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RESEARCH ARTICLE

Nano-curcumin: A potent enhancer of body antioxidant system in diabetic mice

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Abstract

Nano preparation of drug to be helpful in targeted delivery, which avoids any unwanted damage of adjacent healthy tissues. Antidiabetic compounds from natural and synthetic sources have been found to successful management of diabetes. Antioxidants are compound that protect cell against the damaging effects of reactive oxygen species (ROS). 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. Since the aim of this study was to investigate the effectsof curcumin nanoparticles (Nano-curcumin) on antioxidative enzymes i.e Glutathione peroxidase (GPx), Superoxide dismutase (SOD) and Catalase (CAT) in pancreas of diabetic mice. For the present investigation mice (*Mus musculus)* used as experimental animal.Mice were divided into four groups viz, a) Control group b) Diabetic group c) Recovery group I- Diabetic mice treated with curcumin d) Recovery group II - Diabetic mice treated with curcumin and nano-curcumin. The activity of antioxidative enzymes in the pancreas was recorded at the end of experiment. There was decrease in antioxidative enzymes in pancreas of diabetic mice compared to control. After the treatment of curcumin and curcumin nanoparticles significant increase in levels of antioxidative enzymes in recovery group I and II was observed. Moreover as compare to free curcumin nano-curcumin showed better results in enhancement of antioxidative enzymes. Thus it proves that nano-curcumin found to be potent antioxidative compound to reduced oxidative stress induced during the diabetes.

Keywords: Nano-curcumin; Antioxidative enzymes; Pancreas; oxidative stress; ROS

Introduction

Nanotechnology is the advance branch of science which has been implemented in agriculture, food and medical science and its related products. Nano preparation of drug to be helpful in targeted delivery which avoids any unwanted damage of adjacent healthy tissues. [1-3]. Various studies showed that nanoparticles are smoothly enters through cell membranes in organisms and get interacted quickly with biological systems because of its number of advantages such as targeted drug delivery, bioavailability, less immunogenicity and also overcomes traditional therapy problems like less bioavailability and adverse effects [4,5].

Diabetes mellitus (DM) is a disorder having critical priority because of its worldwide appearance by the International Diabetes Institute (IDI) [6]. It is estimated that about 300 million (5.4%) adult population worldwide have this disease by the year 2025 [7]. Diabetes is ranked third after cancer, cerebrocardiovascular diseases in the list of epidemic diseases. Diabetes also known one of the economic disorder because it puts severe financial burden on the victim and their family concerns.

Oxidative damage to DNA, proteins and lipids can ultimately lead to outcomes such as disorganization, dysfunction and destruction of membranes, enzymes and proteins. Specifically, peroxidation of membrane lipids may cause impairment of membrane function, decreased fluidity, inactivation of membrane bound receptors and enzymes, increased permeability to ions and possibly eventually membrane rupture. The oxidative stress is particularly severe it can produce cell death.

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In the diabetic state increased oxidative stress results in rapid damage of islets as compare in other cells [8-10] it is due to the low expression of antioxidant enzymes such as SOD, CAT and GPx as compared with other tissues [11], it means that particularly the β-cells are susceptible to or weakly protected against oxidative stress. Hyperglycemia increases glucose load in diabetic case results in efficient production of reactive oxygen species indicating hyperglycemia is a major cause of ROS generation [12, 13] and protein glycation [14] which causes increase in oxidative stress leads to development of diabetic related complications [15-18]. Lipid peroxidation results in formation of Malondialdehyde [MDA], which is a secondary product of this process and MDA is known to cause cross-linkage of membrane entities containing amino group's results in the membrane fragile [19].

Alloxan is responsible for necrosis of beta cells of pancreas and induces free radicals production which leads to pathogenesis of both experimental and human diabetes mellitus [20]. Various scientists reported that H_2O_2 and free radicals like O_2 and OH produced due to alloxan which are responsible for cellular damage and death. Hence, alloxan was considered sufficient for the study of pathology of DM [21]. Alloxan easily and rapidly accumulates in pancreatic beta-cell [22] and accumulated alloxan is responsible for abnormal change in membrane potential and ion channels in pancreatic beta-cells [23].

Antioxidant play a crucial job in the scavenging the free radical, neutralizes ROS and defend against oxidative stress in human body [9]. Superoxide dismutase (SOD), Catalase (CAT) and Glutathion peroxidase (GPx) are endogenous antioxidant enzymes which are responsible for neutralization of harmful oxygen radicals [24].

It has been reported that many herbs and plants possess hypoglycemic activity when taken orally [25]. Many medicinal plants shows antidiabetic prospective or bioactive compounds such as glycosides, alkaloids, terpenoids, carotenoids and flavonoids are confirmed to be effective in both preclinical and clinical studies [26, 27]. Curcumin is component of turmeric which is responsible for yellow coloration of turmeric [28]. It is an active component of the perennial herb *Curcuma longa*. Curcumin is a popular spice in Asian cuisine whose beneficial effects on glycemic control have been used in ayurvedic [29]. Therefore this study was carried out to evaluate antidiabetic potential of curcumin nanoparticles on alloxan induced diabetic mice.

Materials and Methods

Chemicals used

Alloxan was purchased from Sigma-Aldrich Company (India). All experimental chemicals were used of analytical grade and purchased from Sigma-Aldrich (India).

Preparation of Curcumin nanoparticles

Curcumin nanoparticles were synthesized by drug encapsulation method described by Jaiswal *et al.* (2004) [30].

Experimental animal

In present research work, healthy Swiss albino male mice (*Mus Musculus*) of 3-4 months age and weighing about 35-45 gm were used. Mice were maintained in departmental animal house (1825/PO/EReBi/S/15/CPCSEA) under standard laboratory conditions 12:12 hr L: D cycle light, 21$\pm$2ºC temperature and 55$\pm$5% relative humidity. Mice were fed by standard rodent pelleted diet Nutrinix std-1020 (Nutrivet Life Sciences, Pune) and water *ad libitum*. Prior to study all approvals related to animal study were taken by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India. All these animals were maintained and treated as per guidelines set by the Institutional Animal Ethical Committee (IAEC).

Experimental design 24 male mice were used in present investigation. Mice were divided into four groups and 6 animals kept in each.

Control group: Mice were fed standard diet throughout the experiment and injected with 0.5 ml citrate buffer intraperitoneally (IP), pH 4.5.

Diabetic group: Mice were injected with single dose of Alloxan (150mg/kg body weight) intraperitoneally (IP); in citrate buffer; pH 4.5

Recovery group I (Alloxan + Curcumin group): Diabetic mice were given curcumin (150mg/kg body weight, dissolved in 0.5ml citrate buffer, pH 4.5) intraperitoneally for 20 days.

Recover group II (Alloxan + Curcumin nanoparticles): Diabetic mice were given curcumin nanoparticles (150mg/kg body weight, dissolved in 0.5ml citrate buffer, pH 4.5) intraperitoneally for 20 days.

After 20 days of treatment, the mice were kept in fasting condition for overnight. Blood glucose was measured using glucometer (Accucheck). After which the mice were sacrificed by cervical dislocation. Pancreatic tissue were dissected out, weighted and used for biochemical analysis.

Biochemical analysis

i. Superoxide Dismutase (SOD) Assay Superoxide dismutase assay was carried out according to method Beauchamp and Fridovich (1971) [31]. The substrate NBT reduced to blue colour formazone dye by superoxide radical. The amount of colour formed was measured at 560 nm on UV-spectrophotometer (Shimadzu). One Unit (U) of SOD is defined as the amount of enzyme required to inhibit NBT by 50%. The calculated SOD activity was expressed as Unit SOD/mg protein.

ii. Catalase (CAT) Assay Catalase assay was carried out by Luck method (1974) [32]. The enzyme source (0.05ml) was added to the reaction mixture containing 3ml phosphate buffer (pH 7.0), hydrogen peroxide (H_2O_2) and the enzyme activity was measured at 240nm on UV-spectrophotometer (Shimadzu). The activity of the enzyme is expressed in unit enzyme/mg protein.

iii. Glutathione peroxidase (GPx) assay GPx assay was carried out by Beers and Sizer method (1952) [33]. All the procedure was same as that of estimation of Catalase. The reaction mixture contained 3 ml of phosphate buffer with H2O2 and 0.05 ml enzyme source, 0.01 ml of sodium azide (1mM) was added to inhibit Catalase activity. The absorbance was measured on UV-spectrophotometer (Shimadzu) at 240 nm. Activity of GPx expressed in unit/mg protein.

iv. Lipid peroxidation assay Lipid peroxidation level was determined by Thiobarbituric Acid (TBA) reaction according to Wills (1966) method [34]. Tissue homogenate (2mg/ml) were prepared in chilled mortar and pestle using 75mM potassium phosphate buffer pH 7.0. Malondialdehyde (MDA) is the end product of fatty acid peroxidation, reacts with TBA gives pink colored complex which has maximum absorbance at 532 nm. The concentration of MDA was expressed as nmol MDA/mg wet tissue.

Statistical Analysis Statistical analysis was done by one-way ANOVA, Turkey's HSD test and all values were expressed as mean $\pm$SD.

Results

The effect of curcumin nanoparticles on pancreatic tissue antioxidant was studied. In table 1, Significant decreased activity of SOD, CAT and GPx were observed in pancreas of diabetic mice as compare to control group mice (1:2, P<0.01). Whereas after treatment of curcumin nanoparticles for 20 days the antioxidants were increased significantly by reducing oxidative stress in recovery group II as compared to diabetic group (2:3, P<0.01). This increase was twofold as compare to diabetic group mice. Also curcumin nanoparticles shows good efficiency in oxidative stress as compare to only curcumin treated mice i.e. recovery I group (3:4, P<0.01).

In table 2, Blood glucose level of diabetic groups is significantly increased due to oxidative stress of alloxan as compare to control (1:2, P<0.01). While after curcumin nanoparticle treatment for 20 days the blood glucose level was significantly decreased in recovery group II as compared to diabetic group (2:3, P<0.01). Along with this lipid peroxidation was also found to be significantly raised in diabetic group as compare to control group (1:2, P<0.01). Whereas after curcumin nanoparticles treatment for 20 days the significant decreased in lipid peroxidation was observed in recovery group II as compared to diabetic group (2:3, P<0.01). This decrease was also significant as compare to curcumin treated mice i.e. recover group I (3:4, P<0.01).

Discussion

Reactive oxygen species (ROS) causes oxidative stress results in damage to pancreatic and liver cells subsequently leads to diabetes mellitus [35, 36] and [37]. Many traditional plants have been reported that these are effective agents with hypoglycaemic and antioxidative properties in diabetic mice. In present research we had synthesize curcumin nanoparticles for the treatment of diabetes due to poor bioavailability and slow effects of curcumin shown by various workers. The poor solubility, instability in physiological fluids, and low bioavailability of curcumin are the major obstacles for achieving its good results [38]. Therefore, the present investigation was carried out to find out the antidiabetic and antioxidative outcomes of curcumin nanoparticles for treatment of diabetes.

Lenzen et al., 2008 reported that decrease in insulin secretion as well as glucose uptake by body cells leads to rise in blood glucose, cholesterol and triglycerides and protein contain was decrease results from because of alloxan induced beta cell destruction [37]. Increased blood glucose level responsible for generation of reactive oxygen species, which cause lipid peroxidation and membrane damage, also increases oxidative stress in many organs, especially in the pancreas and liver [39]. In our study, curcumin nanoparticles treatment at a dose 150 mg/kg body weight/day for 20 days significantly decreases fasting blood glucose level in recovery group II compared to diabetes group. This clearly shows that there may be fortification of β cells from oxidative damage and stimulation for increase in insulin secretions.

Presence of endogenous antioxidative system like SOD, CAT and GPx and non-endogenous system such as vitamins E and C in DM can protect and eradicates ROS and boost antioxidative response in body[40][41]. ROS has been known to produce cellular and tissue injury through covalent binding, DNA strand breaking, lipid peroxidation (LPO) and augment fibrosis. In the

Table 1 Effect of Curcumin nanoparticles onactivity of superoxide dismutase (SOD), Catalase (CAT) and Glutathioneperoxidase (GPx) (Enzyme activity expressed in unit/mg protein) in Pancreas of alloxan induceddiabetic mice. Values are mean ±S.D(Numbers in parenthesis denotes number of animals).

Sr. No	Group (n=6)	SOD activity	Statistical Significance	CAT activity	Statistical Significance	GPx activity	Statistical Significance
1.	Control	42.7328± 1.726	1:2, P<0.01	1.1194± 0.0417	1:2, P<0.01	2.3669± 0.1593	1:2, P<0.01
2.	Diabetic	26.7480± 3.285	1:4, non significant	0.8260± 0.0477	1:4, non significant	1.6850± 0.2052	1:4, non significant
3.	Recovery I	35.4200± 1.810	2:3, P<0.01	0.9479± 0.0537	2:3, P<0.01	2.0733± 0.0737	2:3, P<0.01
4.	Recovery II	43.9618± 1.477	3:4, P<0.01	1.0545± 0.0529	3:4 P<0.01	2.3284± 0.0316	3:4, P<0.01

P<0.01=Significant, P>0.5= Non significant

Table 2 Effect of curcumin nanoparticles on bloodglucose, Lipid peroxidation in Pancreas of alloxan induced diabeticmice. Values are mean ±S.D (Numbers inparenthesis denotes number of animals).

Sr. No	Group (n=6)	Blood glucose (mg/dl)	Statistical Significance	Lipid Peroxidation in Pancreas	Statistical Significance
1.	Control	99.4 ± 5.5045	1:2, P<0.01	28.6624 ± 4.0534	1:2, P<0.01
2.	Diabetic	370.8 ± 59.5164	1:4, non significant	53.5923 ± 6.5360	1:4 non significant
3.	Recovery I	130.2 ± 6.3797	2:3, P<0.01	40.7808 ± 4.3088	2:3, P<0.01
4.	Recovery II	103.4 ± 6.3482	3:4, P<0.01	33.2483 ± 4.7961	3:4, P<0.01

P<0.01=Significant, P>0.5= Non significant

present investigation level of MDA was increased and activities antioxidative enzymes i.e. SOD, CAT and GPx was decreased in pancreas of diabetic mice similar results suggested by Daunde et al., 2018 in Trigonelline nanoparticles treatment in HFD-STZ induced diabetic mice [42]. This results from may be due to the production of ROS that can responsible for dysfunctioning of these enzymes and decreased enzymatic antioxidant levels in the pancreas of mice. Administration of curcumin nanoparticles to diabetic group significantly decreased the levels of lipid peroxidation and increased the activity of SOD, CAT and GPx by safeguarding pancreas from ROS. This showed that free radical decreasing ability of curcumin nanoparticles could put forth an advantageous action against oxidative stress.

Alkaloid and flavonoids are known potential antioxidant in the treatment of alloxan induced oxidative stress diabetic [43]. Curcumin is a polyphenolic compound reduces glycemia and hyperlipidemia as well as due to its anti-inflammatory and antioxidant properties it has beneficial effects on diabetic complications [44]. It is possible that the reduction in alloxan induced oxidative stress in pancreas of recovery group II is chiefly due to its antioxidant activity of curcumin nanoparticles. Curcumin nanoparticles may act by scavenging ROS metabolites due to the presence of antioxidative property or by increasing the level of endogenous antioxidant enzymes.

Conclusion

The present investigation suggests that the curcumin nanoparticles enhance body antioxidant activity and develop antioxidant status, which may have hypoglycemic properties with protective effect on pancreatic tissues against oxidative stress. Moreover based on the outcome of biochemical analysis curcumin nanoparticles supposed to be considered as the best medication on diabetes.

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