Title: Formulation and evaluation of solid self-nanoemulsifying drug delivery system loaded with curcumin and quercetin for the treatment of type 2 diabetes mellitus

OBJECTIVES

- To formulate and optimize Liquid self-nanoemulsifying drug delivery system (L-SNEDDS) of curcumin (CUR) and quercetin (QUE)
- To solidify L-SNEDDS into solid powder and pellets
- To characterize the developed formulations
- To perform stability studies of developed formulations
- To carry out pharmacokinetic and pharmacodynamic evaluation of developed formulation on streptozotocin (STZ) induced diabetic rats

Introduction: Currently available antihyperglycemic agents used alone or in combination give only symptomatic relief by controlling the high blood glucose level (BGL) rather than treating the actual cause of type 2 diabetes mellitus (T2DM) i.e., insulin resistance (IR). Since IR is reported to be caused due to genetic as well as certain lifestyle factors such as high fat diet, lack of exercise, stress, obesity and changes in the gut microbiota leading to increased lipopolysaccharide (LPS), therefore it is important to design a formulation that could possibly be able to overcome the aforementioned issues. CUR and QUE are well reported for reduction in serum glucose levels. Moreover, they are also reported to reduce the high fat level of the body by different mechanisms. CUR directly interacts with adipocytes, pancreatic cells, hepatic stellate cells, macrophages, and muscle cells. It suppresses the proinflammatory transcription factors NF-kβ, signal transducer and activators of transcription-3, and Wnt/β-catenin, and it activates peroxisome proliferator-activated receptor-γ and Nrf2 cell-signaling pathways, thus leading to the down-regulation of adipokines, including tumor necrosis factor alpha (TNF-α), interleukin-6 (IL-6), resistin, leptin, and monocyte chemotactic protein-1, and the upregulation of adiponectin and other gene products. These CUR induced alterations reverse IR, hyperglycemia, hyperlipidemia, and other symptoms linked to T2DM. QUE inhibits adipogenesis through stimulating the mitogen activated protein kinase (MAPK) signal pathway. At the same time QUE induces the apoptosis of mature adipocytes by controlling the important extracellular signal-regulated kinase (ERK) and janus-kinase (JNK) pathways. The antidiabetic activities of QUE involve the stimulation of glucose uptake through an MAPK insulin-dependent mechanism.

Despite being potential candidates for the treatment of DM, successful positioning of CUR and QUE in market in the form of suitable oral dosage form, either alone or in combination is very limited due to their poor aqueous solubility and oral bioavailability. In order to overcome these challenges, in the present study SNEDDS have been proposed due to its benefits of improving oral bioavailability of poorly water soluble drugs by increasing their aqueous solubility, protecting their gastric degradation and enhancing their GI permeability. In addition to that two other components viz. probiotics and mushroom polysaccharides will also be added in the formulation to achieve dual role i.e. as antidiabetic agents due to their effect on gut microbiota, circulating LPS, obesity, inflammation and dyslipidemia and as solidifying agents along with other solid carriers to prepare stable solid SNEDDS. Overall, it is anticipated that the proposed composition containing nano CUR and QUE and synbiotics would offer a unique strategy to manage T2DM. **Figure 1** describes the overall hypothesis of this research work.

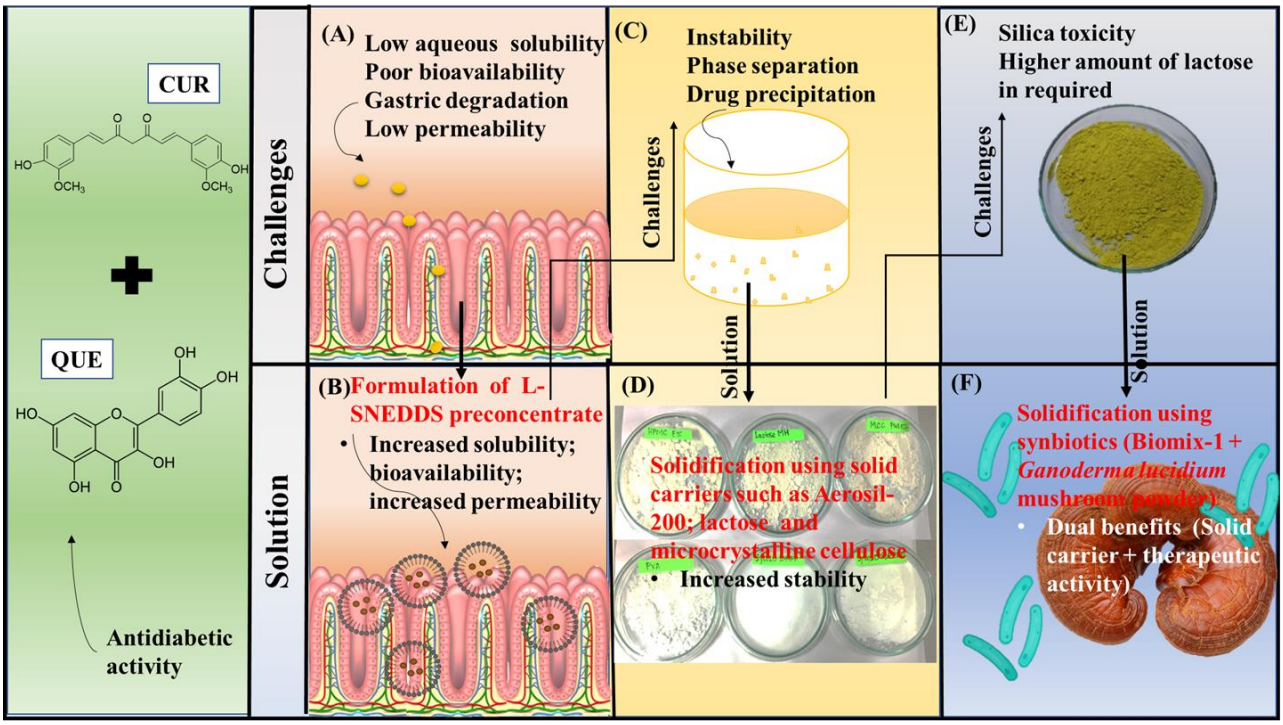


Fig.1. Hypothesis of the research

Methodology: To achieve these objectives initially the L-SNEDDS were formulated by loading CUR and QUE in isotropic mixture of Transcutol P, Tween 80, Labrafil M1944CS and Capmul MCM. Further the L-SNEDDS preconcentrate were solidified using *Ganoderma lucidum* mushroom extract powder (GLEP) and probiotics as solid carriers and Aerosil-200 (A-200) as coating agent. Spray drying technology was used for solidification and conversion of L-SNEDDS into spray dried solid SNEDDS (SD-S-SNEDDS) powder. The solidification process was optimized using Box Behnken Design (BBD). Further in order to enhance the stability of solid powder it was converted into pellets using extrusion spheronizartion technique. The pelletization process was optimized using BBD. The optimized formulations of L-SNEDDS, SD-S-SNEDDS powder and SD-S-SNEDDS pellets were characterized for droplet size, zeta potential and drug loading. The optimized SD-S-SNEDDS pellets were further evaluated for Caco 2 cell line permeability study, toxicity study, pharmacokinetic studies and pharmacodynamic studies.

Results and Discussion: It was seen that the drug loading for all the three optimized batches was above 95% for both CUR and QUE. The droplet size and zeta potential of optimized batch of L-SNEDDS, SD-S-SNEDDS powder and SD-S-SNEDDS pellets was found to be 63.46 ± 2.12 nm; -14.8± 3.11 mV, 70.08 ± 3.15 nm; -42.6± 1.58 mV and 72.46 ± 2.16 nm; -38.7 ± 1.34 mV respectively. In all the three optimized formulations it was seen that more than 90% drugs were released in first five minutes. These results indicated that the integrity of L-SNEDDS was preserved upon solidification and further their conversion into pellets. Moreover, the optimized formulations were thermodynamically stable as there was no phase separation after storage at 40 °C for 48 h, centrifugation, heating /cooling cycles and after freeze/thaw cycles.

The micromeritic studies indicated that both the optimized formulations i.e., SD-S-SNEDDS powder and pellets possessed excellent flow properties as indicated from angle of repose (AOR) (21.97° ± 1.21 for SD-S-SNEDDS powder and 20.9 ± 0.87° for SD-S-SNEDDS pellets). The optimized solid formulations were further characterized for differential scanning calorimetry (DSC), powder X ray diffractometry (PXRD), and scanning electron microscopy (SEM). The observations revealed that the L-SNEDDS were successfully adsorbed onto the porous surface of GLEP, probiotics and A-200. The L-SNEDDS and SD-S-SNEDDS pellets were also characterized for transmission electron microscopy (TEM). The TEM images revealed spherical and unagglomerated droplets in nanometer range with average droplet diameter of 54.23 ± 3.22

nm for L-SNEDDS and 82.54 ± 3.22 nm for SD-S-SNEDDS pellets. The results almost correlated with the results of droplet size obtained through dynamic light scattering.

From the dissolution results it was seen that there were about 4.5 folds enhancement in the dissolution rate of CUR as well as QUE when both the drugs were loaded into SNEDDS pellets due to their complete solubility in the emulsion. A non-significant difference in dissolution and permeability profiles of L-SNEDDS, SD-S-SNEDDS powder and SD-S-SNEDDS pellets indicated that the dissolution rate of both the drugs remained unaltered upon processing of L-SNEDDS preconcentrate during spray drying followed by extrusion and spheronization process. From the results of *in vitro* cell line toxicity, it was revealed that more than 85% cells were found viable in all cases at all three concentrations i.e., 25, 12.5 and 6.25 µg/mL. The pellets were found stable at accelerated temperature and humidity conditions for 6 months (40°C ± 2°C/ 75 % R.H. ± 5% R.H).

Further the % α-glucosidase inhibition test revealed that the inhibition rate for CUR and QUE at a concentration of 25 µg/mL and PB-GLEP ratio (1:1) was 30.56 ± 1.22 %, 26.35 ± 1.35%, and 47.57 ± 4.19%, respectively. The combination of these three i.e., CUR, QUE and GLEP-PB (1:1) in the form of SNEDDS showed 62.46 ± 3.45% α-glucosidase inhibition, which was about 2.04, 2.37 and 1.31 folds more as compared to their individual effect, indicating the synergistic effect of co-administration of these nutraceuticals.

The area under curve (AUCs) of SD-S-SNEDDS pellets of CUR were found to be 44.9 (alone) and 50.23 (combination) folds increased as compared to unprocessed CUR alone and in combination respectively. The AUCs of SD-S-SNEDDS pellets of QUE were found to be 5.03 (alone) and 5.57 (combination) folds higher than unprocessed QUE alone and in combination **(Figure 2).**

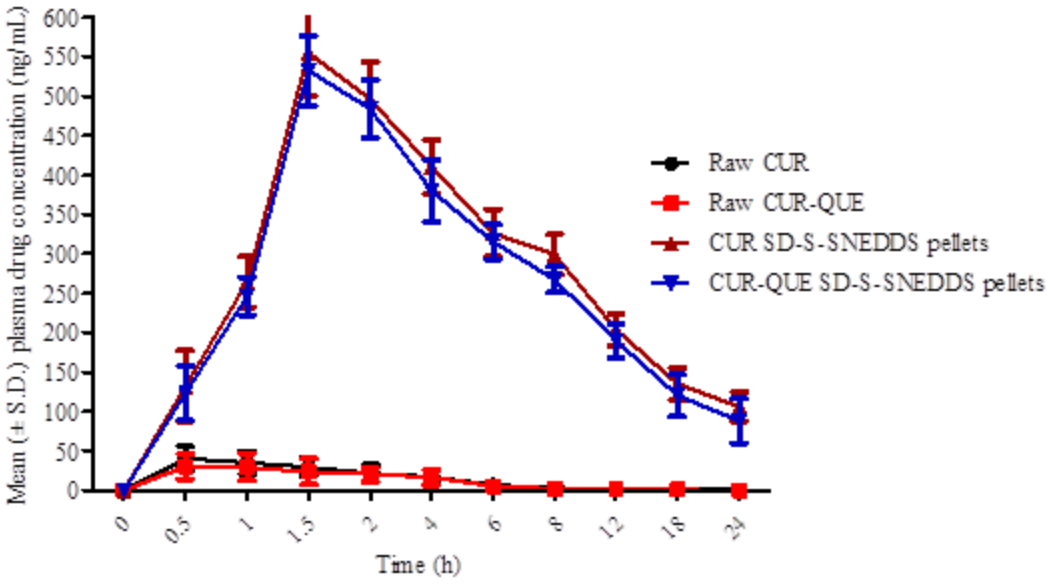


Fig.2a. Mean (± S.D.) plasma vs time profile of CUR in its raw form and SD-S-SNEDDS pellets

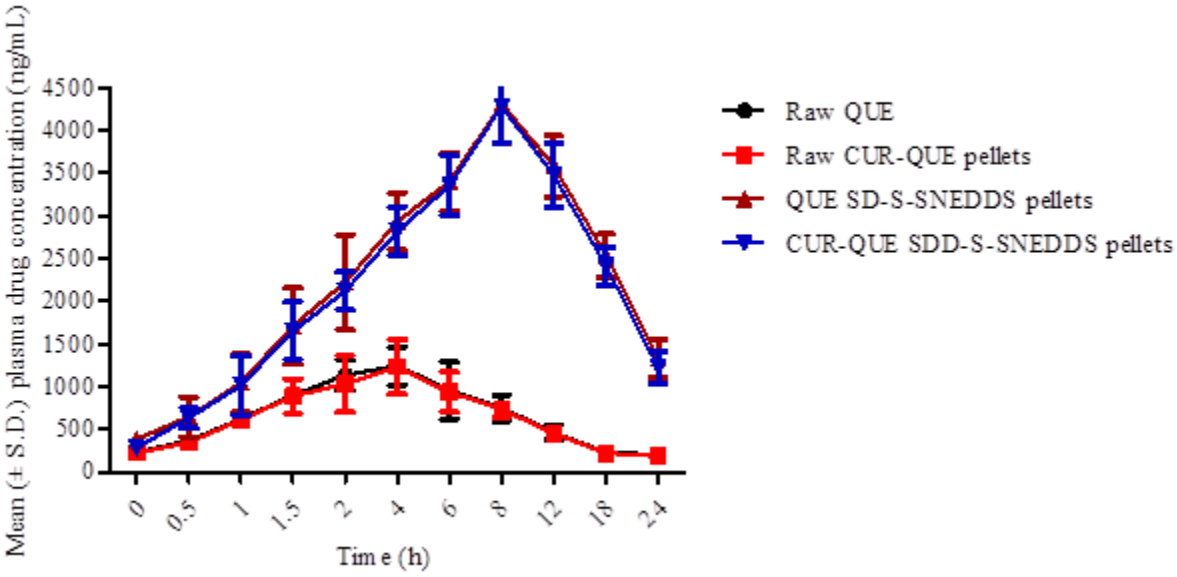


Fig.2b. Mean (± S.D.) plasma vs time profile of QUE in its raw form and SD-S-SNEDDS

Furthermore the *in vivo* pharmacodynamic study conducted for 42 days using high fat diet-streptozotocin (HFD-STZ) induced diabetic rats indicated excellent recovery/reversal in the rats treated with CUR-QUE-SD-S-SNEDDS pellets (group 13 and group 14) in a dose dependent

manner for body weight, BGL **(Figure 3),** lipid profile, kidney function test, liver function test, and anti-oxidant biomarkers post induction of HFD-STZ.

Similar observations were noted with histopathological changes obtained for rats 13 and 14 as compared to rats of experimental control that received HFD-STZ induction. The recovery was confirmed by the loss of vacuolar changes and steatosis in pancreatic and liver cells with better recovery in cell counts in rats of group 13 and 14 **(Figure 4),** whereas rats of experimental control showed significantly high steatosis in liver and vacuolar changes in pancreas along with lymphocyte infiltration. Overall, the study entailed successful development of a formulation containing two potent flavonoids (curcumin and quercetin) in the form SNEDDS.

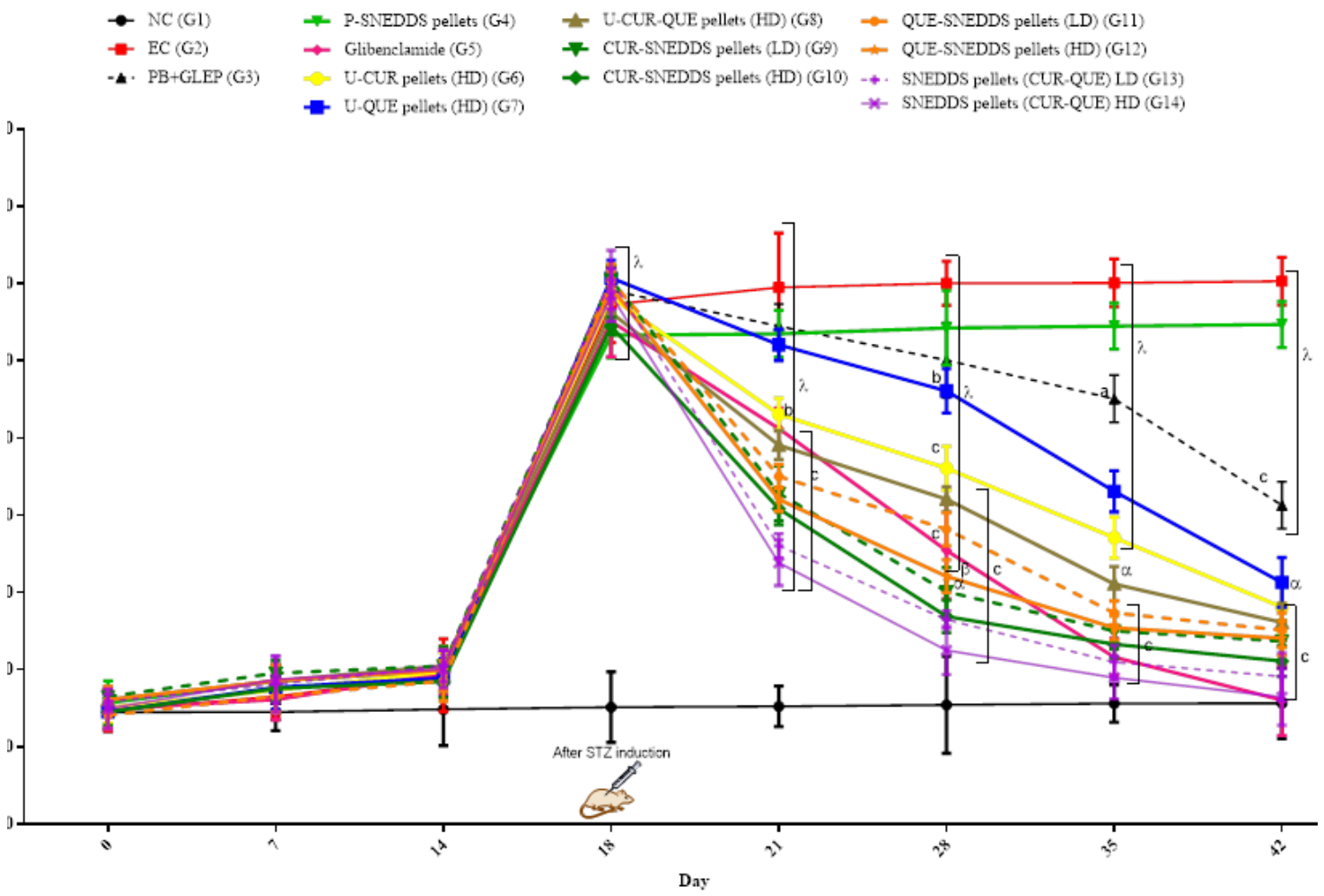


Fig.3. Variation in BGL of rats in different groups during in vivo studies

(NC: Normal control, EC: Experimental control, P-SNEDDS: Placebo SNEDDS; U-CUR: Unprocessed curcumin, U-QUE: Unprocessed quercetin, LD: Low dose; HD: High dose)

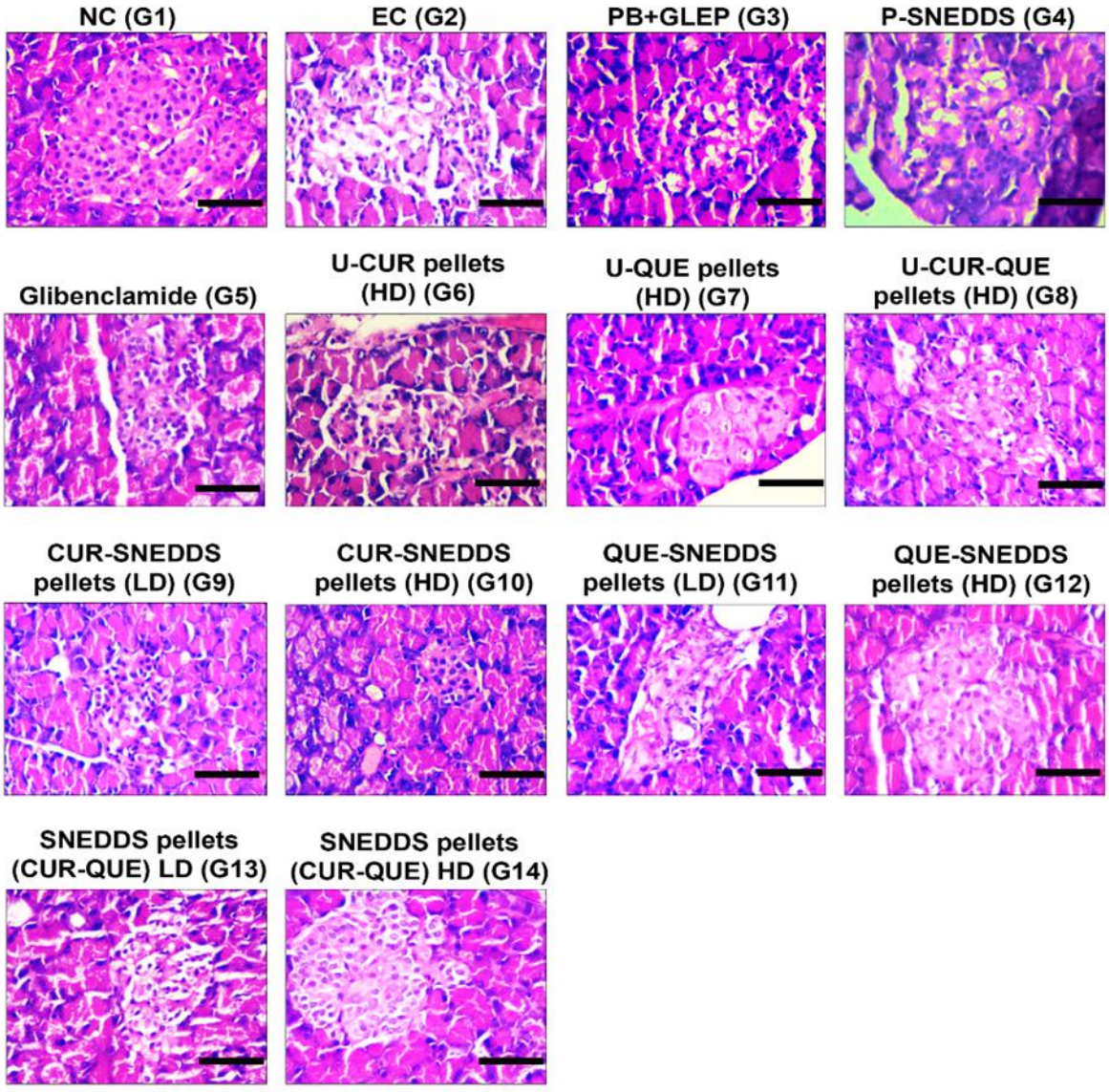


Fig.4. Histopathology images of pancreas for all groups

(NC: Normal control, EC: Experimental control, P-SNEDDS: Placebo SNEDDS; U-CUR: Unprocessed curcumin, U-QUE: Unprocessed quercetin, LD: Low dose; HD: High dose)

Impact of research in the benefit to mankind:

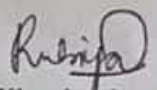
Benefit to society

India is one of the leading countries where maximum number of people are suffering from T2DM. Hence, developed technology could be scaled up in collaboration with pharmaceutical industries that are formulating diabetic medicines. The scaled-up product could be subjected for clinical trial in association with medical hospitals and research centers such as Post Graduate Institute of Medical Education and Research, Chandigarh, India so that the formulation could reach behind the bed side of patients.

Benefit to the researchers

Upon successful development of formulation, the research work carried out in laboratory can be presented at International Conferences organized by various federations such as International Diabetes Federation, AAPS PharmSciTech etc. where the Indigenous research could get more awareness about it. Moreover, the research work can be published in SCI indexed high impact journals e.g. Advances in Wound Care, International Journal of Pharmaceutics.

Signature of the nominee



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