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MODULATORY EFFECT OF CURCUMIN NANOPARTICLES ON ISLETS OF LANGERHANS OF ALLOXAN INDUCED DIABETIC MICE

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Abstract: Over the past few years, nanotechnology has emerged in the field of medical science. Nanoparticles are used in targeted drug delivery systems. Curcumin, active principle of *Curcuma longa*, is used in many diseases due to its potential health benefits. However, it has poor bioavailability, poor solubility and low absorption. To overcome these problems, curcumin nanoparticles *i.e*. nanocurcumin was prepared by double emulsion solvent evaporation method. The present study revealed the effects of nanocurcumin on blood glucose level and histology of islets of Langerhans of pancreas in alloxan induced diabetic mice. 20 albino male mice were divided into 4 groups. (n=5): Control, Diabetic, Recovery I and Recovery II group. Control group was given subcutaneous injection of 0.15 M acetate buffer for 15 days. Subcutaneous injection of alloxan -150 mg/kg body weight was given to induce diabetes. Diabetic mice were given intraperitoneal injection of curcumin-150 mg/kg/day for 15 days in Recovery I group. Diabetic mice were given intraperitoneal injection of nanocurcumin-150 mg/kg/day for 15 days in Recovery II group. At the end of experimentation, blood glucose level and histology of pancreas was studied. In diabetic mice, blood glucose level was significantly increased, but after nanocurcumin administration, blood glucose level was decreased near to normal. Histological assessment of islets of Langerhans showed complete recovery of damaged cells and the restoration of the original size of the islets of Langerhans after nanocurcumin administration. This study showed that nanocurcumin had more curative effects than curcumin. Thus, nanocurcumin reduces blood sugar level and modulates the degenerative changes in islets of Langerhans of diabetic mice.

Keywords: Nanocurcumin, Islets of Langerhans, alloxan, histology

I. INTRODUCTION:

Diabetes mellitus [DM] is one of the major chronic non-infectious diseases in the world with a continuous enhancement in number of patients leads to a much more rigorous burden on the human population. There were 422 million people in the world suffering from the DM (Global Report on Diabetes, WHO, 2016). Hallmark feature of diabetes is elevated blood glucose concentrations due to destruction of pancreatic β-cells which are responsible for insulin production is associated with type 1 diabetes and through the failure of insulin responsiveness in its target tissues like adipose and muscle is associated with type 2 diabetes. Alloxan is a chemical compound used to induce experimental diabetes by leading the β cells of islets of the Langerhans to swell and finally degenerate.

Many unfavorable effects such as hypoglycemia, drug resistance and weight gain are caused due to various antidiabetic agents (Samreen, 2009; Tahrani *et al*., 2010). Considering such drawbacks, it is great deal in the development of more efficient and safer antidiabetic agents discovered from natural source. In Indian medicine system, it has been proved that a long time flora based medications are the major sources of drugs for the treatment of DM (Akhtar *et al.*, 1984), because herbal product exerts very less or no toxic effect and have great potential with less side effects as compared to modern medicine (Brinker, 1998).

Curcumin has been identified as the active principle of turmeric (*Curcuma longa*). Though curcumin has many beneficial effects against health problems, it has limited use due to its poor bioavailability as concluded by number of its pharmacokinetic studies. The poor solubility, instability in physiological fluids, low absorption and low bioavailability of curcumin are the major problems for achieving its optimum theranostic values. As a result, it has become very important to formulate an analogue of curcumin with better bioavailability in future (Rahimi et *al*., 2016).

Nanotechnology is a multidisciplinary scientific field which involves creation and utilization of materials, devices, or systems on the nanometer scale. Nanoparticles represent a promising drug delivery system of controlled and targeted release (Hoang *et al*., 2013). Recently many researchers are working on formulation of nanoparticles using polymer like poly [lactide-co-glycolic acid] [PLGA], because of its excellent properties like biocompatibility, biodegradability and high stability in biological fluids and during long term storage. It has been proved that PLGA nanoformulation of curcumin shows 22-fold greater bioavailability in rat studies as compared to conventional curcumin (Tsai *et al*., 2011). The present study was conducted to investigate the curative effect of curcumin nanoparticles i.e. nanocurcumin in diabetes mellitus.

II. MATERIALS AND METHODS:

A. MATERIALS:

Animals:

For present investigation male albino mice (*Mus musculus* Linn.) of 3 months age and weighing about 25-30 gm were used. All the animals were maintained in departmental animal house (CPCSEA/233), with optimal condition such as 12 hr light and 12 hr dark cycles at temperature of 26ºC ±2ºC. The animals were kept in standard plastic cages. (Dimensions-29 x 22 x 14 cm). Each group of animal consist 4-5 animals per cage provided with rice husk bed and proper care has been taken during course of experiment. The animals were supplied with standard and nutritive diet (Nutrinix std- 1020, Nutrivet life sciences, Pune) and water was given ad libitum. The experimental work and animal handling was conducted in accordance with the guidelines set by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India and all protocols of experiments were approved by the Institutional Animal Ethics Committee (IAEC).

Experimental Groups:

Mice were divided into 4 groups (n = 5)

1. Control Group:
   Three months male mice were given subcutaneous injection of 0.15 M acetate buffer for 15 days.
2. Diabetic Group:
   Three months male mice were given single subcutaneous injection of Alloxan 150 mg/kg body weight.
3. Recovery I Group:
   Diabetic mice were given intraperitoneal injection of Curcumin at a dose of 150 mg/kg/day for 15 days.
4. Recovery II Group:
   Diabetic mice were given intraperitoneal injection of Nanocurcumin at a dose of 150 mg/kg/day for 15 days.

B. METHODS:

Synthesis of Curcumin Nanoparticles i.e. Nanocurcumin:

Oil in water double emulsion solvent evaporation method was used for the preparation of Curcumin Nanoparticles i.e. nanocurcumin. (Jaiswal *et al*., 2004)

Blood Glucose Level:

Fasting blood glucose level was measured by collecting a drop of blood from tail vein of mouse using sharp scissor. Blood glucose level was measured using Accuchek blood glucose monitoring glucometer (Roche diagnostics India Pvt. Ltd.) The obtained results were expressed in terms of milligram per deciliter of blood (mg/dl).

Histological Study:

After completion of dose, mice from all groups were sacrificed by cervical dislocation and pancreas was removed by taking abdominal incision. Pancreas was fixed in 10% formalin solution. Fixed tissue further processed and embedded in paraffin wax. Sections were cut at 5 µ thickness and stained with Hematoxylin - Eosin. (Harris, 1986)

Statistical Analysis:

All values were expressed as mean ± SD. Statistical analysis was carried out by one-way ANOVA, Tukey's HSD test.

III. RESULTS:

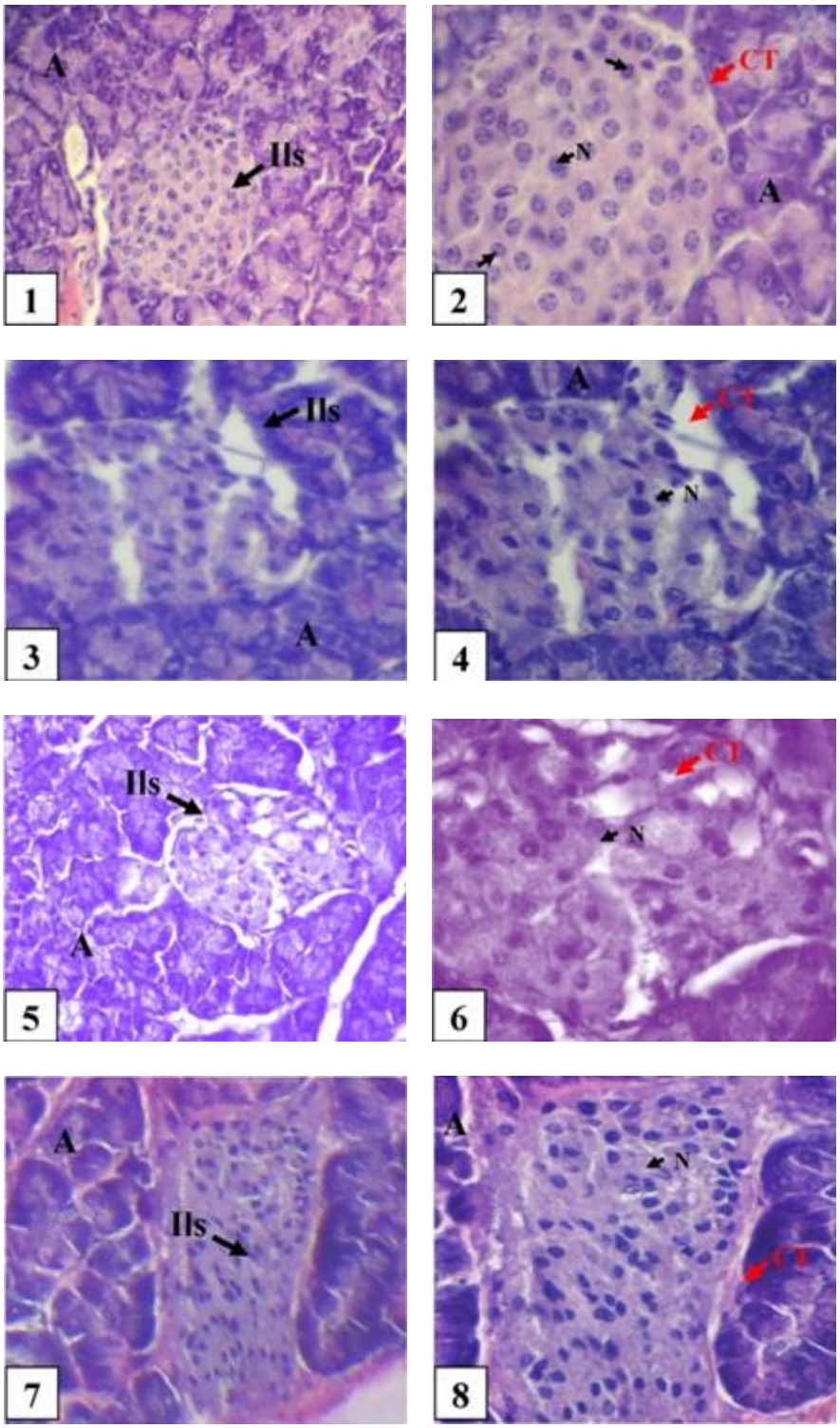
Measurement of fasting blood glucose level:

Table no. I: Effect of curcumin nanoparticles on blood glucose level (in mg/dl) of alloxan induced diabetic mice.

Sr. No.	Groups (n=5)	Blood glucose level in mg/dl	Statistical significance
1	Control	99.4 ± 5.5045	1:2 P<0.01 2:3 P<0.01 2:4 P<0.01 3:4 P<0.01
2	Diabetic	370.8 ± 59.5164	
3	Recovery I	130.2 ± 6.3797	
4	Recovery II	103.4 ± 6.3482	
P<0.01= Significant, P>0.05= Non significant			

Table no. I shows the effect of curcumin nanoparticles on blood glucose level (in mg/dl) of alloxan induced diabetic mice. The blood glucose level in alloxan induced diabetic mice group was significantly raised as compared to control group (1:2, P< 0.01). The blood glucose level in recovery group I was significantly decreased as compared to alloxan induced diabetic mice group (2:3, P< 0.01), whereas, the reduction in blood glucose level in recovery group II was also significant (2:4, P< 0.01). When blood glucose level of mice from recovery group-I and II group were compared, significant decrease in blood glucose in mice from recovery group- II was observed (3:4, P- P<0.01).

Effect curcumin nanoparticles on islets of Langerhans of pancreas in diabetic mice by Hematoxylin-eosin technique



Islets of Langerhans of Pancreas stained by Hematoxylin-Eosin (HE)

Figure 1 and 2: T.S. of Islets of Langerhans of pancreas of Control group mice stained with HE at 400X, 1000 X respectively.
Figure 3 and 4: T.S. of Islets of Langerhans of pancreas of Diabetic group mice stained with HE at 400X, 1000 X respectively.
Figure 5 and 6: T.S. of Islets of Langerhans of pancreas of Recovery-I group mice stained with HE at 400X, 1000 X respectively.
Figure 7 and 8: T.S. of Islets of Langerhans of pancreas of Recovery-II group mice stained with HE at 400X, 1000 X respectively.
Captions: A- Acinar cells, N- Nucleus, Ils- Islets of Langerhans, CT- Reticular Connective Tissue

Histological structure of pancreas of control group mice showed normal architecture with intact islets surrounded by thin layer of reticular connective tissue capsule separating it from adjacent exocrine tissue and maintains the integrity of islets. The islets of Langerhans appeared as lightly stained rounded clusters of islets cells. Islets of Langerhans show uniform rounded cells with darkly stained nuclei with violet colour and lightly stained cytoplasm with pinkish red colour. In alloxon induced diabetic mice group,

histological structure of pancreas showed destructed or damaged as well as relatively small and irregular structure of islets of Langerhans compared to control group. Mostly hydropic degeneration and degranulation in the cytoplasm was observed. The nucleus shows degenerative nature due to necrosis. While some of the cells were observed with pyknotic nuclei had a dark eosinophilic cytoplasm. The reticular connective tissue was destructed due to oxidative stress which loses their intact nature or integrity.

Histological observation of pancreas from recovery group I stained with hematoxylin-eosin showed slight recovered structure of islets with a large proportion of islet cells. Integrity of reticular connective tissue was recovered. Normal rounded structure of cells was observed with no any destructive changes. Islets were stained uniformly with dual stain as compared with alloxon induced diabetic mice. Sections of pancreas from recovery group II showed lightly stained elongated islets of similar size to that detected in control group. Pancreas tissue showed complete recovery of damaged cells and the restoration of the original size and shape of the islets to that of the control group. Also reticular connective tissue around islets was recovered and showed intact structure. Cells of islets uniformly stained with hematoxylin and eosin. Complete recovery of damaged islets and the restoration of the original size of the islets of Langerhans was observed after nanocurcumin administration than that of curcumin.

IV. DISCUSSION:

At present, the management of diabetes mainly involves a constant reduction in hyperglycemia by the use of thiazolidinedions, biguanides, sulfonylureas, D-phenylalanine derivatives, meglitinides and alpha glucoside inhibitors in addition to insulin. However, side effects and the efficacy of these compounds are debatable and there is a demand for new compounds for the treatment of diabetes (Thirunavukkarasu *et al.*, 2003). Hence plants have been recommended as a rich, yet unexplored source of potentially functional antidiabetic drugs.

Administration of curcumin to diabetic mice has been shown to lower blood glucose levels and partially restore the activities of key enzymes of carbohydrates and lipid metabolism to near normal levels in various animal models (Seo *et al*., 2008). Earlier studies have shown the presence of curcuminoide can contribute to improve health, insulin sensitivity, and lower blood glucose levels in animal models of diabetes (Zhang, 2012). In the present study curcumin and curcumin nanoparticles at a dose of 150 mg/kg/day for 15 days lowered the fasting blood glucose level as compared to diabetic group.

In the present research work, the treatment of mice with alloxan only at dose level of 150mg/kg produced signs of disintegration of islets cells. Abunasef, 2006 demonstrated that the vacuolation were appeared in the centre of islets after the treatment of male rat with alloxan at dose level of 180mg/kg. It induced brutal degenerative changes of islet cells with diverse grades of nuclear degeneration. These changes were occupying mostly the centre of islets. The dense connective tissue stroma was observed around some pancreatic ducts and blood vessels. Benoit- Bancamano *et al.,* 2005 reported that the pancreatic lesions are consisting of diffused vacuolation of islets of Langerhans with both acute and chronic onset of diabetes. In the present investigation, the alloxan induced diabetic mice showed apparent reduction of size and number of islets. In this study, it is important to note that administration of curcumin and curcumin nanoparticles to diabetic mice i.e. in recovery-I group and recovery-II group, respectively had recovered many of the light microscopic alterations of pancreatic islets. Recovery-II group showed increased size and number of islets as compared to Recovery-I group. This also suggests that a curcumin nanoparticles at 150mg/kg dose has the ability of inducing the quiescent cells to proliferate to replace the lost cells as compared to curcumin alone at 150 mg/kg.

V. CONCLUSION:

Reduction in fasting blood glucose level and restoration of islets of Langerhans upon curcumin nanoparticles administration showed more curative effects than Curcumin.

VI. ACKNOWLEDGEMENT:

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