# Nano-curcumin safely prevents streptozotocin-induced inflammation and apoptosis in pancreatic ß-cells for effective management of type 1 diabetes mellitus

Raghu Ganugula[1], Meenakshi Arora[1], Patcharawalai Jaisamut[1,2], Ruedeekorn Wiwattanapatapee[3], Heather G Jørgensen[4], Vinod P. Venkatpurwar[5], Beiyan Zhou[6], Aline Rodrigues Hoffmann[7], Rita Basu[8], Shaodong Guo[9], and Majeti N. V. Ravi Kumar[1*]

[1]Department of Pharmaceutical Sciences, Irma Lerma Rangel College of Pharmacy, Texas A&M University, College Station TX, USA

[2]Faculty of Traditional Thai Medicine, Prince of Songkla University, Hat-Yai, Songkhla, Thailand

[3]Department of Pharmaceutical Technology, Faculty of Pharmaceutical Sciences, Prince of Songkla University, Hat-Yai, Songkhla, Thailand

[4]Paul O'Gorman Leukaemia Research Centre, Institute of Cancer Sciences, University of Glasgow, Glasgow, UK, G12 0ZD

[5]Strathclyde Institute of Pharmacy and Biomedical Sciences, University of Strathclyde, Glasgow, G4 0RE, UK

[6]Department of Immunology, University of Connecticut Health Center,

Farmington, CT, USA

[7]Department of Veterinary Pathobiology, Texas A&M University,

College Station, TX, USA

[8]The Integrated Carbohydrate Physiology and Translation Laboratory, Mayo Clinic, Rochester, MN, USA

[9]Department of Nutrition and Food Science, Texas A&M University,

College Station, TX, USA

*Author for correspondence E-mail: mnvrkumar@tamhsc.edu Phone: +1-979-436-0721

Short title: nCUR to manage type 1 diabetes mellitus

Keywords: curcumin; cytokines; nanoparticles; oral bioavailability; type 1 diabetes mellitus.

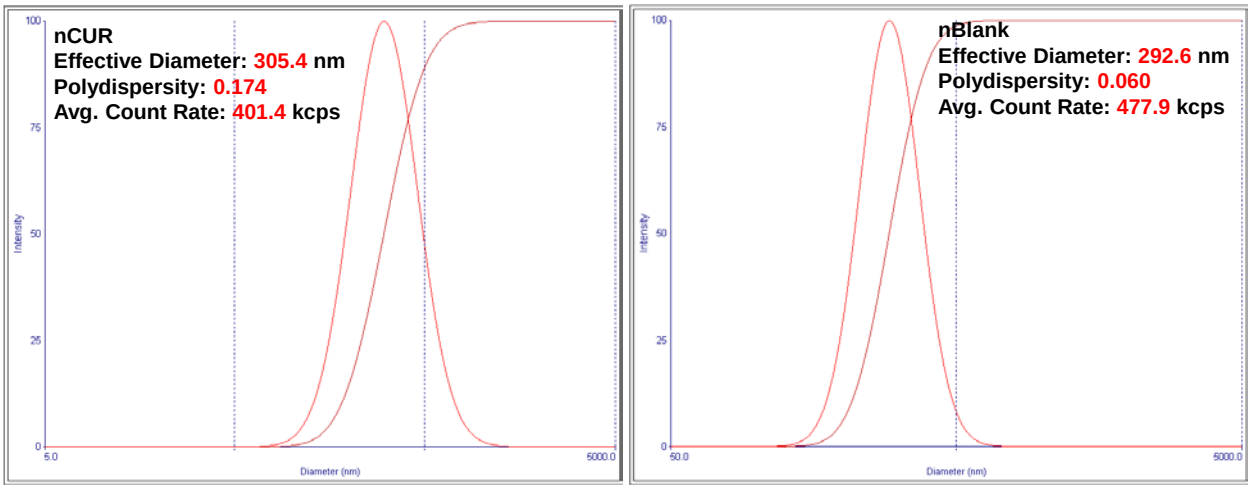


***Figure S1.*** Representative dynamic light scattering particle size distribution of nCUR and nBlank used in the study.

***Table S1.*** *Randomization of rats for subacute toxicity study*

| ***Group*** | ***Treatment mg/kg/day*** |
|---|---|
| ***Control*** | ***-----*** |
| ***CUR 500*** | ***500*** |
| ***nCUR 100*** | ***100*** |
| ***nCUR 50*** | ***50*** |
| ***nCUR 25*** | ***25*** |
| ***nBlank[a]*** | ***Eqv. to 100 of CUR*** |

***[a]CUR-void nanosystems***

***Table S2.*** Pre-treatment with CUR/nCUR (10 or 50 mg/kg) orally prior to STZ challenge does not regulate inflammatory cytokines in the plasma. Plasma was analyzed 72 h post STZ challenge using multiplex assay. Unlike with pancreas tissue homogenates (c.f. Figure 2), plasma analysis showed no significant differences between groups

| | Control | STZ | CUR10 | CUR50 | nCUR10 | nCUR50 | nBlank |
|---|---|---|---|---|---|---|---|
| G-CSF | 11.87±0.97 | 11.59±0.49 | 12.15±0.84 | 11.87±0.97 | 12.99±0.00 | 12.71±0.49 | 11.31±1.68 |
| GM-CSF | 8.70±0.96 | 9.25±0.96 | 10.36±0.96 | 9.81±1.67 | 10.36±0.96 | 9.81±0.00 | 9.25±0.96 |
| IL10 | 8.30±0.96 | 8.30±0.96 | 8.30±0.96 | 8.30±0.96 | 7.75±1.66 | 7.75±0.00 | 8.02±0.48 |
| IL12p70 | 10.87±0.96 | 11.42±0.00 | 10.87±1.92 | 10.87±1.92 | 10.87±0.96 | 10.59±0.83 | 11.42±0.00 |
| IL13 | 12.06±2.28 | 13.06±1.49 | 12.06±0.86 | 12.56±0.86 | 12.56±0.86 | 11.82±0.43 | 12.06±0.86 |
| IL17A | 13.77±1.92 | 14.88±0.00 | 14.33±0.96 | 13.77±0.96 | 13.77±0.96 | 14.05±0.83 | 12.66±0.96 |
| IL1α | 13.00±0.00 | 13.33±1.53 | 14.33±1.15 | 14.33±1.15 | 13.00±0.00 | 13.00±0.00 | 13.00±2.00 |
| IL1β | 10.83±2.89 | 9.72±0.96 | 11.39±0.96 | 10.28±0.96 | 9.72±0.96 | 9.17±1.67 | 9.72±0.96 |
| IL2 | 8.56±0.96 | 9.11±0.96 | 9.11±0.96 | 10.78±0.96 | 9.11±0.96 | 8.56±0.96 | 9.39±1.27 |
| IL4 | 6.22±0.96 | 7.33±0.00 | 7.33±0.00 | 6.78±0.96 | 7.33±0.00 | 7.33±0.00 | 7.61±1.27 |
| IL5 | 9.06±0.48 | 9.61±1.27 | 8.78±0.96 | 9.89±0.96 | 9.06±0.48 | 9.33±0.00 | 9.33±0.00 |
| IL6 | 12.61±0.96 | 12.61±0.96 | 13.44±0.48 | 14.00±0.83 | 12.61±0.96 | 12.89±0.48 | 13.17±0.00 |
| INFγ | 20.51±4.19 | 18.56±3.15 | 16.06±0.96 | 18.56±0.48 | 17.73±0.96 | 17.73±2.55 | 16.62±0.00 |
| TNFα | 17.19±2.55 | 17.19±0.96 | 17.19±0.96 | 17.19±0.96 | 17.47±1.44 | 16.08±0.96 | 18.30±1.67 |

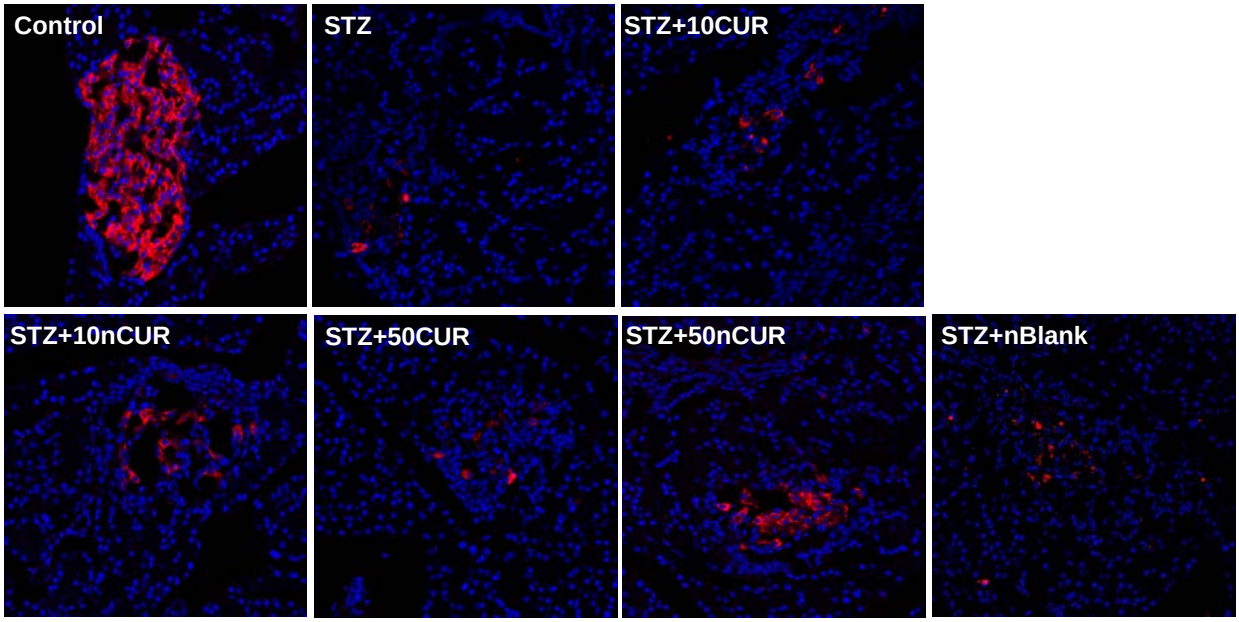


***Figure S2.*** Pre-treatment with CUR/nCUR 10 or 50 mg/kg orally prior STZ challenge and pancreatic tissue sections were immunostained for insulin.

### *General observation, body weight and food intake*

Study-2: General animal health and wellbeing was assessed daily for signs of acute toxicity, such as abnormal locomotor activity, labored breathing or pale appearance. Weights were noted at the same time each day on a calibrated instrument. A known amount of standard feed (pellets) was offered to each cage with the remainder weighed to monitor consumption and replaced fresh daily at the same fixed time in the day. Over the study period, no abnormal variances in activity or signs of acute or ocular toxicity were observed.

### *Hematological parameters*

Study-2: A full differential blood count and analysis was performed using the Hemavet950FS instrument (Drew Scientific Inc., USA). All hematologic parameters measured including hemoglobin (Hb), red blood corpuscles count (RBC), white blood corpuscles count (WBC), and differential leukocyte count (DLC) were within normal reference ranges indicating safety of both CUR as well as their nanosystems (nCUR/nBlank) on chronic administration (Tables S3-6).

### *Oxidative stress, inflammation and antioxidant enzyme levels*

Standard markers of oxidative stress/inflammation were quantified using commercial ELISA kits (Cayman Chemicals Ltd.). Specifically the effect of treatment on fibrinogen (FBG), C-reactive protein (CRP), ceruloplasmin (CP), and endogenous antioxidants superoxide dismutase (SOD) and catalases (CAT) was assayed. All the markers assessed did not change across all the treatment groups compared to control (Table S7).

### *Histopathological evaluation*

Study-2: Organs (heart, kidney, testis, spleen, lung, and small intestine) harvested on sacrifice were washed, weighed, formalin fixed then embedded in paraffin blocks before staining of 5 micron thick cut sections with hematoxylin and eosin. Microscopic histopathological examination confirmed tissue integrity with no notable architectural tissue damage, necrosis or inflammatory infiltrate (Figure S3).

**Table S3.** Absolute white cell counts at the end of the study

| Groups | WBC (K/µL) | NE (K/µL) | LY (K/µL) | MO (K/µL) | EO (K/µL) | BA (K/µL) |
|---|---|---|---|---|---|---|
| **Control** | 5.46 ± 1.07 | 1.16 ± 0.37 | 3.98 ± 0.90 | 0.27 ± 0.05 | 0.04 ± 0.03 | 0.01 ± 0.01 |
| **CUR 500** | 10.41 ± 3.71 | 3.20 ± 1.71 | 6.49 ± 1.91 | 0.49 ± 0.14 | 0.19 ± 0.13 | 0.04 ± 0.04 |
| **nCUR 100** | 11.88 ± 2.19 | 2.29 ± 1.02 | 8.95 ± 1.62 | 0.52 ± 0.18 | 0.10 ± 0.09 | 0.04 ± 0.05 |
| **nCUR 50** | 14.24 ± 2.73 | 3.31 ± 1.02 | 10.25 ± 1.83 | 0.54 ± 0.07 | 0.14 ± 0.06 | 0.01 ± 0.01 |
| **nCUR 25** | 13.05 ± 3.51 | 2.44 ± 1.11 | 10.07 ± 2.80 | 0.53 ± 0.22 | 0.07 ± 0.03 | 0.01 ± 0.01 |
| **nBlank** | 13.33 ± 3.28 | 4.00 ± 1.49 | 8.47 ± 1.70 | 0.65 ± 0.27 | 0.17 ± 0.05 | 0.05 ± 0.04 |
| **Normal Range** | 2.9-20.9 | 0.3-8.5 | 3.8-15.3 | 0.0-1.4 | 0.0-0.3 | 0.0-0.1 |

**Table S4.** Erythrocyte numbers at the end of the study

| Groups | RBC (M/µL) | Hb (g/dL) | HCT (%) | MCV (fL) | MCH (pg) | MCHC (g/dL) | RDW (%) |
|---|---|---|---|---|---|---|---|
| **Control** | 5.73 ± 0.40 | 10.78 ± 0.79 | 32.63 ± 1.31 | 57.88 ± 1.79 | 18.85 ± 1.00 | 33.00 ± 1.39 | 13.28 ± 0.80 |
| **CUR 500** | 6.12 ± 1.06 | 10.98 ± 2.91 | 34.20 ± 7.14 | 55.65 ± 2.00 | 17.75 ± 1.59 | 31.85 ± 1.84 | 14.03 ± 0.19 |
| **nCUR 100** | 6.17 ± 1.36 | 11.90 ± 2.84 | 34.38 ± 8.12 | 57.05 ± 2.18 | 19.25 ± 0.79 | 33.70 ± 1.27 | 14.75 ± 0.44 |
| **nCUR 50** | 6.73 ± 1.64 | 13.43 ± 3.97 | 40.30 ± 11.04 | 59.60 ± 1.89 | 19.78 ± 1.15 | 33.15 ± 0.90 | 14.18 ± 0.29 |
| **nCUR 25** | 5.63 ± 0.42 | 10.65 ± 0.93 | 33.30 ± 2.91 | 59.20 ± 2.43 | 18.95 ± 0.77 | 32.00 ± 1.15 | 13.95 ± 0.35 |
| **nBlank** | 6.79 ± 1.49 | 14.13 ± 3.61 | 40.70 ± 8.22 | 60.13 ± 1.81 | 20.70 ± 1.31 | 34.47 ± 2.17 | 13.80 ± 0.53 |
| **Normal Range** | 4.60-9.19 | 10.0-16.7 | 34.0-53.0 | 50.0-77.8 | 16.0-23.1 | 28.1-34.1 | 12.0-27.0 |

**Table S5.** Differential white cell counts at the end of the study

| Parameters | NE | LY | MO | EO | BA |
|---|---|---|---|---|---|
| **Control** | 21.14 ± 6.32 | 72.81 ± 7.00 | 5.04 ± 1.17 | 0.78 ± 0.31 | 0.11 ± 0.06 |
| **CUR500** | 29.60 ± 5.96 | 63.46 ± 5.27 | 4.91 ± 1.14 | 1.64 ± 0.91 | 0.41 ± 0.42 |
| **nCUR100** | 18.82 ± 6.75 | 75.61 ± 8.65 | 4.36 ± 1.42 | 0.87 ± 0.89 | 0.34 ± 0.47 |
| **nCUR50** | 22.97 ± 3.65 | 72.17 ± 4.15 | 3.83 ± 0.39 | 0.96 ± 0.47 | 0.08 ± 0.06 |
| **nCUR25** | 18.00 ± 4.26 | 77.17 ± 5.45 | 4.25 ± 1.72 | 0.53 ± 0.14 | 0.04 ± 0.04 |
| **nBlank** | 29.30 ± 5.80 | 64.25 ± 5.46 | 4.85 ± 1.20 | 1.26 ± 0.13 | 0.34 ± 0.27 |
| **Normal Range** | 5.3-38.1 | 56.7-93.1 | 0.0-7.7 | 0.0-3.4 | 0.0-0.4 |

**Table S6.** Platelet (PLT) count and mean platelet volume (MPV) at the end of the study

| Parameters | PLT (K/µL) | MPV (fL) |
|---|---|---|
| **Control** | 781.25 ± 134.22 | 5.9 ± 0.28 |
| **CUR500** | 763.25 ± 164.58 | 5.3 ± 0.47 |
| **nCUR100** | 637.25 ± 146.32 | 5.5 ± 0.15 |
| **nCUR50** | 737.25 ± 198.86 | 5.4 ± 0.31 |
| **nCUR25** | 580.00 ± 59.03 | 5.5 ± 0.57 |
| **nBlank** | 671.33 ± 115.64 | 5.4 ± 0.37 |
| **Normal range** | 685-1436 | 5.0-20.0 |

**Table S7.** Plasma markers indicative of oxidative stress and inflammation, and antioxidant enzyme levels at the end of the study

| Group | CRP (µg/ml) | FBG (µg/ml) | CP (µg/ml) | SOD (nmol/min/ml) | CAT (U/ml) |
|---|---|---|---|---|---|
| **Control** | 1714.97 ± 25.39 | 941.28 ± 110.20 | 450.32 ± 109.38 | 1086.37 ± 46.00 | 125.54 ± 31.79 |
| **CUR500** | 1713.69 ± 16.09 | 822.85 ± 113.77 | 459.90 ± 146.14 | 1137.52 ± 152.96 | 143.17 ± 30.81 |
| **nCUR100** | 1710.49 ± 39.35 | 970.33 ± 82.57 | 467.68 ± 76.75 | 1078.92 ± 246.34 | 139.33 ± 14.70 |
| **nCUR50** | 1715.61± 24.15 | 1067.09 ± 49.28 | 406.64 ± 74.52 | 1117.52 ± 123.91 | 127.29 ± 33.51 |
| **nCUR25** | 1698.33 ± 38.11 | 1036.47 ± 45.48 | 412.62 ± 115.99 | 1049.35 ± 112.37 | 131.21 ± 11.81 |
| **nBlank** | 1704.09 ± 35.51 | 1023.07 ± 135.15 | 279.77 ± 104.25 | 1094.78 ± 84.82 | 127.43 ± 10.18 |

**Table S8.** Curcumin concentration in plasma at the end of the study, 24 h post last dose

| Dose | Plasma curcumin Con (ng/ml) |
|---|---|
| CUR 500 | 83.03±52.82 |
| nCUR 100 | 1160.52±206.86 |
| nCUR 50 | 150.82±58.55 |
| nCUR 25 | 59.46±11.96 |

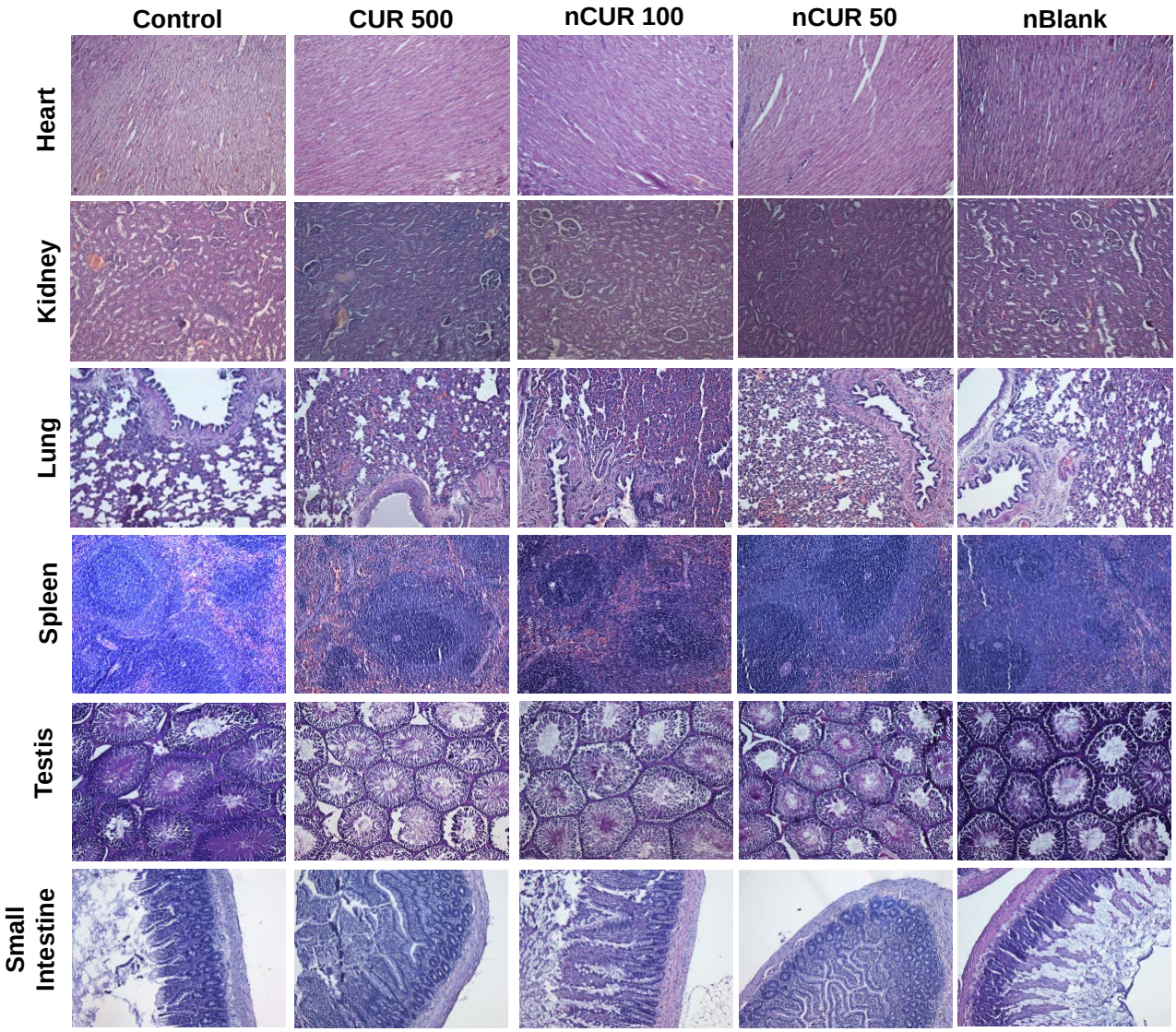


**Figure S3.** Histological images of organs after 28 days subacute toxicity study.

Graphical Abstract