ABSTRACT

Colon cancer is reported as third most prevalent malignancy worldwide, while sericin being an antioxidant, is now used in biomedical applications due to its biochemical characteristics and has shown potential efficacy to treat colon cancer. This study was designed to investigate the antiproliferative and apoptotic potential of sericin and sericin chitosan conjugated silver nanoparticles against CRC cells and was intended to investigate the anticancer potential of Sericin chitosan conjugated silver nanoparticles against colon cancer, induced by 1,2 dimethylhydrazine (DMH) in mice through biochemical markers and histopathological analysis. Sericin was isolated by the degumming process followed by the characterization by using FTIR, UV, XRD, and SEM techniques to confirm the isolation process and successful synthesis of SChiAgNPs. To investigate the antiproliferative and apoptotic activity of sericin and sericin chitosan conjugated silver nanoparticles (SChiAgNPs), three human CRC cell lines (SW480, SW620, HCT116) were used. MTT assay was carried out to analyze the antiproliferative activities, while expression profiling of the genes *i.e.,* GADD45A, BCL2 and TNF was assessed by qRT-PCR analysis. In mice model, sericin silver nanoparticles (SAgNPs) and sericin chitosan silver nanoparticles (SChiAgNPs) were synthesized and characterized by using FTIR, UV, XRD, and SEM techniques to confirm the isolation process and successful synthesis of SAgNPs and SChiAgNPs. The BALB/ c male mice were divided into 13 groups. Group 1: Control, Group 2: DMH (20 mg/kg) (injected (IP) thrice a week for 14 weeks). Groups 3,4,5: Sericin (S) (100mg/kg), Sericin silver nanoparticles (SAgNPs) (100mg/kg), and Sericin Chitosan silver nanoparticles (SChiAgNPs) (100 mg/kg) were given orally for 14 weeks respectively. Groups 6,7,8,9 were given DMH (IP)+5-Fu (IP), DMH(IP)+S (orally), DMH (IP)+SAgNPs (orally), DMH (IP)+SChiAgNPs (orally) respectively. Groups 10,11,12,13 were considered as treatment groups and were given 5-Fu (5mg/kg) (IP), (S) (100mg/kg) (orally), (SAgNPs) (100mg/kg) (orally), (SChiAgNPs) (100mg/kg) (orally) for a period of first 7 weeks. The UV absorption peaks obtained at 435 nm and 463 nm indicated the formation of SAgNPs and SChiAgNPs formation respectively.

FTIR spectra peaks obtained, indicate N-H stretching of primary and secondary amine group), (N-H stretching of amine salt) (N=C=S stretching of thiocyanate compound), (C=C stretching of alkene), (N-O stretching of nitro compound), (S=O stretching of sulfonyl chloride), (C-N stretching of amine) and (C-O-O stretching) for sericin, SAgNPs, and SChiAgNPs, confirming, the presence of theses functional groups. The XRD pattern revealed that SAgNPs and SChiAgNPs had structure crystalline structures. EDX characterization peaks for SAgNPs indicated the presence of silver along with other elements including; calcium, oxygen carbon, while EDX characterization peaks for SChiAgNPs indicated the presence of silver along with other elements including; oxygen, carbon, sodium, phosphorus, Sulphur and chlorine. SEM analysis showed that SAgNPs are of spherical shape, while the SChiAgNPs displayed the rectangular shape. The nanoparticles were moderately monodispersed of size about 73.8 nm with PDI 0.23 was observed for SAgNPs and size of 65.8 nm with PDI 0.22 was observed for SChiAgNPs. At the end of the trial, mice were euthanized. Blood samples and colon tissue were used for the analysis of biochemical markers *i.e.,* CEA, CA19-9, TIMP-1, and IL-6, IL-8, IL-27, SOD, CAT, GR, GSH, GST, MDA and MMP-7 via ELISA and histopathological analysis. Sericin (S-Ext) and SChiAgNPs showed significant antiproliferative activities in SW480, SW620 and HCT116 cells. Overall, there was 29-34% inhibition of viability for sericin extracted and 35-43% for SChiAgNPs in the three cell lines in comparison to untreated control. Expression profiling indicated the significant stimulation of GADD45A, BCL-2 and TNF genes expression in SW480, SW620 and HCT 116 cells. The GADD45A showed induction by 1.43-1.71fold in SW480, 1.09-1.56fold in SW620 and 1.25-4.55fold in HCT116 cells in response to treatment groups. The BCL2 showed the induction by 1.35-2.53, 1.38-3.1 and 2.32-3.76fold in SW480, SW620 and HCT116 cells, respectively. TNF was induced by a factor of 3.9-6.43, 2.53-5.41 and 2.7-5.31fold in in SW480, SW620 and HCT116 cells, respectively after the exposure with compounds. In mice model, the obtained results indicated significantly elevated levels of CEA, CA19-9, TIMP-1, IL-6, IL-8, IL-27, MDA, and MMP-7 in DMH treated group ($p \leq 0.001$) which were decreased significantly in SChiAg(T) ($p \leq 0.001$). In contrast, levels of SOD, GR, GSH, CAT and GST were reduced significantly in DMH treated group, which were increased significantly in SChiAg(T) ($p \leq 0.001$).

The level of CEA was significantly elevated in the DMH-group (6.5±0.2ng/ml) as compared to control group (1.5±0.3 ng/ml) (p ≤0.001). In treatment groups, CEA level was significantly reduced as 5-Fu(T) (4.7±0.2 ng/ml), SChiAg(T) (3.9±0.2 ng/ml), SAg(T) (4.8±0.2 ng/ml) and S(T) (5.1±0.2ng/ml). The level of CA19-9 was significantly elevated in DMH-group (71±3.1 U/mL) as compared to control group (26±1.7 U/mL) (p ≤0.001). In treatment groups, CA19-9 level was significantly reduced as 5-Fu(T) (52±1.8 U/mL) SChiAg (T) (45±1.4 U/mL), SAg(T) (53±1.7 U/mL) and S(T) (61±2.3 U/mL). The level of TIMP-1 was significantly elevated in DMH-group (650±14 ng/mL) as compared to control group (198±16ng/mL) (p ≤0.001). In treatment groups, TIMP-1 level was significantly reduced as 5-Fu(T) (415±14 ng/mL), SChiAg(T) (383±18 ng/mL), SAg (T) (400±16 ng/mL) and S(T) (569±17 ng/mL). The level of MMP-7 was significantly elevated in DMH-group (30.6±1.5 ng/mL) as compared to control group (9.6±1.1 ng/mL) (p ≤0.001). In treatment groups, MMP-7 level was significantly reduced as 5-Fu(T) (22±1.5 ng/mL) and SChiAg(T) (19.8±1.4 ng/mL). The level of IL-6 was significantly elevated in DMH-group (27.6±1.6 pg/mL) as compared to control group (8.8±0.8 pg/mL) (p ≤0.001). In treatment groups, IL-6 level was significantly reduced as 5-Fu(T) (20.4±0.9 pg/mL) and SChiAg(T) (17.8±0.9 pg/mL). The level of IL-8 was significantly elevated in DMH-group (68.6±2.1 pg/ml) as compared to control group (21.8±2.0 pg/ml) (p ≤0.001). In treatment groups, IL-8 level was significantly reduced as 5-Fu(T) (46±2.3 pg/ml), SChiAg(T) (42±3.0 pg/ml), SAg(T) (47.4±1.8 pg/ml) and S(T) (55.6±1.8 pg/ml). The level of IL-27 was significantly elevated in DMH-group (35.6±2.1 pg/ml) as compared to control group (11.3±1.0 pg/ml) (p ≤0.001). In treatment groups, IL-27 level was significantly reduced as 5-Fu(T) (23±1.0 pg/ml), SChiAg(T) (19.2±1.1 pg/ml) and SAg(T) (26.2±1.1pg/ml). The level of MDA was significantly elevated in DMH-group (3.4±0.2 nmol/mg) as compared to control group (1.0±0.2 nmol/mg) (p ≤0.001). In treatment groups, MDA level was significantly reduced as 5-Fu(T) (2.3±0.1 nmol/mg), SChiAg(T) (2.1±0.1 nmol/mg) and SAg(T) (2.3±0.1 nmol/mg). The level of SOD was significantly decreased in DMH-group (9.0±0.7 U/mg) as compared to control group (18.4±0.8 U/mg) (p ≤0.001).

In treatment groups, SOD level was significantly reduced as 5-Fu(T) (13.7±0.7 U/mg), SChiAg(T) (14.7±0.8 U/mg), SAg(T) (13.4±0.8 U/mg) and S(T) (12.6±0.9 U/mg). The level of CAT was significantly decreased in the group of DMH subjected group of mice (13.1±0.7 nmol/mg) as compared to control group (29.0±0.9 nmol/mg) ($p \leq 0.001$). In treatment group, CAT level was significantly reduced as 5-Fu(T) (18.2±0.7 nmol/mg), SChiAg(T) (19.7±0.9 nmol/mg) and SAg(T) (17.5±0.9 nmol/mg). The level of GR was significantly decreased in DMH-group (3.3±0.4 nmol/mg) as compared to control group (12.4±0.7 nmol/mg) ($p \leq 0.001$). In treatment groups, GR level was significantly reduced as 5-Fu(T) (6.9±0.3), SChiAg(T) (7.4±0.4 nmol/mg), SAg(T) (6.4±0.4 nmol/mg) and S (T) (5.9±0.38 nmol/mg). The level of GST was significantly decreased in DMH-group (5.0±0.4 µmol/mg) as compared to control group (9.2±0.7 µmol/mg) ($p \leq 0.001$). In treatment groups, GST level was significantly reduced as 5-Fu(T) (9.1±0.4 µmol/mg), SChiAg(T) (10.6±0.4 µmol/mg), SAg (T) (8.9±0.6 µmol/mg) and S(T) (7.6±0.5 µmol/mg). The level of GSH was significantly decreased in DMH-group (122±7.5 nmol/g) as compared to control group (234±8.1 nmol/g) ($p \leq 0.001$). In treatment groups, GSH level was significantly reduced as 5-Fu(T) (165±6.7 nmol/g), SChiAg(T) (178±7.2 nmol/g) SAg(T) (160±7.1 nmol/g) and S(T) (157±7.2 nmol/g). The histopathological analysis of proximal and distal parts of colon tissue of DMH-treated group showed low grade dysplasia (LGD), and high-grade dysplasia (HGD) while SChiAgNPs improved the histopathological changes induced by DMH. Sericin and S-ChiAgNPs, showed significant growth inhibition and gene expression profiling modifications in the CRC cells and in mice model, the findings provide evidence about sericin and its nano-particle conjugates as potential anticancer medicine for CRC. The findings suggest that sericin chitosan conjugated silver nanoparticles showed their efficacy against DMH-induced colon cancer, making a potential future in anticancer research field.