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Antidiabetic, hypolipidaemic and antioxidant activity of *Dodonaea viscosa* aerial parts in streptozotocin-induced diabetic rats

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Abstract

We evaluated the antidiabetic and antioxidant activity of *Dodonaea viscosa* in streptozotocin-induced diabetic rats. The water extract (DVW) and polar fraction of ethanol extract (DVE-4) in a single-dose one-day study showed dose-dependent reduction in glucose levels with maximum effect of 42.16% and 72.9% respectively at 6 h post drug treatment ($p<0.001$). DVE-4 was more active than DVW and glibenclamide. In a multi-dose fifteen-day study, lower doses of DVW (400 mg/kg) and DVE-4 (200 mg/kg) exhibited higher percentage reduction in glucose levels compared to glibenclamide treated group. Altered levels of lipids, TBARS, non-enzymatic and enzymatic antioxidants were restored by DVW (400 mg/kg) and DVE-4 (200 mg/kg) in diabetic rats. In vitro steady state and time resolved studies reveled that DVW and DVE-4 showed comparable antioxidant ability in steady state and kinetic studies suggesting its possible role in observed antidiabetic and hypolipidaemic activities.

Keywords: Antidiabetic, Antioxidant enzymes, *Dodonaea viscosa*, Free radical scavenger, Hypolipidaemic, Streptozotocin

Introduction

Diabetes mellitus is a multifaceted, dynamic expression of pathological disequilibria, resulting in various micro and macro vascular complications. Large numbers of people around the world suffering from diabetes especially over the past two decades, from 30 million to 230 million [1]. Oxidation and production of free radicals are an integral part of normal cell metabolism. An imbalance between reactive oxygen species (ROS) and the antioxidant defense mechanisms (Enzymatic and non-enzymatic) of a cell leads to excessive production of oxygen metabolites, creating a condition frequently termed as 'oxidative stress' [2]. Excessive oxidative stress has been implicated in the pathology and complications of diabetes mellitus [3]. The increased blood glucose levels in diabetes produce superoxide anions, which generate hydroxyl radicals via Haber-Weiss reaction, resulting in peroxidation of membrane lipids and protein glycation. This leads to oxidative damage to cell membranes. These radicals further damage other important biomolecules including carbohydrates, proteins and DNA [4].

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Streptozotocin (STZ) selectively destroy β-cells of pancreas by generating excess ROS and carbonium ion (CH_3^+) leading to DNA breaks by alkylating DNA bases. The N-nitroso-N methyl urea portion of the molecule exhibits diabetogenic activity. Glucose may act as carrier for this cytotoxic group [5].

Evaluation of plant products to treat diabetes mellitus is of growing interest as they contain many bioactive substances with therapeutic potential. *Dodonaea viscosa* (L). Jacq., (Family: Sapindaceae) popularly known as *aliar* and *vilayati mehandi* in India, is an evergreen shrub abundantly growing in Western Ghats of Karnataka, India. This species has been used in traditional ayurvedic system of medicine to heal simple ulcer, fracture [6], soreness, snakebite pain, relief of gum and teeth pain [7]. Experimental studies have demonstrated antimicrobial, anti-inflammatory [8], anti-ulcer [9], wound healing [10], local anaesthetic and smooth muscle relaxant activities of the title plant [11]. The hypoglycemic effect of the title plant is indicated by Aswal et al. while screening 294 plants of Indian origin [12]. As a part of our preliminary work, we found both ethanol and water extracts (400-800 mg/kg) did not significantly reduce hyperglycemia induced by glucose load (2g/kg) in normal rats. However both the extracts showed reduction in blood glucose levels of STZ-induced diabetic rats indicating that they are active only in diabetic conditions. In literature no exhaustive scientific data regarding detailed antioxidant and antidiabetic effect of *D. viscosa* are available. In the present study, we addressed these points.

Material and methods

Experimental animals

Male wistar rats 7-8 weeks old and weighing 200-250g were used. The animals were obtained from the inbred animal colony of central animal house, Kasturba Medical College, MAHE, Manipal, India. The animals were maintained under controlled conditions of temperature (23 ± 2°C), humidity (50 ± 5%) and 12-h light-dark cycles. The animals were randomized into experimental and control groups and housed two each in sanitized polypropylene cages containing sterile paddy husk as bedding. They had free access to standard pellets as basal diet and water *ad libitum*. All the animal studies conducted were approved by the Institutional Ethical Committee, Kasturba Medical College [vide letter # IAEC/KMC/06/2004-2005], Manipal, according to prescribed guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

Chemicals

Streptozotocin (STZ), thiobarbituric acid (TBA), 5,5'-dithio-bis-2-nitrobenzoic acid (DTNB, Ellman's reagent), 1-chloro-2, 4-dinitrobenzene (CDNB), reduced glutathione (GSH), superoxide dismutase (SOD), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azinobis(3-ethylbenzothiazoline-6-sulphonic acid) ($ABTS^{2-}$), ascorbic acid (ASC), Deoxy-D-ribose, egg phospatidylcholine, nicotinamide adenine dinucleotide (NADH), nitroblue tetrazolium (NBT), butylated hydroxytoluene (BHT), butylated hydroxyl anisole (BHA) and calf thymus DNA were purchased form Sigma chemical Co, USA. Mannitol was procured from SD fine chemicals, Mumbai, India. Trolox and phenazine methosulphate (PMS) were procured from Himedia, Mumbai, India. All chemicals used were of analytical grade. IOLAR grade nitrogen and N_2O were used for degassing the samples. Nanopure water from Millipore Milli-Q system was used for preparing solutions and all solutions were prepared fresh.

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 kept in the college herbarium.

Extraction and fractionation

The shade dried and coarse powdered material (1 kg) was extracted exhaustively with ethanol in a soxhlet apparatus, subsequently extracted with water on a hot water bath. The extracts were concentrated to a small volume and evaporated to dryness in a vacuum desiccator (Osworld, J.R industrial corporation, Mumbai) to yield free flowing ethanol extract [DVE] (Herb: extract = 5) and water extract [DVW] (Herb: extract = 8).

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. Extract coated silica gel was extracted successively using solvents of increasing polarity (4×400 ml each) to yield the following fractions of different polarities: [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].

Acute toxicity studies

Healthy male wistar rats, starved overnight (12 h), were divided into 18 groups of 6 each and were orally fed with increasing doses (10, 30, 100, 300, 1000 and 3000mg/kg) of DVW, DVE and DVE-4 were studied to determine the safe doses by up and down staircase method. The animals were observed continuously for one hour, then frequently for 4 hours and later at the end of 24 h. After administration of the drug, Irwin test was conducted, where the animals were observed for behavioral changes. Further, animals were observed daily for 30 days, and mortality was recorded [13]. To know multiple dose toxicity of extract/fraction, highest dose was fed once daily for 15 days and observed for incidences of mortality for a period of 30 days.

Induction of diabetes mellitus

Diabetes mellitus was induced in wistar rats by single intraperitoneal injection of freshly prepared solution of STZ (45mg/kg body weight) in 0.1M citrate buffer of pH 4.5, in a volume of 3 ml/kg after overnight fasting for 12 h [14]. Rats showing blood glucose level > 200 mg/dl 72 h after STZ administration were considered diabetic and included in the study. Diabetic rats were randomized into different groups based on their blood glucose levels. Glibenclamide was used as positive control in all the protocols described below.

Protocol 1: The effect of single oral dose of 400 mg/kg of DVE and DVW on hyperglycemia at different time intervals was studied in 12 h fasted STZ-induced diabetic animals (5 groups of 5 animals each). Blood samples were collected from the caudal vein by means of a small incision at the end of the tail at 0, 2, 4, 6 and 10 h after drug administration (single-dose one-day study). Blood glucose was estimated by the enzymatic glucose oxidase method using a commercial glucometer (Accu-chek® Active, Roche diagnostic, Mannheim, Germany). Percentage reduction in glycemia was calculated with respect to the initial (0 h) level according to: percent reduction in glycemia = [(Gi-Gt)/Gi] x 100; Where Gi is initial glycemia and Gt is glycemia at 2, 4, 6 and 10 h [15].

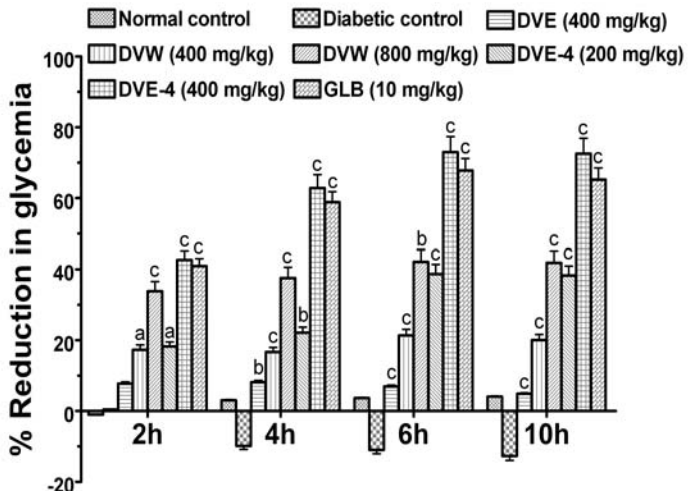

Figure 1. Effect of DVW and DVE-4 on Serum glucose levels in STZ induced diabetic rats [Single dose one-day study]. Bar graph represents the percentage reduction in glycaemia with respect to the initial (0 h) level. Each bar represent mean ± S.E.M; N=5 in each group, [a] $p<0.05$, [b] $p<0.01$, [c] $p<0.001$ compared to diabetic control of the same time interval.

The above groups of animals were further treated with single daily doses for another 15 days in order to evaluate the chronic effect of DVW and DVE treatment on hyperglycemia (Multiple-dose fifteen-day study). Blood glucose was estimated on days 0, 7, 10 and 15 by the enzymatic glucose oxidase method using a commercial glucometer. Percentage reduction in glycemia was calculated with respect to the initial (0 day) level.

Protocol 2: As described in protocol 1, the effect of single oral dose administration of DVE-2 to DVE-4 at 400 mg/kg was studied (6 groups of 5 animals each). DVE-4 was found to be more active, and therefore was further administered once daily for 15 days and percent reduction in glycemia was evaluated.

Protocol 3: Effect of different doses of DVW (400 and 800 mg/kg) and DVE-4 (200 and 400 mg/kg) were studied as described in protocol 1 (7 groups of 5 animals each).

Estimation of biochemical parameters: At the end of 15 days of treatment, the animals (protocol 1 to 3) were fasted overnight and blood samples were collected from retro-orbital plexus. Serum was separated and analysed spectrophotometrically for triglyceride, cholesterol, HDL-cholesterol (HDL-c), using diagnostic reagent kit (Nicholas piramal India Ltd., Mumbai). VLDL-cholesterol (VLDL-c) and LDL-cholesterol (LDL-c) in serum were calculated as per Friedewald's equation. VLDL-c = [TG/5]; LDL-c = [Total cholesterol – (TG/5) - HDL-c]. The markers of dyslipidemia such as TC/HDL-c and LDL-c/HDL-c ratios were also calculated.

Animals were sacrificed by cervical dislocation, livers excised; reduced glutathione, total thiols, TBARS, GST, catalase and SOD were evaluated as described in our recent publication [16].

In vitro free radical scavenging studies

Reaction with DPPH radical: For steady state measurements, 100 μM DPPH in methanol was added to DVW and DVE-4 (5-100 μg/ml) in methanol/water, mixed well and the absorbance was measured at 517 nm with or without the extracts as described earlier [17]. Kinetics of DPPH reaction with the DVW and DVE-4 was studied using stopped-flow kinetic spectrometer Model SX 18 MV (Applied Photophysics, UK) in single mixing mode using two syringes. In this experiment, syringe I contained 100 μM DPPH in methanol and syringe II contained solution of DVW and DVE-4 (26-260 μg/ml) (separately). With a time delay of 1.3 seconds, both the solutions in syringe I and II were mixed and the relative change in the absorbance at 517 nm as a function of time at 25°C was measured. Analysis of the kinetic traces was carried out with an exponential function using the built in software. At least three independent runs were used to determine the observed decay rate constant at different concentrations [18].

Reaction with ABTS radical anion: For steady state measurements, 100 μM $ABTS^{\bullet-}$ was mixed with DVW and DVE-4 (6.9-47 μg/ml) and the decrease in absorbance was measured at 734 nm as described earlier [17]. Kinetics of $ABTS^{\bullet-}$ reaction with DVW and DVE-4 was also studied using stopped-flow spectrometer in single mixing mode. In this experiment, syringe I contained 200 μM $ABTS^{\bullet-}$ in methanol and syringe II contained solution of DVW and DVE-4 (26-260 μg/ml) (separately). Time dependent absorbance changes at 645 nm were measured [19].

Reaction with hydroxyl radical: Steady state $^{\bullet}OH$ scavenging activity of DVW and DVE-4 (34-667 μg/ml) was measured by degradation of deoxy-D-ribose method [20]. Reaction of DVW and DVE-4 with $^{\bullet}OH$ was carried out using nanosecond pulse radiolysis technique employing high-energy electron pulses (50ns, 7MeV) obtained from a linear electron accelerator and the transients detected by kinetic spectrometry [21]. Competition kinetics of $^{\bullet}OH$ scavenging by DVW and DVE-4 against 250 μM KSCN at pH 6.8 were studied by monitoring $(SCN)_2^{\bullet-}$ absorbance at 500 nm [22].

Lipid peroxidation assay: The ability of DVW and DVE-4 (25-125 μg/ml) to inhibit Fe^{3+}-ASC induced lipid peroxidation was studied using phosphatidylcholine liposomes with Trolox as a reference standard [19].

Reaction with superoxide radical anion: Superoxide radical anion are generated in PMS-NADH systems by oxidation of NADH and assayed by the reduction of NBT to blue formazan. The effect of different concentration (29-581 μg/ml) of DVW and DVE-4 was studied as described earlier [23].

Total antioxidant capacity: The total antioxidant assay based on the reduction of molybdate-VI (Mo^{VI}) to molybdate-V (Mo^{V}) by DVW and DVE-4. This was done to study the activity, expressed as the number of equivalents of ascorbic acid (ASC) using standard plot [24].

Calf thymus DNA Protection studies: Effect of DVW and DVE-4 (800μg/ml) on Fenton reaction-induced calf thymus DNA damage was studied and the protection was assessed under transilluminated UV light [24].

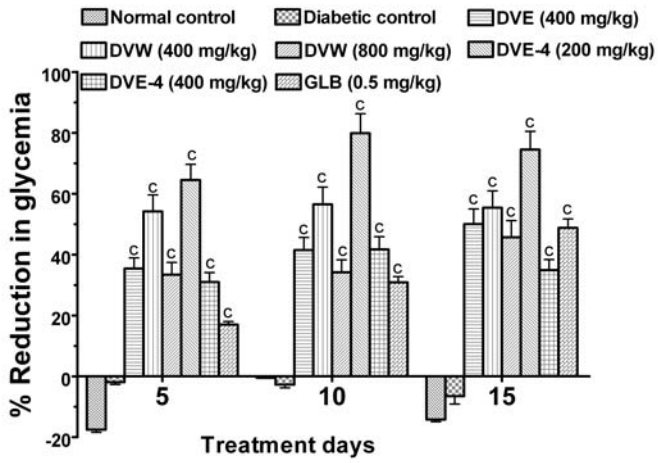

Fig. 2. Effect of DVW and DVE-4 on Serum glucose levels in STZ induced diabetic rats [Multidose fifteen-day study]. Bar graph represents the percentage reduction in glycaemia with respect to the initial (day '0') level. Each bar represent mean ± S.E.M; N=5 in each group, [a] $p<0.05$, [b] $p<0.01$, [c] $p<0.001$ compared to diabetic control of the same time interval.

Statistical evaluation

The data was expressed as mean ± S.E.M. Statistical comparisons were performed by one-way analysis of variance (ANOVA) followed by Tukey's post-test using GraphPad Prism version 4.00. The results were considered statistically significant if the *p*-values were 0.05 or less.

Results

Acute toxicity studies

Animals showed good tolerance to testing single doses of extracts/fractions in doses as high as 3 g/kg were found to be non-lethal. Highest studied doses extracts/fractions did not produce any noticeable signs of toxicity and mortality after once daily administration for 15 days.

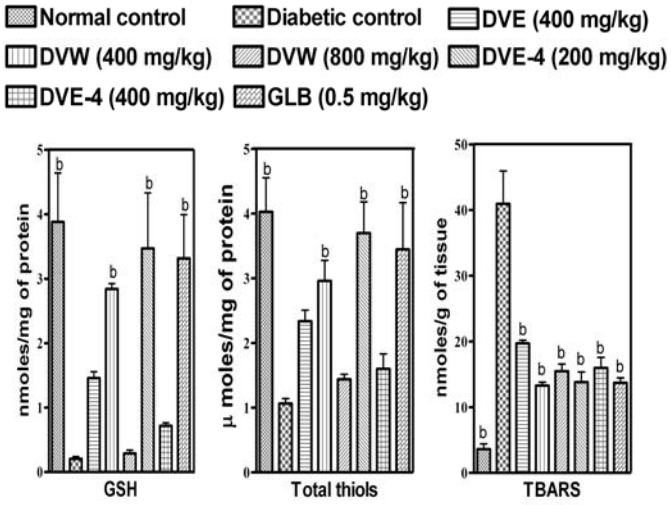

Figure 3. Estimation of non-enzymatic antioxidants and TBARS in the liver homogenates of normal and experimental diabetic animals [Multidose fifteen-day study]. Each bar represent mean ± S.E.M; N=5 in each group, [b] $p<0.01$ compared to diabetic control (ANOVA followed by Tukey's post-test).

Antidiabetic studies

Protocol 1: Single dose of DVW (400 mg/kg) exhibited greater hypoglycemic activity than DVE (400 mg/kg) in STZ-induced diabetic rats. DVW treatment significantly ($p<0.05$ to $p<0.001$) reduced blood glucose levels at different time intervals as compared to diabetic control group. The maximum reduction in glucose levels (21.4%) was observed 6 h after administration of DVW (Figure 2). Repeated administration of DVE and DVW for 15 days, showed significant ($p<0.001$) reduction in blood glucose when

compared to diabetic control group. Treatment of both DVE and DVW exhibited comparable hypoglycemia on 7th, 10th and 15th days to glibenclamide treated group (0.5 mg/kg) (Figure 3). From the above data we found that DVW is most active. Further, DVE was also found to be active in multi-dose fifteen-day study. Therefore, DVE was subjected to bioactive fractionation.

Table 1. Comparison of the IC_{50} in µg/ml of DVW and DVE-4 against various free radicals and their total antioxidant capacity. Standards used are ASC for 1 & 2 (2.78±0.09 and 1.64±0.05 µg/ml respectively); Mannitol for 3 (2950.0±3.5 µg/ml); BHA for 4 (1.46±0.17 µg/ml); Trolox for 6 (7.99±0.2 µg/ml)

Systems	DVW	DVE-4
DPPH scavenging (µg/ml)	49.09±2.4	27.13±1.1
$ABTS^{\bullet-}$ scavenging (µg/ml)	18.11±1.0	20.12±2.1
$^{\bullet}OH$ scavenging (µg/ml)	279.95±3.3	279.27±2.9
$O_2^{\bullet-}$ scavenging (µg/ml)	49.15±2.3	47.08±1.9
Ascorbic acid equivalents (mg)/ mg of extract	0.94±0.02	1.05±0.06
Lipid peroxidation Inhibition (µg/ml)	68.02±1.4	54.53±1.7

All values reported as Mean±S.E.M (n=3).

Protocol 2: DVE-4 was found to be more active than all other tested fractions (DVE-2 to DVE-4) in single-dose one-day study (data was not significant for DVE-2 and DVE-3). DVE-4 (400 mg/kg) exhibited significant (p<0.001) maximum reduction in glucose levels (72.9%) at 6 h post administration compared to diabetic control group. At the same time intervals, DVE-4 treated diabetic rats also showed higher hypoglycemic activity compared to DVW (21.4%) and glibenclamide (67.8%) treated groups (Figure 2). Maximum reduction (p<0.001) in blood glucose (41.7%) was observed on 10th day during the 15-day single dose regime of DVE-4 (Figure 3). Protocol 3 was followed further to study dose-response of DVW and DVE-4.

Protocol 3: Acute DVW (400 and 800 mg/kg) and DVE-4 (200 and 400 mg/kg) treatment produced dose-dependent fall in blood glucose in STZ-induced diabetic rats (Figure 2). However, in a 15-day study, the activity was observed only up to a dose of 400 mg /kg of DVW and 200 mg/kg of DVE-4. (Figure 3)

Estimation of biochemical parameters: Serum triglyceride (TG), total cholesterol (TC), VLDL-c and LDL-c levels were significantly (p<0.001) increased whereas HDL-c was decreased in diabetic rats compared to normal rats. Treatment of diabetic rats with DVE (400 mg/kg), DVW (400 mg/kg) and DVE-4 (200 mg/kg) for 15 days resulted in marked decrease in serum TG, TC, VLDL-c and LDL-c levels and increase in HDL-c levels compared to diabetic control group (Table 2).

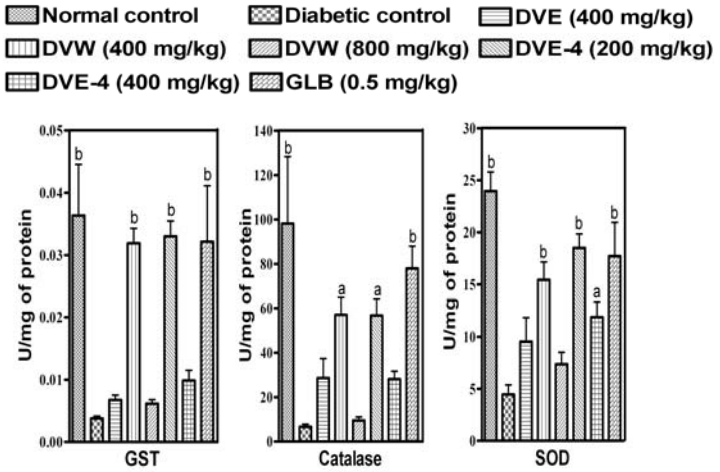

Figure 4. Estimation of enzymatic antioxidants in the liver homogenates of normal and experimental diabetic animals [Multidose fifteen-day study]. Each bar represent mean ± S.E.M; N=5 in each group, [a]p<0.05 and [b]p<0.01 compared to diabetic control (ANOVA followed by Tukey's post-test).

The markers of dyslipidemia such as TC/HDL-c and LDL-c/HDL-c ratios were significantly elevated in the diabetic group with respect to the

control (Table 2) but supplementation of DVW (400 mg/kg) and DVE-4 (200 mg/kg) for 15 days resulted in a significant fall in these dyslipidemic markers, which were restored to near-normal values (Table 2).

Endogenous antioxidant status: Non-enzymatic antioxidants such as reduced glutathione and total thiols were significantly (p<0.01) lower whereas TBARS levels were increased in untreated diabetic rats compared to normal rats. All tested extracts/fraction markedly ameliorated the deleterious effect of STZ. DVW (400 mg/kg) and DVE-4 (200 mg/kg) treated diabetic rats showed significant (p<0.01) increase in reduced glutathione, total thiols and reduced TBARS compared to untreated diabetic rats (Figure 4).

Enzymatic antioxidants such as GST, catalase and SOD were significantly (p<0.01) decreased in diabetic rats compared to normal rats. DVW (400 mg/kg) and DVE-4 (200 mg/kg) treated diabetic group showed significant increase in all the tested enzyme levels compared to diabetic rats (Figure 5).

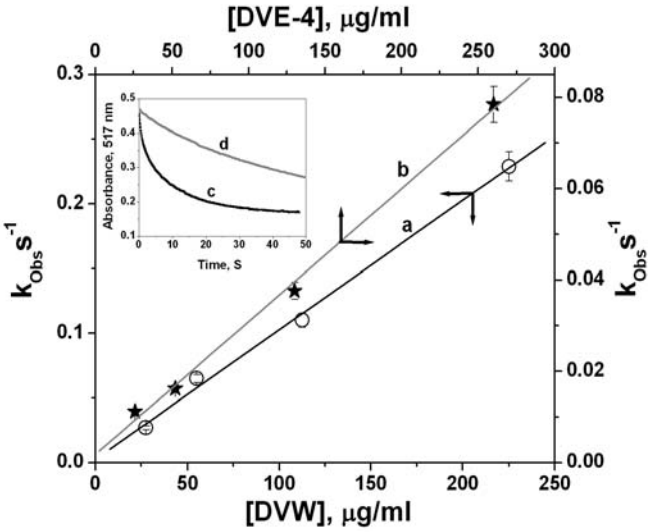

Figure 5. Variations of k_{obs} for DPPH decay at 517 nm as a function of different concentrations of (a) DVW (b) DVE-4. Inset: absorption-time plot showing decay of 50 μM DPPH at 517nm in presence of (c) 112.5 μg/ml of DVW (d) 130 μg/ml of DVE-4.

In vitro free radical scavenging studies

Reaction with DPPH radical: DVW and DVE-4 were found to show DPPH (50μM) scavenging activity under steady state conditions with IC_{50} levels of 49.09 and 27.13 μg/ml respectively. Standard ascorbic acid (ASC) under similar conditions showed an IC_{50} of 2.78 μg/ml (Table 1). For stopped-flow kinetic spectrometric studies, the concentration of DPPH was kept at 50 μM. In the presence of the extract/fraction the absorption due to the DPPH radical (monitored at 517 nm) decayed completely in 50 seconds (Inset Figure 6). This absorption-time plot was fitted to a single exponential function to obtain observed decay rate constant (k_{obs}), which was found to increase linearly with DVW and DVE-4 concentrations from 26-260 μg/ml. The k_{obs} for the decay of DPPH radical in presence of DVW and DVE-4 at 130 μg/ml were found to be 0.133 s^{-1} and 0.039 s^{-1} respectively (Figure 6).

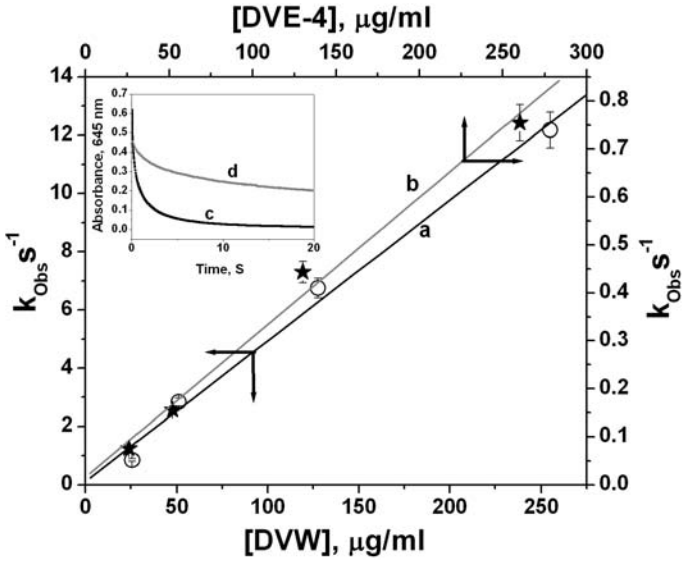

Figure 6. Variations of k_{obs} for $ABTS^{\bullet-}$ decay at 645 nm as a function of different concentrations of (a) DVW (b) DVE-4. Inset: absorption-time plot showing decay of 100 μM DPPH at 645 nm in presence of (c) 127.5 μg/ml of DVW (d) 130 μg/ml of DVE-4.

Reaction with ABTS radical anion: Both DVW and DVE-4 were found to have comparable

ability to scavenge $ABTS^{\bullet-}$ (100μM) under steady state conditions with an IC_{50} 18.11 and 20.12 μg/ml respectively. ASC showed an IC_{50} of 1.64 μg/ml under the same experimental conditions (Table 1). For stopped-flow kinetic spectrometric studies, the concentration of $ABTS^{\bullet-}$ was kept at 100 μM. In the presence of the extract/fraction, the absorbance of $ABTS^{\bullet-}$ decayed completely in 20 seconds (Inset Figure 7). This absorption time-plot was fitted to a single exponential function to get observed decay rate constant (k_{obs}), which was found to increase with concentration of DVW and DVE-4 (26-260 μg/ml). The k_{obs} for the decay of $ABTS^{\bullet-}$ in presence of DVW and DVE-4 at 130 μg/ml was found to be 6.36 s^{-1} and 0.39 s^{-1} respectively (Figure 7).

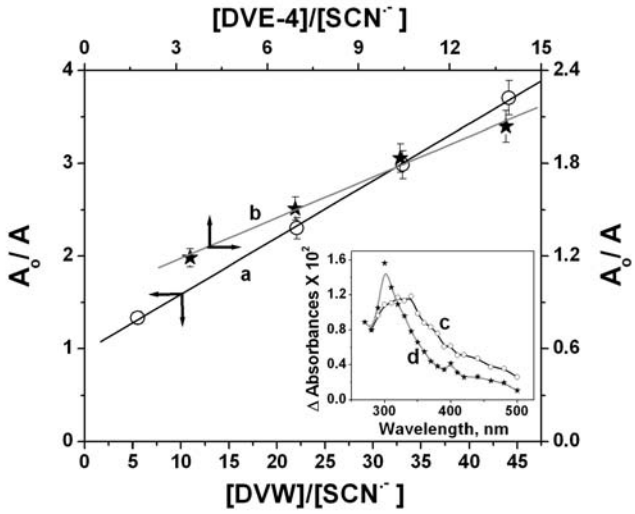

Figure 7. The $^{\bullet}OH$ scavenging ability of (a) DVW and (b) DVE-4 as determined by competition kinetics with KSCN, monitoring $(SCN)_2^{\bullet-}$ at 500 nm. Slope of this curve gives the rate constant. Inset shows absorption spectrum of transient species obtained by $^{\bullet}OH$ attack on DVW and DVE-4 during pulse radiolysis of aqueous solutions containing 80 μg/ml of (c) DVW and (d) DVE-4 at pH 6.8 and saturated with N_2O. Dose per pulse = 18.5 Gy, spectra taken 10 μs after the pulse.

Reaction with hydroxyl radical: DVW and DVE-4 exhibited concentration dependent $^{\bullet}OH$ (generated through Fenton reaction) scavenging with similar IC_{50} values of 279.95 and 279.27 μg/ml respectively. Standard mannitol showed an IC_{50} of 2.95 mg/ml under the same experimental conditions (Table 1). The reactivity, in nanosecond time scales towards $^{\bullet}OH$, by both DVW and DVE-4 were studied using pulse radiolysis. Transient spectra showed a peak at 310 nm (Inset Figure 8). The spectra do not indicate any detectable standard polyphenolic antioxidants such as gallic acid, flavonoids, which probably indicates the presence of low molecular weight phenolics. The ability of DVW and DVE-4 to scavenge $^{\bullet}OH$ was determined by studying the competition kinetics with 250 μM KSCN towards $^{\bullet}OH$ and monitoring the absorbance of the $(SCN)_2^{\bullet-}$ at 500 nm. Percentage relative rates of hydroxyl scavenging by DVW and DVE-4 in comparison to KSCN were calculated (Figure 8). DVW and DVE-4 were found to be 0.064 and 0.088 times as reactive as SCN^- towards $^{\bullet}OH$ respectively.

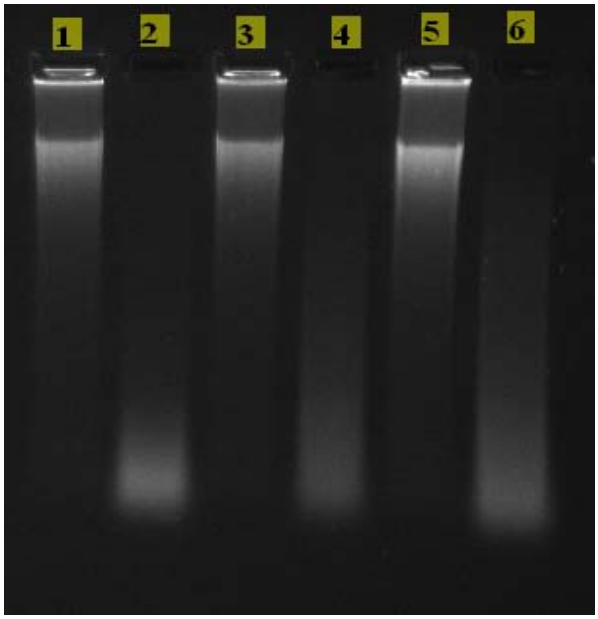

Fig. 8. Calf thymus DNA protection studies by DVW and DVE-4. Lane 1: Control DNA; Lane 2: Fenton reaction (FR) induced DNA damage; Lane 3: DVW and DNA; Lane 4: DVW+ DNA+ FR; Lane 5: DVE-4 and DNA; Lane 6: DVE-4+ DNA+ FR.

Table 2. Effect of DVW and DVE-4 on lipid profiles in normal and experimental diabetic animals [Multidose fifteen-day study]

Serum Parameters	Normal control	Diabetic control	DVE [400mg/kg]	DVW [400mg/kg]	DVW [800mg/kg]	DVE-4 [200mg/kg]	DVE-4 [400mg/kg]	GLB [10mg/kg]
Triglycerides (mg/dl)	71.4±5.4 [c]	140.9±7.9	99.5±7.0 [b]	84.5±7.8 [c]	92.2±8.4 [b]	73.6±8.1 [c]	90.6±8.4 [c]	76.2±6.4 [c]
Cholesterol (mg/dl)	74.4±6.6 [c]	122.6±8.5	108.2±7.6	84.0±4.3 [b]	99.8±6.3	80.6±5.4 [b]	97.4±5.9	78.2±7.8 [c]
HDL-c (mg/dl)	29.0±3.3 [c]	16.2±1.3	16.8±2.4	24.0±2.6	17.2±1.9	26.2±2.0	21.4±2.5	27.4±3.7 [a]
VLDL-c (mg/dl)	14.3±1.1 [c]	28.2±1.6	19.9±1.4 [b]	16.9±1.6 [c]	18.4±1.7 [b]	14.7±1.6 [c]	18.1±1.7 [c]	15.2±1.3 [c]
LDL-c (mg/dl)	31.1±4.8 [c]	78.2±7.5	70.3±9.3	43.1±6.6 [b]	64.2±6.0	39.7±4.6 [c]	57.9±4.8	35.6±3.8 [c]
TC/HDL-c ratio	2.6±0.1 [b]	7.7±0.5	7.3±1.6	3.8±0.7 [b]	6.1±0.7	3.1±0.3 [b]	4.8±0.6	2.9±0.2 [b]
TC/HDL-c ratio	1.1±0.1 [b]	4.9±0.4	4.9±1.4	2.0±0.6 [a]	3.9±1.0	1.6±0.2[b]	2.9±0.5	1.3±0.1[b]

Each value represents Mean ± S.E.M., n=5. [a] $p<0.05$, [b] $p<0.01$ and [c] $p<0.001$ compared to diabetic control. One-way ANOVA followed by Tukey's post-test.

Lipid peroxidation assay: Table 1 shows the Inhibition of Fe^{3+}/ascorbate induced lipid peroxidation in liposomes containing egg phospatidylcholine as assessed by the formation of TBARS by DVW and DVE-4 with IC_{50} 68.02 and 54.53 μg/ml respectively. The IC_{50} of Trolox under the same experimental conditions was found to be 7.99 μg /ml.

Reaction with superoxide radical anion: Superoxide radical anion derived from dissolved oxygen by phenazine methosulphate-NADH coupling reaction reduces NBT^{+2} to NBT^{+1}. The decrease in absorbance at 560nm with antioxidant indicates the quenching of $O_2^{\bullet-}$ in the reaction mixture. Both DVW and DVE-4 were found to have comparable $O_2^{\bullet-}$ scavenging ability under steady state conditions with an IC_{50} 49.15 and 47.08 μg/ml respectively. The IC_{50} of BHA under the same experimental conditions was found to be 1.46 μg /ml (Table 1).

Total antioxidant capacity: Both the tested extract and fraction were found to possess high antioxidant capacity (reduction of Mo^{VI} to Mo^{V}) of 0.94 and 1.05 mg of ASC equivalents per milligram of the DVW and DVE-4 respectively (Table 1).

Calf thymus DNA Protection studies: DVW and DVE-4 did not damage the DNA themselves as indicated in lanes 3 and 5. Both DVW and DVE-4 (800μg/ml) marginally protected the hydroxyl radical-induced DNA damage as evident from lane 4 and 6 respectively.

Discussion

Diabetes mellitus, a common heterogenous metabolic syndrome, is prevalent throughout the world and has been projected to become one of the world's main cause of morbidity and mortality within the next 25 years [25]. Evaluation of plant products to treat diabetes mellitus is of growing interest as they contain

many bioactive substances with therapeutic potential. In recent years several authors evaluated the efficacy of different medicinal plants in the modulation of oxidative stress associated with diabetes mellitus [26-27]. To our knowledge, this is the first study in which a follow up was made on the effect of extract/fractions of *D. viscosa* on the levels of glucose, lipid profile, non-enzymatic and enzymatic antioxidants in STZ-induced diabetic animals.

The present data indicates that *D. viscosa* extract/fraction significantly reduced hyperglycemia in both single dose and multidose diabetic studies. The efficacy of the extract/fraction is comparable to standard glibenclamide, and could be mediated by improving the glycemic control mechanisms and increasing insulin secretion from remnant pancreatic β-cells in diabetic rats.

Diabetes mellitus is often linked with abnormal lipid metabolism. The impairment of insulin secretion results in enhanced metabolism of lipids from the adipose tissue to the plasma [28]. It has been demonstrated that insulin deficiency in diabetes leads to a variety of disruption in metabolic and regulatory processes, which in turn lead to accumulation of lipids [29]. It has also been shown that insulin significantly normalizes lipid levels in diabetic rats [30]. The extract supplementation also results in significant attenuation in serum TG, TC, VLDL-c, and LDL-c. These effects may be due to low activity of cholesterol biosynthesis enzymes or low levels of lipolysis. Increased TC/HDL-c and LDL-c/HDL-c ratios are well known markers of dyslipidemia in STZ-induced diabetic rats [31]. DVW and DVE-4 supplementation restored dyslipidemic markers to normal values. Therefore, normalization of lipids in diabetic rats treated with the *Dodonaea viscosa* may be partly due to its stimulatory effect on insulin secretion from pancreatic β-cells.

Hyperglycemia is a well-known cause for elevated free radical concentration and this can lead to increased lipid peroxidation (TBARS). According to Randle's glucose-fatty acid hypothesis, oxidation of excessive free fatty acid causes production of reactive oxygen species including hydrogen peroxide, which inhibit glucose utilization by the tissue. These reactive oxygen species may cause damage to cellular structures and impair glucose metabolism [32]. Elevated free radical concentration and lipid peroxidation decreases the antioxidant defense in the biological systems. Reduced glutathione (GSH) has an important role in the generation of cellular redox state and consequently, the imbalance in reduced glutathione to oxidized glutathione ratio is a putative indicator of cellular oxidative stress. The generation of oxygen radicals by increased levels of glucose causes tissue GSH depletion which attempts to overcome the deleterious effects of lipid peroxidation. Also glutathione peroxidase utilizes GSH as its cosubstrate while scavenging H_2O_2 formed by the action of SOD. Therefore diminished levels of GSH were observed in untreated diabetic rats. Lowered levels of enzymatic antioxidants such as SOD, catalase and GST have been well documented in the diabetic condition [33]. Treatment with tested extract/ fraction combats the elevated levels of these enzymes, thereby indicating a protective effect.

The phytochemical examination of polar fractions of *D. viscosa* revealed the presence of flavonoids, tannins, saponins and coumarins [9,34]. Several authors have reported flavonoids, phenolics and coumarins as bioactive antidiabetic principles [26,35]. The observed antioxidant and antidiabetic activity of title plant may be attributed to the presence of these bioactive principles and their synergistic properties.

Conclusion

The polar fraction of *D. viscosa* has potent antioxidant, antidiabetic and hypolipidaemic activity in STZ-induced diabetic rats. Collectively, these findings indicate that the antioxidant effects of polar fraction of the title plant may be an important contributor to its antidiabetic potential. The present investigation has also opened avenues for further research especially with reference to the development of

potent phytomedicine for diabetes mellitus from the *Dodonaea viscosa*.

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