ABSTRACT

As of World Health Organization (WHO) 2021, globally 12% of most annually diagnosed cancer cases, breast cancer (BC) became the second most common type among all cancer types. Despite the availability of multi-directional therapeutic strategies, advanced stages of breast cancer are difficult to treat and thus impose major healthcare burdens. This situation reinforces the need to identify new potential therapeutic compounds with better clinical features. The current research was performed to estimate the anti-breast cancer activity of silk protein sericin and its nanoparticles (NPs) against an experimental animal mice model. *Bombyx mori* silk cocoon is a natural source of silk sericin a globular-like protein that is used against various biological properties including anti-oxidant, Ultra-Violet (UV) resistant, antibacterial, and antitumor actions. To better recognize its therapeutic perspective, a detailed analysis of the mechanistic properties of sericin (S) and sericin conjugated-silver nanoparticles (S-AgNO3 NPs) consider important. In this current research, we isolated sericin by degumming process and formation of sericin-silver NPs confirmed by different characterization methods such as Ultra-Violet-Vis spectra, Fourier-transform infrared spectroscopy (FTIR), Energy Dispersive X-Ray analysis (EDX), X-ray diffraction analysis (XRD), and Scanning electron microscopy (SEM) patterns. After the confirmation of nanoparticles, we determined the sericin and its nanoparticles mediated changes in human breast cancer cells. The antiproliferative activity of sericin and sericin NPs was determined by 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl-tetrazolium bromide (MTT) dye reduction assay. Alterations at molecular levels were investigated by qRT-PCR methodology and with the help of nuclear DNA staining protocol, the apoptotic effects of compounds were studied. The anti-cancer effect of selected compounds under the *in vivo* experimental animal mice model was done by using carcinogenic compounds such as 7, 12-Dimethylbenzne anthracene (DMBA)-induced breast cancer in an animal mice model, and treatment of DMBA-induced cancer was done by using selected doses of SI and -AgNO3 NPs I (100 mg/kg, b.w), and SII and S-AgNO3 NPs II (200 mg/kg, b.w). After 60 days of the experiment, mice were dissected and further analysis such as biochemical, hematological, immunoglobulins and histopathological analysis were estimated. Biochemical parameters including urokinase plasminogen, estrogens (ER), progesterone (PR), fibrinogen, lactate dehydrogenase (LDH), alkaline phosphatas

(ALP), gamma-glutamyl transferase (GGT), ferritin, and glutathione (GSH) levels were measured from the blood serum of mice. Similarly, immunoglobulins and hematological parameters such as immunoglobulins A (IgA), immunoglobulins G (IgG), immunoglobulins M (IgM), red blood cells (RBCs), white blood cells (WBCs), neutrophils, eosinophils, platelets, hemoglobin, monocytes, and lymphocytes were calculated respectively. The histopathological analysis of the liver, kidney, brain, spleen, and mammary tissues was also studied. The UV-Vis analysis showed that the S-$AgNO_3$ NPs gave absorption peaks at the wavelength range of 416-426 nm showing the formation of silver nanoparticles. The image of SEM showed that the majority of S-$AgNO_3$ NPs were spherical, but some had hexagonal and triangular formations with a size range of 57 nm, while characteristics peaks produced by EDX recommended the presence of silver accompanied by some other elements such as carbon, calcium, oxygen, potassium, Aluminum, nitrogen and some other elements. The results declared by FTIR analysis revealed that the formation of silver nanoparticles could not change the configuration of sericin molecules. The XRD pattern of diffraction showed that sericin-silver NPs face-centered cubic crystalline structure due to the reduction of Ag^{+} to metallic Ag^{0}. The characteristics of diffraction peaks at scattering angles (2) of about 78.7°, 64.4°, 55.1°, and 38.2° which are attributed to 311, 220, 200 and 111 planes of the silver respectively. The *in vitro* results were declared after 72 h treatment with sericin and S-$AgNO_3$ NPs and presented significant antiproliferative results in MCF-7 (26%) and MDA-MB-231 (41%) cells. Expression modification showed prominent stimulation of anti-cancer cell cycle arrests genes such as cyclin-dependent kinase inhibitors (CDKN1A, CDKN1B), and GADD family genes. RT-PCR results of the GADD family include GADD45A, B, G, 34, and 153 along with cyclin-dependent kinase inhibitors including CDKN1A, 1B showed pronounced significant induction 3.1- to 19.8-folds against MCF-7 cell line while induction against MDA-MB-231 cell line was 2.5- to 34.3-folds. In addition to this, nuclear DAPI staining showed significant induction of apoptosis and nuclear fragmentation of the MDA-MB-231 cell line at a dosage of 1 mg/mL of both sericin and S-$AgNO_3$ NPs. Meanwhile, in the case of MCF-7 cells, after treatment with sericin and S-$AgNO_3$ NPs (1 mg/mL), the cells changed into a round shape and lost their original spindle in dose-dependent manners. The level of biochemical parameters of individual treated, prevention and post-

treatment groups were significantly reduced as compared to DMBA-cancer inducing groups but higher significant effects were shown by S-$AgNO_3$ NPs II (T) at the highest concentrations (200 mg/kg b.w) as followed: urokinase plasminogen (3.34±0.24 ng/mL), ER (75±1.70 pg/mL), PR (6.22±0.20 ng/mL), fibrinogen (2.42±0.26 g/L), and ferritin (366±3.62 ng/mL). The level of GSH, LDH, ALP, and GGT were significantly reduced in all treatment groups as compared to a carcinogenic group but higher significant effects were shown by S-$AgNO_3$ NPs II (T) at the highest concentrations (200 mg/kg b.w) as followed: GSH (2.42±0.26 umol/L), LDH (493.6±5.78 U/L), ALP (158.4±6.35 U/L) and GGT (60±1.70 U/L). The level of immunoglobulins was also significantly reduced in all post-treatment groups but the highest reduction was shown by S-$AgNO_3$ NPs II (T) as followed: IgA (4.22±0.19 g/L), IgG (70±1.70 g/L) and IgM (4.76±0.12 g/L). The hematological outcomes demonstrated that there was a significant rise in the level of RBCs, platelets, lymphocytes, and hemoglobin in all individual, pre-, and post-treatment groups except SII (T) and S-$AgNO_3$ NPs II (T) groups in the case of RBCs and lymphocytes as compared to DMBA-induced BC group. Meanwhile, a reasonable decline was observed in the level of WBCs, neutrophils, eosinophils along with monocytes during all individual, pre- and post-treatment groups except the SII (T) group in the case of eosinophils along with monocytes and SII (P) group only in monocytes as compared to DMBA-induced BC group. The histopathological study of the mammary tissues, liver, kidneys, brain, and spleen declared that the DMBA group showed cytotoxic effects against all selected organs of mice that were recovered by treatment of selective compounds but highly effective recovery was seen in S-$AgNO_3$ NPs II.