Research Article

Nanoparticles from of *Costus speciosus* Extract Improves the Antidiabetic and Antilipidemic Effects Against STZ-induced Diabetes Mellitus in Albino Rats

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ABSTRACT

Diabetes mellitus is the most common and serious metabolic disorder among people all over the world. Many plants have successfully been used to overcome this problem. *Costus speciosus* is widely used in Asian medicine to treat various diseases including diabetes. Nano-encapsulated form of the plant extracts was used in several studies to increase the efficiency of its biological action. Therefore, the present study was undertaken to investigate the effect of nanoparticles extracts of *Costus speciosus* 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. The protective effect of *Costus speciosus*-NPs was studied on type-II diabetes through determination of serum glucose and lipids levels, enzymes activities, DNA fragmentation and expression alterations of insulin and gluconeogenic genes in diabetic rats induced by Streptozotocin (STZ). Our study showed that oral administration of *Costus speciosus* -NPs significantly decreased the blood glucose, serum total cholesterol, triglyceride, LDL cholesterol, alterations in the expression of insulin (I&II) and gluconeogenic genes, DNA Fragmentation. Also *Costus speciosus* -NPs restored the altered plasma enzyme (AST, ALT, LDH, ALP and ACP) levels to near normal. The results could be concluded that the nanoparticles of *Costus speciosus* extract increased the potential to be developed as an antidiabetic agent.

Keywords: *Costus speciosus,* Nanotechnology, Diabetes, normo-glycemic and hypolipidemic effects, Gene expression, DNA Fragmentation.

INTRODUCTION

Diabetes mellitus is a metabolic disorder caused by complete or relative insufficiency of insulin secretion and/or insulin action[1]. Hyperglycemia is the main cause of complications related to coronary artery disease, cerebrovascular disease, renal failure, blindness, limb amputation, neurological complications and premature death[2]. Hyperlipidemia contributes to the development of cardiovascular complications related to diabetes[3].

Biguanides sulphonylureas and thiazolidinediones became available for treatment of type 2 diabetes and have been effective hypoglycemic agents. However they cause some side effects. Hence search for new drugs without adverse effects is going on in several laboratories around the world[4].

Costus speciosus (Koen ex.Retz.) Sm. Costaceae (Family), is widely used in Ayurveda. The roots are bitter, astringent, acrid, cooling, aphrodisiac, purgative, anthelmintic, depurative, febrifuge, expectorant and tonic and useful in treating burning sensation constipation, leprosy, worm infection, skin diseases, fever, asthma, bronchitis, inflammations and anaemia. Herbal healers use this plant to treat diabetes. It is cultivated in isolated patches in different parts of Saudi Arabia[5].

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[6,7].

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[8,9].

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 nanostructured systems to optimize the properties of plant extracts[10]. 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[11].

Therefore, the present investigation was designed to study the normo-glycemic and hypolipidemic effects of nanoparticles of *Costus speciosus* leaves in STZ-induced diabetic rats. In addition, the DNA fragmentation and expression alterations of Insulin (I&II glycolytic and gluconeogenic genes in STZ-induced diabetic rats treated with *Costus speciosus*-NPs were determined.

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 mellitus was induced by single intraperitoneal injection of freshly prepared STZ (50 mg/kg bw)[12] dissolved in 0.1M citrate buffer (pH 4.5) in a volume of 1 ml/kg bw. Diabetes was developed and stabilized in these STZ-treated rats over a period of 3-4 days. The control animals were administered with citrate buffer (pH 4.5). After 3 days, the blood was collected by sinocular puncture and the plasma glucose level of each rat was determined. Rats with a fasting plasma glucose range of 280–350 mg/dl[13] were considered diabetic and included in the study.

Plant material

Costus speciosus leaves collected from private farm in Jeddah, Saudi Arabia were dried by oven at 50°C. Dry plant material 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[14], the extract of *Costus speciosus* was collected, washed three times with water, dried over anhydrous sodium sulfate and evaporated to dryness. Briefly, a 500 g of the shade-dried powdered leaves of *Costus speciosus* were extracted separately with 70% ethanol by maceration and percolation for 24 h. The process of extraction was repeated twice. The alcohol extract of each plant was pooled together and evaporated under reduced pressure at 45°C till free from solvent. The alcohol free residue of each extract was weighted. Preliminary phytochemical tests were carried out to identify the main constituents of each extract[15].

Formation of *Costus speciosus* Loaded Nanoparticles

Solvent displacement technique of Samadder[16] was deployed under optimal conditions to prepare the poly-lactic-co-glycolic acid (PLGA) encapsulation of *Costus speciosus* extract. Briefly, to prepare the poly-lactic-co-glycolic acid (PLGA) encapsulation of *Costus speciosus*, solvent displacement technique of Samadder[16] deployed under optimal conditions. To 20 mL of an aqueous solution of F68; w/v stabilizer (1% polyoxyethylene-polyoxypropylene), an organic phase mixture containing 10 mg of dried *Costus speciosus* dissolved in 3 mL acetone along with 50 mg PLGA in a dropwise manner (0.5 mL/min) was added. Stirring the mixture continuously was performed at room temperature until complete evaporation of the organic solvent; the redundant stabilizer was removed by centrifugation at 2500 g at 4°C for 30 minutes. The pellet was re-suspended in Milli-Q water and washed three times and the nanoparticles obtained were stored in suspensions at 4°C until further use.

Transmission electron microscopy

The particle size and shape were characterized using high resolution transmission electron microscopy (HR-TEM) JEM 2100 LB_6 under operating voltage of 200 kV to investigate the micrograph of prepared PLGA encapsulation of *Citrus medica* extract under operating voltage of 200 kV for different samples (Fig. 1).
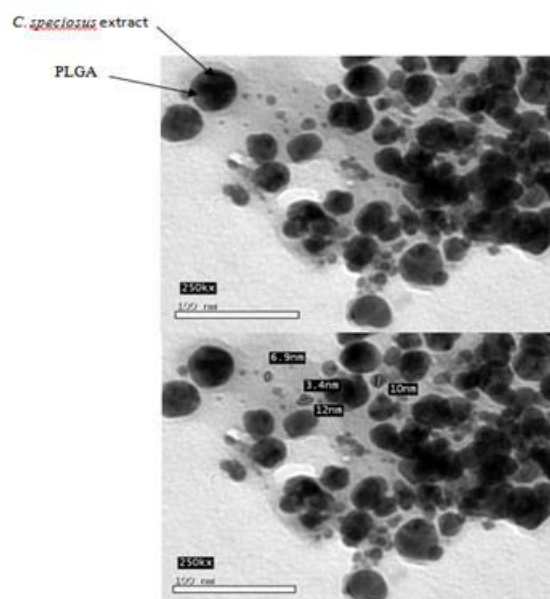

Figure 1: Cross-sectional transmission electron microscopy image of the of poly-lactic-co-glycolic acid (PLGA) encapsulation of *Costus speciosus* nanoparticles.

Experimental Animals

Ninety 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 9 groups. Each group consists of 10 rats:

Group 1 –control: rats were injected 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 – diabetus mellitus induced-rats were administered 10 units insulin subcutaneously[20]; Groups 4-6 – diabetus mellitus induced-rats: rats were treated with 50, 100 and 150 mg/kg bw/day of *Costus speciosus* extract (1/5, 2/5 and 3/5 of the dose used by Gireesh[17] in one dose per oral for 30 days; Groups 7-9 – diabetus mellitus induced-rats were treated with 50, 100 and 150 mg/kg bw/day of *Costus speciosus* extract nanoparticles (1/5, 2/5 and 3/5 of the dose used by Gireesh[17] in one dose per oral for 30 days.

Sample Collections

Blood samples from fasting rats were withdrawn from retro-orbital venous plexus under diethylether anaesthesia 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 pancreas, liver and muscle tissues were used for biochemical analyses and DNA damage as well as gene expression determination were carried out.

Measurement of cholesterol levels

Serum total cholesterol, triglycerides serum HDL-cholesterol and LDL-cholesterol were determined using commercial kits.

Plasma enzyme assessments

Alanine aminotransferase (ALT; EC 2.6.1.2) and aspartate aminotransferase (AST; EC 2.6.1.1) activities were assayed by the method of Reitman and Frankel[18]. Lactate dehydrogenase (LDH, EC 1.1.1.27) activity was determined by the method of Cabaud and Wroblewski[19].

Alkaline phosphatase (ALP; EC 3.1.3.1) activity was measured at 405nm by the formation of paranitrophenol from paranitrophenylphosphate as a substrate[20]. Acid phosphatase (ACP; EC 3.1.3.2) activity was measured using the method of Moss[21]. Protein concentration was assayed by the method of Lowry[22] using bovine serum albumin as a standard.

DNA Fragmentation Analysis

Diphenylamine reaction procedure

Rats liver tissues were used to determine the quantitative profile of the DNA fragmentation. Liver samples were collected immediately after sacrificing the animals. The tissues were lysed in 0.5 ml of lysis buffer containing, 10 mM Tris-HCl (pH 8), 1 mM EDTA, 0.2% triton X-100, centrifuged at 10 000 rpm (Eppendorf) for 20 min at 4°C. The pellets were resuspended in 0.5 ml of lysis buffer. To the pellets (P) and the supernatants (S), 0.5 ml of 25% tri-chloroacetic acid (TCA) was added and incubated at 4°C for 24 h. The samples were then centrifuged for 20 min at

10 000 rpm (Eppendorf) at 4°C and the pellets were suspended in 80 ml of 5% TCA, followed by incubation at 83°C for 20 min. Subsequently, to each sample 160 ml of DPA solution [150 mg DPA in 10 ml glacial acetic acid, 150 ml of sulfuric acid and 50 ml acetaldehyde (16 mg:ml)] was added and incubated at room temperature for 24h[23]. The proportion of fragmented DNA was calculated from absorbance reading at 600 nm wavelength using the formula:

$$\%Fragmented\ DNA = \frac{OD(S)}{OD(S) + OD(P)} \times 100$$

DNA gel Electrophoresis Laddering Assay

Apoptotic DNA fragmentation was qualitatively analyzed by detecting the laddering pattern of nuclear DNA as described by Lu[24]. Briefly, liver tissues were homogenized, washed in PBS, and lysed in 0.5 ml of DNA extraction buffer (50 mM Tris–HCl, 10 mM EDTA. 0.5% Triton, and 100 μg/ml proteinase K, pH 8.0) for overnight at 37 °C. The lysate was then incubated with 100 μg/ml DNase-free RNase for 2h at 37 °C, followed by three extractions of an equal volume of phenol/chloroform (1:1 v/v) and a subsequent re-extraction with chloroform by centrifuging at 15,000 rpm for 5 min at 4 °C. The extracted DNA was precipitated in 2 volume of ice-cold 100% ethanol with 1/10 volume of 3 M sodium acetate, pH 5.2 at –20 °C for 1h, followed by centrifuging at 15,000 rpm for 15 min at 4 °C. After washing with 70% ethanol, the DNA pellet was air-dried and dissolved in 10 mM Tris–HCl/1 mM EDTA, pH 8.0. The DNA was then electrophoresed on 1.5% agarose gel and stained with ethidium bromide in Tris/acetate/EDTA (TAE) buffer (pH 8.5, 2 mM EDTA, and 40 mM Tris–acetate). A 100-bp DNA ladder (Invitrogen, USA) was included as a molecular size marker and DNA fragments were visualized and photographed by exposing the gels to ultraviolet transillumination.

Gene expression analysis

Quantitative RT-PCR

First-strand cDNA synthesis from extracted rat RNA

Total RNA (Poly(A)$^+$ 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 diethylpyrocarbonate (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 photospectrometrically 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.

International Journal of Pharmaceutical Sciences Review and Research
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281

To synthesise first-strand cDNA, 5 µg of complete Poly(A)$^+$ RNA was reverse transcribed into cDNA in a total volume of 20 µL using 1 µL oligo (poly(deoxythymidine)$_{18}$) primer. The composition of the reaction mixture was 50 mmol L^{-1} MgCl$_2$, 10x RT buffer, 200 U µL^{-1} reverse transcriptase (RNase H free, Fermentas, Leon-Rot, Germany), 10 mmol L^{-1} of each dNTP, and 50 µmol L^{-1} 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).

qRT-PCR assay

PCR reactions were set up in 25 µL reaction mixtures containing 12.5 µL 1× SYBR® Premix Ex TaqTM (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[25,26].

The quantitative values of RT-PCR (qRT-PCR) of insulin and gluconeogenic genes (Insulin-I, Insulin-II, GLUT2 and GLUT4) were normalized on the bases of ß-actin expression. The primer sequences of liver cancer related genes are listed in Table 1.

Table 1: List of primers, the primer sequences and the primer melting temperature (Tm)

Gene	Primer	Sequence (5'-3')	Annealing Tm°C
Insulin I	Forward	CCT GTT GGT GCA CTT CCT AC	58
	Reverse	TGC AGT AGT TCT CCA GCT GC	
Insulin II	Forward	CAA CA TGG CCC TGT GGA TGC	60
	Reverse	AGT TGC AGT AGT TCT CCA GC	
GLUT 2	Forward	CATCAAAACGTAGAGCACGGTAA	63.4
	Reverse	TATGGGCATTTAGTCTGCACGTA	
GLUT4	Forward	GCTTGGCTCCCTTCAGTTTG	63.4
	Reverse	CCTACCCAGCCAAGTTGCAT	
β-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.

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[27]:

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

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

The relative quantification of the target to the reference was determined by using the ΔCT method if E for the target (Insulin I&II, GLUT2 and GLUT4) and the reference primers (β-Actin) are the same[27]:

$$Ratio_{(reference/\ target\ gene)} = Ef^{\ CT(reference) - CT(target)}$$

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 *C. speciosus* or *C. speciosus*-NPs on the fasting serum glucose levels in diabetic rats was determined. Diabetic rats revealed extremely high levels of glucose compared with control rats. However, daily treatment of *C. speciosus*-NPs led to a dose dependent fall in serum glucose levels. Administration of DM-rats with medium and high doses of *C. speciosus*-NPs revealed highly decrease in serum glucose levels compared with the DM-rats.

Effect of *C. speciosus* and *C. speciosus*-NPs *on serum lipids and plasma enzymes in normal and diabetic rats*

There was a significant decrease in the level of serum HDL-cholesterol and a significant increase in the levels of total cholesterol, triglycerides and LDL-cholesterol in diabetic rats when compared to normal rats (Table 2). Administration of *C. speciosus* or *C. speciosus*-NPs brought back the levels of serum lipids to near normal (Table 2). Additionally, the protective action of *C. speciosus*-NPs on serum lipids was more effectively compared to *C. speciosus* alone especially with the medium and high doses. The activities of plasma enzymes AST, ALT, LDH, ALP and ACP significantly increased in diabetic rats when compared to normal controls. However, oral administration of *C. speciosus* or *C. speciosus*-NPs for 30 days significantly restored the enzyme levels to near normal in diabetic rats (Table 3). Moreover, the improvement impact of *C. speciosus*-NPs on plasma enzymes was more effectively compared to *C. speciosus* alone especially with the medium and high doses.

International Journal of Pharmaceutical Sciences Review and Research
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282

Table 2: Effect of oral administration of *Costus speciosus* on total cholesterol, triglyceride, LDL cholesterol HDL cholesterol in normal and STZ-induced diabetic male rats for 30 days

Treatment	Total cholesterol (mg/dl)	Triglyceride (mg/dl)	LDL cholesterol (mg/dl)	HDL cholesterol (mg/dl)
Control	91.3 ± 3.4[e]	18.6± 1.1[c]	83.8 ± 4.7c	52.3± 3.5[a]
DM	258± 8.6 [a]	44.3± 2.4[a]	153± 6.2a	31.8± 4.1[c]
DM+Insulin	162.2 ±3.7[b]	27.9± 1.3[b]	101.1 ±4.2b	37.3± 3.1[b]
DM+ *C. speciosus* 50	157.8 ±2.4[bc]	29.2± 1.2[b]	110.3 ±5.2b	34.5± 3.6[b]
DM+ *C. speciosus* 100	142.4 ±4.6 [c]	28.4± 1.1[b]	104.6 ±3.3b	38.2± 4.1[b]
DM+ *C. speciosus* 150	140.8 ±5.2[c]	24.2± 1.2[b]	97.4 ±3.6 b	39.1± 3.2[b]
DM+ *C. speciosus*-*NP*s 50	137.3 ±2.2[dc]	21.8± 1.6[bc]	99.3 ±2.2bc	41.5± 4.4[ab]
DM+ *C. speciosus*-NPs 100	119.5 ±3.5[d]	19.7± 1.2[c]	95.9 ±3.1bc	44.8± 2.6[ab]
DM+ *C. speciosus*-NPs 150	103.4 ±4.1[de]	18.6± 1.1[c]	86.2 ±2.6c	48.9± 5.2[ab]

Table 3: Effect of oral administration of *Costus speciosus* on plasma AST, ALT, LDH, ALP and ACP in normal and STZ-induced diabetic male rats for 30 days.

Treatment	AST (U/dl)	ALT (U/dl)	LDH (U/dl)	ALP (U/dl)	ACP (U/dl)
Control	31.4 ± 1.3[d]	52.4± 2.6[c]	1098.6 ± 33.6[d]	48.6± 3.3[c]	13.5± 0.8[c]
DM	68.5± 3.3[a]	98.6± 4.5[a]	1722.5 ± 51.3[a]	84.3 ±2.7[a]	26.7± 3.1[a]
DM+Insulin	48.4 ±2.1[b]	64.7± 5.2[b]	1253.7 ± 42.8[c]	67.8± 3.0[b]	17.4± 1.1[b]
DM+ *C. speciosus* 50	52.2 ±1.8[b]	72.4± 3.1[b]	1349.4 ± 43.9[b]	69.6± 4.2[b]	19.4± 1.4[b]
DM+ *C. speciosus* 100	48.9 ±2.3[b]	68.6± 4.2[b]	1276.4 ± 41.5[c]	61.5± 3.7[c]	16.8± 2.2[b]
DM+ *C. speciosus* 150	41.6 ±4.3[c]	66.7± 4.3[b]	1211.4 ± 51.4[c]	59.3± 3.5[c]	16.4± 1.6[b]
DM+ *C. speciosus*-*NP*s 50	44.7 ±1.6[bc]	67.3± 4.6[b]	1228.6 ± 36.3[c]	62.6± 6.2[bc]	17.6± 2.1[b]
DM+ *C. speciosus*-NPs 100	38.2 ±2.2[dc]	54.8± 3.1[bc]	1126.7 ± 50.6[d]	58.2± 2.6[c]	15.8± 1.1[bc]
DM+ *C. speciosus*-NPs 150	33.6 ±3.2[d]	49.5± 2.2[c]	1074.2 ± 38.4[d]	46.7± 3.1[c]	14.2± 1.3[c]

AST: Aspartate aminotransferase, ALT: Alanine aminotrasferase, LDH: Lactate dehydrogenase, ALP: Alkaline phosphatase, ACP: Acid phosphatase.

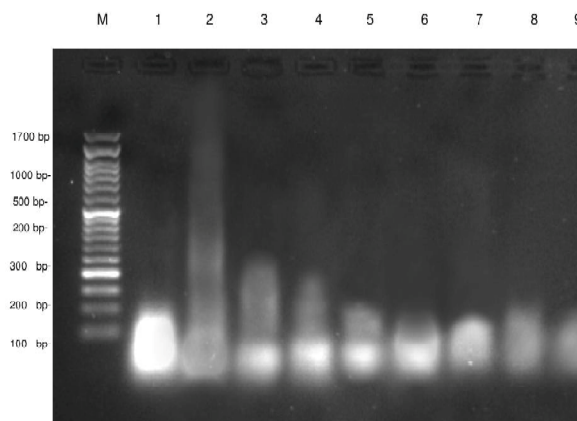### Effect of *C. speciosus* and *C. speciosus*-NPs *on* rates of DNA fragmentation

The results of the DNA fragmentation assay revealed that treatment of diabetic rats with different doses of *C. speciosus* and *Costus speciosus*--NPS induced different rats of DNA fragmentation (Fig. 2 and Table 4).

The rate of DNA fragmentation in control rats induced a low rate of DNA damage (Fig. 2 and Table 4). However, DM- male rats induced a high rate of DNA fragmentation which was 36.4±2.3% compared with 09.0±0.6% in control rats.

Table 4: DNA fragmentation in liver tissues of male DM-rats treated with treated with different doses of *Costus speciosus* analyzed by diphenylamine reaction procedure.

Treatment	% of DNA Fragmentation	
	Range	Mean±SEM
Control	07 – 13	09.0±0.6[d]
DM	34 – 47	36.4±2.3[a]
DM+Insulin	30 – 36	29.1±1.3[b]
DM+ *C. speciosus* 50	25 – 29	27.5±0.9[bc]
DM+ *C. speciosus* 100	22 – 27	26.0±1.3[bc]
DM+ *C. speciosus* 150	24 – 29	24.1±1.1[c]
DM+ *C. speciosus*-*NP*s 50	22 – 29	25.9±1.6[bc]
DM+ *C. speciosus*-NPs 100	19 – 22	23.8±1.0[c]
DM+ *C. speciosus*-NPs 150	21 – 26	20.4±0.9[c]

In contrary, treatment of DM-rats with different doses of *Costus speciosus* or *Costus speciosus*—NPS revealed significantly low rats compared with DM-rats. Moreover, the protective action of *C. speciosus*-NPs on the DNA fragmentation was more effectively compared to *C. speciosus* alone especially with the medium and high doses. Treatment of DM-rats with medium and high doses of *Costus speciosus*—NPS revealed lower rates of fragmentation compared with those in DM-rats treated with *Costus speciosus* alone (Fig. 2 and Table 4).

Figure 2: DNA fragmentation in liver tissues of male DM-rats treated with different doses of *Costus speciosus*. M:

DNA marker. Lane 1 represents PCR products of untreated control samples; lane 2 represents DM-rats; lane 3 represents DM-rats treated with insulin; Lanes 4-6 represents DM-rats treated with 50, 100, 150 mg/kg *Costus speciosus* extract. Lanes 7-9 represent DM-rats treated with 50, 100, 150 mg/kg *Costus speciosus*-NPs.

On the other hand, treatment of DM-rats with insulin induced low rats of DNA fragmentation compared with DM-rats, where the rate of damage was 29.1±1.3% in

DM-rats treated with insulin compared with 36.4±2.3% DNA fragmentation in DM-rats (Fig. 2 and Table 4).

Effect of *C. speciosus* and *C. speciosus*-NPs *on* expression of insulin and gluconeogenic genes

The expression of diabetic-associated insulin (I & II) and gluconeogenic genes, in the streptozitocin-induced diabetic rats treated with *Costus speciosus and Costus speciosus*-NPs was determined using RT-PCR (Figures 3-6).
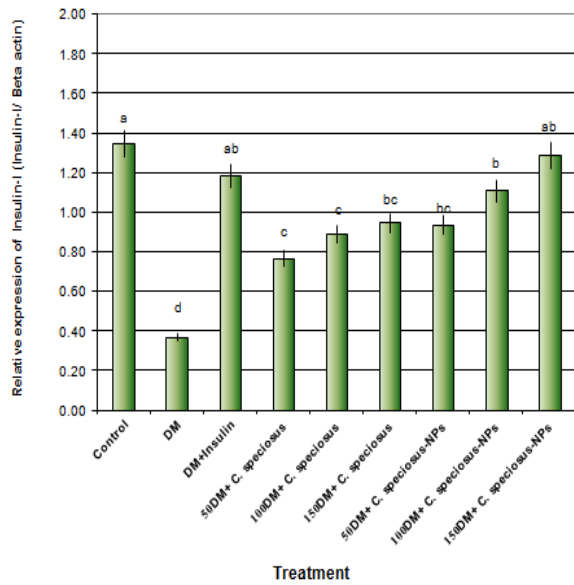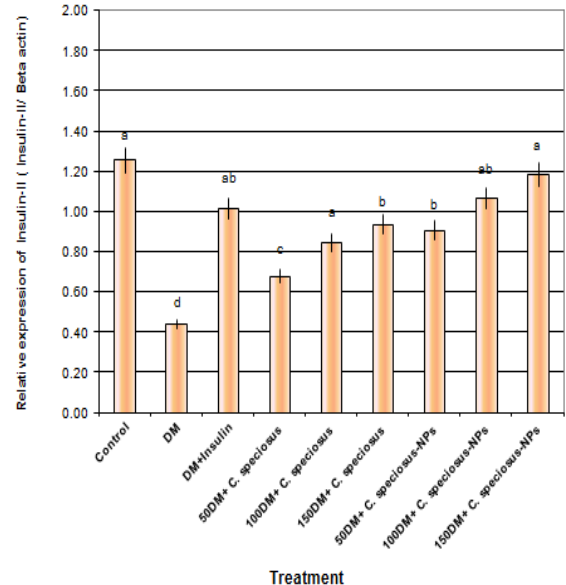

Figure 3: The alterations of Insulin-I mRNA in pancreas tissues isolated of male DM-rats treated with different doses of *Costus speciosus*-NPs. [a,b,c]Mean values within tissue with unlike superscript letters were significantly different (*P*<0.05).

Figure 4: The alterations of Insulin-II mRNA in pancreas tissues isolated of male DM-rats treated with different doses of *Costus speciosus*-NPs. [a,b,c]Mean values within tissue with unlike superscript letters were significantly different (*P*<0.05).
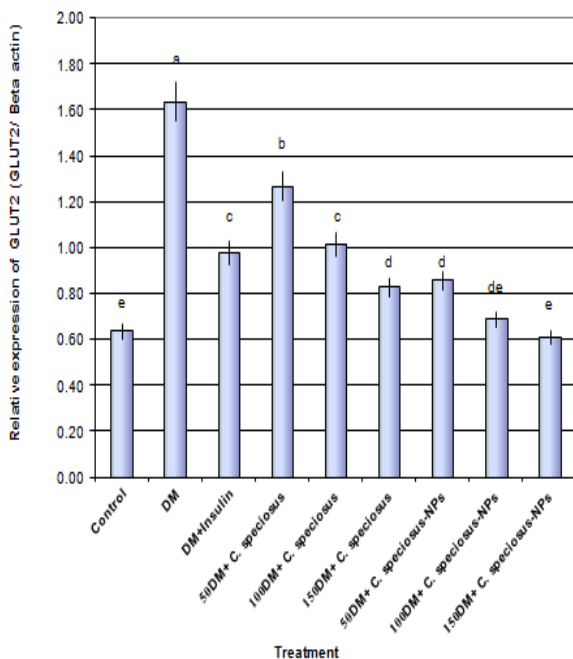

Figure 5: The alterations of GLUT2-mRNA in liver tissues isolated of male DM-rats treated with different doses of *Costus speciosus*-NPs. [a,b,c]Mean values within tissue with unlike superscript letters were significantly different (*P*<0.05).
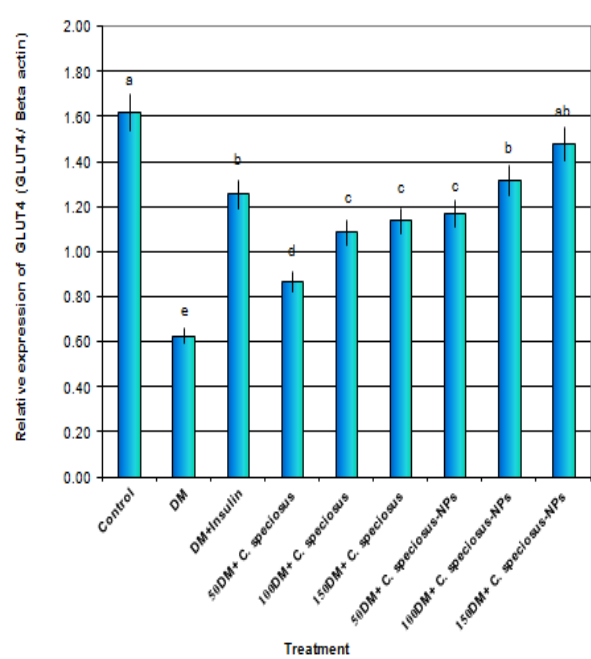

Figure 6: The alterations of GLUT4-mRNA in muscles tissues isolated of male DM-rats treated with different doses of *Costus speciosus*-NPs. [a,b,c]Mean values within tissue with unlike superscript letters were significantly different (*P*<0.05). ect

The results revealed that DM-rats showed significantly lower expression values of pancreatic insulin 1 and II and muscle Glucose transporter type 4 (GLUT4) genes in comparison with the control rats (Figures 3, 4 & 6). While, DM-rats treated with Low, medium and high doses of *Costus speciosus* or *Costus speciosus*-NPs caused significant increase in insulin I and II and GLUT4 expression as compared with the DM-rats. Furthermore, the highest expression levels of insulin I and II as well as GLUT4 genes were showed in DM-rats treated with the medium and high doses of *Costus speciosus*-NPs (Figures 3, 4 & 6). In addition, treatment of DM-rats with insulin increased significantly the expression of insulin I and II and GLUT4 genes, however with low effect compared with *Costus speciosus*-NPs.

Concerning the Glucose transporter type 2 (GLUT2) gene, the present results revealed that DM-rats showed significantly higher expression values of GLUT2- mRNA in comparison with the control rats (Fig. 5). However, DM-rats treated with Low, medium and high doses of *Costus speciosus or Costus speciosus*-NPs caused significant decrease in GLUT2- mRNA expression as compared with the DM-rats. Moreover, lowest expression levels of GLUT2- mRNA genes were showed in DM-rats treated with the medium and high doses of *Costus speciosus*-NPs (Fig. 5). Moreover, DM-rats treated with insulin showed significantly lower expression values of GLUT2- mRNA in comparison with the DM-rats.

DISCUSSION

Novel approach to treat diabetes with flavonoid nanoparticulate system to enhance the antidiabetic activity on animal models has been discussed in this article. Diabetes is the wide spread pandemic disease resulting in increased morbidity and mortality. The blood glucose level of diabetics can be effectively controlled by utilizing currently available antidiabetic agents. The FDA approved anti diabetic agents is used for the therapy to control blood glucose level[28,29]. Incorporation of natural antidiabetic preparation is given privilege to make use of it, as it holds less toxicity and negligible side effects. Almost all the flavonoids having potential for antidiabetic activity but they are limited in usage on account of deprived solubility and bioavailability.

To overcome the problem of solubility a poly (D,L-lactic acid, PLA) nanoparticles of *Costus speciosus* prepared by nanoprecipitation method[30]. Further nanoencapsulated *Costus speciosus* of PLA nanoparticles have better bioavailability and intestinal absorption than *Costus speciosus*. Based on the above fact we propose polymeric nanoparticles increases the bioavailability than the flavonoid alone for safer antidiabetic treatment alternative with synergistic action. On other hand, role of biological antioxidants like super oxide dismutase (SOD), catalase (CAT) etc., in recent times for pancreatic islets isolation and transplantation in animal models has been very effective[31]. This activity reveals the option for utilizing plant biomolecules (flavonoids) in transplantation process involving pancreas with novel technology.

The currently available drug regimens for management of diabetes mellitus have certain drawbacks and therefore there is a need to find safer and more effective antidiabetic drugs[32]. The aim of the present study was to evaluate the normo-glycemic, hypolipidemic, gene expression alteration and anti-genotoxicity effects of *Costus speciosus*-NPs or *Costus speciosus*-NPs in STZ-induced diabetic rats.

The experimental diabetic model used in this study was type II since low dose of STZ (50 mg/kg bw) destroyed half a population of pancreatic beta cells[33]. There were residual beta cells which secreted insufficient insulin causing type II diabetic model[34]. The mechanism by which streptozotocin brings about its diabetic state includes selective destruction of pancreatic beta cells which make cells less active[35] leading to poor glucose utilization by tissues[36]. The increased levels of plasma glucose in STZ-induced diabetic rats were better declined by the administration of *Costus speciosus*-NPs. The reduced glucose levels suggested that *Costus speciosus* or its active ingredient costunolide might exert insulin like effect on peripheral tissues by either promoting glucose uptake metabolism by inhibiting hepatic gluconeogenesis[37,38], or by absorption of glucose into the muscle and adipose tissues[39], through the stimulation of a regeneration process and revitalization of the remaining beta cells[40-42].

The normo-glycemic action of *Costus speciosus* was caused by potentiation of insulin release from the existing beta cells of islets of Langerhans. Since costunolide (active ingredient of *Costus speciosus*) is a sesquiterpene, it might have stimulated the beta islets to secrete insulin and increase the sensitivity of insulin to uptake glucose[43]. Achrekar[44] reported that the water extract of the pulp of *Eugenia jambolana* stimulated the release of insulin both in *in vivo* and *in vitro* studies. The increase in plasma insulin might be attributed to proinsulin leading to insulin conversions, possibly by pancreatic cathapsin B, and/or its secretion[45].

The possible mechanism of action of *Costus speciosus*/costunolide might be that it stimulated the beta islets to secrete insulin by inhibiting the expression of nitric oxide synthase. It has been shown that costunolide inhibited the expression of nitric oxide synthase and thus helped in correcting the secretary defects in diabetes[46,47].

Abnormalities in lipid profile are one of the most common complications in diabetes mellitus found in 40% of diabetic cases[48]. Diabetes causes an increase in the cholesterol, triglycerides, LDL and VLDL[49]. These findings agreed our findings, where a significant increase in the levels of total cholesterol, triglycerides and LDL-cholesterol in diabetic rats was found. High levels of total cholesterol and more importantly LDL-cholesterol in blood are major coronary risk factors in DM disease. The

abnormal high concentration of serum lipids in the diabetic subject is due mainly to the increase in the mobilization of free fatty acids from the peripheral fat depots, since insulin inhibits the hormone sensitive lipase. Insulin deficiency or insulin resistance may be responsible for dyslipidemia, because insulin has an inhibitory action on HMG-coA reductase, a key rate-limiting enzyme responsible for the metabolism of cholesterol-rich LDL particles. Acute insulin deficiency initially causes an increase in free fatty acid mobilization from adipose tissue. This results in increased production of cholesterol rich LDL particle[50-52]. On the other hands our results found that administration of Costus speciosus-NPs increased the level of serum HDL-cholesterol and decreased the levels of total cholesterol, triglycerides and LDL-cholesterol.

Moreover, the current results revealed that the activities of plasma AST, ALT, LDH, ALP and ACP were increased which indicated that diabetes might be induced due to liver dysfunction. Ohaeri[53] also found that liver was necrotized in STZ-induced diabetic rats. Therefore, an increase in the activities of AST, ALT, LDH, ALP and ACP in plasma might be mainly due to the leakage of these enzymes from the liver cytosol into the blood stream[54] which gives an indication on the hepatotoxic effect of STZ. On the other hand, we found that treatment of the diabetic rats with Costus speciosus-NPs caused reduction in the activity of these enzymes in plasma when compared to the diabetic group and consequently alleviated liver damage caused by STZ-induced diabetes. These results are in agreement with those obtained by El-Demerdash[55] in rats.

The current study observed that the rate of DNA damage in DM- male rats induced a high rate of DNA fragmentation with control mice. While, treatment of DM-rats with Costus speciosus-NPs revealed significantly low rats compared with DM-rats. In agreements with our findings Qari[56] found that treatment of C. speciosus has a strong inhibitory role against the genotoxic action of Ethyl methanesulphonate induced DNA damage. These results strongly suggest that the extract of Costus speciosus is not genotoxic, or cytotoxic but might be anti-genotoxic agent.

The protective effect of the Costus speciosus on the molecular mechanism inhibiting changes in the gene expression and genotoxicity is not clear understood. However, several studies suggested the protective effects of Costus speciosus may be attributed to its antioxidant activity.

Free radicals have aroused significant interest among scientists in the past decade. Their broad range of effects in biological systems has drawn the attention of many experimental works. It has been proved that these mechanisms may be important in the pathogenesis of certain diseases and ageing. There are many reports that support the use of antioxidant supplementation in reducing the level of oxidative stress and in slowing or preventing the progress of complications associated with diseases[57]. Many synthetic antioxidant components have

shown toxic and/or mutagenic effects, which have shifted the attention towards the naturally occurring antioxidants. Numerous plant constituents have proven to show free radical scavenging or antioxidant activity[58]. Flavonoids and other phenolic compounds (hydroxyl cinnamic derivatives, catechines, etc.) of plant origin have been reported as scavengers and inhibitors of lipid peroxidation[59]. In the study of Qari[56] reported that superoxide scavenging effect of C. speciosus was demonstrated. They indicated that C. speciosus inhibited the production of superoxide anion radicals by 68.7%. These results are also supported by the previous studies on antioxidant activity of C. speciosus[60].

The protective effect of C. speciosus is due to its antioxidant action, trapping of free radicals, formation of complex with mutagens[61]. The mode of action of anti-mutagenesis may act as modulation of mutagen metabolism by absorbing the xenobiotics, or inhibition of SOS (superactive oxygen species) functions or by altering the activation and detoxification of toxic agents as suggested by similar results obtained by Premkumar; Oda; and Goud[62,63], respectively. Also, the stabilization of the formed phenoxy free radicals is responsible for its free radical scavenging activity and chemopreventive effect mutagens[64]. The modulatory role of C. speciosus in inhibiting mutagenicity and/or cytotoxicity need more studies to understand the mechanism of antigenotoxic action.

The results revealed that DM-rats showed significantly lower expression values GLUT4 gene and higher expression of Insulin (I&II) and GLUT2- mRNA in comparison with the control rats. While, DM-rats treated Costus speciosus-NPs caused significant increase in GLUT4 expression and lower expression values of Insulin (I&II) and GLUT2- mRNA as compared with the DM-rats. To date no data discussed the effect of Costus speciosus-NPs on the expression of insulin and gluconeogenic genes.

However, Kang[65] studied the inhibitory effect of costunolide isolated from costus species on the protein and mRNA expression of interleukin-1beta (IL-1beta) in LPS-stimulated RAW 264.7 cells. They demonstrated that costunolide inhibits IL-1beta gene expression by blocking the activation of MAPKs and DNA binding of AP-1 in LPS-stimulated RAW 264.7 cells. Therefore, the expression alterations of insulin and gluconeogenic genes due to Costus speciosus treatment could be attributed to the active ingredient costunolide.

In conclusion, our results provide novel mechanisms for the plasma glucose-lowering action of nanoparticles of Costus speciosus. The extract produced its anti-hyperglycemic effect. Further it is confirmed that the extract suppressed the transcription of genes involved in pancreatic insulin and hepatic glucose production. In addition, the extract stimulated the expression of GLUT-4 gene in skeletal muscles of streptozitocin-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 streptozitocin which was observed through the reduced expression of GLUT-2 gene. Moreover, oral administration of nanoparticles of *Costus speciosus* decreased serum total cholesterol, triglyceride, LDL cholesterol and at the same time markedly increased HDL cholesterol. Also *Costus speciosus*-NPS restored the altered plasma enzyme (aspartate aminotransferase, alanine aminotrasferase, lactate dehydrogenase, alkaline phosphatase and acid phosphatase) levels to near normal. Altogether, it can be concluded that the nanoparticles of *Costus speciosus* extract could be used as a drug to bring about normo-glycemic and hypolipidemic effect.

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287

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