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Antidiabetic effect of *Dodonaea viscosa* (L). Lacq. aerial parts in high fructose-fed insulin resistant rats: A mechanism based study

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To study the effect and mode of action of water extract (DVW) and polar fraction of ethanol extract (DVE-4) of *D. viscosa* in high-fructose diet induced insulin resistance in male Wistar rats. *D. viscosa*'s effects were evaluated on a battery of targets involved in glucose homeostasis (*in vitro* studies). Rats were rendered insulin resistant by feeding 66% (w/w) fructose and 1.1% (v/w) coconut oil mixed with normal pellet diet (NPD) for six weeks. DVW and DVE4 at different doses were administered simultaneously. At the end of the study, blood glucose, oral glucose tolerance test, lipid profile and insulin were estimated and homeostatic model assessment (HOMA) levels were calculated. In addition, enzymatic and non-enzymatic liver antioxidant levels were also estimated. Quantification of biomarker quercetin was done using HPLC. Fructose diet with DVW, DVE-4 significantly reduced blood glucose, serum insulin, HOMA, lipid profiles and significantly improved glucose tolerance and HDL-c levels. In addition, these extract and fraction also decreased oxidative stress by improving endogenous antioxidants. In different bioassays, DVW and DVE-4 inhibited protein tyrosine phosphatase-1B with IC_{50} 65.8 and 54.9 μg/ml respectively and showed partial inhibition of dipeptidyl peptidase-IV. Moreover, DVW and DVE-4, at 10 μg/ml showed 60 and 54.2% binding to peroxisome proliferator-activated receptor-γ. Further, 2.1% (w/w) of quercetin was quantified in bioactive-DVE-4 using HPLC method. The results provide pharmacological evidence of *D. viscosa* in treatment of prediabetic conditions and these effects may be mediated by interacting with multiple targets operating in diabetes mellitus.

Keywords: *Dodonaea viscosa*, DPP-IV, Homeostatic model assessment, HPLC, PPAR-γ, Prediabetic, PTP-1B, Quercetin

The sudden surge in the incidence of type 2 diabetes is complicated by the fact that it is a multi-factorial disease, frequently associated with a cluster of pathologies including obesity, hypertriglyceridemia, impaired glucose tolerance, insulin resistance and hypertension, collectively referred to as the metabolic syndrome [formerly known as syndrome X and insulin resistance syndrome][1]. There has been a heightened awareness of the metabolic syndrome and its prevention, due to its strong association with premature morbidity and mortality. In particular, these predisposing factors subject the individual to greater cardiovascular risk and Type 2 diabetes[2].

The main driving forces that increase the prevalence of insulin resistance is the dramatic rise in obesity driven by modern Westernized diets and changes in eating habits. Recent research suggests that a high intake of refined carbohydrates may also increase the risk of insulin resistance. In addition, diets specifically high in fructose have been shown to contribute to a metabolic disturbance in animal models resulting in weight gain, hyperlipidemia and hypertension[3]. There is an urgent need for increasing public awareness about the risks associated with high fructose consumption and greater efforts should be made to curb the supplementation of packaged foods with high fructose additives. Moreover, supplementation of plant-derived neutraceuticals could be beneficial.

Dodonaea viscosa (L). Jacq., (Family: Sapindaceae) popularly known as *aliar* and *vilayati mehandi* (Hindi) in India, is an evergreen shrub abundantly growing in Western Ghats of Karnataka state, India. The species has been used in traditional medicine to heal simple ulcer[4], fracture, sore and for snakebite and immediate relief of gum and teeth pain[5]. Antimicrobial, anti-inflammatory[6], anti-ulcer[7],

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wound healing[8], local anaesthetic and smooth muscle relaxant activities[9] of the plant have been reported.

The hypoglycemic effect of *D. viscosa* was reported by Aswal *et al*.,[10] while screening 294 plants of Indian origin. In addition, water extract (DVW) and polar fraction of ethanol extract (DVE-4) of *D. viscosa* (400-800 mg/kg) failed to reduce hyperglycemia induced by glucose load (1.5 g/kg, po) in normal rats. Moreover, repeated administration of DVW and DVE-4 for 15 days also failed to produce hypoglycemia in normal rats. In other words, neither of the two extracts alter normal glycemic control in animals. However both extracts reduced blood glucose levels of streptozotocin (STZ)-induced mild diabetic (MD) condition in rats indicating that they are active only in diabetic conditions[11]. Therefore the action of both extract and fraction may be considered more corrective than disruptive. Further, the lack of hypoglycemic action in hypoinsulinemic model [severe diabetic (SD) or type 1 diabetic model] and beneficial effects in high-fat diet and low-dose streptozotocin-induced type 2 diabetic condition in rats[12] demonstrates that DVW and DVE-4 probably operate by improving the extra-pancreatic glycemic control mechanisms rather than augmenting insulin secretion from remnant pancreatic β-cells.

In the present study, the effects of these extract and fraction have been evaluated in prediabetic condition induced by 66% fructose in rats. Furthermore, attempts were made to isolate bioactive components from active fraction of ethanolic extract of *D. viscosa* (DVE-4). Preliminary phytochemical, co-TLC and chromatographic studies, resulted in the identification of a prominent biomarker, quercetin and its estimation in DVE-4 using HPLC.

Materials and Methods

Chemicals—Bovine serum albumin (BSA), 5,5′-dithio-bis-2-nitrobenzoic acid (DTNB, Ellman's reagent), 1-chloro-2, 4-dinitrobenzene (CDNB), reduced glutathione (GSH), superoxide dismutase (SOD) were purchased form Sigma Chemical Co, USA. Pioglitazone was the gift sample from Amoli Organics, Mumbai.

Plant material—The whole plant of *Dodonaea viscosa* was collected in and around Dharwad, Karnataka during the month of September 2006. The plant was authenticated by Prof. M. Jayaraj, Department of Botany, Karnataka University, Dharwad and voucher specimen (#MCOPS/PC/05/06) of the plant was preserved in the college herbarium.

Extraction and fractionation—The coarse powdered material (4 kg) of the shade-dried plant was extracted exhaustively with ethanol in a Soxhlet apparatus, and subsequently extracted with water on a hot water bath. The extractives were concentrated to a small volume and evaporated to dryness in a vacuum desiccator to yield ethanol extract [DVE] (yield: 333.0 g) and water extract [DVW] (yield: 445.0 g).

Dried DVE (246 g) was fractionated by adsorptive solvent extraction method. Briefly, extract was dissolved in ethanol (200 ml) and adsorbed onto silica gel (120#) and air dried. This was extracted successively using solvents of increasing polarity (4 × 400 ml each) to yield the following fractions: [1] petroleum ether (60-80°C) [DVE-1; yield 52.8 g], [2] chloroform [DVE-2; yield 35.4 g], [3] ethyl acetate [DVE-3; yield 64.3 g] and [4] methanol [DVE-4; yield 89.4 g]. DVE-4 was found to be more active than DVE-1, DVE-2 and DVE-3 in STZ-induced diabetic model[11]. Therefore, the most active fraction (DVE-4) was evaluated for further studies.

Estimation of bioactive-quercetin in DVE-4 using HPLC method

Preparation of sample solution—DVE-4 (10 mg) was dissolved in minimum quantity of methanol and made upto 10 ml with Milli Q water to obtain stock 1 mg/ml DVE-4.

Preparation of standard solutions of quercetin—Stock solution of 1 mg/ml of quercetin was prepared and aliquots (0.125 to 2 ml) of stock solutions were transferred to 10 ml volumetric flasks and the volume of each was adjusted to 10 ml with methanol, to obtain standard solutions containing 200, 100, 50, 25 and 12.5 µg/ml of quercetin respectively.

Preparation of calibration curve of quercetin—Standard solutions (20 µl) of quercetin (0.25 to 4 µg/ml) were injected in triplicate into the reverse phase (RP-C18) packed column from Supelcosil, USA (250 mm × 4.6 mm; particle size 5 µm) using HPLC (LC-10AD*v*P, Shimadzu Corporation, Japan). Isocratic elution using methanol, water, and phosphoric acid (ratio: 41:58.9:0.1%) at a total flow rate of 1.0 ml/min and elution was monitored by using PDA detector. The chromatograms at 280 nm were analyzed and calibration curve was constructed by plotting area under the peak versus corresponding concentration of quercetin.

Quantification of quercetin in the DVE-4—Sample solution (1 mg/ml; 20 µl) was injected in triplicate into the reverse phase packed column. Sample was run and scanned as described above. The peak areas were recorded and the amount of quercetin in sample was calculated using the linear regression equation derived from the calibration curve of standard quercetin.

Animals—Male adult Wistar rats weighing 160-240 g were obtained from the inbred animal colony of central animal house, Kasturba Medical College, Manipal University, Manipal, India. The animals were maintained under controlled conditions of (23° ± 2°C; 50 ± 5% RH and 12:12 h light-dark cycles). The animals were randomized into experimental and control groups and housed individually in sanitized polypropylene cages containing sterile paddy husk as bedding. They had free assess to standard pellets as basal diet and water *ad libitum*. All the studies conducted were approved by the Institutional Ethical Committee, Kasturba Medical College, Manipal [vide letter # IAEC/KMC/06/2004-2005], according to prescribed guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India. We selected male animals for all our studies, since females are shown to be protected from changes in lipid-induced insulin action[13]. Moreover, female rats may possess counter mechanisms that protect against the adverse effects of fructose such as, hyperinsulinemia, insulin resistance and hypertension[14].

Effect of DVW and DVE-4 in high fructose diet-induced prediabetic rat model—After acclimatization, rats were made insulin resistant (pre-diabetic) by feeding 66% (w/w) fructose and 1.1% (v/w) coconut oil mixed with normal pellet diet (NPD) for 6 weeks. The high fructose diets (HFD) were prepared fresh daily and mixed with respective extract/fraction and fed to rat *ad libitum*. The experimental rats were divided into 7 groups of 5 each and treated as follows:

Group 1: NPD-fed rats received 1% sodium carboxy methyl cellulose (Na CMC) [~2 ml/kg/day, po]

Group 2: HFD-fed rats received 1% Na CMC [~2 ml/kg/day, po]

Group 3: HFD-fed rats treated with DVW [1% (w/w)/day, po]

Group 4: HFD-fed rats treated with DVW [2% (w/w)/day, po]

Group 5: HFD-fed rats treated with DVE-4 [0.5% (w/w)/day, po]

Group 6: HFD-fed rats treated with DVE-4 [1% (w/w)/day, po]

Group 7: HFD-fed rats treated with pioglitazone [0.05% (w/w)/day, po]

The dose of DVW/DVE-4/pioglitazone taken by the individual animals was calculated based on average quantity of food consumed by each rat during the study period (Table 1).

Oral glucose tolerance test (OGTT)—An oral glucose tolerance test (OGTT) was carried out on day 42 (after six weeks of HFD-feeding). Glucose (2 g/kg) was administered to 12 h-overnight fasted rats and blood samples were collected from the caudal vein by means of a small incision at the end of the tail at 0 (immediately after glucose load), 30, 60 and 120 min after glucose administration. Blood glucose levels (BGL) was estimated by the enzymatic glucose oxidase method using a commercial glucometer. The results were expressed as integrated area under curve for glucose ($AUC_{glucose}$), which was calculated by trapezoid rule,

$$AUC_{glucose} = \frac{(C_1 + C_2)}{2} \times (t_2 - t_1)$$

Estimation of biochemical parameters—After completion of OGTT, blood samples were collected from retro-orbital plexus. Serum was separated and analysed spectrophotometrically for triglyceride (STG), total cholesterol (STC), HDL-cholesterol (HDL-c), using diagnostic reagent kit (Nicholas Piramal India Ltd., Mumbai). Serum insulin (SI) was

Table 1 — The average food intake and dose of DVW and DVE-4 in HFD fed rats
[Values are mean ± SE of 5 rats in each group]

Treatment groups	Average food intake (g/rat/day)	Average dose (mg/rat/day)
NPD-fed rats	14.6±0.93	-
HFD-fed rats	11.4±1.08	-
HFD-fed rats treated with DVW [1% w/w]	11.0±0.55	110±0.005
HFD-fed rats treated with DVW [2% w/w]	10.4±0.51	208±0.010
HFD-fed rats treated with DVE-4 [0.5% w/w]	10.6±1.21	53±0.006
HFD-fed rats treated with DVE-4 [1% w/w]	11.3±1.41	113±0.014
HFD-fed rats treated with Pioglitazone [0.05% w/w]	10.8±1.85	5.4±0.001

estimated by radioimmunoassay method using the kit from Bhabha Atomic Research Centre, Mumbai, India. Homeostatic model assessment (HOMA) as a measure of insulin resistance was calculated by the formula[15,16]:

$$\text{HOMA} = \frac{\text{insulin } \mu\text{U / ml} \times \text{glucose mmol / L}}{22.5}$$

The markers of dyslipidemia such as TC/HDL-c and LDL-c/HDL-c ratios were also calculated. VLDL-cholesterol (VLDL-c) and LDL-cholesterol (LDL-c) in serum were calculated as per Friedewald's equation[17].

$$\text{VLDL - c} = \frac{\text{Triglyceride}}{5}$$

$$\text{LDL - c} = \text{Total cholesterol} - \frac{\text{Triglyceride}}{5} - \text{HDL - c}$$

Endogenous enzymatic and non-enzymatic antioxidant levels—Animals were sacrificed by cervical dislocation, liver was perfused with saline and whole liver was dissected out. A 10% homogenate of the livers of different groups were prepared with ice cold saline-EDTA and protein content was determined[18]. The homogenate was further subjected to the estimation of non-enzymatic (reduced glutathione and total thiols) and enzymatic antioxidants (catalase, GST, and SOD) using standardized protocols of the laboratory quoted in our previous publication[19]. Lipid peroxidation levels of the liver homogenates were also determined[20].

***In vitro* bioassays**

Protein tyrosine phosphatase-1B [PTP-1B] inhibition assay—This assay is based on the principle of dephosphorylation of para-nitrophenyl phosphate (pNPP) by PTP-1B into yellow chromogen para-nitrophenol (pNP) and phosphate[21]. Inhibition of PTP-1B was followed as per the product brochure from m/s Upstate, USA, Cat. No. 14358. Briefly, 19 µl of pNPP Tyr assay buffer *p*H 7.2 or positive control (RK 682 at a concentration 5.4 to 540 µ*M*) or different concentration of test samples (25 to 100 µg/ml) were added in 384 micro well polystyrene, non-treated plates. To this, 22.5 µl of BSA solution (100 µg/ml) and 3.5 µl of PTP-1B (46.72 U/100 µl) were added, mixed and incubated at 37°C for 10 min. Then 5 µl of pNPP (50 m*M*) was added and further incubated at 37°C for 60 min and measured OD at 405 nm.

PPAR-γ binding assay—PPAR-LBD (PPAR γ-ligand binding domain) was added to a fluorescent PPAR ligand (Fluormone™ PPAR Green) to form a PPAR-LBD/Fluormone™ PPAR Green complex resulted in a high polarization value. This complex is then added to individual test compounds in micro well plates. Competitors displaced the fluorescent Fluormone™ PPAR Green ligand from the PPAR-LBD/Fluormone™ PPAR Green complex, caused the fluorescent ligand to tumble rapidly during its fluorescence lifetime and resulted in a low polarization value. Noncompetitors will not displace the fluorescent ligand from the complex, and therefore the polarization value remains high. The shift in polarization value in the presence of test compounds was used to determine relative affinity of test compounds for the PPAR-LBD[22].

The product brochure of PolarScreen™ PPAR competitor Assay, Green from PANVERA (Invitrogen discovery screening) Cat. No. PV3355 was used. Briefly, 10 µl of Complete Green Buffer (CGB: 30 µl 1*M* DTT to 5970 µl of PPAR γ green buffer) or positive control (rosiglitazone: 0.1 to 25 µ*M*) or test samples (10 µg/ml) were added in 384 micro well polystyrene, non-treated plates. To this, 5 µl of PPAR-LBD (2200 ng/ml) and 5 µl of fluormone PPAR green (10 n*M*) were added, mixed and incubated at room temperature in dark for 2 h and then measured Δ polarization.

Dipeptidyl peptidase-IV (DPP-IV) inhibition assay—DPP-IV cleaves the chromogenic protein (Gly-Pro-p-nitroaniline hydrochloride) at a position next to proline residue to give a yellow chromogen p-nitroaniline, resulting in an increase in absorbance at 405 nm[23]. Briefly, 242.5 µl of tris hydrochloride buffer (100 m*M*) or positive control (Ile-Pro-Ile) or 200 µg/ml DVW/DVE-4 were added to 96 well plates. To this, 7.5 µl of DPP -IV (from procaine kidney) and incubated 37°C for 10 min. Then 10 µl of 1.4 mM Gly-Pro-p-nitroaniline hydrochloride was added and incubated at 37°C for 30 min and OD measured at 405 nm.

Statistical evaluation—All the results were expressed as mean ± SE. Statistical comparisons were performed by one-way ANOVA followed by Tukey's post-test using GraphPad Prism version 4.0, USA.

Results

Estimation of bioactive-quercetin in DVE-4 using HPLC method—Quercetin showed a retention time

15.35 min in presence of other compounds in DVE-4 (Fig. 1). Moreover, the presence of quercetin in DVE-4 was confirmed by comparing the UV spectrum of standard quercetin. The amount of quercetin in DVE-4 was found to be 2.1% (w/w) as quantified by the proposed method using standard plot of quercetin.

Effect of DVW and DVE-4 in high fructose diet-induced prediabetic rat model

Blood glucose, serum insulin and HOMA—At the end of 6 weeks of dietary manipulation, fasting BGL levels of HFD-fed rats (148.6±11.2) were significantly higher (P<0.01) than those in NPD-fed rats (87.1±3.8). Treatment with DVW and DVE-4 dose-dependently abolished the increase in fasting BGL levels induced by HFD, however the effects were not significant (Fig. 2A). However, pioglitazone showed significant (P<0.01) reduction in fasting BGL levels (Fig. 2A).

HFD-fed rats (92.1±8.7) exhibited significantly increased (P<0.001) levels of serum insulin compared to NPD-fed rats (30.5±5.5). The degree of insulin resistance as calculated by HOMA was also higher in HFD-fed rats (Fig. 2B and C). Feeding fructose along with DVW, DVE-4 and pioglitazone for six weeks resulted in significantly reduced serum insulin levels and HOMA values (Fig. 2B and C). This suggested that, treatment of DVW, DVE-4 and pioglitazone decreases insulin resistance caused by high fructose feeding.

Oral glucose tolerance test (OGTT)—Administration of glucose (2 g/kg, po) did not produce any significant change in the BGL levels of NPD-fed rats and AUC for the 120 min interval was not altered. The HFD-fed rats exhibited significant elevation in fasting BGL (at time zero) and showed significant impairment in glucose tolerance to exogenously administered glucose compared to NPD-fed rats (Fig. 3A).

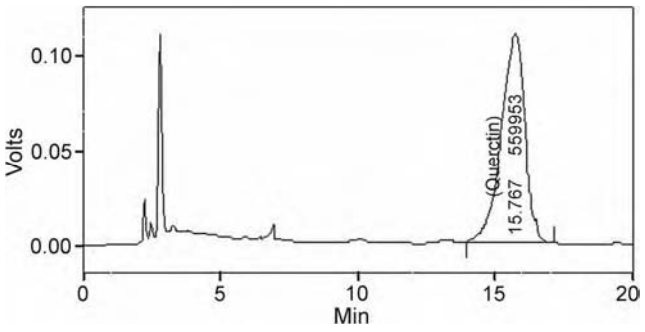

Fig. 1—Reversed-phase HPLC chromatogram of DVE-4 showing the presence of quercetin.

Treatment with different doses of DVW, DVE-4 and pioglitazone showed significant (P<0.01; P<0.001) reduction in BGL levels over the 120 min compared to HFD-fed rats (Fig. 3A). Integrated areas under the

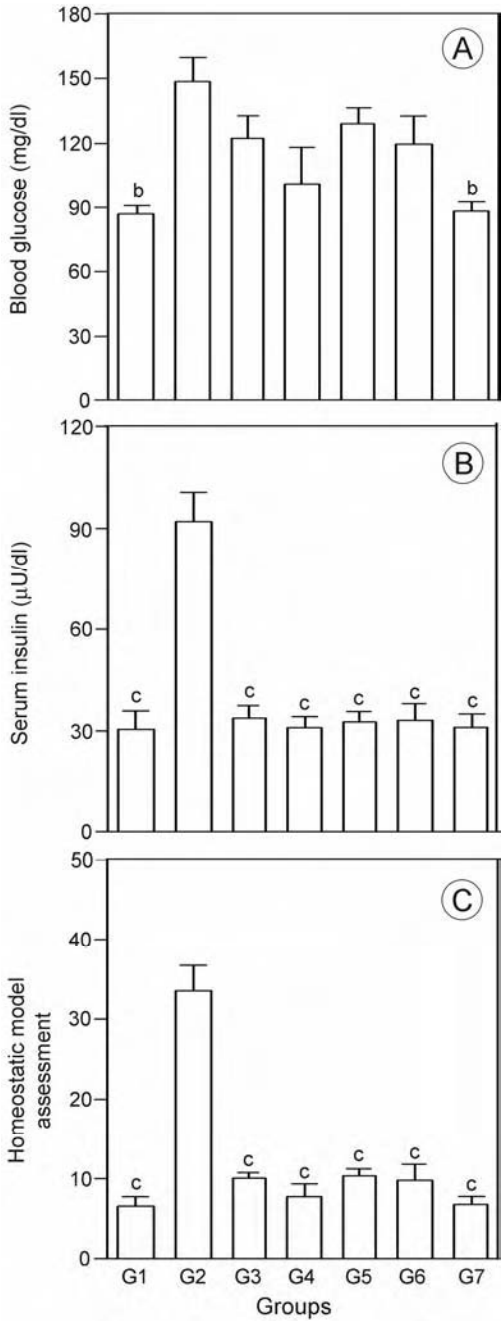

Fig. 2—Effect of 6 week treatment with DVW and DVE-4 on [A] blood glucose [B] serum insulin and [C] homeostatic model assessment (HOMA) levels in HFD rats. [G1 = NPD-fed rats, G2 = HFD-fed rats, G3 = HFD-fed rats treated with DVW (1% w/w), G4 = HFD-fed rats treated with DVW (2% w/w), G5 = HFD-fed rats treated with DVE-4 (0.5% w/w), G6 = HFD-fed rats treated with DVE-4 (1% w/w), G7 = HFD-fed rats treated with pioglitazone (0.05% w/w). Each bar represent mean±SE (n = 5). $^{b}P < 0.01$; $^{c}P < 0.001$ compared with HFD-fed rats].

glucose curve over 120 min ($AUC_{glucose}$) of HFD-fed group was significantly higher (P<0.001) compared to NPD-fed. Treatment with tested doses of DVW, DVE-4 and pioglitazone produced a significantly (P<0.01; P<0.001) decreased $AUC_{glucose}$ compared to HFD-fed control group (Fig. 3B).

Estimation of AUC values indicated that, treatment with pioglitazone, higher doses of DVW and DVE-4 showed 27.7%, 24.6%, 27.4%, and 25.9% decrease in BGL levels respectively compared to HFD diabetic rats (Fig. 3B).

Lipid parameters—The serum TG, HDL-c, VLDL-c and TC/HDL-c values were significantly elevated in HFD-fed rats, whereas serum TC, LDL-c and LDL-c/HDL-c values were not significantly different from NPD-fed rats (Figs 4 and 5). Treatment of DVW, DVE-4 and pioglitazone to the fructose fed rats led to

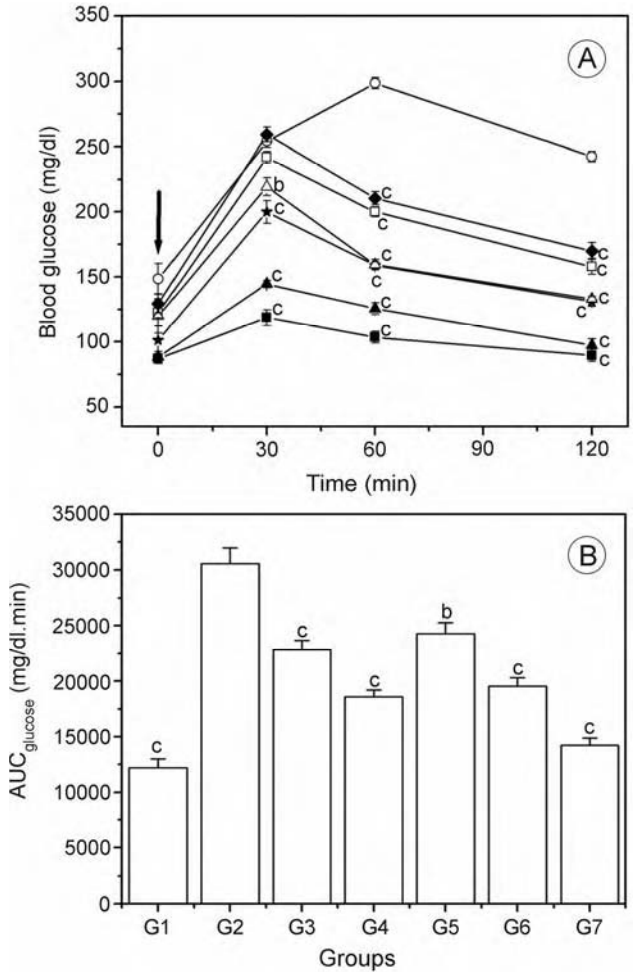

Fig. 3—Effect of 6 week treatment with DVW and DVE-4 on glucose tolerance in HFD rats. [A] blood glucose concentrations were measured prior to, and after p.o. administration of glucose alone (2 g/kg body weight), or in combination with DVW or DVE-4 or pioglitazone. The time of glucose administration is indicated by the arrow (0 min). [B] area under curve for glucose ($AUC_{glucose}$) values for 0-120 min post glucose load. –■–/ G1 = NPD-fed rats, –●–/ G2 = HFD-fed rats, –□–/ G3 = HFD-fed rats treated with DVW (1% w/w), –○–/ G4 = HFD-fed rats treated with DVW (2% w/w), –★–/ G5 = HFD-fed rats treated with DVE-4 (0.5% w/w), –Δ–/ G6 = HFD-fed rats treated with DVE-4 (1% w/w), –▲–/ G7 = HFD-fed rats treated with pioglitazone (0.05% w/w). Each bar represent the mean±SE (n = 5). $^{b}P < 0.01$; $^{c}P < 0.001$ compared with HFD-fed rats.

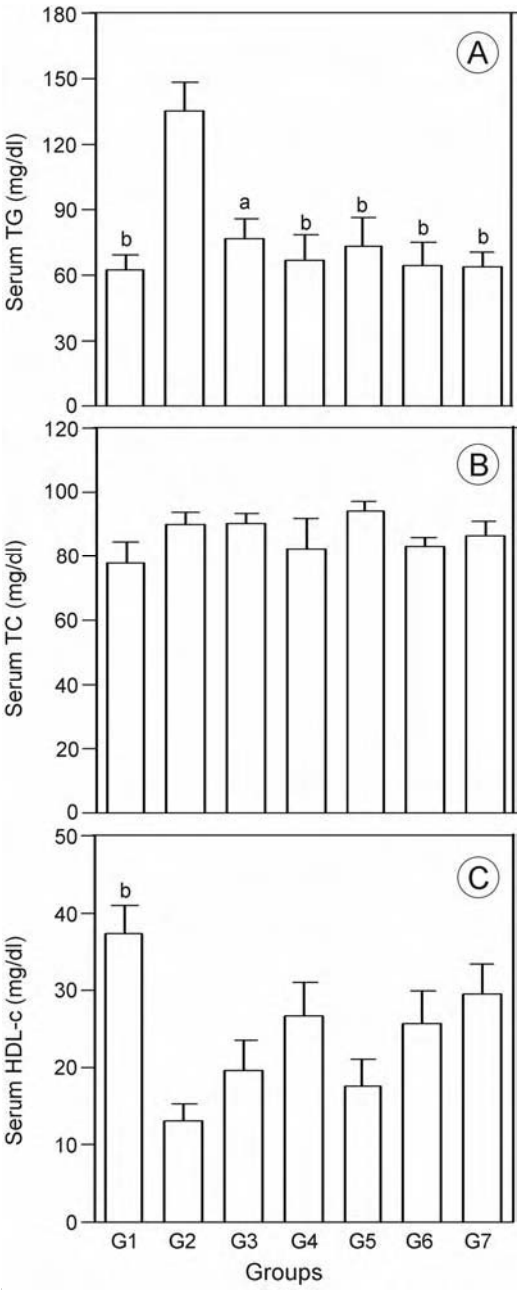

Fig. 4—Effect of 6 week treatment with DVW and DVE-4 on [A] serum TG [B] serum TC [C] serum HDL-c levels in HFD rats. Details of groups are same as in Fig. 2. Each bar represent the mean ± SE (n = 5). $^{a}P < 0.05$; $^{b}P < 0.01$ compared with HFD-fed rats.

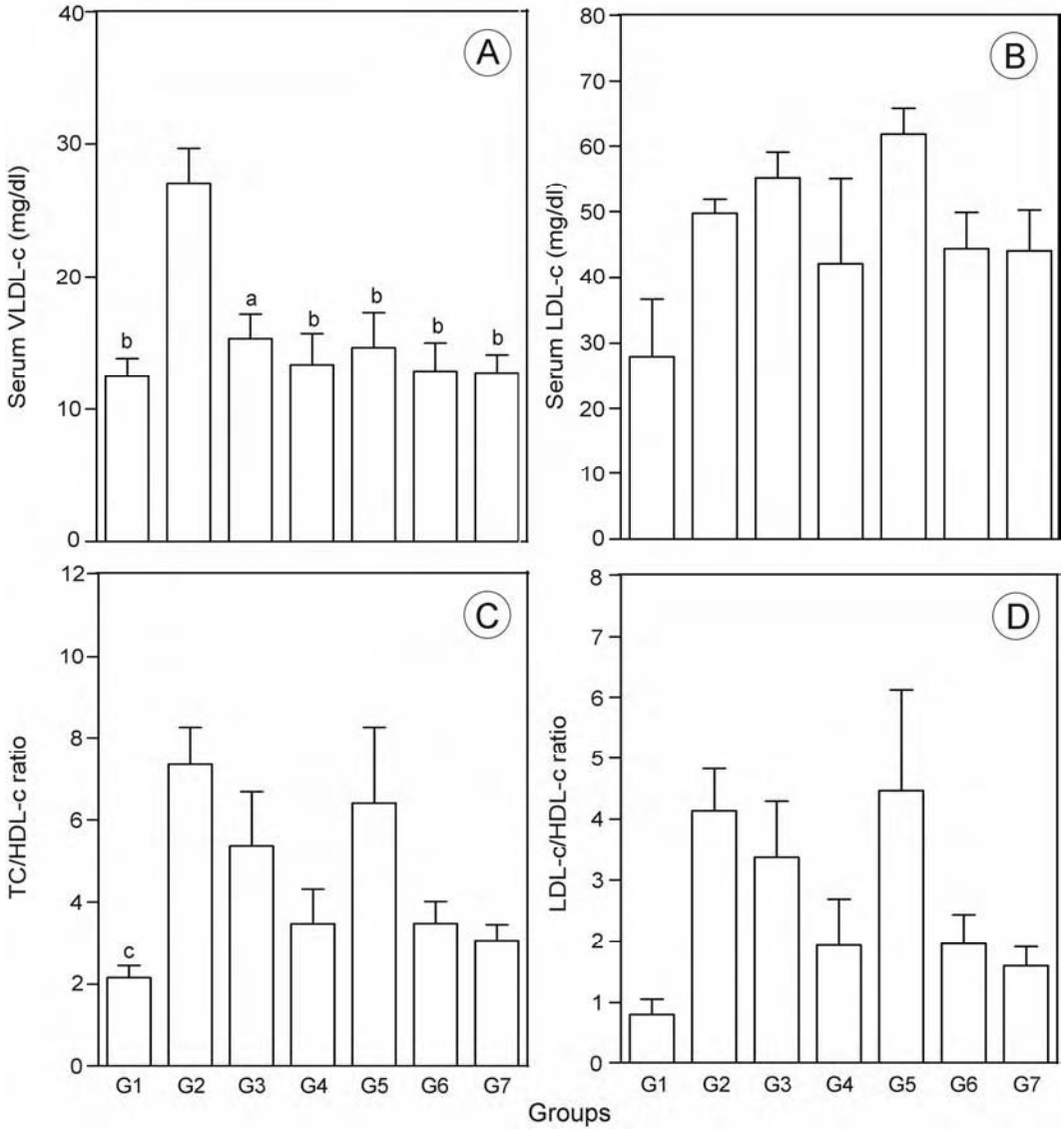

Fig. 5—Effect of 6 week treatment with DVW and DVE-4 on [A] VLDL-c [B] LDL-c [C] TC/HDL-c ratio [D] LDL-c/HDL-c ratio in HFD rats. Details of groups are same as in Fig. 2. Each bar represent the mean ± SE (n = 5). $^{a}P < 0.05$; $^{b}P < 0.01$, $^{c}P < 0.001$ compared with HFD-fed rats.

a significant reduction in STG and VLDL-c levels, whereas the levels of STC, HDL-c, LDL-c, TC/HDL-c and LDL-c/HDL-c were not significantly different from those in the HFD-fed rats (Figs 4 and 5).

Endogenous enzymatic and non-enzymatic antioxidant levels

Reduced glutathione (GSH)—NPD-fed rats showed basal GSH levels of about 11.6±1.0 nmoles/mg of protein. Fructose feeding for six weeks led to significant reduction ($P < 0.001$) in GSH levels (1.87±0.31 nmoles/mg of protein). Treatment with higher doses of DVW (2% w/w) and DVE-4 (1% w/w) to HFD-fed rats showed significant ($P < 0.05$; $P< 0.001$) improvement in GSH levels. Moreover, pioglitazone treatment significantly ($P < 0.001$) improved the GSH levels (Fig. 6A).

Total thiols—Basal total thiol levels in NPD-fed rats were found to be 8.38±0.86 μmoles/mg of protein. HFD-fed rats showed significantly decreased ($P < 0.01$) levels of total thiols (2.07±0.4 μmoles/mg of protein). Moreover, treatment of DVW (2% w/w), DVE-4 (0.5% & 1% w/w) and pioglitazone (0.05% w/w) to HFD-fed rats showed significantly ($P < 0.05$) increased levels (Fig. 6C).

Lipid peroxidation—NPD-fed rats showed basal TBARS levels of about 10.33±1.6 nmoles/g of liver

tissue. HFD-fed rats showed significantly increased ($P < 0.001$) TBARS levels (102.91±8.3 nmoles/g of tissue). Treatment with DVW (2% w/w), DVE-4 (0.5% and 1% w/w) and pioglitazone (0.05% w/w) significantly ($P < 0.05$; $P < 0.001$) abolished the increase in TBARS levels induced by high fructose diet (Fig. 6B).

Glutathione-S-transferase (GST)—Normal basal GST activity in NPD-fed rats was found to be 0.051±0.007 U/mg of protein. HFD-fed rats exhibited significantly reduced ($P< 0.001$) levels of GST (0.0039±0.001 U/mg of protein). All the tested doses of DVW, DVE-4 and pioglitazone significantly ($P < 0.05$; $P < 0.01$; $P < 0.001$) increased the levels of GST (Fig. 6D).

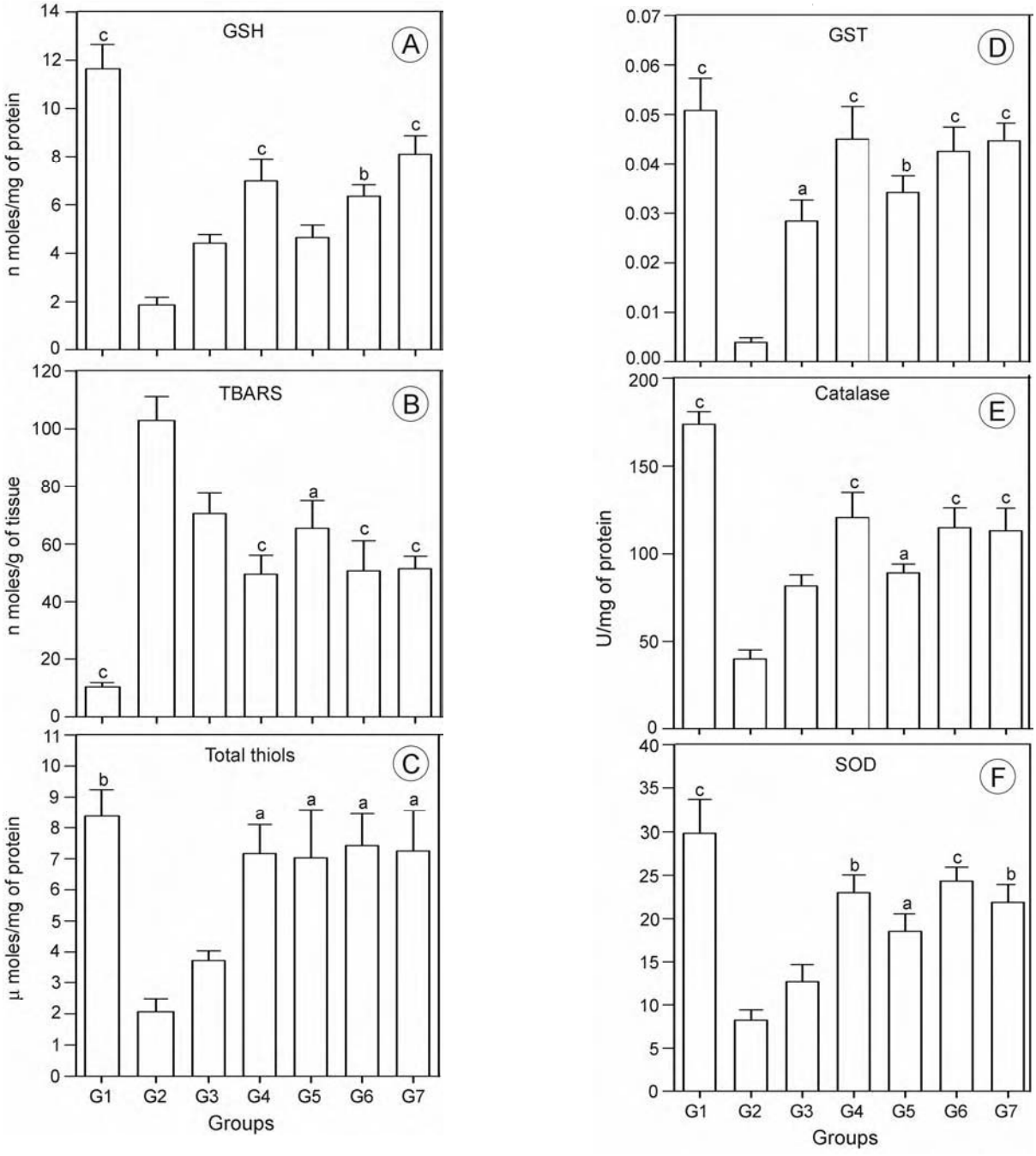

Fig. 6—Effect of 6 week treatment with DVW and DVE-4 on non-enzymatic antioxidants (GSH and total thiols) and TBARS and enzymatic antioxidants (GST, Catalase and SOD) in the liver homogenates of HFD rats. Details of groups are same as in Fig. 2. Each bar represent the mean ± SE (n = 5). *P* values: [a] < 0.05; [b]< 0.01, [c]< 0.001 compared with HFD-fed rats.

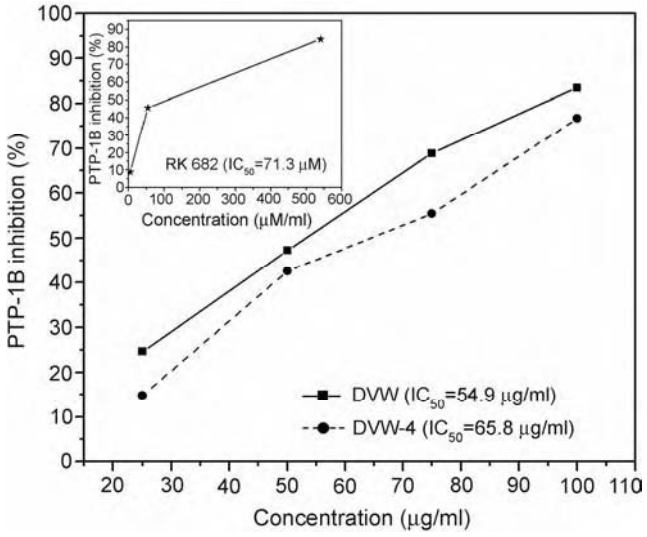

Fig. 7—Percentage protein tyrosine phosphatase-1B (PTP-1B) inhibition by DVW and DVE-4. Inset: Percentage PTP-1B inhibition by standard drug (RK 682).

Catalase—Normal basal level of catalase activity in NPD-fed rats was found to be 173.8±7.1 U/ mg of protein. HFD-fed rats showed significantly decreased ($P < 0.001$) levels of catalase by about 4-folds (i.e. 39.99±5.2). However, treatment with DVW (2% w/w), DVE-4 (0.5% and 1% w/w) and pioglitazone (0.05% w/w) showed significantly ($P < 0.05$; $P < 0.001$) increased levels of catalase to the near normal values (Fig. 6E).

Superoxide dismutase (SOD)—NPD-fed rats showed basal SOD levels of about 29.8±3.8 U/mg of protein. HFD-fed rats exhibited significantly reduced ($P < 0.001$) levels of SOD by about 3.5 folds (8.2±1.18 U/mg of protein). Treatment with DVW (2% w/w), DVE-4 (0.5% and 1% w/w) and pioglitazone (0.05% w/w) showed significantly ($P < 0.05$; $P < 0.01$; $P < 0.001$) increased levels of SOD to the near normal values (Fig. 6F).

***In vitro* bioassay**

Protein tyrosine phosphatase-1B [PTP-1B] inhibition assay—Both DVW and DVE-4 showed dose dependent inhibition of PTP-1B (Fig. 7). Positive control (RK 682) showed an IC_{50} 71.3 µ*M* (inset Fig. 7). DVE-4 showed lower PTP-1B inhibition (IC_{50}: 65.8 µg/ml) than DVW (IC_{50}: 54.9 µg/ml).

PPAR-γ binding assay—Rosiglitazone at 25 µ*M* concentration showed 78.1% binding ability to PPAR γ-LBD and 0.54 µ*M* was required to bind 50% of PPAR γ -LBD. DVW and DVE-4 at concentration 10 µg/ml, showed 60 and 54.2% ability to bind with PPAR γ-LBD respectively.

Dipeptidyl peptidase-IV (DPP-IV) inhibition assay—Standard drug, Ile-pro-Ile exhibited IC_{50} 39 µg/ml. Both DVW and DVE-4 showed very less inhibition of DPP- IV (6.6% and 11.7% respectively) at 200 µg/ml concentration.

Discussion

The fructose-fed rat model provides an ideal means of investigating the causes of a simulated metabolic syndrome (syndrome X) in animals. Further, this model provides convincing evidence that dietary imbalance can initiate the development of metabolic syndrome[24]. Wistar rats fed with high fructose (66% w/w) for 6 weeks resulted in hyperglycemia, hypertriglyceridemia and hyperinsulinemia. These rats also showed impaired glucose tolerance after the oral glucose challenge. The degree of insulin resistance was higher in fructose-fed rats as indicated by higher HOMA values. Insulin resistance in fructose-fed rats has been attributed to a low level of insulin-stimulated glucose oxidation due to modifications in the post-receptor cascade of insulin action. These findings are consistent with reported literature[14,25].

Fructose (66%) feeding leads to hyperinsulinemia and subsequent insulin resistance as shown by the increased circulatory C-peptide concentration, overexpression of GLUT 5 (fructose transporter), lower levels of insulin receptor mRNA and insulin receptor numbers in skeletal muscle and liver of rats. Further, high fructose feeding decreases the autophosphorylation of insulin receptor, probably due to the overexpression of PTP1B in these rats. Moreover, these increased levels of PTP1B resulted in increased mRNA and promoter activity of SREBP-1c (sterol regulatory element binding protein-1c), and subsequent increases in the expression of fatty acid synthase (FAS). PTP1B may therefore regulate the lipogenesis and hypertriglyceridemia associated with insulin resistance syndrome[26].

DVW/DVE-4 mitigated the adverse effects of fructose load on glucose and insulin in fructose fed rats. Moreover, in HFD-fed rats, both DVW and DVE-4 caused a fall in HOMA values, indicating improved insulin sensitivity. Glucose tolerance was improved and the other components of insulin resistance syndrome, such as high levels of STG and VLDL-c, were brought to near normal levels

compared to HFD-fed rats. These favorable effects of DVW and DVE-4 may be attributed to their action on multiple targets including higher affinity for PPAR-γ, inhibition of PTP1B and subsequent reduction in overexpression of SREBP-1c and FAS.

Enhanced lipid peroxidation in HFD rats could be associated with high circulating glucose and TG. Hyperglycemia and hypertriglyceridemia are well known to increase the generation of reactive oxygen species (ROS) and subsequent lipid peroxidation[14]. The catalytic actions of antioxidant enzymes are important for the effective removal of oxygen radicals. Increased generation of free radicals by fructose reduces the activities of antioxidant enzymes such as catalase, GST and SOD. Previous studies have found that supplementation with the antioxidant vitamin E, could improve the oxidative stress and have a beneficial effect on insulin sensitivity[27].

Quercetin, which could be one among the active principles present in the tested extract, was quantified in bioactive-DVE-4 by HPLC method is 2.1% (w/w). Therefore, the amount of quercetin present in tested doses (1 and 2% w/w) of DVE-4 is 2.1 and 4.2% respectively.

Quercetin is reported to be active in different diabetic conditions and is also found to ameliorate oxidative stress in STZ-induced diabetic rats[28]. In addition, quercetin is also reported to increase significantly, hepatic glucokinase activity in diabetic rats[29]. Observed antidiabetic, antioxidant and hypolipidemic activity of the title plant may be attributed to bioactive quercetin (2.1% w/w), as a major constituent. Furthermore, it could also result from synergizing action of a combination of several components interacting with multiple targets of diabetes.

Major outcome of the present study is therefore to provide a platform in the direction of application of whole extracts or enriched fractions of *Dodonaea viscosa*, either to supplement the existing oral antidiabetic drugs or reduce the transformation of prediabetics into diabetics.

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