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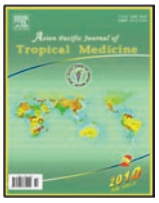

Document heading

Oral delivery of insulin with *Desmodium gangeticum* root aqueous extract protects rat hearts against ischemia reperfusion injury in streptozotocin induced diabetic rats

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ABSTRACT

Objective: To evaluate the effect of insulin administered via oral route with the help of aqueous extract of *Desmodium gangeticum* (DG) root in rendering cardio protection against ischemia reperfusion injury in diabetic rats. **Methods:** Diabetes mellitus was induced in rats by the β –cell toxin, streptozotocin (STZ, 60 mg/kg). Isolated rat (IR) heart was used to investigate the effect of insulin mixed DG pretreatment on ischemia reperfusion injury. Mitochondrial respiratory enzymes and microsomal enzymes were used to assess the metabolic recovery of myocardium. Cardiac marker enzymes were used to find the functional recovery, which were compared with that of the STZ treated IR rats. **Results:** Compared with IR control group, rat treated with insulin mixed DG showed a significant functional and metabolic recovery of myocardium from the insult of ischemia reperfusion. Even though orally administered insulin mixed DG displayed a slow but prolonged hypoglycemic effect, the cardio protection it provided was more significant than when it was given intra peritoneal. Furthermore the above result indicates that insulin mixed DG can overcome the barriers in the gastrointestinal tract and be absorbed. **Conclusions:** The above results indicate the efficacy of insulin mixed DG in protecting the heart from ischemia reperfusion induced injury in diabetic rats. Furthermore the study gives additional information that herbal extracts can be used to transport insulin across the membrane and found to be a feasible approach for developing the oral delivery of insulin, as well as other peptide drugs.

1. Introduction

The agents that can protect myocardium from damaging effects of ischemia/reperfusion are of considerable importance. They can be administered during cardiac surgery or in the case of heart transplantation to minimize the effect of myocardial ischemic damage. Epidemiological studies and clinical trials have clearly shown that both type 1(insulin-dependent) and type 2(noninsulin-dependent) diabetic individuals are more prone to developing ischemic heart disease, including acute myocardial infarction and post infarct complications [1].

There is a close association between hyperglycemia and increased risk of mortality after acute myocardial infarction (AMI) where stress hyperglycemia can be observed in patients with or without diabetes [2]. The deleterious effect of diabetes on cardiac performance has been well established in early studies[3]. Insulin can prevent, or revert, a number of outcomes caused by experimental diabetes. Thus normalization of blood glucose is essential for the prevention of diabetes mellitus (DM)-related microvascular and macrovascular complications and can be done by insulin administration.

But for insulin, rapid enzymatic degradation in the stomach, inactivation and digestion by proteolytic enzymes in the intestinal lumen, and poor permeability across intestinal epithelium because of its high molecular weight and lack of lipophilicity paved the way for its subcutaneous administration. However insulin if administered via oral route is advantageous because it is delivered directly to the liver, its primary site of action, via the portal circulation, a mechanism very similar to endogenous insulin.

Previous experimental data from our lab showed that *Desmodium gangeticum* (DG) aqueous root extract can mediate the absorption of insulin into blood through gastrointestinal tract [4].Insulin therapy is considered to be beneficial not only to AMI but also to critical illness in patients with or without diabetes mellitus. In this study, we focused on delayed action of insulin by supplementing it through oral route with the help of a cardioprotective herbal

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extract[5] namely, *Desmodium gangeticum* aqueous root extract. *Desmodium gangeticum* (L.) DC (Fabaceae) (DG) is a perennial non-climbing shrub which is widely used as medicinal herb in the treatment of ischemic heart diseases [6]. The phyto-chemical analysis of DG reported to have flavonoids, glycosides, pterocarpanoids, lipids, glycolipids and alkaloids[7].

2. Materials and methods

2.1. Preparation of aqueous extract of the roots of Desmodium gangeticum

The plant maintained in the department after collection from the herbal garden was washed and cleaned. The plant material was taxonomically identified by Prof. James Joseph, Head of the Department, Department of Botany, Saint Berchman´s College, Mahatma Gandhi University, Kerala. The voucher specimen A/C no. 3908 was retained in our laboratory for future reference.

One kilogram (1 kg) of fresh secondary roots of DG were sliced and air-dried at room temperature. The sliced, air-dried roots of the plant were milled into fine powder in a warring commercial blender. The powder was soxhlet extracted with 2.5 liters of distilled water at room temperature for 24 hours with shaking. The aqueous extracts were filtered and concentrated to dryness under reduced pressure at (30±1)℃. The resulting aqueous extract was freeze-dried, finally giving 18.66 g [i. e., 1.866% yield] of a light-brown, powdery crude aqueous root extract of DG. Aliquot portions of the crude root aqueous extract residue were weighed and dissolved in distilled water for use on each day of our experiment.

2.2. Animals

Frogs of *Rana hexadactyla* species maintained in the animal house and male Wistar albino rats (150-200 g) housed in cages were maintained in controlled temperature of (23±2) ℃ with 12 h light/dark cycle were used for the studies. The animals were fed with food and water *ad libitum*. The animals were maintained as per the norms of CPCSEA and the experiments were approved by CPCSEA and the local ethics committee.

2.3. Chemicals

Streptozotocin was obtained from Sigma co., St. Louis, MO, USA. Glucose estimation kits were from Ecoline, Merck Ltd. Mumbai, India. All the other reagents and chemicals used in the study were of analytical grade.

2.4. Induction of experimental diabetes

Diabetes mellitus was induced by 60 mg/kg/5 mL of STZ (dissolved in 50 mM Citrate buffer) was once dosed by intraperitoneal injection, and equal volume of buffer was dosed in normal control using the same route.

2.5. Heart preparation

Wistar male rats weighing 250-280 g were anesthetized with 40 mg/kg sodium thiopentenone. After an intravenous injection of 300 U heparin, the heart was rapidly excised via a mid-sternal thoracotomy and arrested in the ice cold Krebs-Henseleit (KH) buffer containing (mM/L) NaCl 118, KCl 4.7, $MgSO_4$1.2, KH_2PO_4 1.2, $CaCl_2$ 1.8, $NaHCO_3$ 25 and Glucose 11. The heart was attached to a Langendorff apparatus via an aorta for retrograde perfusion with KH buffer maintained at 37 ℃and pH=7.4 and saturated with a gas mixture of 95% O_2 and 5% CO_2. The coronary perfusion pressure was maintained at 80 mmHg. The left ventricular pressure developed with ventricle which was filled with Krebs solution and was recorded with a pressure transducer, which in turn was connected to a device amplifier and chart recorder. This left ventricular pressure gave an indication of the mechanical performance of the heart. Coronary flow was measured simply by collecting the perfusate draining from the heart in a graduated cylinder for a defined time. The heart rate was measured by counting the number of contractions (obtained from the left ventricular pressure record) per minute.

2.6. Preparation of insulin mixed DG

Insulin mixed DG was prepared by mixing aqueous extract of DG (0.1 mg/mL) with human insulin (40 IU/mL) in ratio of 1:1(v/v) and kept for 10 minutes at low temperature. The above combination was arrived from the results of pilot study where different combination (v/v) of insulin and DG was utilized for its hypoglycemic effect in glucose administered rats.

2.7. Experimental protocol I

The rats were divided into four groups: group I, normal control; group 2, diabetic control; group 3, reperfusion, and group 4, drug.

Group 1: In normal control group, hearts (n=6) were perfused for 90 minutes with KH buffer and used for the biochemical analysis.

Group 2: In this group, hearts (n=6) of diabetic rats were subjected to similar protocol as followed in group1.

Group 3: The animals in reperfusion group were subdivided into three sub groups after induction of diabetes mellitus as follows:

Group 3.1: Hearts (n=6) in this group were perfused with KH buffer for 20 minutes and were subjected to 30 minutes of global ischemia followed by 15 minutes of reperfusion;

Group 3.2: After 20 minutes equilibration the hearts (n=6) were induced with 30 minute global ischemia followed by 30 minutes reperfusion;

Group 3.3: In this group the hearts (n=6) were subjected to 30 minute global ischemia followed by 45 minutes reperfusion.

Group 4: In this group also, diabetes mellitus induced animals were subdivided into 6 sub-groups:

Group 4.1: Diabetic rats (n=6) in this groups were pretreated with insulin intra peritoneal at a dose of 200 μU/g body weight, one hour before the start of the experiment. Hearts were subjected to 30 minutes of global ischemia after equilibration, followed by 45 minutes reperfusion;

Group 4.2: Diabetic rats (n=6) in this groups were administered with DG at a dose of 50 mg/kg body weight, through intra peritoneal before one hour to the commencement of the experiment. Hearts were subjected to 30 minutes of global ischemia after equilibration and followed by 45 minutes of reperfusion;

Group 4.3: In this group, rat hearts (n= 6) were treated with DG mixed insulin intra peritoneal before one hour of the experiment. Ischemia reperfusion was induced by subjecting the hearts to 30 minutes of global ischemia after equilibration and followed by 45 minutes of reperfusion.

Group 4.4: DM rats (n=6) in this groups were treated with methods similar to that of group 4.1 except the insulin at a dose of 200 μU/g body weight was administered through oral route;

Group 4.5: A similar experimental protocol as that of 4.2 was followed in the rat hearts of this group except for the route administration of drug, DG (50 mg/kg body weight), which was given orally;

Group 4.6: Rat hearts (n=6) in this group were subjected to similar protocol that was followed in group 4.3 except that the drug was administered through oral route.

2.8. Tissue preparation

The heart was excised, rinsed in ice cold isotonic saline, blotted with filter paper, weighed, homogenized in 0.25 M sucrose at 4 ℃ by Polytran homogenizer for 5 seconds at maximum power. The homogenate was centrifuged for 10 min at 600 g, nuclear and cytoskeleton fractions were discarded. The supernatant was centrifuged for 20 min at 15 000×g to pellet mitochondria [8]. The mitochondria were suspended in 0.25 M sucrose containing 10 mM Tris-HCl and 1mM EDTA to a known volume of 3 mL.

The post mitochondrial (supernatant) fraction was further centrifuged at 105 000×g for 60 min to isolate the microsomal fraction [9]. The microsomal pellet suspended in 50 mM Tris- HCl buffer pH 7.5 containing potassium chloride.

2.9. Experimental protocol II

Frog hearts were isolated from specimens of Rana hexadactyla [weighing (22.015±1.200) g (mean±SE)] and connected to a perfusion apparatus as previously described [10]. Experiments were done at room temperature (18 - 21℃). The hearts were perfused with frog-Ringer solution containing NaCl 6.5 g, KCl 0.14 g, $CaCl_2$ 0.12 g, and $NaHCO_2$ 0.2 g, NaH_2PO_4 0.01 g, Glucose 2.0 g in g. per liter. The force of contraction was recorded and the rate of contraction was counted and tabulated.

2.10. Biochemical assays

Assay of isocitrate dehydrogenase (ICDH)[11], malate dehydrogenase (MDH) [12], succinate dehydrogenase (SDH) [13], α ketoglutarate dehydrogenase (α KGDH)[14], NADH dehydrogenase[15] and cytochrome c oxidase [16] were carried out in a UV-1601 Schimadzu spectrophotometer. Protein concentration was measured with Folin phenol reagent, following the procedure described by Lowry [17].

After isolating the sarcoplasmic reticulum (SR), Na^+K^+ ATPase[18], Ca^{2+}ATPase [19], Mg^{2+}ATPase [20] and 5´ nucleotidase [21] were assayed. The activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were estimated by the method of Reitman and Frankel[22]. Lactate dehydrogenase (LDH) and creatine phosphokinase (CPK) were determined by the method of King[23] and Okinaka and his co-worker [24], respectively, using commercially available kit.

2.11. Statistical analysis

All dates are reported as mean±SD. Results were statistically analyzed by a one-way analysis of variance (ANOVA) by SPSS software 12.00, followed by Duncan´s Multiple range Test (DMRT). P< 0.05 was considered to be significant.

3. Result

3.1. Functional recovery of heart after ischemia

Table 1 showed the hemodynamic parameters that suggested significant recovery of ischemic heart from reperfusion induced physiological derangement in diabetic rat hearts pretreated with DG mixed insulin. The recovery of mean arterial pressure (MAP), heart rate (HR) and left ventricular developed pressure were quick in rat hearts pretreated orally with DG mixed insulin than sole administration of insulin or DG root extract. In Figure 1, we presented the time course of rate pressure product (RPP) of hearts going through a cycle of ischemia and reperfusion. These data confirmed that following oral insulin mixed DG administration hearts recovered from 3 minutes isothermic global ischemia with better hemodynamic function than normal and control rat hearts (Table 1).

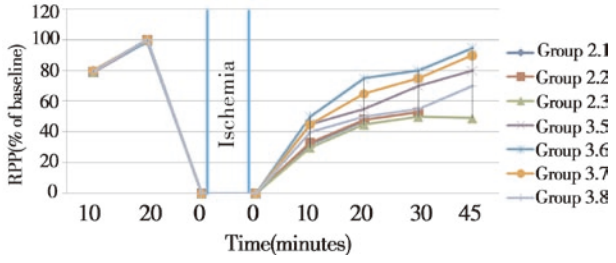

Figure 1. Rate pressure product of hearts subjected to 30 minntes global ischemia followed by reperfusion in diabetes melliitus rats

In perfusate, level of cardiac necrotic marker proteins LDH increased during ischemia reperfusion. But the elevated levels of the enzymes were declined in the drug treated groups (Figure 2). Simultaneous preservation of cardiac enzymes in the tissue complimented the above observation (Figure 3). Interestingly, we observed a better myocardial preservation (limited LDH in perfusate) in oral DG mixed insulin treated diabetic rat than insulin and DG given intravenously or even oral route.

Table 2 showed the activity of mitochondrial respiratory enzymes during experiments. Significant improvement (P<0.1) in the activity of ICDH, SDH, MDH, α KGDH, NADH dH and cytochrome c oxidase was observed in drug treated ischemic reperfused rat hearts. Indeed oral administration of insulin along with DG preserved the above enzymes more efficiently.

The activity of Na^+K^+ ATPase, Ca^{2+} and Mg^{2+} ATPases decreased significantly in the hearts of ischemic reperfused rats compared with normal control rats (Table 3). Pretreatment with drugs [insulin, DG, insulin + DG (oral and intra-peritoneal)] significantly increased the activity of above ATPase in ischemia reperfused rat hearts compared with untreated IR rat (Table 3).

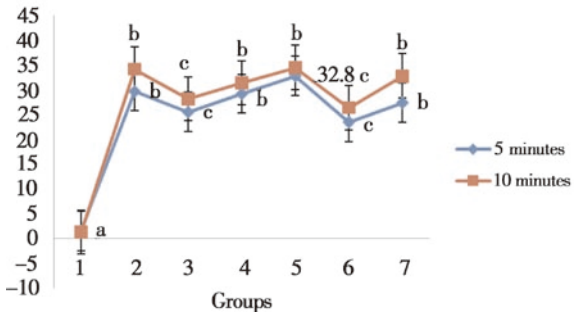

Figure 2. The level of LDH in rat heart perfusate.
Values are mean ± SD for 6 rats in each group. *n*, number of hearts in each group; Values not sharing a common superscript (a,b,c,d,e,f) differ significantly at $P<0.05$) when compared between the groups. LDH (μ moles/min./mg protein)

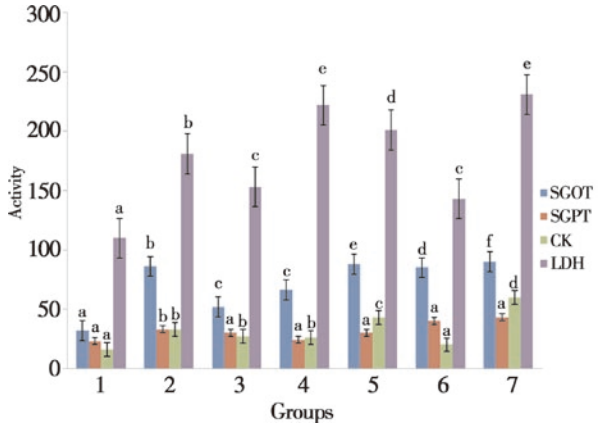

Figure 3. Aetivity of SGOT, SGPT, CK & LDH in the tissue homogenate of isolated rat heart.
Values are Mean ± SD for 6 rats in each group. *n*, number of hearts in each group; Values not sharing a common superscript (a,b,c,d,e,f) differ significantly at P<0.05 when compared between the groups. CK (μ mol phosphorous liberated/min/mg protein), LDH (nmol pyruvate liberated/min/mg protein), AST and ALT (nmol pyruvate liberated/min/mg protein).

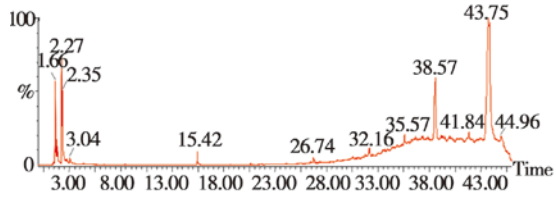

Figure 4. GC Mass spectrum for water extract of *Desmodium gangeticum* root.

GS/MS analysis of DG root aqueous extract resulted in the identification of 22 compounds (Figure 4). Major compounds comprises of phenol,4-[2-(dimethylamino) ethyl]-(Cactine) (R.T 15.41), tri tetracontane (R.T 32.16), docosanoic acid docosyl ester (R.T 35.58), 5 hydroxy -7-(4- methoxyphenyl)-2,2-dimethyl-10-(3-methyl-2-butenyl)-2H, 6H-pyrano [3,2- γ]chromen-6-one (R.T 38.57) and 15-isobutyl-(13 α H)-isocopalane (R.T 44.96). It represented around 35%. Minor compounds such as 2,2-dimethyl N phen ethyl propinamide (R.T 20.65), 5 hydroxymethyldihydrofuran-2-one (R.T 8.38) and 3-methyl-2-(2-oxopropyl)furan (R.T 21.73).

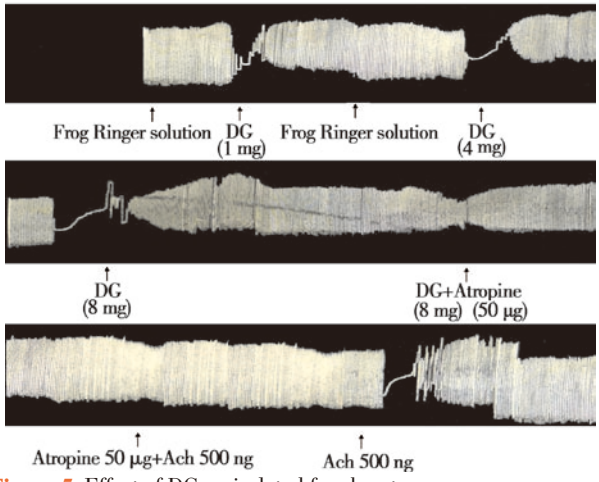

Figure 5. Effect of DG on isolated frog heart.

The inotropic and chronotropic effects of isolated heart were recorded on kymograph paper. The normal rate and contraction of isolated heart was recorded after stabilization for 15 minutes. The different doses 1 mg, 4 mg, and 8 mg body weight of methanol extract of DG were injected directly to the isolated heart along with perfusion. The baseline reading of cardiac flow rate, heart rate and force of contraction were found to be changed with extract, acetyl choline and atropine (Table 4). The negative inotropic and chronotropic effect shown by the extract followed a dose dependent change and the maximum response was produced by 8 mg of the extract. However, the decreased heart rate and force of contractions were recovered partially when 50 μg of atropine was administered along with the extract (Figure 5). Similarly when Ach was treated after atropine, the heart rate and force of contraction again declined.

4. Discussion

The major observations in the present study can be summarized as follows: (1) Myocardial recovery from ischemia reperfusion induced contractile dysfunction was significant in diabetic rat hearts administered orally with insulin mixed DG orally than insulin or DG alone and even insulin mixed DG administered intra-peritoneal. (2) Myocardial protection render by both oral and intra-peritoneal administration of DG mixed insulin was more significant than the administration of DG root extract or insulin alone.

A deleterious effect of diabetes on cardiac performance has been well documented in isolated heart preparation [25], isolated cardiomyocytes [26] and intact anesthetized animals [27]. Besides myocardial dysfunction, it is well known that experimental diabetes affects hemodynamic parameters such as heart rate and arterial pressure [28]. The recovery in hemodynamic parameters of rat hearts administered with insulin or insulin mixed DG indicate insulin mediated protection of ischemia reperfusion injury[29]. Insulin administration reduces the ischemia reperfusion induced elevation in LVDP in drug treated groups. Insulin resistance is characterized by a generalized defect in the insulin signal pathway and was considered to be one of the main causative factors for the exacerbation of ischemia reperfusion injury in diabetic condition. In the present study, the functional

Table 1

Hemodynamic characteristic.

Group	LVDP(mmHg)	CF(mL/min)	HR(b.p/min)	RPP $\pm 10^3$(mmHg.bt min^{-1})	MAP(mmHg)
Group 1	108.11±3.30[a]	9.70±1.40[a]	349.0±26.60[a]	37.14±7.70[a]	122±7[a]
Group 2	104.73±2.10[a]	9.50±1.20[a]	347.0±23.5[a]	36.11±7.10[a]	118±7[a]
Group 3.1	91.21±1.10	9.00±0.91	260.0±19.3*	23.78±3.60*	98±46*
Group 3.2	92.43±4.30	9.10±0.94	266.0±19.2*	24.58±6.10*	100±6*
Group 3.3	96.70±4.60	9.10±1.020	270.0±21.3*	26.15±6.20*	101±7*
Group 4.1	107.20±4.30[a]	9.20±1.14[a]	338.0±34.3[a]	36.24±8.70[a]	114±8[a]
Group 4.2	99.40±3.60[a]	9.20±0.94[a]	303.0±33.2[b]	30.14±6.80[a]	104±5[b]
Group 4.3	106.30±5.90[a]	9.40±1.10[a]	338.0±31.3[a]	35.82±5.10[a]	110±7[b]
Group 4.4	102.10±2.10[a]	9.00±1.00[a]	310.0±32.8[b]	31.65±5.90[a]	101±7[b]
Group 4.5	104.10±4.20[a]	9.20±1.03[a]	291.0±18.3[b]*	30.29±6.50[b]*	103 ±7[b]*
Group 4.6	107.90±5.30[a]	9.60±1.05[a]	344.0±33.5[a]	37.14±7.40[a]	117±6[a]

Values are Mean ± SD for 6 rats in each group. n, number of hearts in each group; LVDP, left ventricular developed pressure; CF, coronary flow; HR, heart rate; RPP, rate pressure product; MAP, mean arterial pressure. Values not sharing a common superscript (a,b) differ significantly at $P<0.05$ when compared between the groups,*$P < 0.05$, compared with control.

Table 2

Effect of DG coated insulin on mitochondrial enzymes in isolated rat heart.

Group	ICDH	SDH	MDH	α KGDH	NADH dH	Cyt.C. oxidase
Group 1	730.1±21.5[a]	244.3±6.6[a]	349.1±14.1[a]	75.2 ±1.6[a]	135.4±3.4[a]	32.4±0.8[a]
Group 2	726.1±22.4[a]	241.2±5.2[a]	344.1±11.8[a]	71.6 ±1.5[a]	131.6±3.3[a]	30.5±0.6[a]
Group 3.1	582.2±19.3[c]	113.4±4.2[c]	217.9±12.5[c]	29.0 ± 0.8[c]	83.0±2.4[c]	14.0±0.3[c]
Group 3.2	594.5±18.6[c]	116.5±4.3[c]	228.5±10.3[c]	31.0 ± 0.9[c]	92.5±2.5[c]	15.6±0.3[c]
Group 3.3	609.2±16.4[c]	123.1±4.2[c]	232.5±111.0[c]	36.0 ± 0.9[c]	95.5±2.1[c]	16.2±0.1[c]
Group 4.1	643.4±17.2[b]	172.8±14.3[b]	321.4±9.3[a]	61.3 ± 1.2[a]	131.2±3.4[a]	11.3±0.2[c]
Group 4.2	612.2±13.7[c]	155.2±16.7[c]	276.5±10.7[c]	46.3±2.3[b]	112.7±2.5[a]	14.2±1.2[c]
Group 4.3	667.8±14.5[a]	188.6±9.1[b]	313.4±14.6[b]	66.7±1.7[a]	127.4±2.3[a]	20.3±0.5[c]
Group 4.4	619.7±14.3[c]	162.9±14.5[b]	284.2±13.8[c]	52.2±1.5[b]	121.2±1.6[c]	19.1±0.5[b]
Group 4.5	624.1±16.1[c]	155.5±16.1[c]	303.8±12.5[b]	57.8±1.2[b]	107.5±2.5[c]	12.5±0.2[c]
Group 4.6	698.4±15.5[a]	202.5±15.7[e]	332.1±12.6[a]	68.4±2.5[a]	130.6±2.4[a]	23.5±1.1[b]

Results are Mean±SD (n=6). Activity is expressed as nmol of NADP reduced per min per mg protein for ICDH, nmol of succinate oxidized per min per mg protein for SDH; nmol of NADH oxidized per min per mg proteins for MDH: nmol of α -keto glutarate formed per hour per mg protein for α KGDH and nmol of NADH oxidized per min per mg protein for NADH dH: change in optical density per minutes per mg protein for cytochrome c oxidase. Values not sharing a common superscript (a,b,c,d,e,f) differ significantly at $P<0.05$) when compared between the groups; Cyt.C. oxidase= Cytochrome oxidase.

Table 3

Effect of DG coated insulin on sarcoplasmic ATPase enzymes in isolated rat heart.

Group	Na^+ K^+ATPase	Ca^{2+} ATPase	Mg^{2+} ATPase	5′-nucleotidase
Normal	0.563± 0.010[a]	0.274 ± 0.020[a]	0.474± 0.010[a]	4.25± 0.15[a]
Group 2	0.561± 0.010[a]	0.270 ± 0.020[a]	0.470± 0.010[a]	4.21± 0.12[a]
Group 3.1	0.509± 0.010[c]	0.229± 0.030[b]	0.369± 0.020[c]	3.99± 0.11[b]
Group 3.2	0.530 ± 0.010[b]	0.241± 0.000[b]	0.381± 0.010[c]	4.02 ± 0.12[b]
Group 3.3	0.534 ± 0.010[b]	0.245± 0.020[b]	0.386 ± 0.010[c]	4.06± 0.12[b]
Group 4.1	0.530± 0.010[b]	0.271 ± 0.010[a]	0.399± 0.010[b]	4.10± 0.12[b]
Group 4.2	0.525± 0.020[b]	0.377± 0.030[c]	0.415± 0.020[b]	3.86± 0.12[c]
Group 4.3	0.525± 0.020[b]	0.240± 0.010[b]	0.390± 0.010[b]	4.03± 0.13[b]
Group 4.4	0.507± 0.010[c]	0.249± 0.010[b]	0.355± 0.030[d]	3.89± 0.10[c]
Group 4.5	0.523± 0.010[b]	0.245 ± 0.020[b]	0.384± 0.010[c]	4.01± 0.09[b]
Group 4.6	0.534± 0.010[b]	0.270± 0.010[a]	0.497± 0.030[a]	4.09± 0.10[b]

Results are Mean±SD (n=6). A ctivity is expressed as μmoles of phosphorus liberated per sec per gram protein for Na^+ K^+ ATPase, Ca^{2+} ATPase and Mg^{2+} ATPase; mmoles of phosphorus released per mg protein per hour for 5′-nucleotidase. Values not sharing a common superscript (a,b,c,d,e,f) differ significantly at $P<0.05$ when compared between the groups.

recovery of the myocardium was better in rats administered with oral insulin mixed DG. According to the reports of Govindarajan[30], DG possesses the ability to reduce insulin resistance in diabetic rats. However, the better cardio protection provided by oral insulin mixed DG than when it was injected, suggested the role of insulin resistance in impaired protective mechanism. Previous studies have also demonstrated that insulin resistance animals were more sensitive to ischemia reperfusion injury [31], and the mechanisms was thought to be associated with decreased GLUT4 glucose transporter content and impaired GLUT4 translocation [32]. Probably, oral administration of insulin with DG might provoke the signaling molecules in small intestine that prevent the initiation of insulin resistance [33]. Not only that insulin secretogenic action of DG [34] has increased the insulin level and thereby renders cardio protection. Similarly decline in CK and LDH activity in the perfusate and tissue homogenate of insulin mixed DG in diabetic rat supported the above findings. In fact, insulin normalizes not only blood glucose levels [35], but most of the metabolic parameters of diabetic rats [36]. Previous studies from isolated papillary muscle and isolated heart preparations [37] showed that insulin reversed, or prevented, contractile dysfunction in diabetic rats.

Mitochondrion seems to be the direct target of the diabetic states which was evident by the depression of pyruvate dehydrogenase complex and F0F1 ATPase [38]. Apparently, the impaired mitochondrial oxidative energy metabolism was exacerbated by myocardial ischemia reperfusion. However, the recovery of mitochondrial energy linked function in diabetic rats undergone ischemia reperfusion by the oral and intra-peritoneal administration of DG mixed insulin suggested the myocardial protection exhibited by combined insulin mixed DG. Paradoxically, insulin and DG administered alone did not give the significant protection towards mitochondrial function in ischemia reperfused rat heart. This result indicates synergic action of insulin and DG root aqueous extract in mediating myocardial tolerance towards ischemia reperfusion.

Studies using several types of diabetic rodent models have demonstrated impaired endothelial-dependent relaxation in both conduit and resistance arteries which can aggravate the pathology related to ischemic reperfusion [39]. Various factors have been proposed to contribute to this defect, including increased release of an endothelium-derived constricting factor, increased protein kinase C activity, inhibition of Na^+/K^+ ATPase activity. Thus the improved microsomal ATPase enzymes in ischemia reperfused diabetic rats indirectly suggested the partial recovery from myocardial endothelium dysfunction, one of the main contributing factors for ischemia reperfusion injury.

On the basis of above results, it could be summarized that insulin administered along with DG not only mediates the intestinal absorption of exogenous insulin, the synergic action of insulin and DG helps the functional recovery of diabetic rat hearts from the insult of ischemia reperfusion. This may be due to the muscarinic effect mediated by the active ingredient of DG, that may activate either M1,M3 or M4 receptors of Islet cells of pancreas, resulted in increased release of insulin [40] . Cholinergic effect on pancreatic beta cells exerts primarily through muscarinic receptors resulted in secretion of insulin [41]. Apparently the same study noted that muscarinic M1 and M3 receptors function differentially and regulate glucose induced insulin secretion, which has clinical significance in glucose homeostasis.

The above results indirectly provide enough evidences for the successful delivery of insulin through oral route when mixed with aqueous extract of DG root. DG extract not only act as vehicle for insulin delivery but also mediate synergic action with insulin and render cardio protection in ischemia reperfused rat hearts.

Table 4
Effect of DG on flow rate, heart rate and force of contraction in frog heart.

Drug & Extract	Flow Volume (mL/min)	Heart Rate (Beats/min)	Amplitude (mm)
Baseline	9.0	62	18
Extract 1 mg	5.0	28	10
Extract 4 mg	3.7	16	08
Extract 8 mg	3.1	14	05
Atropine 50 μg+Extract 8 mg	4.5	58	14
Atropine 50 μg+Ach 500 ng	4.8	60	17
Ach 500 ng	3.0	15	07

Conflict of interest statement

We declare that we have no conflict of interest.

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