Development of siRNA Functionalized Graphene Oxide Based Therapeutic Nanoformulation to Ameliorate Type-2 Diabetes Mellitus (T2DM)

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ABSTRACT

Diabetes mellitus type 2 is a long term metabolic disorder that is characterized by high blood glucose levels, insulin resistance, and relative lack of insulin. There are around 400 million people affected from this disease worldwide. In type 2 diabetic patients, increased hepatic glucose production (HGP) is a major cause of fasting and postprandial hyperglycaemia. The current strategies to alleviate type 2 diabetes mellitus include different drugs e.g. Metformin, Sulfonylureas etc. These strategies are costly and require regular adequate dosage to prevent the increased glucose level in the body. siRNA technology, one of the most promising therapeutic technology in current times and its exploitation in controlling hyperglycaemia has a great potential. In the current study graphene oxide nanosheets has been exploited to deliver therapeutic siRNA in order to control hyperglycaemic condition in type 2 diabetes mellitus. Synthesis and functionalization of graphene oxide nanosheets has been done and further characterized by different spectroscopic **(FTIR, UV-Visible, DLS, TGA, DSC, RAMAN)** and microscopic techniques **(HRTEM, AFM, FESEM)**. Cytotoxicity profiling of functionalized nanosheets has also well studied *in-vitro* via different assay **(MTT assay, Trypan Blue Assay, Propidium idodide (PI) assay)** techniques. Insulin resistant (IR) cell line has also been established and validated by molecular studies **(RT-PCR, Western blotting)** of the important gene markers and also by **FACS and Confocal microscopy** using 2-NBDG **(Fluorescent Glucose). Gel retardation assay** and **Confocal microscopy** clearly reveals the strong electrostatic binding of fluorescent siRNA to graphene nanosheets and delivery of siRNA *in-vitro* condition respectively. Molecular studies **(RT-PCR and Western)** showed many-fold decrease in the genetic and protein markers responsible for gluconeogenesis in the treated samples of in-vitro. Furthermore, C57/BL6 mice model was used as a model of type 2 diabetes and for all *in-vivo* studies (**Molecular studies, Animal imaging, Histology and diabetic related blood parameters)**. All the findings concludes the effectiveness of the synthesized therapeutic nanoformulation in controlling hyperglycaemic state in case of type 2 diabetes mellitus.