Nano-scaling of Trigonelline Improves Antioxidative Status of hfd-stz Induced Diabetic Mice.

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Abstract: In medical sciences nanotechnology helps us to produce nano-scale medication or medicine. 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. In present investigation we synthesized Trigonelline nanoparticles (TNPs) successfully. Trigonelline is the active plant alkaloid chemically known as N-methylnicotinic acid (C7H7NO2) which was first isolated from the fenugreek seed. Diabetic Mellitus is a metabolic disease associated with impaired uptake and metabolism of glucose. Hyperglycemic condition in diabetic case leads oxidative stress in body and finally results in damage to the various biomolecules in the body such as lipid protein and nucleic acid. The present investigation was aims to study the antioxidative effect of TNPs on Streptozotocin induced diabetic mice (Mus musculus). Mice were divided into three groups viz, a) Control group (Group I) b) Diabetic group (Group II) c) Recovery group (Group III). Diabetic mice treated with TNPs 100 mg/kg body weight intraperitoneally. The activity of antioxidative enzymes i.e. Superoxide dismutase (SOD), Catalase (CAT), Glutathion peroxidase (GPx) and lipid peroxidation in the liver and pancreas was recorded at the end of experiment. There was decrease in antioxidative enzymes and increase in lipid peroxidation in pancreas of diabetic mice as compare to control (Group I). After the treatment of TNPs significant increase in levels of antioxidative enzymes and significant decrease in lipid peroxidation in liver and pancreas of recovery group (Group III) was observed. The study suggest that nano-scaling of trigonelline reduces oxidative stress improves antioxidative status of diabetic mice.

Keywords: Nanotechnology, nanoparticles, trigonelline, TNPs, antioxidants.

1. INTRODUCTION

In medical sciences nanotechnology helps us to produce nano-scale medication or medicine. In medical science these nano-scale medication or devices can smoothly enters through blood vessel walls, or even penetrates through most of body cells membranes because of its nano-scale formulation up to 20-50 nm. Nanoformulation of drug to be used in targeted delivery, which avoid any unnecessary involvement or damage of surrounding healthy tissues [1] [2] [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 recognised as critical priority because of its epidemic nature 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]. In ranking of epidemic diseases DM comes at third position after cancer, cerebrovascular and cardiovascular diseases. It is not only killer of lives but also puts severe financial burden on the victim and their family concerns [8]. Diabetes mellitus (DM) is a common metabolic as well as multifactorial disease with many side effects [9] [10]. It is characterized by hyperglycemic conditions i.e. elevated blood glucose level resulting in insulin resistance along with insulin hyposecretion caused due to the failure of pancreatic beta cells [11]. Diabetes also responsible for abnormal protein and lipid metabolism [12]. Hyperglycemia increases glucose load in diabetic case results in excess production of reactive oxygen species, indicating hyperglycemia is a major cause of ROS generation [13] [14] and protein glycation [15] which causes increase in oxidative stress leads to development of diabetic related complications [16] [17] [18] [19]. Streptozotocin (STZ) is a secondary metabolite synthesized by Streptomycyes achoromagens. Food and Drug Administration (FDA) approved STZ as a therapeutic drug in the treatment of pancreatic cancer [20]. It is responsible for nitric oxide production, alkylation and DNA fragmentation in beta cells of pancreas which leads to inhibition of glucose induced insulin secretion by beta cells of pancreas. Because of this mechanism of STZ, it is widely used in experimental diabetic studies [21]. Imbalance between...
radical generating and radical scavenging mechanism results in increase production of ROS [22] [23]. DM is responsible for production of lipid peroxides and causes impairment in glutathione metabolism as well as defects in the antioxidant enzymes [24] [25]. Antioxidant play a crucial job in the scavenging the free radical, neutralizes ROS and defend against oxidative stress in human body [26]. Superoxide dismutase (SOD), Catalase (CAT) and Glutathione peroxidase (GPx) are endogenous antioxidant enzymes which are responsible for neutralization of harmful oxygen radicals [27]. Hence, the drugs having antidiabetic and antioxidant property useful for the treatment of the diabetic patient [28]. Thiazolidinediones, Sulfonylureas, biguanides, alpha-glucosidase and meglitinides are allopathic drugs existing in the market are used for the treatment of diabetes and associated complications. For management of diabetes these drugs play a crucial role, but have boundaries due to undesirable side effects such as weight gain, hypoglycemia, and inability to prevent pancreas degeneration [29] [30]. Therefore, it is intense need to find out newer anti-diabetic agents that have high therapeutic effectiveness with least side effects [31].

Trigonelline is the active plant alkaloid chemically known as N-methyl nicotinic acid (C7H7NO2) which was first isolated from the fenugreek seed. Number of previous studies with good results showed that trigonelline have antidiabetic [32], antioxidative, antiviral, antibacterial, antimigraine, antihyperlipidimic properties and also beneficial in prevention and treatment of central nervous system diseases [33] [34]. It is reported that trigonelline suppresses oxidative stress in kidney in STZ induced neonatal diabetic rats [35]. Therefore this study was carried out to evaluate antidiabetic potential of TNPs on STZ-induced diabetic mice.

II. MATERIALS AND METHODS

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

B. Preparation of TNPs
TNP’s were synthesized by drug encapsulation method described by Jaiswal et al (2004) [36].

C. Experimental animal
In present study, healthy Swiss albino male mice (*Mus Musculus*) of 3-4 months age and weighing about 35-45 gm were used. Mice were kept in departmental animal house (1825/PO/ERReBi/S/15/CPCSEA) under standard laboratory conditions 12:12 hr L: D cycle light, 21±2ºC temperature and 55±5% relative humidity. Mice were fed by standard rodent pelleted diet Nutrinix std-1020 (Nutrivet Life Sciences, Pune), High fat diet (HFD) (VRK nutritional solutions, Sangli.) 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).

D. Experimental Design
18 adult mice were used in present experiment. Animals were divided into three groups and 6 animals kept in each.

1) Control group: Mice were injected with 0.5 ml citrate buffer intraperitoneally (IP), pH 4.5 for 15days.
2) Diabetic group (HFD+STZ): Mice were injected with multi low dose of Streptozotocin (STZ) (40mg/kg body weight) intraperitoneally (IP); in citrate buffer; pH 4.5 for five consecutive days.
3) Recovery group (HFD+STZ+TNPs): Diabetic mice* were given TNPs (100mg/kg body weight, dissolved in 0.5ml citrate buffer, pH 4.5) intraperitoneally for 15 days. (*The development of hyperglycemia in mice was confirmed by elevated fasting blood glucose (FBG) level after one weeks of STZ injection. The mice having FBG higher than 200mg/dl were considered as diabetic and selected for studies. The TNPs treatment was started after diabetes confirmation.)

After 15 days of TNPs treatment, the mice were fasted overnight. Blood glucose levels were measured. Then the mice were sacrificed by cervical dislocation. Liver and pancreas were dissected out, weighted and used for biochemical analysis.

E. Biochemical Analysis

1) Superoxide Dismutase (SOD) Assay: Superoxide dismutase assay was done carried out according to method Beauchamp and Fridovich (1971) [37]. 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.

2) Catalase (CAT) Assay: Catalase assay was carried out by Luck method (1974) [38]. The enzyme source (0.05ml) was added to the reaction mixture containing 3ml phosphate buffer (pH 7.0), hydrogen peroxide (H2O2) and the enzyme activity was measured at 240nm on UV-spectrophotometer (Shimadzu). The activity of the enzyme is expressed in unit enzyme/mg protein

3) Glutathione peroxidase (GPx) assay: GPx assay was carried out by Beers and Sizer method (1952) [39]. 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.

4) Blood glucose: Fasting blood glucose was measured by collecting a drop of blood from the tail after incision with a sharp blade. The blood glucose level was determined by using a rapid glucose analyzer with a glucose strip inserted in Accuchek blood glucose monitoring glucometer (Roche diagnostics India Pvt. Ltd.). The results were expressed in terms of milligram per deciliter of blood [40].

5) Lipid peroxidation assay: Lipid peroxidation level was determined by Thiobarbituric Acid (TBA) reaction according to Wills (1966) method [41]. 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.

F. Statistical Analysis
Statistical analysis was carried out by one-way ANOVA, Turkey’s HSD test and all values were expressed as mean ±SD.

III. RESULTS
Table No I: Effect of TNPs on activity of superoxide dismutase (SOD), Catalase (CAT) and Glutathione peroxidase (GPx) (Enzyme activity expressed in unit/mg protein) in Liver of STZ induced diabetic mice. Values are mean ±S.D (Numbers in parenthesis denotes number of animals).

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Group (n=6)</th>
<th>SOD activity</th>
<th>Statistical Significance</th>
<th>CAT activity</th>
<th>Statistical Significance</th>
<th>GPx activity</th>
<th>Statistical Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>68.4987 ±2.024</td>
<td>1:2, P&lt;0.01</td>
<td>11.0048 ±0.2373</td>
<td>1:2, P&lt;0.01</td>
<td>11.8002 ±0.1608</td>
<td>1:2, P&lt;0.01</td>
</tr>
<tr>
<td>2</td>
<td>Diabetic</td>
<td>37.4857 ±5.9604</td>
<td>1:3, non significant</td>
<td>3.961 ±0.1391</td>
<td>1:3, non significant</td>
<td>6.0538 ±0.2592</td>
<td>1:3, non significant</td>
</tr>
<tr>
<td>3</td>
<td>Recovery</td>
<td>68.5257 ±2.2091</td>
<td>2:3, P&lt;0.01</td>
<td>9.207 ±0.1966</td>
<td>2:3, P&lt;0.01</td>
<td>9.8381 ±0.4896</td>
<td>2:3, P&lt;0.01</td>
</tr>
</tbody>
</table>

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

Table No II: Effect of TNPs on activity of superoxide dismutase (SOD), Catalase (CAT) and Glutathione peroxidase (GPx) (Enzyme activity expressed in unit/mg protein) in Pancreas of STZ induced diabetic mice. Values are mean ±S.D (Numbers in parenthesis denotes number of animals).

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Group (n=6)</th>
<th>SOD activity</th>
<th>Statistical Significance</th>
<th>CAT activity</th>
<th>Statistical Significance</th>
<th>GPx activity</th>
<th>Statistical Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>46.3889 ±9.3092</td>
<td>1:2, P&lt;0.01</td>
<td>2.9415 ±0.0603</td>
<td>1:2, P&lt;0.01</td>
<td>2.4986 ±0.1612</td>
<td>1:2, P&lt;0.01</td>
</tr>
<tr>
<td>2</td>
<td>Diabetic</td>
<td>28.5344 ±5.4564</td>
<td>1:3 non significant</td>
<td>1.2485 ±0.2644</td>
<td>1:3, non significant</td>
<td>1.3591 ±0.1519</td>
<td>1:3, non significant</td>
</tr>
<tr>
<td>3</td>
<td>Recovery</td>
<td>59.5692 ±9.8668</td>
<td>2:3, P&lt;0.01</td>
<td>2.6555 ±0.1358</td>
<td>2:3, P&lt;0.01</td>
<td>2.2016 ±0.1891</td>
<td>2:3, P&lt;0.01</td>
</tr>
</tbody>
</table>

P<0.01=Significant, P>0.5= Non significant
Lipid peroxides and hydroperoxides are the secondary products of oxidative stress and are the positive effects of specific plant on T2DM. In present investigation we observed that diabetes mellitus can scavenge free radicals and increase the response of antioxidant defence systems to oxidative stress in the body. Lipid peroxides and hydroperoxides are the secondary products of oxidative stress and are result of the toxic effect of ROS produced during LPO in diabetes. In the present study level of MDA was increased and activities of antioxidative enzymes i.e.

A. The effect of TNP on antioxidative enzymes in liver And Pancreas was Studied and Illustrates In Table I And II.

In table I, diabetic mice showed twofold decrease in SOD and GPx activity, whereas threefold decrease in CAT activity in liver as compared to control group (1:2, P<0.01). While after TNP treatment for 15 days the activity of SOD, CAT and GPx was significantly increased in liver of recovery group as compared to diabetic group (2:3, P<0.01).

In table II, diabetic mice showed two fold decrease in activity of SOD, whereas activity of CAT and GPx were reduced to its half value from its normal value in pancreas as compare to control group. Similarly, after treatment with TNP for 15 days there was twofold increase in the activity of SOD, CAT and GPx observed in pancreas of recovery group as compare to control group (2:3, P<0.01).

B. The effect of TNP on Blood Glucose level And Lipid Peroxidation in Liver and Pancreas was Studied and Illustrates in Table III.

In table III, Diabetic mice showed threefold increase in the level of blood glucose as compare to control (1:2, P<0.01). Whereas after TNP treatment for 15 days, twofold decrease in blood glucose level was observed in recovery group as compared to diabetic group (2:3, P<0.01). Lipid peroxidation was also increased twofold in liver and pancreas of diabetic mice as compare to control group (1:2, P<0.01). Similarly, after treatment of TNP for 15 days the lipid peroxidation in liver was decreased threefold and in pancreas it was decreased twofold in recovery group as compared to diabetic group (2:3, P<0.01).

IV. DISCUSSION

Diabetes mellitus is associated with generation of reactive oxygen species leading to oxidative damage particularly in pancreas and liver [42] [43] and [21]. Most of T2DM patients also have insulin resistance, which is the major cause of hypertension, obesity, hyperglycemia, and decreased HDL and high triglyceride levels [44]. Although many individual traditional plants have long been reported to be effective agents with hypoglycemic, hypolipidemic and antioxidative properties in diabetic mice, only some researchers are able to show the positive effects of specific plant on T2DM. In present investigation we have synthesized TNP for the treatment of diabetes, as the crude extract or active component have less bioavailability and slow effect. Therefore, the present study was carried out to investigate the antioxidative efficacy of TNP to ratify their use as a treatment for diabetes.

The STZ diabetic mice exhibited persistent hyperglycemia which is the main diabetogenic factor and contributes to the increase in oxygen free radicals by autoxidation of glucose. Hyperglycemia also generates reactive oxygen species, which in turn, cause lipid peroxidation and membrane damage, also increases oxidative stress in many organs, especially in the liver [3]. In our study, administration of TNP at a dose 100 mg/kg body weight/day for 2 weeks decreased fasting blood glucose level in recovery group compared to DM group.

Hyperglycemia results in the generation of free radicals which can exhaust antioxidant defences thus leading to the disruption of cellular functions, oxidative damage to membranes and enhanced susceptibility to LPO [45]. Many researches demonstrated the presence of antioxidant enzymes such as SOD, CAT, and GPx and non-enzymatic antioxidants such as vitamins E and C in diabetes mellitus can scavenge free radicals and increase the response of antioxidant defence systems to oxidative stress in the body [46][47]. Lipid peroxides and hydroperoxides are the secondary products of oxidative stress and are result of the toxic effect of ROS produced during LPO in diabetes [48]. In the present study level of MDA was increased and activities of antioxidative enzymes i.e.

### Table No III: Effect of TNP on blood glucose, Lipid peroxidation in Liver and Pancreas of STZ induced diabetic mice. Values are mean ±S.D (Numbers in parenthesis denotes number of animals).

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Group (n=6)</th>
<th>Blood glucose (mg/dl)</th>
<th>Statistical Significance</th>
<th>Lipid Peroxidation in liver</th>
<th>Statistical Significance</th>
<th>Lipid Peroxidation in Pancreas</th>
<th>Statistical Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>99 ±6.1237</td>
<td>1:2, P&lt;0.01</td>
<td>34.6153 ±4.0795</td>
<td>1:2, P&lt;0.01</td>
<td>26.2307 ±6.1262</td>
<td>1:2, P&lt;0.01</td>
</tr>
<tr>
<td>2</td>
<td>Diabetic</td>
<td>326.4 ±14.3631</td>
<td>1:3, non significant</td>
<td>65.7692 ±6.579</td>
<td>1:3, non significant</td>
<td>49.6153 ±8.7495</td>
<td>1:3 non significant</td>
</tr>
<tr>
<td>3</td>
<td>Recovery</td>
<td>135 ±8.5147</td>
<td>2:3, P&lt;0.01</td>
<td>28.8461 ±7.0658</td>
<td>2:3, P&lt;0.01</td>
<td>23.0769 ±4.0795</td>
<td>2:3, P&lt;0.01</td>
</tr>
</tbody>
</table>

P<0.01=Significant, P>0.5= Non significant
SOD, CAT and GPx was decreased in liver and pancreas of diabetic mice. This may be due to the production of reactive oxygen free radicals that can themselves reduce the activity of these enzymes and decrease enzymatic antioxidant levels in the liver and pancreas of mice. Administration of TNPs to diabetic mice significantly decreased the levels of MDA and increased the activity of antioxidative enzymes by protecting tissue from ROS. The increased activities of antioxidative enzymes may act as an added compensation mechanism to maintain the cell integrity and protection against free radical damage. This showed that free radical decreasing ability of TNPs could exert a beneficial action against oxidative stress. Thus our results indicates that there may be protection of β cells from damaging effects of free radicals or stimulation to existing β cells which leads into increase in insulin secretions or there may be increase in number of β cells due to division and differentiation of pancreatic stem cells.

V. CONCLUSION

The present study suggests that the TNPs possess potent antioxidative activity and improve antioxidative status, which have hypoglycemic properties with protective effect on liver and pancreas tissues against ROS. Moreover based on the outcome of biochemical analysis TNPs supposed to be considered as a best nano-scale device for future studies as well as medication on diabetes.

REFERENCES


