ABSTRACT

Aging and age-related disorders have become a prominent worldwide issue for researchers. Oxidative stress caused by the overproduction of reactive oxygen species is the most significant cause of aging. In aging infections are leading causes of mortality and morbidity due to immunosenescence, and the multidrug resistance phenomenon has become the main cause for concern and there has been an inadequate achievement in the development of novel antibiotics to treat bacterial infections. Various mediators with anti-inflammatory and anti-oxidant properties might be probable competitors for the improvement of innovative anti-aging treatments. The aim of current study was to elucidate the antibacterial and antiaging effect of silk proteins and vitamin C against D-Galactose-induced aging in mice. The antibacterial activity of synthetic antimicrobial agents has been found remarkable, but most of them have several side effects and are effective against only a few microbes. Therefore, we developed a novel, eco-friendly, and cost-effective silk proteins conjugated nanoparticles to evaluate the antibacterial activity against human bacterial pathogens such as sericin-silver nanoparticles (S-AgNPs) and silk fibroin chitosan blend zinc oxide nanoparticles (SF-CH-ZnONPs) by using sericin and fibroin as a stabilizer, dispersant, and reductant of silver ions and zinc ions instead of noxious compounds. The antibacterial activities of S-AgNPs were elucidated against *Clostridium difficile, proteus mirabilis*, and *Bacillus licheniformis* at different concentrations (1-6 mg/ml) through the agar well diffusion method. These sericin-coated silver nanoparticles (S-AgNPs) significantly subdued the growth of *C. difficile* (18.7±0.9 mm), *P. mirabilis* (12.3±0.3 mm), and *B. licheniformis* (10.7±0.9 mm). The antibacterial activity of silk fibroin-chitosan blend ZnONPs at numerous concentrations 2-8 mg/ml, various pH values (4, 8,12) and temperatures (4°C, 37°C, and 50°C) against Gram-negative: *Klebsiella Pneumoniae*, *E. coli*, *Serratia rubidiaea*, and *Proteus mirabilis*) and Gram-positive bacterial strains: (*Staphylococcus aureus*, *Bacillus thuringensis*, *Bacillus licheniformis*, and *Clostridium difficile*) were also assessed. Silk fibroin-chitosan blend ZnONPs inhibited the growth of Gram-negative bacterial strains and Gram-positive bacterial strains at all concentrations but the highest zones of inhibition were found at 8 mg/ml and the lowest zones of inhibition were found at 2 mg/ml. Zones of inhibition were bacterial strain as well as concentration-dependent. Silk fibroin-chitosan blend ZnONPs showed the highest zone of inhibition against *S. rubidiaea* (16.7±0.9 mm), and *S. aureus* (17.3±1.2 mm). Similarly, growth

inhibition of all bacterial strains by vitamin C was concentration-dependent. Vitamin C highest significantly inhibited the growth of Gram-positive bacteria *Bacillus licheniformis* (25.3±0.9 mm) in comparison to other bacterial strains. Synthesis of nanoparticles was confirmed by using various characterization techniques such as UV visible spectrophotometer, analysis of particle size and morphology through scanning electron microscopy (SEM), Transmission electron microscopy (TEM), and Fourier transform infrared spectroscopy (FTIR). The absorption peaks of S-AgNPs were recorded through (UV–Vis spectrometer) at the range of 416-426 nm. and the shape of nanoparticles was spherical.

In vitro antioxidant activity of silk proteins was measured by the 2,2-Diphenyl-1-picrylhydrazyl radical scavenging activity (DPPH), total flavonoid content (TFC), Ferric ion reducing antioxidant power assay (FRAP), total phenolic content (TPC), and total flavonoid content (TFC) in silk proteins (sericin, fibroin) at various concentrations (0.5mg/ml, 1mg/ml and 2 mg/ml). The ability of silk sericin and fibroin to reduce Fe^{3+} to Fe^{2+} was investigated by the use of the FRAP assay. Maximum conversion of Fe^{3+} to Fe^{2+} was shown by silk sericin (0.69±0.25 mg FeSO4/g DW) and silk fibroin (0.70± 0.10 mg FeSO4/g DW) at the highest concentration (2mg/ml), which is an indication of greater reducing power. The free radical foraging activity of the silk sericin, fibroin, and ascorbic acid is specified by DPPH % inhibition. Silk sericin showed the highest inhibition activity (73.33±4.41%) at 2mg/ml. Free radical scavenging of silk fibroin also raised 59.00±3.06% to 71.33±5.24% as the concentration elevated from 0.5 to 2 mg/ml.

D-galactose (250 mg/kg body weight) was dissolved in 0.9 percent normal saline and injected 0.3 ml intraperitoneally for 60 consecutive days to produce a natural aging mice model. The antiaging effect of sericin (Ser), fibroin (Fib), sericin nanoparticles (SNPs), fibroin nanoparticles (FNPs), and Vitamin C were evaluated through various biochemical parameters and measurement of telomere length. Our outcomes showed that oral administration (200 mg/kg body weight) of sericin, fibroin, SNPs, and FNPs and Vitamin C (150 mg/kg body weight) for 60 days gradually recovered the organ indices, a bodyweight of mice and improved the morphological/histological changes of the brain, kidney, and liver in D-Gal induced aging mice. Intraperitoneal injection of D-Gal (250 mg/kg bodyweight) for 60 days caused the highest significant reduction in the level of antioxidant enzymes such as superoxide dismutase (SOD), glutathione

peroxidase (GSH-Px), catalase (CAT), and glutathione reductase (GSH-Rx). Whereas, oral administration of 200 mg/kg bodyweight of sericin, fibroin, SNPs, FNPs, and 150 mg/kg bodyweight of Vitamin C improved/increased the level of antioxidant enzymes. The highest significant upsurge (21.7±1.4 µg/l) has been found in cortisol level in D-Gal treated mice in comparison to control or young mice (6.6±1.4 µg/l). After treatment with sericin fibroin, SNPs, FNPs, and Vitamin C highest significant reduction in the level of cortisol was seen. Similarly, the level of Tri-iodothyronine (T3) reduced in the D-Gal treated group but the level of T3 sustained in normal range in prevention/pre-treatment groups of sericin, fibroin SNPs, FNPs, metformin, and Vitamin C treated group. Lipid profiles such as low-density lipoprotein (LDL), high-density lipoprotein (HDL), and total cholesterol were measured. Upsurge in LDL and total cholesterol and decline in HDL was found in D-Gal treated mice when compared with control or young mice. Whereas when mice were orally treated with sericin fibroin, SNPs, FNPs, and Vitamin C the values were found within the range of normal control group in prevention groups in comparison to post-treatment groups. When the level of follicle-stimulating hormone (FSH) was measured highest significant upsurge has been found in D-Gal treated group in comparison to control mice. However, sericin, fibroin SNPs, FNPs, metformin, and Vitamin C restored the level of (FSH) in treatment groups. Real-time/qPCR data showed that the level of telomere length related protein/ gene TERT significantly downregulated (10.43±0.1) in the D-Gal-treated group (250 mg/kg b. w.) as compared to the control group (21.97±0.5). The highest significant upregulation or fold change was found in TERT gene in sericin, fibroin, SNPs, FNPs, and Vitamin C-treated groups. These results demonstrate that silk proteins have an obvious anti-aging activity in D-Gal-induced aging mice, enhancing the antioxidant defenses, reducing oxidative stress, and improving the immune function of aging model mice.