5.3 RESULTS

5.3.5 Cytotoxicity of bacteriocin from Lactococcus garvieae by MTT assay.

The present investigation undertaken to test the anticancer activity of bacteriocin on cell lines through cytotoxicity test by MTT assay. The experimental setup included five cell lines.

5.3.5.1 Cytotoxicity of bacteriocin on MDA-MB - Human Adenocarcinoma mammary gland cancer cell line (Table 1; Fig 1).

The cytotoxicity was carried out on five cell lines. The cytotoxicity of bacteriocin against MDA-MB - Human adenocarcinoma, mammary gland at a concentration of 10 μg/ml, 20 μg/ml and 30 μg/ml showed optical density of 0.187, 0.386 and 0.598 and no cell lysis was observed at 10 μg/ml, there was 25% and 50 % cell lysis was noticed at a concentration of 20 and 30 μg/ml. The (inhibitory concentration) IC 50 value of bacteriocin against MDA-MB - Human adenocarcinoma was found to be 30 μg/ml.

5.3.5.2 Cytotoxicity of bacteriocin on LNCap-FGC - Human carcinoma Prostate cancer cell line (Table 2; Fig 2).

Further, the cytotoxicity of against LNCap-FGC - Human carcinoma Prostate cell line was sensitive at different degrees to the toxic bacteriocin at a concentration of 10 μg/ml, 20 μg/ml and 30 μg/ml showed optical density of 0.291, 0.571 and 0.722, there was 25, 50 and 75% cell lysis was noticed at a concentration of 10 and 20 and 30 μg/ml of bacteriocin. No cell lysis in control cell line was observed showing O.D value of 0.175. The IC 50 value of
bacteriocin against LNCap-FGC - Human carcinoma Prostate was found to be 20 μg/ml.

The bacteriocin was further tested on cell lines such as K-562- Human chronic myelogenous leukemia, Bone marrow, Hela - Human cervix, A549 - human lung carcinoma at a concentration of 10, 20 and 30 μg/ml of bacteriocin, but showed no cell lysis or cytotoxicity.

5.3.6 Determination of apoptotic properties of bacteriocin in PC3 human Prostrate carcinoma cell line by DAPI staining. (Table 3; Graph 3; Fig. 3).

The cytotoxicity of bacteriocin from L. garvieae against LNCap human prostrate cell line showed good cytotoxicity at 10, 20, 30 μl in the above parameter. Further, in continuation of the anticancer study the apoptosis was carried out on the PC3 human prostate cancer cell line, which was carried out by DAPI staining.

The cytotoxicity of bacteriocin was carried out at five concentrations viz. 50, 25, 12.5, 6.25 and at 3.125 μg/ml against PC3 human cancer cell line that showed the 17.72, 12.64, 8.69, 6.09 and 3.66 % cytotoxicity. The cytotoxicity of bacteriocin was calculated for its CTC 50 value and which depicted that >50 μg/ml was required to inhibit 50 % of cells. The bacteriocin that showed good cytotoxic effect at higher concentration in comparison to standard doxorubicin at 2 μg/ml was choosen for apoptosis activity.

The bacteriocin at 50 and 25 μg/ml was used for induction of apoptosis in PC3 human cancer cell line and standard apoptotic drug doxorubicin was used at concentration of 2 μg/ml. The bacteriocin showed apoptosis in cancer cells which was depicted though DAPI, a nuclear staining method. The cancer cells upon
bacteriocin treatment showed cell plasma membrane blebbing, cell shrinkage, no discharge of cellular components, the cells are smaller in size, the cytoplasm is dense and may be the organelles are more tightly packed.

From, these outcomes it was confirmed that the bacteriocin from *L. garvieae*, which was isolated from cows milk had a potent anticancer property.