C., ALI, S. (2019): Silk of Spider and *Bombyx mori*: An Overview. *Punjab University Journal of Zoology* **34**(2): 221–229.

[33] TERADA, S., SASAKI, M., YANAGIHARA, K., YAMADA, H. (2005): Preparation of silk protein sericin as mitogenic factor for better mammalian cell culture. *Journal of Bioscience Bioengineering* **100**(6): 667–671. doi: 10.1263/jbb.100.667

[34] TSUBOUCHI, K., IGARASHI, Y., TAKASU, Y., YAMADA, H. (2005): Sericin enhances attachment of cultured human skin fibroblasts. *Bioscience Biotechnology and Biochemistry* **69**(2): 403–405. doi: 10.1271/bbb.69.403

[35] VOLLRATH, F., SELDEN, P. (2007): The role of behavior in the evolution of spiders, silks, and webs. *Annual Review of Ecology, Evolution, and Systematics* **38**: 819–846. doi: 10.1146/ANNUREV.ECOLSYS.37.091305.110221

[36] WEI, L., HUANG, C., YANG, H., LI, M., YANG, J., QIAO, X., MU, L., XIONG, F., WU, J., XU, W. (2015): A potent anti-inflammatory peptide from the salivary glands of horsefly. *Parasites & Vectors* **8**: 556. doi: 10.1186/s13071-015-1149-y

[37] ZHENG, J., LEE, H. L., HAM, Y. W., SONG, H. S., SONG, M. J., HONG, J. T. (2015): Anti-cancer effect of bee venom on colon cancer cell growth by activation of death receptors and inhibition of nuclear factor kappa B. *Oncotarget* **6**(42): 44437–51. doi: 10.18632/oncotarget.6295