Role Of Polyphenon-60 Encapsulated In Chitosan Nanoparticles In The Amelioration Of Cardiac Injury In Streptozotocin-Treated Rats

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Abstract: Objective: To evaluate the cardio protective effect of chitosan-encapsulated polyphenon-60 in nanoformulation (chito-nanoPP-60) on STZ-induced cardiac injury and attempts to understand the mechanism underlying the effect of this nanoformulation in terms of antiapoptotic and antioxidant ability.

Methods: Streptozotocin (STZ) was injected intraperitoneally into male rats (40 mg/kg), and after diabetic induction chito-nanoPP-60 (75 mg/kg body wt) was orally administered for 21 days.

Results: Chito-nanoPP-60 with size of <200nm was prepared by ionotropic gelation technique. Chito-nanoPP-60 significantly ameliorated hyperglycemia, HbA1c and lipid fraction levels toward the control levels compared with the STZ-treated rats. Chito-nanoPP-60 normalized the increase in the troponin level and the activities of CK-MB and LDH in the serum indicating maintenance of heart integrity. This is accompanied with amelioration of the oxidative stress in the heart and considerably minimized the number of apoptotic cells while preventing the increase in Bax, caspases-3 and 9 and enhancing the expression of antiapoptotic protein Bcl-2.

Conclusion: This new nanoformulation containing green tea polyphenols present good therapeutic modality for cardioprotection in case of diabetes and similar diseases characterized by increased oxidative stress and apoptosis.

Key words: Chitosan nanoparticle, Heart, Apoptosis, Oxidative stress, Diabetes, Streptozotocin, Polyphenols, Green tea.

1. Introduction

Diabetic heart injury is a major public health issue, affecting people of different ages. Redox imbalance resulting from hyperglycemia plays a central role in the heart injury and cardiomyopathy process [1]. Type-1 diabetes is characterized by cardiomyopathy predominantly due to cell death by apoptosis leading to morbidity and mortality [2]. Both types of diabetes are associated with an exponential increase in generation of reactive oxygen species (ROS) and oxidative damage [3]. Based on that, studies have been directed to the potential therapeutic application of antioxidants for prevention and possible treatment of diabetic cardiomyopathy.

Cardioprotection in case of diabetes require constant monitoring of glucose level and regulating redox state through uptake of polyphenol-rich fruits and vegetables to attain normoglycemia and healthy heart. Catechins are group of flavonoids that constitute the majority of soluble solids of green tea (*Camellia sinensis*) and characterized by their antioxidant potential. These include epicatechin (EC), epicatechingallate (ECG), epigallocatechin (EGC) and epigallocatechingallate (EGCG) [4]. Polyphenon 60 contains 60 % pure polyphenols [5]. These polyphenols showed beneficial effects in the treatment and prevention of disorders associated with diabetes such as hypercholesterolemia, antihyperglycemia, oxidative stress [6, 7]. Moreover, catechins showed an antioxidant impact by enhancing antioxidant enzymes, inhibiting pro-oxidant enzymes and scavenging free radicals [8].

Despite these reported beneficial effects in experimental and preclinical studies, green tea polyphenols has only shown limited potential in clinic settings due to poor systemic delivery and bioavailability [9]. Such limitation can be overcome by using nanocarrier of biopolymer which can enable the adhesion of the nanoformulation to the mucosal surface and help facilitate drug permeation. The concept of nanochemoprevention for enhancing the outcome of green tea catechins for chemopreventive intervention was introduced [10, 11], which improve the pharmacokinetic and pharmacodynamics profiles of natural chemopreventive agents.
Chitosan is a polymer of natural origin, used as structural material in hydrogels. It is a nontoxic compound derived from the deacetylation of chitin [12]. Chitosan nanoparticles can be used for oral delivery of drugs because of their characteristic mucoadhesive properties [13]. Chitosan can stay in the gastrointestinal tract for a longer time because it has a positive charge on its amino groups. Therefore, the present study selected chitosan to encapsulate green tea polyphenol-60 in nanoformulation as a potential nanochemoprotective agent in streptozotocin-induced diabetic cardiomyopathy. Based on the association of elevated level of oxidative stress and cardiac injury, the present study aimed to evaluate the cardioprotective effect of chitosan encapsulated PP-60 in nanoformulation (chito-nanoPP-60) on STZ-induced cardiac injury and attempts to understand the role of this nanoformulation in terms of antiapoptotic and antioxidant ability.

2. Materials and Methods

Green tea polyphenol (polyphenol-60) and streptozotocin (STZ) were purchased from Sigma (Sigma, St. Louis, MO, USA). All other chemicals were of highest analytical grade.

2.1. Preparation of polyphenol-60 chitosan-nanoparticles

The ionotropic gelation technique was used to prepare chito-PP-60 nanoparticles as previously described [14]. PP-60, 20% (W/V) was added to chitosan solution before adding tripolyphosphate (TPP) solution.

2.2. Characterization of nanoparticles

The measurements of particle size and zeta potential of nanoparticles were performed on a Zetasizer Nano-ZS (Malvern Instrumentation Co., Westborough, MA) on the basis of Dynamic light scattering (DLS) techniques.

2.3. Size measurement by transmission electron microscope

The size and morphology of chito-nanoPP-60 were examined by transmission electron microscopy (TEM) using a JEOL JEM-100CX TEM (JEOL USA, Peabody, MA).

2.4. Animals and experimental protocol

All experiments were performed on adult male Wistar rats with an average weight of 250 ±10 g. All experimental procedures were carried out in accordance with the guide for care and use of laboratory animals published by the US National Research council. Rats were housed in steel mesh cages and maintained for two weeks acclimatization period on commercial standard diet and tap water. For the experimental induction of diabetes, STZ was freshly prepared in citrate buffer, pH 4.7. A single dose of 40 mg/kg STZ was injected intraperitoneally to rats. Two days after STZ injection, only rats with fasting blood glucose concentration higher than 200 mg/dl were considered as diabetic. A total of 45 rats were categorized into 5 groups, 9 rats in each group. Group 1 was normal controls. Rats in group 2 administrated chitosan-polyphenol-60-nanoparticles (75 mg/kg body wt) by oral intubation daily for 15 days. Group 3, rats administrated orally with void chitosan nanoparticles. Group 4, rats received single intraperitoneally injection with streptozotocin. Group 5, rats received single intraperitoneally injection with streptozotocin followed by daily oral administration of chitosan-polyphenol-60-nanoparticle (75 mg/kg body wt) for 15 days. Rats were sacrificed after 15 days.

2.5. Collection of blood samples

At the end of the experimental period overnight fasted rats were sacrificed under light anesthesia, blood was collected by cardiac puncture and serum was separated and stored in small aliquots at -20 ºC for further analysis.

2.6. Collection of heart samples and tissue homogenate

Hearts were quickly extracted, rinsed with sterile cold isotonic saline solution. The heart samples were divided into two parts, one part stored at -5ºC in clean tubes containing 5 ml of sterile cold isotonic saline for determination of antioxidants. The other parts were stored at -20 ºC for flow cytometry assays.

Heart samples were homogenized in ice cold homogenate buffer Tris-HCl buffer (0.1 M) pH 7.4 and used for the determination of the biochemical parameters. The homogenates were centrifuged at 4000 rpm for 10 minutes at -4 °C to remove cell debris. The resulting supernatants were stored at -20 ºC for biochemical assays.

2.7. Analysis

The blood glucose and HbA1c levels were estimated using kits supplied by Spinreact (St. Esteve d'en Bas Girona, Spain) and Vitro (Hannover, Germany) respectively. The profile of the serum lipids, including total lipids, triglyceride, total cholesterol, high-density lipoprotein (HDL), very...
low-density lipoproteins (VLDL) was assayed following the kit’s instructions Spinreact (St. Esteve d'en Bas Girona, Spain).

The heart function was estimated by determining the creatine kinase (CK-MB) and lactic dehydrogenase (LDH) activities using kits purchased from Spectrum (Hannover, Germany). Troponin T in serum was assessed using kits provided by Bioscience (San Diego, CA, USA). The lipid peroxidation was estimated by measuring the amount of malondialdehyde (MDA) while the antioxidant levels were estimated by determining superoxide dismutase (SOD) activity, catalase (CAT) activity, and glutathione (GSH) content using kits supplied by Biodiagnostic (Cairo, Egypt).

2.8. Histopathological examination

The ventricle was excised and fixed in 4% buffered paraformaldehyde, dehydrated in ascending grades of ethyl alcohol, cleared in xylene and mounted in molten para plast. Four micron histological section were cut, stained with hematoxylin and eosin and examined under bright field light microscopy and photographed.

2.9. Statistical analysis

The data are presented as the mean ± SEM. The statistical analyses were performed by t tests followed by Student’s t-distribution. Statistical significance was considered as $P < 0.05$.

3. Results

3.1. Size, zeta potential and morphology of chitosan nanoparticles

A representative diagram of the size and size distribution of chito-nanoPP-60 by dynamic light scattering is shown in Figure 1A. It was observed that the size distribution of chito-nanoPP-60 nanoparticles was 229 nm in diameter. The size and morphology were further confirmed by TEM as shown in Figure 2, which also supported the fact that the nanoparticles had a size of <200 nm in diameter as evidenced by dynamic light scattering in Figure 1A. Additionally, from the TEM picture, it was clear that the nanoparticles were spherical in shape. Figure 1B shows that the surfaces of chitosan nanoparticles have a positive charge at about 15mV.

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**Table 1: Size and Intensity Distribution**

<table>
<thead>
<tr>
<th>Peak</th>
<th>Size (d.nm)</th>
<th>%Intensity</th>
<th>St Dev. (d.nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak1</td>
<td>229.6</td>
<td>63.4</td>
<td>15.43</td>
</tr>
<tr>
<td>Peak2</td>
<td>4.179</td>
<td>36.6</td>
<td>0.06719</td>
</tr>
<tr>
<td>Peak3</td>
<td>0.000</td>
<td>0.0</td>
<td>0.000</td>
</tr>
</tbody>
</table>

**Figure 1:**

- **A**: Size and size distribution measurement by dynamic light scattering of chito-nanoPP-60. (A) The size distribution by dynamic light scattering of chitosan nanoparticles. This graph represents the size statistics of nanoparticles by intensity. (B) Zeta potential distribution of the nanoparticles.

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**Figure 2:**

- **A**: Zeta Potential (mV): 15.0
- **B**: Zeta Deviation (mV): 4.12
- **C**: Conductivity (mS/cm): 0.490

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3.2. Effect of chito-nanoPP-60 on Blood glucose and HbA1c

The level of blood glucose and HbA1c of the diabetic rats were significantly increased in STZ-treated rats compared with that of the control group. The treatment of the diabetic rats with chito-nanoPP-60 resulted in a marked amelioration of hyperglycemia and the level of HbA1c compared with the diabetic group. The treatment with either void or PP-60-loaded chito-nano particle showed insignificant changes in serum glucose and HbA1c levels from the control values (Figure 3A & B).

3.3. Effect of chito-nanoPP-60 on the level of lipid profile

The lipid profile, including the levels of total lipids, triglycerides, total cholesterol, low-density lipoprotein (LDL) and very-low lipoprotein (VLDL), was significantly higher, whereas the level of high-density lipoprotein (HDL) was significantly lower in the diabetic rats than normal control group. The treatment of the diabetic rats with chito-nanoPP-60 caused marked amelioration of the lipid fractions toward the control levels compared with the diabetic group (Table 1). The treatment with either void or PP-60-loaded chito-nano particle showed insignificant changes in serum lipid fraction from the control values.

Table 1. Effect of streptozotocin (STZ) and chitosan-encapsulated polyphenon-60 in nanoformulation (chito-nanoPP-60) on the lipid profile [total lipids, cholesterol, triglycerides, high-density lipoprotein, and very-low-density lipoprotein] expressed as mg/dl in the serum in rats in different groups.

The values are expressed as the mean ± SEM (n=9). * Significant at $P<0.05$, **## Significant at $P<0.01$, and ### Significant at $P<0.001$. ##*, **, & *** indicate comparisons with respect to the control group. #, ##&, and ### indicate comparisons with respect to the diabetic group.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Chito-nanoPP-60</th>
<th>Chito-nano</th>
<th>STZ</th>
<th>STZ+Chito-nanoPP-60</th>
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<tr>
<td>Control</td>
<td></td>
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<tr>
<td>Chito-nanoPP-60</td>
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<td>Chito-nano</td>
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<td>STZ</td>
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<tr>
<td>STZ+Chito-nanoPP-60</td>
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</table>
3.4. Effect of chito-nanoPP-60 on the level of Cardiac markers

The diabetic rats showed significantly higher activities of CK-MB and LDH as well as troponin T levels in the serum compared with the control values. The oral treatment with chito-nanoPP-60 after diabetic induction normalized the increase in these functional markers and demonstrated insignificant changes compared with those of the control group (Figure 4 A, B&C). The treatment with either void or PP-60-loaded chito-nano particle showed insignificant changes in activities of the serum CK-MB and LDH as well as troponin T levels from the control values.

<table>
<thead>
<tr>
<th></th>
<th>Cholesterol (mg/dl)</th>
<th>Total lipid (mg/dl)</th>
<th>HDL (mg/dl)</th>
<th>VLDL (mg/dl)</th>
<th>Triglyceride (mg/dl)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>62.42 ±1.81</td>
<td>1004 ±53.5</td>
<td>47.87 ±3.58</td>
<td>3.76 ±0.194</td>
<td>18.84 ±0.97</td>
</tr>
<tr>
<td></td>
<td>62.84 ±2.90</td>
<td>789.4 ±35.6</td>
<td>41.36 ±4.66</td>
<td>3.25 ±0.18</td>
<td>16.28 ±0.90</td>
</tr>
<tr>
<td></td>
<td>52.06 ±0.36</td>
<td>990.4 ±94.5</td>
<td>47.50 ±5.08</td>
<td>4.37 ±0.193</td>
<td>21.94 ±0.98</td>
</tr>
<tr>
<td></td>
<td>94.00 ±3.79</td>
<td>1818 ±113.8</td>
<td>27.10 ±2.25</td>
<td>15.94 ±0.54</td>
<td>79.83 ±2.72</td>
</tr>
<tr>
<td></td>
<td>63.46 ±1.97</td>
<td>1141 ±25.8</td>
<td>35.94 ±1.77</td>
<td>7.86 ±0.63</td>
<td>39.32 ±3.15</td>
</tr>
</tbody>
</table>

|                  | * Significant at P<0.05, **### significant at P<0.001, * , ** & *** indicate comparisons with respect to the control group. #, ##& ### indicate comparisons with respect to the diabetic group. |

3.5. Effect of chito-nanoPP-60 on Lipid peroxidation and antioxidants

Table 2 display changes in levels of lipid peroxidation and antioxidants in heart of different rat groups. The levels of the MDA were significantly increased in the hearts of the STZ-induced diabetic rats. Significant decreases were demonstrated in the SOD and CAT activities and the GSH content in the hearts of STZ-treated rats. These changes appeared similar to control levels when chito-nanoPP-60 was
administered and were significantly better than those of the diabetic rats. The treatment with either void or PP-60-loaded chito-nano particle showed insignificant changes in lipid peroxidation and GSH levels and activities of SOD and CAT in heart compared with the control values.

Table 2. Effect of streptozotocin (STZ) and chitosan-encapsulated polyphenon-60 in nanoformulation on the levels of the lipid peroxidation product (MDA; nmol/g.tissue), superoxide dismutase (SOD; nmol/g.tissue) and catalase (CAT; nmol/g.tissue) glutathione (GSH; mg/g.tissue) in the hearts of rats in different groups. The values are expressed as the mean ± SEM (n=9). *## Significant at \( P<0.05 \), **## significant at \( P<0.01 \), and ***#### significant at \( P<0.001 \). *##*, **##*, and ***##* indicate comparisons with respect to the control group. ##, ##*, and ### indicate comparisons with respect to the diabetic group.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Chito-nanoPP-60</th>
<th>Chito-nanoPP-60</th>
<th>STZ</th>
<th>STZ+Chito-nanoPP-60</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipid peroxidation</td>
<td>286 ±26.5</td>
<td>289 ±11.3</td>
<td>220 ±11.0</td>
<td>439 ±18.4**</td>
<td>276 ±24.5</td>
</tr>
<tr>
<td>nmol/g.tissue</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Catalase</td>
<td>4.04 ±0.33</td>
<td>4.08 ±0.22</td>
<td>5.00 ±0.17</td>
<td>2.64 ±0.09*</td>
<td>3.80 ±0.23</td>
</tr>
<tr>
<td>nmol/g.tissue</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>SOD</td>
<td>1307 ±100.6</td>
<td>1141 ±124.3</td>
<td>1606 ±79.3</td>
<td>631 ±66.1**</td>
<td>1097 ±59.4</td>
</tr>
<tr>
<td>nmol/g.tissue</td>
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<tr>
<td>GSH</td>
<td>42.28 ±3.64</td>
<td>44.34 ±3.52</td>
<td>49.50 ±1.44</td>
<td>25.02 ±1.26*</td>
<td>35.02 ±2.01</td>
</tr>
<tr>
<td>mg/g.tissue</td>
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3.6. Effect of chito-nanoPP-60 on apoptosis

The percentages of apoptotic cells were significantly higher in the hearts of diabetic rats than in those of the controls (Figure 5). The chito-nanoPP-60 treatment considerably minimized the number of apoptotic cells compared to the diabetic rats. The treatment with either void or PP-60-loaded chito-nano particle showed insignificant changes in the number of apoptotic cells and live cells compared to the control values.
Fig. 5. Effects of streptozotocin (STZ) and chitosan-encapsulated polyphenon-60 in nanoformulation (chito-nanoPP-60) on the percentage of apoptosis in rats in different groups. Flow cytometry results showing examples of the data generated by FACS are at the top of each histogram. The values are expressed as the mean ± SEM (n=9). * Significant at $P<0.05$, ** Significant at $P<0.01$, and *** Significant at $P<0.001$. $\#$, ## & ### indicate comparisons with respect to the control group. $\#$, ## & ### indicate comparisons with respect to the diabetic group.

3.7. Effect of chito-nanoPP-60 on the level of caspase 3, caspase 9, Bcl2 and Bax

The analysis of the flow cytometry data revealed that the anti-apoptotic protein Bcl-2 was significantly reduced, while the apoptotic protein Bax was increased in the hearts of STZ-induced diabetic rats (Figures 6A & B). Chito-nanoPP-60 treatment considerably normalized the STZ-induced changes in the expression of these proteins.

Fig. 6. Effects of streptozotocin (STZ) and chitosan-encapsulated polyphenon-60 in nanoformulation (chito-nanoPP-60) on the percentage of Bcl-2 (%)(A) and Bax (%) (B) in rats in different groups. Flow cytometry results showing examples of the data generated by FACS are at the side of each histogram. The values are expressed as the mean ±SEM (n=9). * Significant at $P<0.05$, ** Significant at $P<0.01$, and *** Significant at $P<0.001$. $\#$, ## & ### indicate comparisons with respect to the control group. $\#$, ## & ### indicate comparisons with respect to the diabetic group.
In Figure (7A & B), the expression levels of caspases-3 and 9 were significantly up-regulated in the hearts of the STZ-induced diabetic rats. Chito-nanoPP-60 administration significantly normalized these apoptotic proteins.

Fig.7. Effects of streptozotocin (STZ) and and chitosan-encapsulated polyphenon-60 in nanoformulation (chito-nanoPP-60) on caspases 3 (%) (A) and caspase 9 (%) (B) expressions in rat hearts in rats in different groups. Flow cytometry data showing examples of the data generated by FACS are at the side of each histogram. The values are expressed as the mean ± SEM (n=9). * &## Significant at $P<0.05$, ** &### significant at $P<0.01$, and *** &#### significant at $P<0.001$. *, ** & *** indicate comparisons with respect to the control group. #, ### & #### indicate comparisons with respect to the diabetic group.

3.8. The histo-pathological investigation

The histological examination of heart sections from the control and chito-nanoPP-60 treated animals demonstrated the uniform size and regular arrangement of the cardiac muscle fibers, with centrally located round or oval nuclei. The histopathological investigation of the heart sections of STZ-diabetic rats showed a disorganized array of the myocardial structure, myofibrillar discontinuation, myocyte degeneration, and pyknotic nuclei. Chito-nanoPP-60 treatment of STZ-induced diabetic rats markedly ameliorated these changes in the STZ-injected rat hearts and revealed remarkable less disorganization of the architecture of most of the cardiac muscle fibers, with centrally located vesicular nuclei (Figure 8).
4. Discussion

Nanotechnology has the potential to make drug carrier loaded with active compounds into a single nanosized particle that would be capable of reaching target tissue and cells more effective to achieve maximum therapeutic effect. Chitosan nanoparticles were synthesized in aqueous conditions by promoting the interaction of the NH$_2$ group present in chitosan with the phosphate group present in TPP. This nanoformulation can be used as a carrier system for many of the bioactive compounds that have sensitivity to acidic pH [15]. Therefore, this particular formulation of chitosan-polyphenol-60-nanoparticles has been chosen. It can produce a size ~200 nm with spherical morphology. Shape and size of nanoparticles play an essential role in cellular uptake and can thus modulate toxicity. Spherical nanoparticles are better taken into cells than rod shaped ones [16]. It has been shown that 100 to 200 nm sized particles accumulated 4-fold more effectively than those of 300 nm or smaller than 50 nm [17].

The obstacles that limit the use of natural product’s active components in drug delivery are the poor bioavailability and its degradation in the gastrointestinal canal. The therapeutic potential of green tea catechins following oral consumption is limited by their low bioavailability, attributed to poor stability and intestinal absorption [18]. The selected chitosan nanoparticles provide a formulation for green tea catechins to be embedded in the polymeric network [15], and therefore green tea catechins can be protected from degradation in the rough milieu of the stomach and the gastrointestinal tract [14]. This chitosan encapsulated catechin nanoparticles was suggested to enhance and prolong the intestinal absorption of PP-60. Reports have shown that using nanoparticles with biomolecules such as chitosan can improve biocompatibility and decrease possible toxicity [19]. This is consistent with reported work that chitosan nanoparticles enhance the plasma exposure of (-)-epigallocatechin gallate in mice through an enhancement in intestinal stability [18]. Chitosan nanoparticle can adhere to the mucosa of the intestinal membrane and open up the tight junction between epithelial cells [12].

STZ-induced diabetes is an established model to study the physiopathology and therapeutic mechanisms in diabetic subjects. Administration of STZ causes pancreatic β cell destruction and induction of changes similar to type 1 diabetes including hyperglycemia and hypoinsulinemia with body weight loss [20]. In support with previous studies the present study demonstrated that hyperglycemia in STZ-treated rats is associated with significant increase in HbA1c, whereas chito-nanoPP-60 treatment resulted in a remarkable reduction in blood glucose and HbA1c levels. These results are also in agreement with antidabetic role of green tea polyphenols in several other studies. Green tea has been reported to improve insulin-stimulated glucose uptake and insulin binding of adipocytes, an effect associated with increased expression of GLUT4 [21]. In addition, green tea catechins controlled the dietary glucose uptake by inhibiting the activity of sodium-dependent glucose transporter SGLUT1 in small intestine [22]. Down-regulation of gene expression of gluconeogenic enzymes by EGCG was demonstrated in hepatocytes in vitro [23].

In the present study, chito-nanoPP-60 given orally improved lipid profile compared with the diabetic rats. These results are in accordance with previous studies in which green tea extract exerted hypolipidmic effect and normalized triglycerides and cholesterol levels in plasma in obese patients [24]. Green tea polyphenol supplementation has also been reported to contribute to significant decreases in the total and low-density lipoprotein cholesterol and triglycerides, but an increase in high-density lipoprotein cholesterol [25]. Catechin component of green tea also showed anti-obesity effects which explained by its ability to affect lipid metabolism on intestinal mucosa and liver leading to reduced increased post-prandial fat oxidation [26]. Lowering the levels of risk factors including lipids through dietary polyphenol treatment appears to be the mainstay for decreasing the threat of cardiac disease and related complications [27, 28]. Accordingly, the present results indicate that polyphenol-60 in nanoformulation has a beneficial effect for

![Image](http://www.onlinejournal.in)
contractile dysfunction. The effect of chito-nanoPP-cardiac myocytes has been shown to produce cells in the same rats. Caspase-3 activation in paralleled with a decrease in the number of apoptotic in the heart compared with diabetic rats. This is caspases-3 & 9 while effectively upregulating Bcl-2 chito-nanoPP-60 inhibited the increase Bax and after chito-nanoPP-60 treatment has been expression in the heart of STZ-induced diabetic rats the levels of apoptosis and related regulatory protein demonstrating the efficiency of this nano fromula for protection of cardiomyocyte integrity.

To explain the mechanism underlying the action of chito-nanoPP-60 on cardiac damage in diabetic rats, the levels of apoptosis and related regulatory protein expression in the heart of STZ-induced diabetic rats after chito-nanoPP-60 treatment has been determined. The oral treatment of diabetic rats with chito-nanoPP-60 inhibited the increase Bax and caspases-3 & 9 while effectively upregulating Bcl-2 in the heart compared with diabetic rats. This is paralleled with a decrease in the number of apoptotic cells in the same rats. Caspase-3 activation in cardiac myocytes has been shown to produce contractile dysfunction. The effect of chito-nanoPP-60 may be attributed to the effective antioxidant and scavenging free radical potential of catechins. These data are in accordance with reports which showed that green tea catechins markedly suppress oxidative stress in different cells and tissues, including human leucocytes [34], human mucosa cell cultures [35] and liver cells [36]. Enhanced Bcl-2 expression in heart exerts anti-apoptotic effects through its antioxidant influence on intracellular ROS [37-39]. Moreover, Bcl-2 may decrease lipid peroxidation by enhancing cell resistance to ROS and preventing ROS production [40]. Therefore, it has been postulated that the anti-apoptotic effect of chito-nanoPP-60 by inhibiting caspases-3&9, and the increasing Bcl-2 may be related to its antioxidant impact. It appears that PP-60 in chitosan nano formulation decreases apoptosis by blocking pro-apoptotic protein and enhancing anti-apoptotic expressions.

Taken together, we firstly demonstrated that the PP-60 encapsulated in chitosan nanoparticles suppressed risk factors of cardiomyopathy by exerting anti-diabetic and hypolipidemic effects. Moreover, the cardioprotective effect chito-nanoPP-60 against diabetes induced cardiac apoptosis involves control of redox state and the main steps of the intrinsic signal pathway of apoptosis. Chito-nanoPP-60 might be a potential agent in preventing and treating myocardial disorders associated with diabetes.

5. References


apoptosis and protected the testes and sperm quality against bisphenol A-induced oxidative toxicity. *Toxicology and industrial health*, 2014.
