Abstract: <strong>Introduction:</strong><br />
Hemophilia B is bleeding disorder characterized by a deficiency in coagulation factor IX. Carboxylation of glutamate is essential for production of active hFIX is done by γ-carboxylase enzyme which is inhibited by Calumenin. RNAi has now gained popularity in a variety of biological systems as a methodology of choice for knocking down gene expression. Our main goal in the present study is designing artificial miRNAs against human Calumenin and studying the application of artificial miRNAs to silence the inhibition of γ-carboxylase activity by calumenin.<br />

**Methods:**<br />
Calumenin variants Sequences were obtained from NCBI . Using dharmacon, we designed siRNA for common sequences. The sequence of siRNA was replaced in the miR-30 endogenous miRNA pri-precursor backbone. Then this sequence analyzed by mfold program. Artificial miRNAs were synthesized by Shingene company in pUC57. We were attached artificial miRNA at the 3' end of hFIX cDNA in PCDNA3 vector by molecular techniques.<br />

**Results:**<br />
The result of mfold showed that the designed sequences could form stem–loop structures. Cloning of artificial miRNA at the 3’ end of hFIX cDNA was confirmed by digestion and electrophoresis. After verification, the recombinant plasmids are considered for transient expression of the hFIX in mammalian cell line in parallel with cell transfected with parental hFIX expression plasmids.<br />

**Conclusion:**<br />
Based on the results reported previously, which showed a 5-fold increase in γ–carboxylase activity, following the application of RNAi, an improvement of the hFIX expression efficiency is expected after expression of the artificial miRNA. Hemophilia B, artificial miRNA, calumenin.