Biomedical Current Insights
DOI: https://doi.org/10.63626/zrb7jf61
ISSN Online: 3104-414X
ISSN Print: XXXX-XXXX

Research Article

Cytotoxic effects and expressional modifications by sericin in liver cancer cells

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Abstract
Background: Liver cancer is the 3rd leading cause of cancer related deaths worldwide with increased risk in males than females. Many natural derivatives are being used as anticancer agents due to their antioxidant and cytotoxic potential. Silk sericin, extracted by the degumming of silkworm cocoon, has emerged as a novel potential anticancer compound. Due to increased elasticity, stability and ease of access made it an important protein for research in cancer studies. In this study, cytotoxic effects of sericin and its impact on expressional changes of selected genes in liver cancer cells were investigated.
Methods: Cultured HepG2 cells were treated with extracted sericin by the degumming of local cocoons and commercially available purified sericin (0.03-1 mg/ml) for 24-72 hours. MTT dye reduction assay was used to determine the inhibitory effects of sericin on cultured HepG2 cells. Expressional analysis of GADD45A, GADD45B and CDKN1A was done by real-time PCR. Fold changes were determined by Livak method while comparing the results with untreated controls.
Results: The cytotoxic effect of sericin (pure and extracted) was concentration dependent as seen by increasing concentration, the suppression of proliferation became more pronounced. The effects were more intense after the mid-interval exposure period (48 hour). GADD45A was inhibited moderately whereas GADD45B and CDKN1A were induced with increasing concentration of sericin.
Conclusion: Proliferation of HepG2 cells was inhibited by sericin exposure. Sericin also showed potential to regulate the expression of genes involved in DNA repair, cell cycle progression, DNA damage and apoptosis.
Key Words: Silkworm, Sericin, Liver cancer, Anticancer, Therapy

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INTRODUCTION

One of the most common malignancies ranking sixth in the world is liver cancer [1] [2]. It is an aggressive tumor that originates in the liver. Primary liver cancer is referred as hepatocellular carcinoma (HCC) and is the 3rd leading cause of cancer-related deaths worldwide and ranks fifth and seventh among males and females respectively [3]. Increased alcohol consumption and obesity are surpassing chronic viral hepatitis B and C as leading cause of liver cancer throughout the world [4]. Further exogenous risk factors include cigarette smoking, ingestion of aflatoxin-contaminated food, and liver fluke infestation [5]. Preferable treatment options for liver cancer include hepatic resection and liver transplantation due to its poor prognosis and diagnosis at late stages. Additional treatment options include ablation therapy, radiation therapy and multi-kinase inhibitors such as sorafenib and regorafenib [6].

An economically significant worm called silkworm (*Bombyx mori)*, feeds on mulberry leaves while in its larval stage and spins a cocoon of silk around. The cocoon of this worm is composed of spherical proteins called fibroin and sericin [7] [8]. Sericin (20–30%) which is globular in nature is water-soluble glue-like protein, encases fibroin fibers and fibroin (70–80%), the structural part of the silk fibers [9]. Sericin has three different fractions known as A, B and C; that may be identified on structural and molecular weight of amino acids and also by the hydrophilic nature of sericin [10]. It contains eighteen amino acids, eight of which are involved in different metabolic pathways of the human body including serine (ample amount), glycine (16%) and aspartic acid (40%). Mechanical stability and elasticity of sericin is increased by the presence of carboxyl-, hydroxyl- and amino group [11]. Biological (such as wound healing, antioxidant, anti-aging, bio-membranes), biomedical (tissue engineering), therapeutic (anti-bacterial, anti-diabetic,

anti-hypertensive, anti-tumor) and pharmacological (drug delivery) utilization of sericin protein are due to the co-polymerization and cross-linking properties of amino acids that make up this protein [12].

Potential mechanism of action of sericin includes but is not limited to its interaction with ROS. It protects from hydrogen peroxide (H_2O_2) along with ultraviolet radiation which induces oxidative stress damage thus preventing cancer development in different body organs. Sericin also contains flavonoids and carotenoids that have antioxidant and anti-tyrosinase [13] [14]. Due to its cytotoxic effects and nanocarrier drug formulations, sericin has proven its impact in anti-cancer research [15]. Silkworm powder decreases signal transducer and activator of transcription 3 (STAT3) phosphorylation, suppressing IL-6 mRNA levels in liver cancer and attenuated the expression of hepatic carcinogen-related target genes, such as hypoxia inducible factor-1 alpha (HIF-1), c-Myc, p53, and organic cation transporter-1 thereby reducing carcinogenesis through STAT3 signaling pathway in DEN-induced chronic liver cancer rat model [16] [17].

The Growth Arrest and DNA Damage-inducible 45 (GADD45) family of proteins consisting of three members, GADD45A, GADD45B and GADD45G are critical stress sensors that are activated by DNA damage and stress signals linked to various cellular responses, including DNA repair, cell cycle arrest, cell growth regulation and apoptosis [18]. They modulate tumor formation in response to oncogenic stress, terminal differentiation and apoptotic cytokines playing a critical role in carcinogenic process [19]. Cyclin dependent kinase inhibitor 1A (CDKN1A) is responsible for different molecular and biological functions including cell cycle progression, regulation of G1/S, G2/S checkpoints, cell migration/invasion, apoptosis, DNA repair and stem cell reprogramming [20]. But the role of CDKN1A is multifaceted and unclear because its activity depends on the status of P53 gene.CDKN1A overexpression in case of P53 loss of function mutation or by stabilizing cyclin D1-CDK4 complexes leading to more aggressive behavior of cancer cells that are capable of escaping cell block, senescence and apoptosis [21] [22]. Due to this reason, many tumors including HCC show CDKN1A expression changes that correlates with high malignancy, poor prognosis, and drug resistance [23].

This study aimed to determine the cytotoxic effects of sericin in liver cancer cells (HepG2) and to investigate the role of sericin on gene's expression involved in cell cycle progression, DNA damage and apoptosis.

METHODS

Cell Culture and Sericin

Available liver cancer cell line (HepG2), originally obtained from American type culture collection (ATCC), was supplemented with FBS (10%), L-glutamine (2mM), streptomycin (100ug/ml) and penicillin (100 IU/ml) and maintained at standard humidified conditions (5% CO_2, 37°C) for growth purposes. Sericin extraction was done by degumming of the cocoons of silkworms, washing them with deionized water and autoclaving at 121°C for 45-60 min followed by filtering the solution to remove fibroin. This filtrate was lyophilized to obtain sericin powder (S-Extract) and stored 4°C until further use. Purified sericin (S-Pure) was obtained from Sigma company (S5201-5G) to compare the effects of purified sericin in parallel.

Cytotoxicity Assay

The cells were cultured in 96-well plates (3000cells/well/100µl media) for overnight. On the next day, the cells were exposed to different concentrations (0.03-1.0mg/dl) of extracted and pure sericin for different time intervals (24, 48, 72 hours). Afterwards, MTT solution (10mg/ml in PBS) was added in each well and was converted to formazan crystals by viable cells to determine the cytotoxic effects of sericin. These crystals were dissolved by adding DMSO (50µl/well) and intensity of color reflected the number of viable cells present in solution. Untreated cells were grown in parallel and act as control in all experiments.

Treatment for Expression Analysis

Hep2G cell line was cultured in RPMI-1640 in 6-well plate (150,000 cells/well/2ml medium) and exposed to low and high concentrations (0.25-1mg/ml) of S-Extract and S-Pure for 48 hours.

RNA Extraction and Quantification

RNA extraction was done using Thermofisher Scientific (K0731) kit by adding 350µl RLT buffer for cell lysis. After vortex for 10-15 sec, 350µl of 70% ethanol was added and mixed by pipetting for few seconds. The lysate was transferred to the spin column after centrifugation at 10,000rpm for 30 sec. 700µl of RW1 was added and centrifuged again. After that, 500µl of RPE buffer was added and centrifuged again for 30 sec. This step was repeated with centrifugation for 2 minutes. The column was placed in a 1.5ml tube, 50µl elution buffer was added and centrifuged after 2 minutes. RNA quantification was done by using Nanodrop ND 2000. Extracted RNA was stored at -80°C until further use.

cDNA Synthesis
cDNA was synthesized by reverse transcribing 500ng RNA/sample using Thermofisher Scientific kit (K1622). Total reaction mix of 20µl was prepared by adding 4µl reaction buffer, 1µl Ribo, 2µl dNTP mix, 1µl OligodT, 1µl RevertAid, 500ng RNA and nuclease free water up to 12µl to make up the total volume. The cycling conditions were set at 42°C for 60min and terminate at 70°C for 5minutes. Synthesized cDNA was stored at -20°C for further use.

Primer Designing and Optimization
Primers for selected genes (GADD45A, GADD45B, CDKN1A and GAPDH) were designed by choosing gene sequences from NCBI GenBank and using Primer3 software (Table 1). Gradient PCR with different temperatures was used for primer optimization. The temperature settings for gradient PCR were as follows: starting with initial denaturation at 95°C for 5 minutes followed by 35 cycles of 95°C for 20 sec, 56°C, 59°C, 62°C for 30 sec, 72°C for 40 sec and final extension at 72°C for 7 min. The product was loaded in 2% Agarose gel for gel electrophoresis at 100volts for 45 min and visualized with the help of Gel Documentation.

Real-Time PCR
qRT-PCR was done in triplicate to analyze the expression of selected genes (GADD45A, GADD45B, CDKN1A) in liver cancer cell line HepG2. For this purpose, prepared cDNA samples along with SybrGreen and gene specific optimized primers were used along with QuantStudio3 Thermal cycler. GAPDH was used as reference gene for data normalization. Livak method was used to calculate the fold change.

Table 1: Primer sequences of genes

Genes	Primer Sequence		Product Size
GADD45A	F	CGCTAGGCTTAATCGCGTT	135
	R	TTCGAAGCTGGTTCGAATGC	
GADD45B	F	TGAATGTGGACCCAGACAGC	245
	R	GTCCGTGTGAGGGTTCGT	
CDKNI1A	F	GCTTCATGCCAGCTACTTCC	260
	R	CTGTGCTCACTTCAGGGTCA	
GAPDH	F	ACGGATTTGGTCGTATTGGG	214
	R	CGCTCCTGGAAGATGGTGAT	

RESULTS

Cytotoxic Effects on HepG2 Cells
Visual findings demonstrated a decrease in cellular proliferation in response to sericin exposure. The number of viable cells in a well were directly correlated with the development of purple color intensity after the solvent addition. Inhibitory effects were also calculated numerically as percentages of untreated controls. Cytotoxic effects were almost concentration dependent since the suppression of cellular proliferation became more pronounced as concentration was raised (Figure 1). The effects were more intense after the mid-interval exposure period (48 hour). Furthermore, the effects of extracted and purified sericin were also compared. Overall, sericin substantially decreased cell growth and exhibited lethal effects against liver cancer cells (HepG2).

RNA Quantification and cDNA Verification
RNA quantification of treated and untreated HepG2 cells was done spectrophotometrically using Nanodrop ND 2000 and purity was calculated at 260/280 ratio. A good quality and quantity of RNA was achieved as shown in Table 2. Extracted RNA was reverse transcribed to cDNA, verified using GAPDH primer on a conventional PCR and visualized by the help of gel electrophoresis. Primers for selected genes were optimized using gradient PCR at different temperatures (56°C, 59°C and 62°C). Prominent bands for all three genes were seen at all temperatures after agarose gel electrophoresis.

Expressional changes in GADD45A, GADD45B and CDKN1A
Real-Time PCR was performed by using SybrGreen master mix for selected genes by using synthesized cDNA from cultured liver cell line (HepG2) treated with sericin at 0.25, 0.5 and 1.0mg/ml concentrations. Sericin induced the expression of GADD family of genes in liver cancer cells. GADD45A gene was inhibited to moderate levels in HepG2 cells whereas GADD45B was expressed to a maximum of 1.7fold by sericin induction. There was a minimum difference seen in expression change of GADD family at different concentrations between S-Extract and S-Pure. CDKN1A was fractionally inhibited at lower concentration (0.25mg/ml) in case of S-Pure whereas

moderately induced at 0.25mg/ml concentration of extracted sericin. Higher concentrations (0.5 and 1.0mg/ml) of both extracted and pure sericin shows similar fold change with minimum differences.

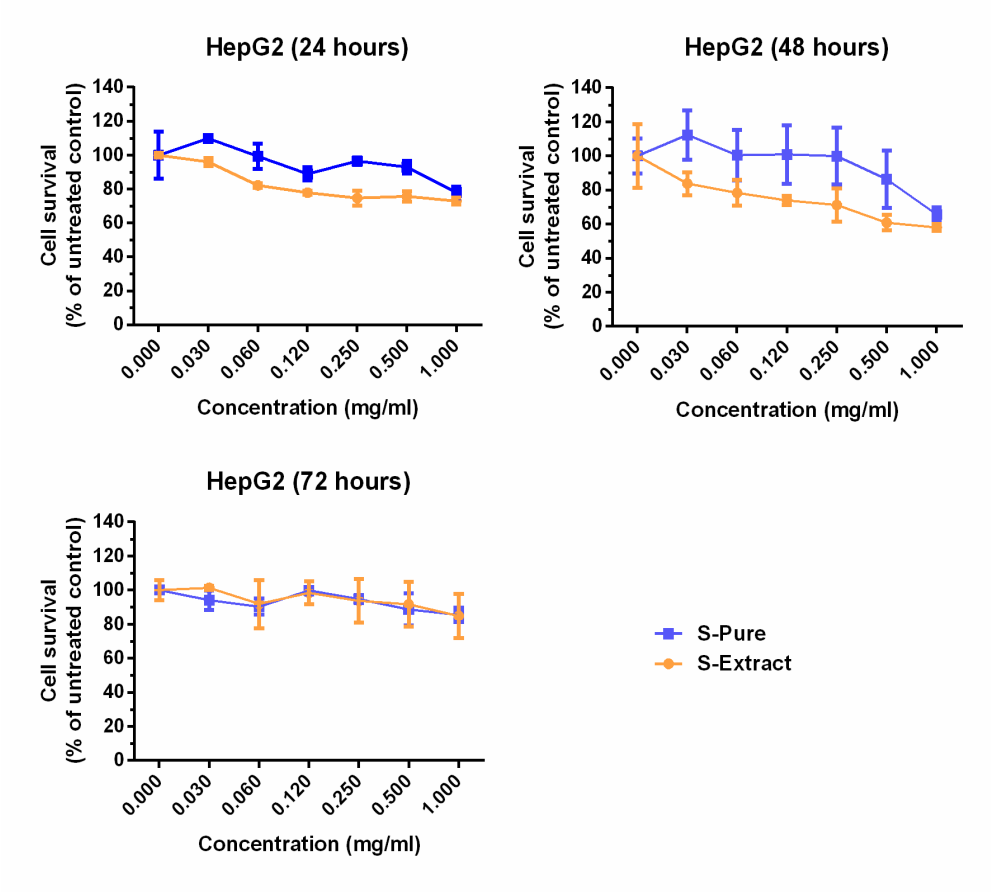

Figure 1: MTT result of HepG2 cell line after 24-, 48- and 72- hours treatment with S-Pure or S-Extract.

Table 2: RNA concentration of Sericin Treated and Untreated HEPG2 cell line

Cell line	Treatment (mg/ml)	RNA Concentration	260/280 ratio
HEPG2	Untreated Control	689.4 ng/µl	2.10
	Sericin S-Pure (0.25)	541.2 ng/µl	2.09
	Sericin S-Pure (0.5)	448.5 ng/µl	2.10
	Sericin S-Pure (1.0)	607 ng/µl	2.09
	Sericin S-Extract (0.25)	412.8 ng/µl	2.09
	Sericin S-Extract (0.5)	396 ng/µl	2.09
	Sericin S-Extract (1.0)	461.4 ng/µl	2.09

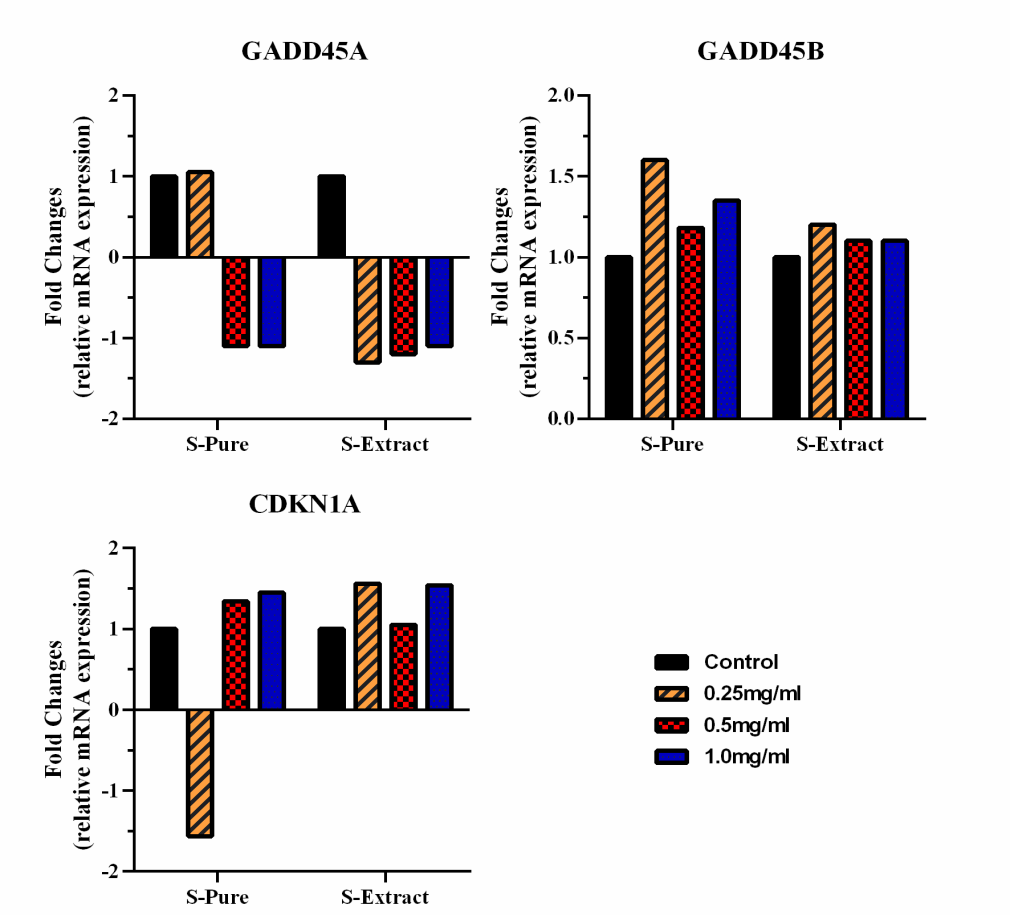

Figure 2: Expressional modulation of GADD45A, GADD45B and CDKN1A in HepG2 cell line identified by Real-Time PCR. Livak method was used to identify the fold differences.

DISCUSSION

Cancer is the most serious and complex disease that endangers the health, prosperity, and lives of people and it results from several variances in certain genetic patterns [11]. One of the most prominent causes of cancer mortality globally is liver cancer, which has a relatively high frequency across all malignancies. It has always been very difficult to treat cancer [24]. Despite recent major advancements, conventional therapeutic methods including surgery, chemotherapy, and radiation have been in use. An emerging treatment option includes the use of natural derivatives as anticancer agents to improve the cancer prognosis and quality of life of cancer patient [15]. Due to its ease of cultivation and high extraction yield, silk produced from *B. mori* (silkworms) cocoons is the most common form of silk sericin, representing ~20–30 wt.% of the total weight [25].

Sericin can also function as a pro-oxidant B because it contain polyhydroxy amino acids like serine as well as polyphenol and flavonoids as secondary metabolites that have various biomedical properties including anticancer activity [26]. Silk sericin has shown anti-cancer effects on the cancer cell lines. Silk sericin suppresses the proliferation related PI3K/Akt pathway, arresting the cell cycle in G_0/G_1 phase and promotes apoptosis [27]. In previous studies, human cancer cells (A431, SAS, and MCF-7) were examined for anticancer activity of sericin in terms of cytotoxicity, ROS levels, cell cycle arrest and mitochondrial membrane potential [14].

There was some therapeutic evidence for treating cancer with sericin. Various studies found the apoptotic effects of sericin on human cancer cells, such as treating human colon cancer cells (SW480) with sericin led to induction of apoptosis, decreased cell viability, caspase-3 activity, and decreased Bcl-2 expression [28]. These findings indicate that Sericin holds the potential to instigate apoptosis in diverse cancer cell types, suggesting its prospective therapeutic applications in cancer treatment.

In a study from 2024, cytotoxic effects of sericin were assessed using different concentrations of sericin (0, 0.125, 0.25, 0.5, and 1mg/ml) and showed no considerable results. Furthermore, RNA-Seq Analysis of many differentially expressed genes were done with low (0.125mg/ml) and high (1.0mg/ml)

concentrations of sericin in HepG2 cells and fold change was observed in both groups [12].

In this study, we took human liver cancer cell line (HepG2) and studied the cytotoxic effects of sericin in these cells via MTT dye reduction assay. The HepG2 cell line was cultured in 96 well plate under suitable conditions and were exposed to different concentrations of the natural compound sericin with various concentrations (0.25-1.0mg/ml for 24, 48 and 72 hours). For comparison purposes, the cancer cell line was also exposed to the commercially available purified sericin obtained from Sigma company. It was clear from the obtained result that sericin showed an effective anti-proliferating effect in these cells as compared to the no cytotoxic results obtained by Jantaravinid et al., The comparable results from commercially available sericin and our local extracts also confirmed the reliable extraction process adopted in this study. Overall, the cytotoxic effects were mild and concentration dependent on HepG2 cells. In a study conducted by Ratanabunyong et al., in 2024, they observed the effects of sericin treatment on colorectal cancer cell line (HCT116). There was minimal impact on apoptosis when concentration of sericin equal to IC50 was utilized. However, when the dosage was increased to 100 µg/mL, the detection of apoptosis increased by over 80%. This indicates that sericin exhibited a dose-dependent effect on inducing apoptosis in HCT116 colon cancer cells [10].

Identifying the molecular basis of any functional outcome is an interesting and much needed area of investigation. To figure out the potential reason behind the anti-proliferative effects of sericin, expression analysis of the important cell cycle regulator gene (CDKN1A) and cell stress and death related genes (GADD45 family) were selected in this study. CDKN1A gene was induced in HepG2 cells in response to the sericin exposure, which may have contributed to slowing down the cell cycle machinery and cellular proliferation rate. Only the lowest concentration of S-Pure inhibited the CDKN1A expression. As far as GADD family of genes was concerned, GADD45B was induced in HepG2 cancer cell line in response to treatment with either S-Pure or S-Extract. GADD45A was inhibited with high concentrations of pure sericin and extracted sericin and only induced with lower concentration of pure sericin in HEPG2 cells. All in all, the expression of cell cycle (CDKN) and stress related genes (GADD45) showed alterations in expression levels in response to sericin.

To conclude, liver cancer cell line (HepG2) is responsive to sericin exposure as witnessed by inhibition of proliferation and cytotoxic effects after exposure to this protein. At molecular levels, sericin can interfere with the expression levels of important genes including CDKN1A, GADD45A and GADD45B. Furthermore, there were a few limitations including that we have only examined the cytotoxic effects and changes in genetic expressions caused by exposure to sericin in liver cancer cell line (HepG2). A bigger pool of representative cancer cell lines should be used to verify the obtained results. Additionally, only transcriptome levels of expression modulations were examined; proteome levels of validation are also required. Further *in vitro* and *in vivo* studies are needed to confirm the anticancer potential of sericin against liver cancer cell lines.

REFERENCES

1. Zeng L-P, Qin Y-Q, Lu X-M, Feng Z-B, Fang X-L. Identify GADD45G as a potential target of 4-methoxydalbergione in treatment of liver cancer: bioinformatics analysis and in vivo experiment. World Journal of Surgical Oncology. 2023;21(1):324.
2. Mani RJ, Anand M, Agarwal K, Tiwari A, Amanur Rahman Hashmi Q, Vikram Singh T, et al. A Systematic Review of Molecular Pathway Analysis of Drugs for Potential Use in Liver Cancer Treatment. Drugs and Drug Candidates. 2023;2(2):210-31.
3. Anwanwan D, Singh SK, Singh S, Saikam V, Singh R. Challenges in liver cancer and possible treatment approaches. Biochimica et Biophysica Acta (BBA)-Reviews on Cancer. 2020;1873(1):188314.
4. Barsouk A, Thandra KC, Saginala K, Rawla P, Barsouk A. Chemical risk factors of primary liver cancer: an update. Hepatic Medicine: Evidence and Research. 2021:179-88.
5. Rumgay H, Arnold M, Ferlay J, Lesi O, Cabasag CJ, Vignat J, et al. Global burden of primary liver cancer in 2020 and predictions to 2040. Journal of hepatology. 2022;77(6):1598-606.
6. Huang Y, Wang S, Ke A, Guo K. Ferroptosis and its interaction with tumor immune microenvironment in liver cancer. Biochimica et Biophysica Acta (BBA)-Reviews on Cancer. 2023;1878(1):188848.
7. Elahi M, Ali S, Tahir HM, Mushtaq R, Bhatti MF. Sericin and fibroin nanoparticles—natural product for cancer therapy: a comprehensive review. International Journal of Polymeric Materials and Polymeric Biomaterials. 2021;70(4):256-69.
8. Jo Y-Y, Kweon H, Oh J-H. Sericin for tissue engineering. Applied Sciences. 2020;10(23):8457.
9. Mumtaz S, Ali S, Pervaiz A, Qureshi MZ, Kanwal K, Saleem T. Apoptotic and antiproliferative effects of silk protein sericin conjugated-AgNO3 nanoparticles in human breast cancer cells. Saudi Journal of Biological Sciences. 2023;30(2):103551.
10. Ratanabunyong S, Siriwaseree J, Wanaragthai P, Krobthong S, Yingchutrakul Y, Kuaprasert B, et al. Exploring the apoptotic effects of sericin on HCT116 cells through comprehensive nanostring

transcriptomics and proteomics analysis. Scientific Reports. 2024;14(1):2366.
11. Dan AK, Aamna B, De S, Pereira-Silva M, Sahu R, Paiva-Santos AC, et al. Sericin nanoparticles: Future nanocarrier for target-specific delivery of chemotherapeutic drugs. Journal of Molecular Liquids. 2022;368:120717.
12. Jantaravinid J, Tirawanchai N, Ampawong S, Kengkoom K, Somkasetrin A, Nakhonsri V, et al. Transcriptomic screening of novel targets of sericin in human hepatocellular carcinoma cells. Scientific Reports. 2024;14(1):5455.
13. Mumtaz S, Ali S, Pervaiz A, Awan UA, Nauroze T, Kanwal L, et al. Toxicological effects of dimethlybenzeneanthracene in Balb C mice and pharmacological intervention by silk sericin–conjugated silver nanoparticles. Science Progress. 2024;107(1):00368504231221670.
14. Kumar JP, Mandal BB. Silk sericin induced pro-oxidative stress leads to apoptosis in human cancer cells. Food and Chemical Toxicology. 2019;123:275-87.
15. Baci G-M, Baciu E-D, Cucu A-A, Muscă A-S, Giurgiu AI, Moise AR, et al. Sericultural by-products: The potential for alternative therapy in cancer drug design. Molecules. 2023;28(2):850.
16. Lee D-Y, Yun S-M, Song M-Y, Ji S-D, Son J-G, Kim E-H. Administration of steamed and freeze-dried mature silkworm larval powder prevents hepatic fibrosis and hepatocellular carcinogenesis by blocking TGF-β/STAT3 signaling cascades in rats. Cells. 2020;9(3):568.
17. Cho H-D, Min H-J, Won Y-S, Ahn H-Y, Cho Y-S, Seo K-I. Solid state fermentation process with Aspergillus kawachii enhances the cancer-suppressive potential of silkworm larva in hepatocellular carcinoma cells. BMC complementary and alternative medicine. 2019;19:1-15.
18. Patel K, Murray MG, Whelan KA. Roles for GADD45 in development and cancer. Gadd45 stress sensor genes. 2022:23-39.
19. Palomer X, Salvador JM, Griñán-Ferré C, Barroso E, Pallàs M, Vázquez-Carrera M. GADD45A: With or without You. Medicinal Research Reviews. 2024;44(4):1375-403.
20. Shi L, Zhang W, Ma Q, Li W, Zhao H, Zhong L, et al. A multidimensional Pan-cancer analysis of CDKN1A identifies CDKN1A as an Immunological and Prognostic Biomarker. bioRxiv. 2024:2024.09. 03.610958.
21. Manousakis E, Miralles CM, Esquerda MG, Wright RH. CDKN1A/p21 in Breast Cancer: Part of the Problem, or Part of the Solution? International Journal of Molecular Sciences. 2023;24(24):17488.
22. Zamagni A, Pasini A, Pirini F, Ravaioli S, Giordano E, Tesei A, et al. CDKN1A upregulation and cisplatin-pemetrexed resistance in non-small cell lung cancer cells. International Journal of Oncology. 2020;56(6):1574-84.
23. Khan MGM, Boufaied N, Yeganeh M, Kandhi R, Petkiewicz S, Sharma A, et al. SOCS1 deficiency promotes hepatocellular carcinoma via SOCS3-dependent CDKN1A induction and NRF2 activation. Cancers. 2023;15(3):905.
24. Seno A, Afify SM, Hassan G, Seno M. Induced pluripotent stem cells as the source of cancer stem cells providing novel concepts of cancer: gaps between in vitro and in vivo in induced pluripotent stem cells modeling. iPSC Derived Progenitors: Elsevier; 2022. p. 265-88.
25. Reizabal A, Fidalgo-Marijuan A, Gonçalves R, Gutiérrez-Pardo A, Aguesse F, Pérez-Álvarez L, et al. Silk fibroin and sericin polymer blends for sustainable battery separators. Journal of Colloid and Interface Science. 2022;611:366-76.
26. Abdelgalil RM, Khattab SN, Ebrahim S, Elkhodairy KA, Teleb M, Bekhit AA, et al. Engineered sericin-tagged layered double hydroxides for combined delivery of pemetrexed and ZnO quantum dots as biocompatible cancer nanotheranostics. ACS omega. 2023;8(6):5655-71.
27. Kumari N, Pullaguri N, Sahu V, Ealla KKR. Research and therapeutic applications of silk proteins in cancer. Journal of Biomaterials Applications. 2023;38(1):39-50.
28. El-Fakharany EM, Abu-Elreesh GM, Kamoun EA, Zaki S, Abd-EL-Haleem DA. In vitro assessment of the bioactivities of sericin protein extracted from a bacterial silk-like biopolymer. RSC advances. 2020;10(9):5098-107.

Author Contributions: UAM and ZQA performed the experiments, SI supervised the manuscript writing.
Competing Interests: Not Applicable
Data Availability Statement: The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.