



## Nano-Encapsulated form of *Phyllanthus emblica* Extract Increases its Therapeutic Effects as Antidiabetic and Antioxidant in Rats

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### ABSTRACT

Recently, there has been increasing interest in *Phyllanthus emblica* due to its chemical composition and the potential health benefits. The present study was undertaken to investigate the effect of nanoparticles extracts of *Phyllanthus emblica* on diabetes, because nano-structured systems could promote sustained release of active constituents, reduce the required dose, low toxicity, decrease side effects, and improve activity. Therefore, the protective effect of *P. emblica*-NPs was studied on type-II diabetes through determination of serum glucose levels, expression alterations of glycolytic and gluconeogenic genes, DNA damage and liver-specific antioxidant glutathione peroxidase (GPx) enzyme in diabetic rats induced by Streptozotocin (STZ). Our study showed that *P. emblica*-NPs significantly decreased the blood glucose alterations in the expression of glycolytic and gluconeogenic genes, DNA damage and increased the activity of GPx. The results could be concluded that the nanoparticles of *P. emblica* extract increased the potential to be developed as an antidiabetic agent.

**Keywords:** Diabetes, DNA damage, Gene expression, Nanotechnology, *Phyllanthus emblica*.

### INTRODUCTION

The incidence of diabetes is considered to be high worldwide.<sup>1</sup> The incidence of diabetes is considered to be high worldwide.<sup>1</sup> According to the International Diabetes Federation, there are 246 million people with diabetes on the globe and this figure will rise to 380 million by the year 2025.<sup>2</sup> Diabetes is a group of metabolic disorders that result in hyperglycemia due to decreased insulin production (type-I) or insufficient insulin utilization (type-II).<sup>3</sup> Of these, type-II (NIDDM; Non- Insulin - Dependent - Diabetes Mellitus; Adult on set) diabetes is the major problem of today and it accounts for nearly 95% of total diabetic population.<sup>4</sup> The commonly practiced treatments of diabetes include oral antidiabetic drugs, insulin injections (in severe cases) and management through diet and physical exercise.<sup>5</sup> Beside these, people especially in Asian and African countries, still use extracts of different medicinal plants with or without the advice of Doctors or Ayurvedic practitioners. Few of these medicinal plants have been scientifically investigated. *Phyllanthus emblica* Linn, is one of the medicinal plants used in diabetic therapy.<sup>6</sup>

*Phyllanthus emblica* (*Embllica officinals* Gaertn; family: Euphorbiaceae) Linn, commonly known as Amla, is widely distributed in Asia and Africa.<sup>7</sup> Its fruit is commonly used for the treatment of anorexia, constipation, piles, leucorrhea, inflammatory bowels, cough, hemorrhoids, fever, thirst, toxicity of blood and atherosclerosis.<sup>8</sup> This fruit is acrid, cooling, refrigerant, diuretic, laxatives. It is a very rich source of vitamin C and is also used as a medicine to prevent aging (rejuvenation) due to its strong antioxidant properties.<sup>9</sup>

Although nanotechnology contributions are advantageous for several medicinal areas, it is essential to highlight some of the disadvantages. Clinical researchers have mentioned some negative factors, such as toxicity of metal nanoparticles through easy inhalability of nanoparticles which can result in dangerous lung diseases, and often lead to other diseases that can lead to changes in homeostasis, or even death.<sup>10, 11</sup> However, the strategy of applying nanotechnology to plant extracts has been widely cited in the literature, because nano-structured systems could potentiate action of plant extracts, promote sustained release of active constituents, reduce the required dose, low toxicity, decrease side effects, and improve activity.<sup>12, 13</sup>

Moreover, several studies have been used nano-encapsulated form of the plant extracts to increase the efficiency of its biological action. Kesarwani and Gupta published a review that mentioned several studies which employed nano structured systems to optimize the properties of plant extracts.<sup>14</sup> Bhattacharya and Ghosh used lipid-based systems incorporated green tea and ginseng (*Panax ginseng* CA Meyer) (Araliaceae) extracts, in various formulations, to increase the absorption of the active components.<sup>15</sup>

Therefore, the present investigation was designed to determine the protective effect of nanoparticles of fruit extract of *P. emblica* Linn on diabetic rats including the expression alterations of glycolytic and gluconeogenic genes, DNA damage and changes in antioxidant enzymes.



## MATERIALS AND METHODS

### Drugs and chemicals

Streptozotocin (STZ) was purchased from Sigma–Aldrich (USA). Reagents for RT-PCR were purchased from Invitrogen (Paisley, UK) and Fermentas (Leon-Rot, Germany).

### Induction of experimental diabetes

Diabetes was induced in 12 h fasted rats with single i.p. injection of STZ (50 mg/kg,<sup>16</sup> dissolved in citrate buffer (0.01 M, pH 4.5). Normal control group was injected with citrate buffer alone. Animals were considered diabetic when their blood glucose level exceeded 250 mg/dl<sup>17</sup> and were included in the study after 72 h of STZ injection.

### Plant material

*Phyllanthus embelica* fruits collected from private farm in Jeddah, Saudi Arabia were dried by oven at 50°C. Dry plant material (10 kg) was grinded and boiled in water for 30 min, filtered and evaporated by evaporator. The extract was dried by freeze dry as water extract of PE (PEW). The percentage of yield obtained as 43.4%. The samples have been preserved in the refrigerator (-20°C). Authentication of plant materials was identified by comparing against the specimens deposited King Abdulaziz University, where herbarium vouchers have been kept.

### Preparation of the extract

According to Tasanarong et al.<sup>18</sup>, the ether extract of *Phyllanthus embelica* was collected, washed three times with water, dried over anhydrous sodium sulfate and evaporated to dryness.

### Formation of *Phyllanthus embelica* Loaded Nanoparticles (SON)

Solvent displacement technique of Samadder et al.<sup>19</sup> was deployed under optimal conditions to prepare the poly-lactic-co-glycolic acid (PLGA) encapsulation of *Phyllanthus embelica* extract.

### Experimental Animals

Sixty adult albino male rats (100-120 g, purchased from the Animal House Colony, Giza, Egypt) were maintained on standard laboratory diet (protein, 16.04%; fat, 3.63%; fiber, 4.1%; and metabolic energy, 0.012 MJ) and water *ad libitum* at the Animal House Laboratory, National Research Center, Dokki, Giza, Egypt. After an acclimation period of 1 week, animals were divided into several groups (10 rats/ group) and housed individually in filter-top polycarbonate cages, housed in a temperature-controlled (23 ± 1°C) and artificially illuminated (12 h dark/light cycle) room free from any source of chemical contamination. All animals received humane care in compliance with the guidelines of the Animal Care and Use Committee of National Research Center, Egypt.

### Experimental design

Animals were divided into following 6 groups. Each group consists of 10 rats:

Group 1 – control: oral saline (C); Group 2- rats were injected by single i.p. dose of STZ (50 mg/kg dissolved in citrate buffer to induce diabetes; Group 3 – diabetes mellitus induced-rats were administered 10 units insulin subcutaneously<sup>20</sup>; Group 4 – diabetes mellitus induced-rats: 25 mg/kg bw/day of *Phyllanthus embelica* nanoparticles (1/8 of the dose used by Qureshi et al.<sup>6</sup> in one dose per oral for 30 days; Group 5 – diabetes mellitus induced-rats were treated with 50 mg/kg bw/day of *Phyllanthus embelica* nanoparticles (1/4 of the dose used by Qureshi et al.<sup>6</sup> in one dose per oral for 30 days; Group 6 – diabetes mellitus induced-rats were treated with 100 mg/kg bw/day of *Phyllanthus embelica* nanoparticles (1/2 of the dose used by Qureshi et al.<sup>6</sup> in one dose per oral for 30 days.

### Sample Collections

Blood samples from fasting rats were withdrawn from retro-orbital venous plexus under diethyl ether anesthesia in dry clean centrifuge tubes and left to clot. The animals were anesthetized with ether, and blood was collected from retro-orbital puncture. Serum was then separated for the estimation of glucose. At the end of the treatment, the animals were sacrificed and liver and muscle tissues were used for gene expression, genotoxicity and biochemical analyses.

#### a) Quantitative RT-PCR

##### First-strand cDNA synthesis from extracted rat RNA

Total RNA (Poly(A)<sup>+</sup> RNA) was extracted from 50 mg of liver and muscles tissues using the standard TRIzol extraction method (Invitrogen, Paisley, UK) and recovered in 100 µL diethyl pyrocarbonate (DEPC)-treated water by passing the solution a few times through a pipette tip.

Total RNA was treated with one unit of RQ1 RNase-free DNase (Invitrogen, Karlsruhe, Germany) to digest DNA residues, re-suspended in DEPC-treated water, and quantified photo spectrometrically at 260 nm. Total RNA was assessed for purity from the ratio between quantifications at 260 nm and 280 nm, and was between 1.8 and 2.1. Integrity was verified with the ethidium bromide-stain analysis of 28S and 18S bands using formaldehyde-containing agarose gel electrophoresis. Aliquots were either used immediately for reverse transcription (RT) or stored at -80 °C.

To synthesize first-strand cDNA, 5 µg of complete Poly(A)<sup>+</sup> RNA was reverse transcribed into cDNA in a total volume of 20 µL using 1 µL oligo (poly(deoxythymidine)<sub>18</sub>) primer (El-Makawy, 2008). The composition of the reaction mixture was 50 mmol L<sup>-1</sup> MgCl<sub>2</sub>, 10x RT buffer, 200 U µL<sup>-1</sup> reverse transcriptase (RNase H free, Fermentas, Leon-Rot, Germany), 10 mmol L<sup>-1</sup> of each dNTP, and 50 µmol L<sup>-1</sup> of oligo(dT) primer. RT reaction was carried out at 25 °C for



10 min, followed by 1 h at 42 °C, and completed with denaturation at 99 °C for 5 min. Reaction tubes containing RT preparations were then flash-cooled in an ice chamber until used for DNA amplification through polymerase chain reaction (PCR) (Ali et al., 2008).

#### qRT-PCR assay

PCR reactions were set up in 25 µL reaction mixtures containing 12.5 µL 1× SYBR® Premix Ex Taq™ (TaKaRa, Biotech. Co. Ltd., Germany), 0.5 µL 0.2 µM sense primers, 0.5 µL 0.2 µM antisense primer, 6.5 µL distilled water, and 5 µL of cDNA template. The reaction program was allocated to 3 steps. First step was at 95.0°C for 3 min. Second step consisted of 40 cycles in which each cycle divided to 3 steps: (a) at 95.0°C for 15 sec; (b) at 55.0°C for 30 sec; and (c) at 72.0°C for 30 sec. The third step consisted of 71 cycles which started at 60.0°C and then increased about 0.5°C every 10 sec up to 95.0°C. At the end of each qRT-PCR a melting curve analysis was performed at 95.0°C to check the quality of the used primers. Each experiment included a distilled water control. Table 1 lists the specific gene primer sequences and PCR cycling conditions.<sup>21, 22</sup>

The quantitative values of RT-PCR (qRT-PCR) of glycolytic and gluconeogenic genes (GK, Glc-6-Pase, GLUT2 and GLUT4) were normalized on the bases of β-actin expression. The primer sequences of liver cancer related genes are listed in Table 1.

At the end of each qRT-PCR a melting curve analysis was performed at 95.0 °C to check the quality of the used primers.

#### Calculation of Gene Expression

First the amplification efficiency (Ef) was calculated from the slope of the standard curve using the following formulae:<sup>23</sup>

$$Ef = 10^{-1/\text{slope}}$$

$$\text{Efficiency (\%)} = (Ef - 1) \times 100$$

The relative quantification of the target to the reference was determined by using the

ΔCT method if E for the target (GK, Glc-6-Pase, GLUT2 and GLUT4) and the reference primers (β-Actin) are the same.<sup>23</sup>

$$\text{Ratio}_{(\text{reference}/\text{target gene})} = Ef^{CT(\text{reference}) - CT(\text{target})}$$

#### b) Comet assay for DNA strand break determination

Isolated hepatic cells of all groups of rats were subjected to the modified single-cell gel electrophoresis or comet assay.<sup>24</sup> To obtain the cells, a small piece of the liver was washed with an excess of ice-cold Hank's balanced salt solution (HBSS) and minced quickly into approximately 1 mm<sup>3</sup> pieces while immersed in HBSS, with a pair of stainless steel scissors. After several washings with cold phosphate-buffered saline (to remove red blood cells), the minced liver was dispersed into single cells using a

pipette. In brief, the protocol for electrophoresis involved embedding of the isolated cells in agarose gel on microscopic slides and lysing them with detergent at high salt concentrations overnight (in the cold). The cells were treated with alkali for 20 min to denature the DNA and electrophoresis under alkaline conditions (30 min) at 300 mA, 25 V. The slides were stained with ethidium bromide and examined using a fluorescence microscope (Olympus BX60 F-3) with a green filter at × 40 magnifications. For each experimental condition, about 100 cells (about 25 cells per fish) were examined to determine the percentage of cells with DNA damage that appear like comets. The non overlapping cells were randomly selected and were visually assigned a score on an arbitrary scale of 0–3 (i.e., class 0 = no detectable DNA damage and no tail; class 1 = tail with a length less than the diameter of the nucleus; class 2 = tail with length between 1× and 2× the nuclear diameter; and class 3 = tail longer than 2× the diameter of the nucleus) based on perceived comet tail length migration and relative proportion of DNA in the nucleus. A total damage score for each slide was derived by multiplying the number of cells assigned to each class of damage by the numeric value of the class and summing up the values. Slides were analyzed by one observer to minimize the scoring variability.<sup>24</sup>

#### Determination of glutathione peroxidase (GPx) activity

Glutathione peroxidase activity measurements were carried out by a procedure according to Miranda et al.<sup>25</sup> The reaction mixture consisted of 8 mM H<sub>2</sub>O<sub>2</sub>, 40 mM guaiacol, 50 mM sodium acetate buffer, pH 5.5, and a suitable amount of the enzyme preparation. The change in absorbance at 470 nm due to guaiacol oxidation was followed at 30 s intervals. One unit of glutathione peroxidase activity was defined as the amount of enzyme which increases the O.D. 1.0/min under standard assay conditions.

#### Statistical Analysis

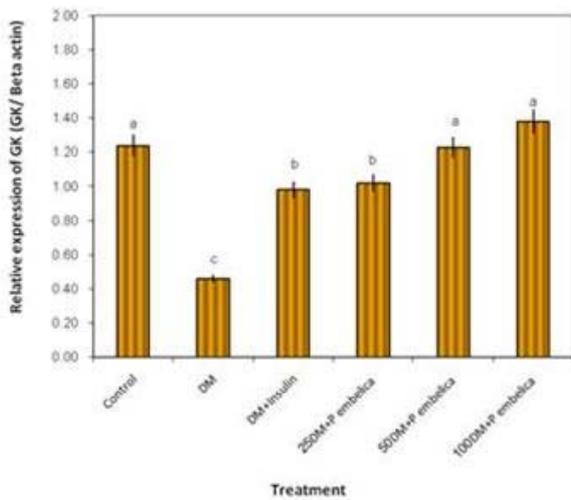
All results were expressed as Mean±S.E of the mean. Data were analyzed by one way analysis of variance (ANOVA) using the Statistical Package for the Social Sciences (SPSS) program, version 11 followed by least significant difference (LSD) to compare significance between groups. Difference was considered significant when P < 0.05.

## RESULTS

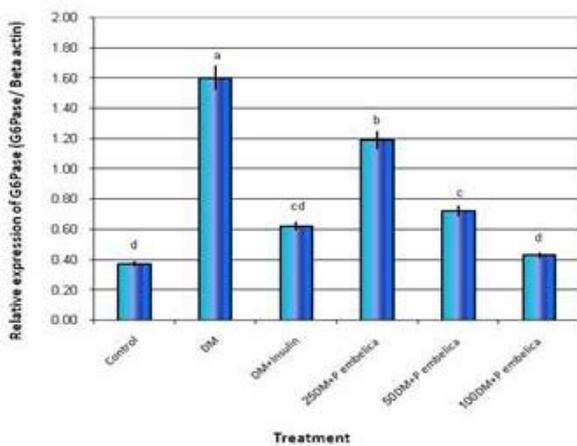
#### Serum glucose levels

The antihyperglycemic effect of the *P embelica*-NPs on the fasting serum glucose levels in diabetic rats is shown in Table 2. Diabetic rats revealed extremely high levels (P<0.001) of glucose compared with control rats. However, daily treatment of *P embelica*-NPs led to a dose dependent fall in serum glucose levels. Administration of DM-rats with high dose of *P embelica*-NPs revealed highly significant decrease in serum glucose levels compared with the DM-rats.

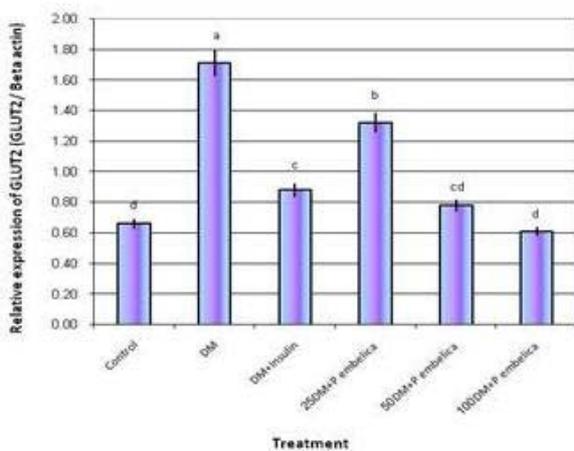




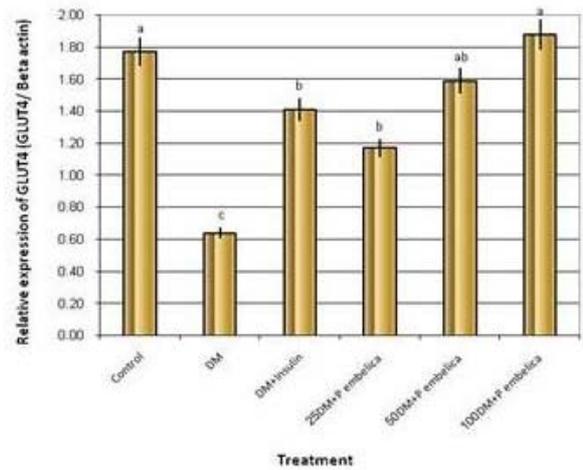
**Figure 1:** The alterations of GK-mRNA in liver tissues isolated of male rats treated with different dose of *P. embellica*-NPs. <sup>a,b,c</sup> Mean values within tissue with unlike superscript letters were significantly different ( $P<0.05$ ).



**Figure 2:** The alterations of G6Pase-mRNA in liver tissues isolated of male rats treated with different dose of *P. embellica*-NPs. <sup>a,b,c</sup> Mean values within tissue with unlike superscript letters were significantly different ( $P<0.05$ ).



**Figure 3:** The alterations of GLUT2-mRNA in liver tissues isolated of male rats treated with different dose of *P. embellica*-NPs. <sup>a,b,c</sup> Mean values within tissue with unlike superscript letters were significantly different ( $P<0.05$ ).



**Figure 4:** The alterations of GLUT4-mRNA in muscles tissues isolated of male rats treated with different dose of *P. embellica*-NPs. <sup>a,b,c</sup> Mean values within tissue with unlike superscript letters were significantly different ( $P<0.05$ ).

**Table 1:** List of primers, the primer sequences and the primer melting temperature ( $T_m$ )

Gene	Primer	Sequence (5'-3')	Annealing $T_m$ °C
GK	Forward	CATATGTGCTCCGAGACTAG	61.7
	Reverse	CTTGTACACGGAGCCATCCA	
G6Pase	Forward	GGATCTACCTTGCGGCTCACT	62.7
	Reverse	TGTAGATGCCCGGATGTG	
GLUT 2	Forward	CATCAAACGTAGAGCACGGTAA	63.4
	Reverse	TATGGGCATTTAGTCTGCACGTA	
GLUT4	Forward	GCTTGGCTCCCTTCAGTTTG	63.4
	Reverse	CCTACCCAGCCAAGTTGCAT	
$\beta$ -actin	Forward	GTG GGC CGC TCT AGG CAC CAA	64.5
	Reverse	CTC TTT GAT GTC ACG CAC GAT TTC	

GK: Glucokinase, G6Pase: Glucose-6-phosphatase, GLUT2: Glucose transporter type 2, GLUT4: Glucose transporter type 4.

**Table 2:** Glucose levels in diabetic rats treated with different doses of *P. embellica*-NPS

Treatment	Glucose level (mg/dl)	
	Day 0*	Day 30
Control	86.1 ± 13.6	84.2 ± 14.2 <sup>c</sup>
DM	369.2 ± 19.1	385.6 ± 22.6 <sup>a</sup>
DM+ Insulin	362.4 ± 20.9	124.2 ± 12.5 <sup>b</sup>
DM+ P embellica 25	364.7 ± 21.4	132.6 ± 10.8 <sup>b</sup>
DM+ P embellica 50	364.8 ± 20.4	112.7 ± 11.3 <sup>bc</sup>
DM+ P embellica 100	366.2 ± 22.5	87.9 ± 9.2 <sup>c</sup>

DM: Diabetus mellitus; <sup>a, b</sup> Mean values within column with unlike superscript letters were significantly different ( $P<0.05$ ).

**Expression of glycolytic and gluconeogenic genes**

The expression of diabetic-associated, glycolytic and gluconeogenic genes, in the streptozotocin-induced



diabetic rats treated with *P. embelica*-NPs was determined using RT-PCR (Figures 1-4).

The results revealed that DM-rats showed significantly lower expression values of hepatic Glucokinase (GK) and muscle Glucose transporter type 4 (GLUT4) genes in comparison with the control rats (Figures 1 & 4). While, DM-rats treated with Low, medium and high doses of *P. embelica*-NPs caused significant increase in GK and GLUT4 expression as compared with the DM- rats. Furthermore, highest expression levels of GK and GLUT4 genes were showed in DM-rats treated with the high dose of *P. embelica*-NPs (Figures 1 & 4). In addition, treatment of DM-rats with insulin increased significantly the expression of GK and GLUT4 genes.

Regarding Glucose-6-phosphatase (G6Pase) and Glucose transporter type 2 (GLUT2) genes, the present results revealed that DM-rats showed significantly higher expression values of G6Pase and GLUT2- mRNA in comparison with the control rats (Figures 2&3). However, DM-rats treated with insulin showed significantly lower expression values of G6Pase and GLUT2- mRNA in comparison with the DM-rats. Moreover, DM-rats treated with Low, medium and high doses of *P. embelica*-NPs caused significant decrease in G6Pase and GLUT2- mRNA expression as compared with the DM-rats. Moreover, lowest expression levels of G6Pase and GLUT2- mRNA

genes were showed in DM-rats treated with the high dose of *P. embelica*-NPs (Figures 2&3).

### DNA Damage detected by Comet assay

The results of the comet assay revealed that treatment of diabetic rats with different doses of *P. embelica*-NPS induced different rats of DNA damage (Table 3).

The rate of DNA damage in control rats induced a low rate of DNA damage (Table 3). However, the rate of DNA damage in DM- male rats induced a high rate of DNA damage which was 17.4% compared with 4.6% in control mice.

In contrary, treatment of DM-rats with different doses of *P. embelica*—NPS revealed significantly low rats compared with DM-rats. Treatment of DM-rats with low, medium and high doses of *P. embelica*—NPS revealed 8.8, 7.6 and 4.4% DNA damage compared with 17.4% DNA damage in DM-rats.

On the other hand, treatment of DM-rats with insulin induced low rats of DNA damage compared with DM-rats, where the rate of damage was 4.6% in DM-rats treated with insulin compared with 17.4% DNA damage in DM-rats. (Table 3).

**Table 3:** Visual score of DNA damage in diabetic rats treated with different doses of *P. embelica*--NPS using comet assay.

Treatment	Number of animals	No. of cells		Class <sup>‡</sup> of comet				DNA damaged cells (%)
		Analyzed(*)	Total comets	0	1	2	3	
Control	5	500	23	477	17	6	0	4.6
DM	5	500	87	413	26	27	34	17.4
DM+Insulin	5	500	46	454	27	11	8	9.2
DM+P embelica 25	5	500	44	456	22	13	9	8.8
DM+P embelica 50	5	500	38	462	17	15	6	7.6
DM+P embelica 100	5	500	22	478	16	6	0	4.4

‡: Class 0= no tail; 1= tail length < diameter of nucleus; 2= tail length between 1X and 2X the diameter of nucleus; and 3= tail length > 2X the diameter of nucleus. (\*): No of cells analyzed were 100 per an animal.

**Table 4:** The amount of glutathione peroxidase activity in diabetic rats treated with different doses of *P. embelica*-NPS

Treatment	Glutathione peroxidase activity (U/mg tissues/min)
Control	6.7±0.08 <sup>a</sup>
DM	2.5±0.05 <sup>c</sup>
DM+Insulin	4.6±0.07 <sup>b</sup>
DM+P embelica 25	4.1±0.04 <sup>b</sup>
DM+P embelica 50	5.8±0.06 <sup>ab</sup>
DM+P embelica 100	7.2±0.04 <sup>a</sup>

Data are presented as mean ± SEM. <sup>a,b,c</sup>Mean values within tissue with unlike superscript letters were significantly different ( $P<0.05$ ).

### Effects of *P. embelica*-NPS on GPx levels in DM-rats

Table 4 shows the protective effect of *P. embelica*—NPS on STZ-induced alteration in the antioxidant enzyme. Comparing with the control group, GPx activity levels were relatively similar with *P. embelica*-NPS treatment. Moreover, the high dose of *P. embelica*-NPS revealed GPx activity levels more than those in healthy rats. However, the activity level of GPx decreased significantly in DM-rats compared with control group. When compared with the DM-rats group, GPx concentration elevated 1.84, 1.64, 2.32, and 2.88-fold in DM+insulin, DM+ *P. embelica*-NPS25, DM+ *P. embelica*—NPS50 and DM+ *P. embelica*-NPS100 groups, respectively (Table 4).

### DISCUSSION

Nanosized drug delivery systems for herbal drugs can potentially enhance the biological activity and overcome



problems associated with plant medicines. However, significant challenges remain for implementation of clinically viable therapies in this field. Diabetes disease is one of the major health problems throughout the world especially in adults of age above 35 years in both sexes.<sup>3</sup> In spite of the presence of number of synthetic oral antidiabetic drugs in the market, researchers are now diverted their attention to different herbs and medicinal plants in order to find out new active principle with less side effects and better antidiabetic activity.<sup>26</sup> Therefore, *P. embelica* (Amla) Linn was selected for the present study in order to provide some help in patronizing indigenous drugs.

The antihyperglycemic effect of the *P. embelica*-NPs on the fasting serum glucose levels in diabetic rats is shown in Table 2. Diabetic rats revealed extremely high levels ( $P < 0.001$ ) of glucose compared with control rats. However, daily treatment of *P. embelica*-NPs led to a dose dependent fall in serum glucose levels. Administration of DM-rats with high dose of *P. embelica*-NPs revealed highly significant decrease in serum glucose levels compared with the DM-rats.

The results of the present study revealed that nano-encapsulated form of fruit extract of *P. embelica* has a potent antidiabetic activity by showing a significant fall in blood glucose level of diabetic rats treated with *P. embelica*-NPs. Maximum decrease in blood glucose level was observed with high dose of *P. embelica*-NPs which revealed highly significant decrease in serum glucose levels compared with the DM-rats.

In agreement with our observations, Qureshi and Hasnain<sup>27</sup> reported that treatment of diabetic rats induced by alloxan with aqueous fruit extract of *P. embelica* decreased significantly blood glucose levels.

The antidiabetic effect of aqueous fruit extract might be extra-pancreatic either by inhibiting glycogenolysis, hepatic gluconeogenesis and glucose absorption from intestine or by increasing glucose absorption in cells of peripheral tissues (muscles and adipose tissues) and hepatic glycogenesis.<sup>28</sup> However, it was very interesting that almost same antidiabetic effect was observed by chlorpropamide, which is known to produce its effect by stimulating the release of endogenous insulin<sup>28</sup> This finding supports the earlier reports of few *Phyllanthus* species, which were found to involve in regeneration and rejuvenation of  $\beta$  - cells leading to an increased insulin production and secretion.<sup>29</sup> Abnormalities to lipid metabolism may be secondary to diabetes and it was also confirmed in STZ-induced diabetic rats, where an increase in plasma glucose level was accompanied by an increase in TG levels.

The results revealed that DM-rats showed significantly lower expression values of GK and GLUT4 genes and higher expression of G6Pase and GLUT2- mRNA in comparison with the control rats. While, DM-rats treated *P. embelica*-NPs caused significant increase in GK and

GLUT4 expression and lower expression values of G6Pase and GLUT2- mRNA as compared with the DM-rats. To date no data discussed the effect of *P. embelica* on the expression of glycolytic and gluconeogenic genes. However, Yahayo et al.,<sup>30</sup> studied the effect of *P. embelica* on the molecular mechanism of antimetastatic activity. They determined the gene expression of matrix metalloproteinases, MMP2, and MMP9 using RT-PCR assay. The mRNA levels of both genes were significantly down-regulated after pretreatment with *P. embelica*. This study suggested that there is a high potential to use *P. embelica* extracts clinically as an optional adjuvant therapeutic drug for therapeutic intervention strategies in cancer therapy or chemoprevention.

The current study observed that the rate of DNA damage in DM- male rats induced a high rate of DNA damage compared with control mice. While, treatment of DM-rats with *P. embelica*—NPS revealed significantly low rats compared with DM-rats. Similar results were showed in the study of Madhavi et al.<sup>31</sup> They found that when male mice primed with *Phyllanthus* fruit extract a reduction in the frequency of sperm head abnormalities was observed. They suggested that *Phyllanthus emblica* plays a key role in inhibition of heavy metal genotoxicity in mammals.

The protective effect of the *P. embelica* on the molecular mechanism inhibiting changes in the gene expression and genotoxicity is not clear understood. However, several studies suggested the protective effects of *P. embelica* may be attributed to its antioxidant activity. The high antioxidant activities of *P. embelica* extract in the *in vitro* radical scavenging assays have previously shown potential sources of natural antioxidants.<sup>32,33</sup> Furthermore, the inhibitory effect of this extract on UV-induced ROS and collagen damage in normal human dermal fibroblasts suggest promising cosmeceutical benefits against photoaging.<sup>34</sup> In agreement with these findings, our study revealed also that high dose of *P. embelica*-NPS revealed GPx activity levels more than those in healthy rats. When compared with the DM-rats group, GPx concentration elevated 2.88-fold in DM+ *P. embelica*-NPS100.

## CONCLUSION

Our results provide novel mechanisms for the plasma glucose-lowering action of *P. embelica*-NPS. The extract produced its anti-hyperglycemic effect. Further it is confirmed that the extract suppressed the transcription of genes involved in hepatic glucose production, such as Glc-6- Pase. In addition, the extract stimulated the hepatic GK gene expression, which consequently leads to an increased expression of GLUT-4 gene expression in skeletal muscles of streptozotocin-induced diabetic rats. Altogether, the extract potentially displayed antidiabetic activity by inhibiting hepatic glucose production and promoting glucose utilization. The extract also nullifies the hyperglycemic effects of streptozotocin which was observed through the reduced expression of GLUT-2 gene. Altogether, it can be concluded that the



nanoparticles of *P. embelica* extract increased the potential to be developed as an antidiabetic agent.

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