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SUSTAINED RELEASE CURCUMIN NANOPARTICLES PREPARED THROUGH BIODEGRADABLE POLYMER FOR EVALUATION OF ANTI-DIABETIC ACTIVITY IN ANIMAL MODEL

K SHAILAJA[1]*, S MADHU[2], AND K SENTHILKUMARAN[3]

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Abstract

Curcumin, known as the 'wonder of drug' is proven to be efficacious in a variety of chronic diseases including diabetes. Type 2 diabetes is more common among diabetes also known as insulin resistance or hyperglycemia. Hyperglycemia leads to formation of glycation end products which is considered as highly toxic to human body. Conventional therapy fails to treat diabetes effectively and the side effects are a major challenge. Curcumin, herbal medicine has various pharmacological activity is still used as an adjuvant and not as first-line therapeutic agent because of its poor solubility and bioavailability. The present study was framed to assess the effectiveness of curcumin administered as nanoformulation on blood glucose level, insulin, HbA1c, liver and kidney function test in Streptozocin induced diabetic rats. Thirty wistar rats of both genders were distributed into five groups: negative control, positive control, and three diabetic groups treated with Curcumin, Curcumin nanoparticles and Metformin respectively. Streptozocin induced beta cell damage leads to decrease in insulin secretion which was found to be significantly improved in treated groups compared to untreated groups. Also, there was a significant improvement in the liver and kidney parameters in treated groups. From this the study concludes that curcumin administered in the form of nanoparticles had prolonged action and effective against streptozocin induced diabetic rats.

Keywords: Streptozocin, nanocurcumin, oxidative stress, β cell dysfunction, insulin resistance

[1]Department of Pharmacy Practice, C L Baid Metha College of Pharmacy, Chennai-600097, India.

[2]Department of Pharmacology, C L Baid Metha College of Pharmacy, Chennai-600097, India.

[3]Department of Pharmaceutics, K K College of Pharmacy, Chennai-600122, India.

***Address for Correspondence:** Shailaja K. M Pharm., (Ph D), Associate Professor, Department of Pharmacy Practice, C. L. Baid Metha College of Pharmacy, Affiliated to "The Tamilnadu Dr. MGR Medical University", Chennai, India. Mail: shailajampharm@gmail.com

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Introduction

In India, the diabetes mellitus (DM) burden is increasing daily and is one of the main epidemic non-communicable diseases. IDF (International Diabetes Federation) estimated, 425 million people suffered from diabetes all over the world in 2017 and it will reach a maximum of 629 million by 2045 [1]. Among various types of DM, type 2 diabetes is the most common type, WHO (World Health Organisation) estimated 77 million people are suffering from type 2 diabetes who are above 18 years of age and nearly 25 million are at a high risk of developing diabetes in the future (prediabetes). Its prevalence is high and its complications have contributed incredibly to the burden of mortality with the greatest amounts in low-and middle-income countries and increased the healthcare cost all over the world [1,2]. The pathogenesis in type 2 diabetes, is insufficient insulin secretion by Beta cells, if insulin is produced by the pancreas, it is not taken by the cells, which is known as "insulin resistance" characterized by hyperglycemia [3]. If the hyperglycemia is not treated for a prolonged period of time, it causes an elevation of advanced glycation end products (AGEs) which activate a cascade of reactive oxygen species (ROS) production and activation of pro-inflammatory cytokines and causes oxidative stress, in turn, leads to secondary complications [4-6]. Hence it is not a curable disease, and managing with diabetic medications without the side effects is a major challenge. However, most of the medications promote hypoglycaemia, weight gain, gastrointestinal disturbances [7,8], renal failure, diarrhea, and hypersensitivity [9]. Gupta et al have reported severe side effects like hypoglycaemic coma and hepatorenal disorders by synthetic antidiabetic agents [10]. Due to the lack of treatment and side effects of synthetic drugs recent attention towards herbal drugs has progressively increased with in-depth research to develop alternative medicine [11,12]. Natural compounds have a significant role in the treatment of chronic diseases, which are affordable, and easily accessible with fewer side effects [13]. One such compound is curcumin, which is known as the "wonder of the drug" obtained from Curcuma Longa and has various pharmacological and biological activities [14]. FDA-approved curcumin is a safer drug to be used in human beings even at high doses. Though it is well known for its various therapeutic activities due to poor solubility and bioavailability when administered orally it is still not claimed as a frontline therapeutic agent [15]. The first animal model (Sprague-Dawley rats) used to investigate the pharmacokinetics and pharmacodynamics of curcumin is by Wahlstrom and Blennow in 1978 [16]. Various technologies have been applied for the transition of curcumin from the kitchen to clinical trials, administered with milk, yogurt, and vegetable oil, and administration with piperine increased the plasma concentration of curcumin as well enhanced the bioavailability [17]. Recently, nanomedicine has been proven to enhance the solubility and bioavailability of such compounds through various drug delivery systems (liposomes, micelles, dendrimers, cyclodextrins, phospholipid complex, SLN-solid lipid nanoparticles, polymeric nanoparticles, and nanocapsules). Also, it is important to consider the route of administration equally, since the oral route is the preferred route by patients who require repeated dosing for a chronic period [18,19]. This study aimed to prepare the sustained-release curcumin nanoparticles using biodegradable polymer chitosan and to assess its efficacy in diabetic-induced rats. Hence, animal models are considered a useful tool for research. The results of curcumin nanoparticles were compared with plain curcumin administered in the same route to diabetic-induced rats on blood glucose levels, insulin, HbA1C, liver, and renal parameters. This study will shed a future scope to treat diabetics in humans without side effects.

Materials and Methods

Chemicals

Curcumin from Sigma Aldrich, Dimethylsulphoxide (DMSO) from Merck, Chitosan, tripolyphosphate (TPP), ethanol, glacial acetic acid, L6 cell line procured from NCCS, well plates- Techno Plastic from S V Scientific, penicillin, streptomycin, Fetal Bovine Serum (FBS), Dulbecco's modified eagle medium (DMEM), from Himedia, MTT reagent from Sigma Aldrich, acetonitrile of HPLC grade. All other chemicals were of analytical grade.

Preparation of Curcumin loaded Chitosan nanoparticles [20]

Curcumin was obtained from Sigma Aldrich and the CUR-NPs were prepared by ionic gelation method. Curcumin is poorly soluble in water; the curcumin solution was prepared by dissolving curcumin in dimethylsulphoxide and acetone. To enhance the solubility of curcumin two solvents were used instead of one solvent. This solution was added to the polymeric stabilizer (Chitosan) in glacial acetic acid solution under magnetic stirring at 500rpm, at room temperature (25±2ºC). The amino groups present in the chitosan interact with the methoxy hydroxyl groups in curcumin and form curcumin nanoparticles. To this, 3%w/v of tripolyphosphate in water was added to cross-link the desolvated Cur-NPs, and the cross-linking process was performed for over 24h. Tripolyphosphate (chelating agent) is a polyanion found to bind with the amino groups of chitosan and form a tight covalent bond, therefore the mobility of the chitosan is limited which increases the stability. The obtained suspension was subjected to differential centrifugation for 5 cycles and the pellet was dispersed to the original volume in distilled water. Each redispersion step was carried out using a bath sonicator. The obtained suspension was lyophilized using a freeze dryer. The Nanoparticles were collected and stored in a well-closed container until further use.

Characterization of Curcumin Nanoparticles

Characterization of CUR-NPs was evaluated by TEM analysis by diluting the samples with a suitable dispersion medium. Fourier Transforms Infrared (FT-IR) spectroscopy was performed to assess the compatibility of the drugs and the excipients. Moreover, Dynamic Light Scattering was performed to assess the average particle size, size distribution, and polydispersity index by Zetasizer (Malvern Instruments).

Evaluation of cytotoxic effects of optimized nanoparticles on rats.

For the cytotoxicity study, the L6 cell line (rat skeletal muscle cell line) was procured from NCCS, and the stock cell was cultured in DMEM medium supplemented with 10% inactivated FBS, penicillin (100 IU/ml), streptomycin (100 µg/ml) in a humidified atmosphere of 5% CO_2 at 37ºC until confluent.

Procedure [21]

100µl of cell suspension of density 1 × 10^4 cells/well were placed into each well of 96-well plates and incubated for 24h. After 24h, when a partial monolayer was formed, the supernatant was flicked off, washed the monolayer once with medium, and 100µl of different concentrations 6.25, 12.5, 25, 50, and 100µg/ml of the test sample (CUR, CUR-NPs, and Metformin) was added to the partial monolayer in microtiter plates. The plate was then incubated at 37ºC for 48h in a 5% CO_2 atmosphere. After incubation, the test solutions in the wells were discarded and 100µl of [3-(4, 5-dimethylthiazol-2-yl]- 2, 5-diphenyl tetrazolium bromide] MTT (1mg/ml of MTT in PBS) was added to each well. The plate was incubated for 4h at 37ºC in a 5% CO_2 atmosphere. The supernatant was removed and 100µl of DMSO was added and the plate was gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 570 nm. The MTT test was performed to assess the number of viable cells after 48hrs and the percentage of viability was calculated using the following formula:

% of viability = Sample abs/Control abs x 100

Glucose uptake by L6 Rat skeletal muscle cells [22]

The glucose utilization in L6 cells (Rat skeletal muscle cell line) was determined by the minor modification method described by van de Venter *et al.* 2018. The L6 cells were dislodged by brief exposure to 0.25% Trypsin in phosphate-buffered saline, counted, suspended in a new growth medium, and then seeded at a density of 6000 cells per well into a 96-well culture plate and allowed to adhere and grow in a humidified incubator with 5% CO_2 at 37˚C for 48 hrs. Two cell-free rows were served as blanks. On day three after seeding, without changing the medium, different concentrations (6.25, 12.5, 25, and 50 μg/ml) of test samples (CUR and CUR-NPs) and standard Metformin were added to each well. After 48 h incubation, the spent culture medium was removed by aspiration and replaced with a 25 μl incubation buffer (DMEM medium diluted with PBS, 0.1% BSA, and 8 mm glucose) and further

incubated for an additional 3 h at 37°C. The negative control (untreated) contained only the incubation buffer without samples. After incubation, 10 μl of the incubation medium was removed from each well and transferred into a new 96-well plate into which 200 μl of glucose oxidase reagent was added to determine the concentration of glucose in the medium. After 15min of incubation at 37°C, the absorbance was measured at 492 nm using a microtiter plate reader. The amount of glucose utilized was calculated as the difference between the untreated control and treated wells.

% Glucose uptake = [Absorbance of control cells - Absorbance of treated cells/ Absorbance of control cells] x 100

Animals

Six to eight weeks old healthy Wistar rats of either sex, weighing 180-220g were obtained from the animal house of C L Baid Metha College of Pharmacy, India, were employed for acute and sub-acute toxicity studies as well as glucose estimation and insulin by ELISA, HbA1c by immunological assay, liver, and kidney function by the standard assay kits. The animals were housed in polypropylene cages with a maximum of three animals of same sex per cage under standard laboratory conditions, room temperature at 25±2°C and relative humidity (55%±5%) was retained during a cycle of 12/12 dark and light cycle. Animals were kept on free access to water and diet for 12 hours of the experiment. All animal experiments were approved by the Institutional Animal Ethics Committee (IAEC) of C L Baid Metha College of Pharmacy, India (reference no. 02/321/PO/Re/S/CPCSEA).

Induction of diabetes [23]

All experimental rats were fasting overnight and diabetes was induced by single-dose administration of intraperitoneal injection of 50mg/kg Streptozotocin (Himedia) freshly prepared and dissolved in 0.1 mL sodium citrate buffer (pH 4.5). Streptozocin induces diabetes within 3-7 days. Glucose levels were measured using a glucometer by glucose oxidase and mono oxidase methods. Rats with high blood glucose levels >300 mg/dL were considered as diabetic and included for the study.

Experimental design

A total of 30 rats were categorized into five groups each consisting of 3 males and 3 females. Group I (negative control) was treated with saline, Group II (positive control- STZ 50mg/kg) was treated with saline, Group III (STZ 50mg/kg + 100mg/kg pure curcumin p.o.), Group IV (STZ 50mg/kg + 100mg/kg CUR NPs p.o.) and Group V (STZ 50mg/kg + 500mg/kg Metformin p.o.). The compound's treated groups (CUR, CUR-NPs and Metformin dissolved in distilled water) were administered orally once daily for 21 days from the 7th day after STZ injection to establish diabetes (Table 1). Blood samples were collected frequently at different time intervals (0, 7, 14, 21 and 28th days) and analyzed for blood glucose, insulin, HbA1c, and other biochemical parameters such as SGOT, SGPT, total bilirubin, serum urea, and serum creatinine were measured. Initial value and final valve were compared to measure the effectiveness of CUR, CUR-NPs and Metformin-treated groups with untreated groups.

Biochemical parameters

Serum blood glucose was estimated by the glucose oxidase and mono oxidase method using a glucometer, serum insulin concentration by enzyme-linked immunosorbent assay (ELISA), HBA1c by immunological assay, serum SGOT, SGPT, bilirubin, urea, creatinine by the standard assay kits (DiaSys, Germany, and EKF-diagnostic GmbH, Germany).

Statistical analysis

All data were analyzed using SPSS software, and the results were expressed as mean ±SD. Comparisons among the groups were made by ANOVA (analysis of variance). $P<0.05$ was considered statistically significant.

Results

Characterization of Optimized CUR-NPs

CUR-NPs were prepared successfully by the Ionic gelation method based on the optimization by the Box Behenken method. Optimized CUR-NPs were characterized by TEM and found that the particles were spherical in shape and had a narrow distribution of particles. Whereas PDI was found to be 0.321±0.045%. Further, FTIR showed absorption peaks between 400 and 4000cm^{-1}.

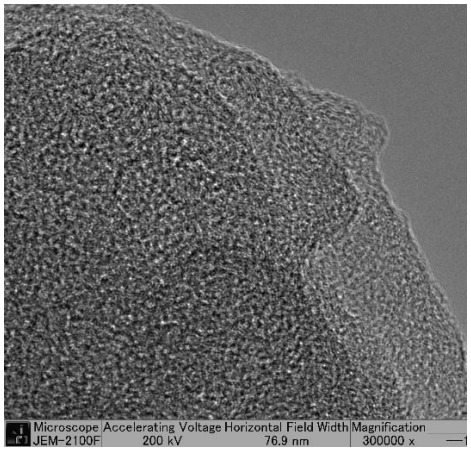

Figure 1. Characterization of CUR-NPs by TEM

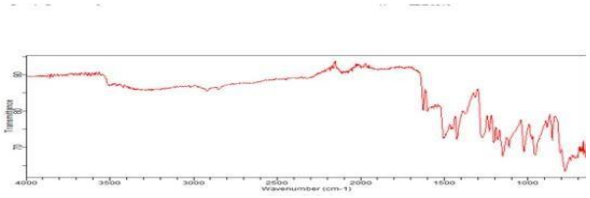

Figure 2. FTIR spectrum of CUR

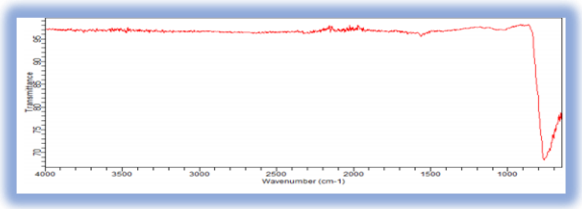

Figure 3. FTIR spectrum of Chitosan

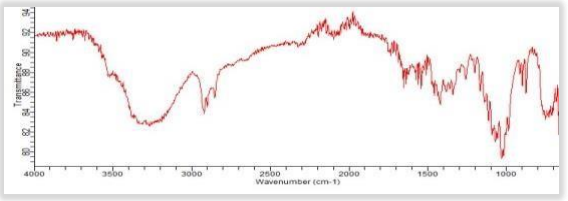

Figure 4. FTIR spectrum of CUR-NPs

FTIR spectrum of Curcumin shows stretching vibration at 1628 cm^{-1} attributed predominantly to the overlapping stretching vibrations of alkenes (C=C) and carbonyl (C=0) character. Infrared of curcumin ligand show stretching vibration at 3200-3500 cm^{-1} due to OH groups, C=C aromatic stretching vibration at 1427 cm^{-1}, and high-intensity band at 1512 cm^{-1} attributed to the mixed vibration including stretching carbonyl bond vibration ν(C=0), in-plane bending vibration around aliphatic δ CC-C, δ CC=0 and in-plane blending vibration around aromatic δ CC-H of keto and enol configurations and stretching vibration around aromatic νCC bonds of keto and enolic form of curcumin [24]. Furthermore, significant intense band at 1277 cm^{-1} attributed to the bending vibration of the ν(C-0) phenolic band. In the IR spectra of the [M(Cur)(phen)]NO3 complexes as shown in figure.3, the νC=0 band of the free curcumin is shifted from 1628 cm^{-1} to 1625-1598 cm^{-1} depending on the metal used [25,26]. The presence of an intense band at 1277cm^{-1} region which attributed to υ(CO) of phenolic group, confirmed the absence of –OH phenolic in the chelation process. The υ (OH) of the two phenolic groups in curcumin showed broad band in the 3200- 3500 cm^{-1} region. The IR spectra of the complexes exhibited new bands at 460-479 and 551-555 cm^{-1} are assigned to υ(M-O) and υ(M-N) stretching frequency, respectively [27]. The band due to ring vibrations of the uncoordinated phen observed at 1618 cm^{-1} was shifted to 1583- 1599 cm^{-1} in the complexes indicating the participation of phen in the coordination. We can observe the infrared spectrum oh chitosan. A strong band in the region 3291- 361 cm^{-1} corresponds to N-H and O-H stretching, as well as the intramolecular hydrogen bonds. The absorption bands at around 2921 and 2877 cm^{-1} can be attributed to C-H symmetric and asymmetric stretching, respectively. These bands are found in other polysaccharide spectra, such as xylan [26], glucan [28] and carrageenans [29]. The presence of residual N-acetyl groups was confirmed by the bands at around 1645 cm^{-1} (C=0 stretching of amide I) 1325cm^{-1} (C-N stretching of amide III), respectively.

Evaluation of cytotoxic effects of optimized nanoparticles on rats.

The toxicity of optimized CUR-NPs and CUR was examined in L6 rat skeletal muscle cell lines at different concentrations and were determined after 48 h. The viability of the samples was measured at different concentrations (100. 50, 25, 12.5 and 6.25μg/ml), the experiments were performedas triplicate and the values were expressed in terms of mean ±SD. The viability for curcumin was defined as (95.6, 86.1, 80.9, 76.6 and 69.7 %) at concentrations of (6.25, 12.5, 25, 50, and 100 μg/ml), for nano curcumin (93.97, 83.63, 77.40, 69.89 and 66.28 %) respectively. The viability of cells (L6 cells) for curcumin was found to be 69.7% at a concentration of

100 µg/ml and, for CUR-NPs 66.28%. On comparing both samples, CUR-NPs exhibited effective activity in the cell line. On increasing the concentration of CUR-NPs and curcumin the cell viability was found to decrease but no significant harmful effect on the cells. But the cell viability started to decrease with increasing concentrations. The values of nano curcumin at different concentrations were found to be closer to curcumin which indicates that nano curcumin is safer for healthy hepatocyte cells.

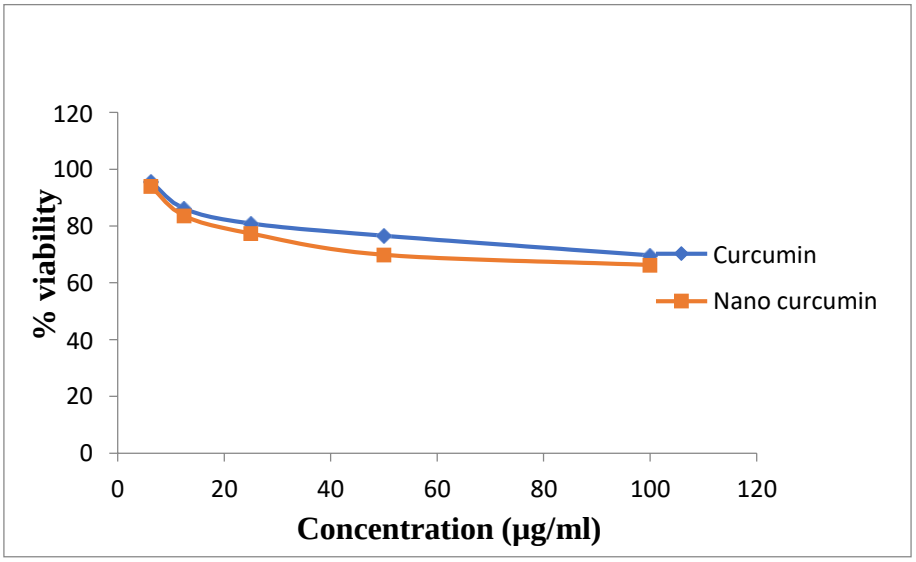

Figure 5. Evaluation of CUR-NPs and CUR cytotoxicity against L6 rat skeletal muscle cell line by MTT assay.

Effects of CUR-NPs and CUR on glucose uptake

In L6 rat skeletal muscle cells, different concentrations (6.25, 12.5, 25 & 50µg/ml) of CUR and CUR-NPs were assessed, and found that both lowered blood glucose levels at all the concentrations tested. Metformin was used as a positive control and was found to produce better stimulation of glucose uptake in L6 cells with a response of 36.92 (50µg/ml). whereas CUR stimulates glucose uptake by 20.90 and CUR-NPs by 25.79. Among various concentrations of CUR and CUR-NPs, 50µg/ml of encapsulated curcumin enhanced maximum glucose uptake. From this, it is well known that curcumin has beneficial effects on hyperglycemia in type 2 diabetes. L.-X. Na et al conducted a study on curcumin improves insulin resistance in skeletal muscles of rats and found that curcumin improved insulin resistance through increasing the oxidation of fatty acids and glucose [30].

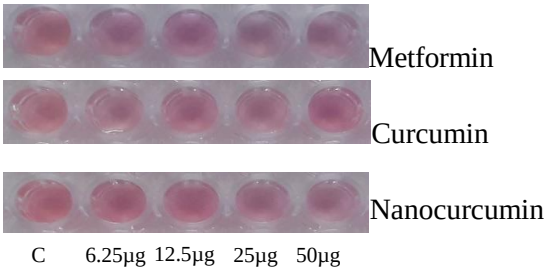

Figure 6. Effects of Metformin, CUR and CUR-NPs on glucose uptake

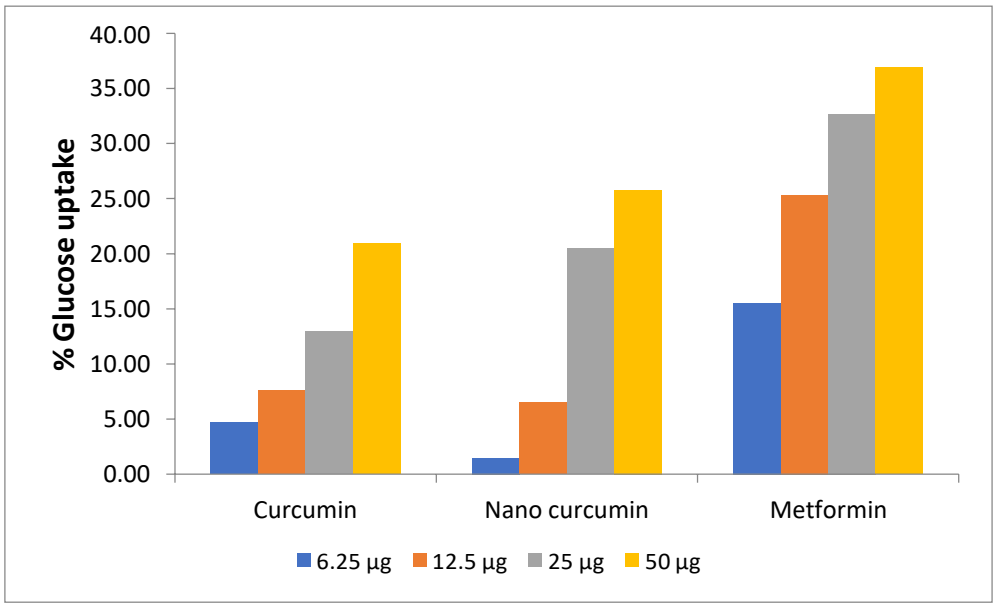

Figure 7. Effects of different concentrations of CUR, CUR-NPs and Metformin on glucose uptake

Effects of CUR, CUR-NPs, and Metformin on serum glucose, insulin, and HBA1c of STZ-induced diabetic rats

The effect of optimized CUR-NPs and CUR on the blood glucose levels, of STZ-induced diabetic rats, was presented in Table 1. After STZ administration, the mean values of blood glucose levels were taken at different time intervals (0, 7, 14, 21, and 28 days) and revealed that the mean blood glucose levels of Group II (untreated diabetic rats) were found to be higher at 328.07±4.55mg/dL during the entire period of the study. When compared with group I (non-diabetic control) blood glucose levels (103.44±4.04mg/dL), group II blood glucose levels were found to be higher (328.07±4.55mg/dL) and were statistically significant with p-value <0.001. The mean blood glucose levels of group III, group IV, and group V rats treated with 100mg/kg of CUR, 100mg/kg of CUR-NPs, and 500mg/kg Metformin once daily for 21 days were found to be reduced (335.67±8.62 to 198.33±4.16mg/dL, 334.67±10.97 to 176.33±4.51mg/dL and 321.33±2.65 to 162.21±2.14mg/dl). On comparing these values, it clearly indicated that curcumin administered in the form of nanoparticles was more efficient than plain curcumin in lowering blood glucose levels and the values were closer to the values of standard drug and found to be statistically significant with a p-value (<0.001).

Table 1: Effects of CUR-NPs and CUR on serum blood glucose of STZ-induced diabetic and control rats.

S.No	Treatment	Blood glucose level (mg/dl)				
		0 day	7 days	14 days	21 days	28 days
1.	Group I – Non-diabetic control	100.16± 3.31	102.90±4.55	109.54±5.05	104.50±4.97	103.44±4.04
2.	Group II – diabetic control [STZ 50mg/kg]	108±3.54	324.06±4.75	325.11±4.56	327.59±4.95	328.07±4.55
3.	Group III – STZ 50mg/kg+ pure CUR 100mg/kg	107.33±4.16	335.67±8.62	254.33±11.24	214.67±7.37	198.33±4.16
4.	Group IV- STZ 50mg/kg+	112.33±3.79	334.67±10.97	228.33±6.03	189.33±7.09	176.33±4.51

	optimized CUR NPs 100mg/kg					
5.	Group V-Metformin 500mg/kg/p.o	106.23±1.23	321.33±2.65	197.41±2.13	176.77±3.71	162.21±2.14

Values are expressed as mean±SD at n=6, P<0.05 is considered as statistically significant

In addition, the effect of nano curcumin on insulin levels, HbA1c, urea, creatinine, SGOT, SGPT, and bilirubin levels was also measured and shown in Table 2.

Table 2. Effects of CUR, CUR-NPs, and Metformin on Biochemical parameters of STZ induced diabetic and control rats.

Variables		Group I	Group II	Group III	Group IV	Group V
Insulin	Stat	13.31±0.23	9.12±0.42	8.21±0.25	8.47±0.27	8.01±0.11
	Final	13.21±0.43	8.57±0.31	9.21±0.22	10.24±0.25	8.95±0.21
Urea mg/dl	Stat	16.50±4.09	25.56±4.55	25.38±3.30	25.12±3.12	24.11±0.41
	Final	16.48±4.11	24.03±4.25	19.48±3.32	16.58±3.07	15.42±0.62
Creatinine mg/Dl	Stat	0.60±0.14	1.75±0.16	1.83±0.17	1.80±0.19	1.76±0.11
	Final	0.65±0.12	1.73±0.14	0.71±0.15	0.65±0.18	0.61±0.14
SGOT U/L	Stat	64.56±3.45	245.01±2.15	240.67±2.35	241.73±2.55	237.87±2.16
	Final	63.45±3.03	240.55±3.00	235.67±3.02	230.33±3.51	228.46±2.33
SGPT U/L	Stat	90.04±1.45	162.44±1.65	157.67±1.54	154.67±1.97	148.91±1.28
	Final	89.15±1.39	161.14±1.52	151.33±1.58	147.67±1.34	140.88±1.02
Total Bilirubin mg/dL	Stat	0.17±35	1.67±53	1.53±0.07	1.32±0.08	1.38±0.01
	Final	0.16±41	1.64±23	1.02±0.09	0.93±0.10	0.91±0.01
HBA1c %	5.33±0.32		9.18±0.21	8.87±1.32	7.16±1.35	6.21±1.22

Values are expressed as mean±SD, (n=6) for each group

As shown in Table 2, at the end of the study there were no improvements in the insulin levels in the untreated group. But in the treated group significant changes in insulin levels were observed. HbA1c values were high in treated groups compared to negative control. Likewise, a significant elevation in serum liver profile (SGOT, SGPT, bilirubin), and kidney functions (urea and creatinine) was found in STZ-induced rats compared to healthy rats (P<0.05). The opposite results were seen when the STZ-induced group was treated with CUR, CUR-NPs, and Metformin. A significant reduction in elevated serum liver profiles and creatinine indicated the effectiveness of the treatment with CUR, CUR-NPs, and Metformin.

Discussion

Diabetes mellitus is a global burden characterized by hyperglycemia resulting from beta cell dysfunction of pancreas islets. IDF declared that there is an urgent need for the government and policymakers to take necessary action. Though many drugs for the treatment of diabetes are available on the market, side effects of conventional therapy had diverted into traditional medicine, and plant-based medications have attracted researchers to invent new molecules or existing molecules for the treatment of chronic diseases including diabetes. Curcumin is a compound found to have various biological and pharmacological activities and the first article published on curcumin use in humans was by Oppenheimer in 1937 [31]. Yet it was used as an adjuvant and

as a first-line therapeutic agent in the treatment of chronic diseases because of problems associated with solubility and bioavailability. Though many researchers worked on curcumin there is no clear and sufficient data available for the treatment of diabetes. To overcome these problems small research was planned to prepare CUR-NPs with the biodegradable polymer and investigated the effects of blood glucose levels, insulin resistance, glycated hemoglobin, and liver and kidney functions in STZ-induced diabetic rats. According to the findings, diabetic-induced rats had significantly higher glucose levels compared to the negative control and the treated groups. This is because STZ is a broad-spectrum antibiotic taken up through the cell membrane GLUT2 glucose transporter and causes β cell death [32]. The reason behind hypoinsulinemia, STZ reduces the number of beta cells in the pancreas by producing reactive oxygen species (ROS), which is the major organ that secretes insulin [33]. In our study, we observed that CUR-NPs effectively lowered blood glucose values and improved insulin levels this may be through the inhibition of NF-kB inhibition that destroys the β cells, and the inhibition of NO synthesis. Our findings are consistent with the results of other studies. Chauan et al prepared curcumin-loaded chitosan nanoparticles and found that curcumin enhanced the glucose uptake in muscles by enhancing its solubility [34]. Zhang et al described that CUR enhances GLUT-4 translocation from intracellular compartments to the plasma membrane, and increases glucose uptake in muscle tissues [30]. Kim et al suggested that curcumin stimulates glucose uptake through the activation of AMPK in muscles. These findings enlighten us with a greater understanding of the anti-diabetic effects of curcumin [35]. Shamsi-Goushki et al reported that curcumin and nano curcumin was effective in decreasing insulin resistance and blood glucose levels in diabetic rats [36]. Mantzorou et al proved that CUR has been shown to improve insulin resistance and decrease plasma glucose levels in diabetic-induced rats [37]. In another randomized, double-blind, placebo-controlled clinical trial study by Chuengsaman et al, CUR significantly lowered blood glucose levels compared to the control group and delayed the development of type 2 diabetes [11]. Furthermore, seo et al stated that curcumin exerts anti-diabetic activity by inhibiting ROS synthesis thereby increasing the viability of beta cells [38]. Additionally, curcumin induces the opening of anion channels and depolarizes the beta cells of the pancreas, and increases insulin secretion [38]. In the present study, biochemical markers of the liver (SGOT, SGPT) and kidney (urea and creatinine) were found to be improved in animals treated with CUR and CUR-NPs. These biochemical markers are the indicators of hepatic/renal injury and toxicity. A similar study conducted by Shaymaa Abdulmalek et al stated that curcumin administered in the form of nanoparticles to diabetic-induced rats counteracted the oxidative stress and inflammation in the internal hepatic and renal tissues [21]. Curcumin and liposomal curcumin were found to have hepatoprotective and hypoglycemic effects in STZ-induced DM rats. The study also demonstrated better curcumin efficacy on hepatic enzymes [39]. In a study by G.M. Abu-Taweel et al CUR-NPs had slightly higher anti-diabetic effects compared to free CUR in rats and concluded that CUR-NPs had remarkable antidiabetic activity [40]. The outcome of Shehata et al revealed that CUR-MgO NP had a potential ameliorative effect on the hepatic metabolic alterations in type 2 diabetic rats [41]. Briefly, it clearly indicated that CUR and CUR-NPs are effective in lowering blood glucose levels, improving insulin levels, and improving liver and function kidney function. Compared to CUR, CUR-NPs were found to be more effective due to improved solubility and bioavailability.

Conclusion

According to our study results, it can be concluded that the administration of curcumin as nanoformulations enhances insulin sensitivity, and reduces blood glucose levels, serum liver enzymes SGOT, SGPT, and renal parameters such as urea and creatinine. Also, the effects of CUR-NPs on these parameters are more effective than CUR. The study suggested that the anti-diabetic activity of curcumin was improved with the help of nanotechnology. Nanomedicine can be used in humans without any harmful effects. More studies are required to bring curcumin as a first-line therapeutic agent in the treatment of chronic diseases like diabetes mellitus which is the major risk factor including COVID-19.

AUTHORS CONTRIBUTION STATEMENT

Mrs. K Shailaja designed the whole study including procedures, sample collection, and data analysis. Dr. S Madhu helped in conducting the animal study and Dr. K Senthilkumaran read and approved the final version of the manuscript.

CONFLICT OF INTEREST

The authors do not have any conflicts of interest

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