ORIGINAL ARTICLE

A comparative study on *in vitro* and *in vivo* antioxidant activities of aqueous extract of *Desmodium gangeticum (Leguminosae)* root

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The present study was designed to investigate the *in vitro* and *in vivo* free radical scavenging effect of aqueous extract of *Desmodium gangeticum* (DG) root in different antioxidant models and experimentally induced ischemic reperfusion in an isolated rat heart. The rats were divided into three groups namely control, reperfusion control, and drug treated. For *in vivo* study, ischemic reperfusion injury (IRI) was induced by 30 minutes ischemia followed by reperfusion of Krebs-Henseleit buffer for 15 minutes or 30 minutes or 45 minutes. Oral administration of DG extract (50 and 100 mg/kg once daily for 30 days) was performed in rats of drug group and an increase of enzymatic activity of SOD, catalase and GPx along with an inhibition of lipid per-oxidations were observed. However, a significant rise in lipid peroxidation and reduction in myocardial SOD, catalase, and GPx were observed during IRI. The inhibitory concentrations (IC_{50}) in DPPH, superoxide scavenging activity, hydroxide scavenging activity, nitric oxide scavenging activity and lipid peroxidation were found to be 51.3, 24.6, 52.7, 53.2 and 415 μg/mL respectively. The presence of cactine, an alkaloid used to treat irregular heartbeat, angina pectoris and cardiac neuralgia was reported in DG through GS-MS analysis. The above results suggest that the aqueous extract of DG root exhibit potential free radical scavenging effect that can reduce the oxidative stress exhibited by IRI.

Key words: *Desmodium gangeticum*, GS-MS, *in vitro* antioxidants, myocardial ischemia reperfusion

INTRODUCTION

Free radical-induced per-oxidation has gained much importance because of their involvement in several pathological conditions such as cancer, cardiovascular disease, cataracts, immune system decline, liver diseases, diabetes mellitus, inflammation, renal failure, brain dysfunction and stress among others. Healthy cells can scavenge free radicals effectively by means of antioxidants. An antioxidant can act by scavenging reactive oxygen species by inhibiting their formation, by binding transition metal ions and preventing the formation of hydroxyl and/or decomposition of lipid peroxides, by repair damage or by combination of all.[1]

Recent success in experiments focused on antioxidant therapy in myocardial ischemic reperfusion injury (IRI) suggested the significant role of free radical mediated in the pathology of revascularization injury. At the onset of ischemic reperfusion, a rapid increase in calcium occurs in mitochondria[2] and that resulted in sudden generation of reactive oxygen species that can dramatically upset the balance between reactive oxygen species and antioxidants in human body. Accumulated reactive oxygen species can reduce the natural antioxidants including superoxide dismutase, catalase and glutathione peroxidase. Synthetic antioxidants, such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), have restricted use in foods as they are suspected to be carcinogenic. Therefore, the importance of search for natural antioxidants has greatly increased in the recent years.[3] Natural products can play an important role in two aspects: Enhance the activity of original natural antioxidants and neutralize reactive oxygen species work by non-enzymatic mechanisms.[4] Many natural products like mangolol[5] and honokiol[6] showed their effect on inhibition of ventricular tachycardia, ventricular fibrillation and/or extra systole during the period of ischemic reperfusion injury. Many studies reported that many plants often contain substantial amounts of antioxidants including vitamin C and E, carotenoids, flavonoids and tannins and thus can be utilized to scavenge the excess free radicals from human body.

Desmodium gangeticum (DG) (Leguminosae) is an herb or shrub common on the lower hills and plains throughout India; on the Himalayas it ascends to 5000 feet. The root

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is used as a febrifuge by Hindu physicians. DG is widely used as a medicinal herb in the treatment of ischemic heart diseases also.[7] The root of DG is one of the ingredients of a famous Ayurvedic preparation Dashamoola kwaatha, which is considered to be an antipyretic, alterative and a bitter tonic. It is reported to be beneficial in the treatment of typhoid, biliousness and also as a diuretic and aphrodisiac. The aqueous extract of the root of DG exhibited anti-inflammatory, antibacterial and antifungal activities.[8] There are reports suggesting 19 compounds of various classes that, such as flavonoids, phenolic acids, glycosides, pterocarpanoids, lipids, glycolipids and alkaloids, were isolated and identified from the DG whole plant.[9]

With this observation, the present study was designed to investigate the antioxidant activity of DG root extract in different *in vitro* antioxidant models. However, apart from the direct scavenging activity of the compounds, other factors such as transition metal chelating and uptake into a membrane may play an important role in the antioxidant potential of the extract. Hence, the study also focuses on the *in vivo* free radical scavenging potential of DG root extract by inducing oxidative stress in ischemic reperfused rat heart model. GS-MS analysis was also assigned to predict the reasoning for the activity of the DG root extract as volatile compounds are reported to have cardio protective action against IRI.

MATERIALS AND METHODS

Plant Material

The plant *Desmodium gangeticum (Leguminousea)* after collection from the botanical garden maintained in the botany department of Assumption College, Changanacherry, was washed and cleaned with water. The plant material was taxonomically identified by Professor 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.

Preparation of Aqueous Extract of *Desmodium gangeticum* Roots

One kilogram (1 kg) of fresh secondary roots of DG was 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 L of distilled water at room temperature for 24 hours with shaking. The extracts were filtered and concentrated to dryness under reduced pressure at 30 ± 1°C. The resulting aqueous extract was freeze-dried, finally giving 18.66 g (i. e., 1.866 g/100 g 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.

Dosage Fixation

A pilot study was conducted to determine the effect of DG at four different doses (25, 50, 100 and 150 mg/kg b.wt.) for a period of 30 days in ischemic reperfused rats. Since 50 and 100 mg/kg b.wt. showed significant ($P < 0.05$) effect, we used these doses for further studies.

Chemicals

All chemicals used were of analytical grade.

Animals

Adult male Albino rats of the Wistar strain, weighing approximately 250-280 g were obtained from King Institute of Preventive Medicine, Chennai, India. They were acclimatized to animal-house conditions and were fed commercial pelleted rat chow (Hindustan Lever Ltd., Bangalore, India) and had free access to water (ethically approved by Ministry of Social Justices and Empowerment Government of India). The experimental protocol was approved by the institutional ethics committee.

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 buffer (KH) 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 $C_6H_{12}O_6$ 11. The heart was attached to a Lagendorff apparatus via an aorta for retrograde perfusion with KH buffer maintained at 37°C and pH = 7.4 and saturated with a gas mixture of 95 mL O_2 and 5 mL CO_2. The coronary perfusion pressure was maintained at 80 mmHg. The left ventricular pressure developed with ventricle filled with Kreb solution was recorded with a 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.

Experimental Protocol

The rats were divided into three groups: Group 1, control; group 2, reperfusion, and group 3, drug.

Normal control

In normal control group, hearts were perfused for 90 minutes with KH buffer and used for the biochemical analysis.

Reperfusion

In reperfusion group, the 30 minutes ischemic hearts (n = 6 in each sub groups) were subjected to 15 minutes reperfusion (2.1), 30 minutes reperfusion (2.2) and 45 minutes reperfusion (2.3), respectively.

Drug

The animals in the drug group were subdivided into eight groups.

Group 3.1: Rats (n = 6) in this groups were pretreated orally with DG at a dose of 50 mg/kg b. wt. for 30 days. Hearts were perfused for 90 minutes with KH buffer and used for the biochemical analysis.

Group 3.2: Rats (n = 6) in this groups were pretreated orally with DG at a dose of 100 mg/kg b. wt. for 30 days Hearts were perfused for 90 minutes with KH buffer and used for the biochemical analysis.

Group 3.3: Rats (n = 6) in this groups were pretreated orally with DG at a dose of 50 mg/kg b. wt. for 30 days. Hearts were subjected to 30 minutes of global ischemia after equilibration and followed by 30 minutes of reperfusion.

Group 3.4: Rats (n = 6) in this groups were pretreated orally with DG at a dose of 50 mg/kg b. wt. for 30 days. Hearts were subjected to 30 minutes of global ischemia after equilibration and followed by 45 minutes of reperfusion.

Group 3.5: Rats (n = 6) in this groups were pretreated orally with DG at a dose of 100 mg/kg b. wt. for 30 days. Hearts were subjected to 30 minutes of global ischemia after equilibration and followed by 30minutes of reperfusion.

Group 3.6: Rats (n = 6) in this groups were pretreated orally with DG at a dose of 100 mg/kg b. wt. for 30 days. Hearts were subjected to 30 minutes of global ischemia after equilibration and followed by 45 minutes of reperfusion.

Group 3.7: Rats (n = 6) were perfused with KH buffer for 20 minutes and were infused with standard drug, verapamil (0.2 mg/kg b.wt.) for 30 minutes. They were subjected to 30 minutes global ischemia followed by 30 minutes reperfusion.

Group 3.8: Rats (n = 6) were perfused with KH buffer for 20 minutes and were infused with standard verapamil (0.2 mg/kg b.wt.) for 30 minutes. They were subjected to 30 minutes global ischemia followed by 45 minutes reperfusion.

Tissue Preparation

Preparation of mitochondria

The heart was excised, rinsed in ice-cold isotonic saline, blotted with filter paper, weighed, homogenized in 0.25 M sucrose at 4°C by polytran homogenizer for 5 seconds at maximum power. The homogenate was centrifuged for 10 minutes at 600 × *g*, nuclear and cytoskeleton fractions were discarded. The supernatant was centrifuged for 20 minutes at 15000 × *g* to pellet mitochondria. The mitochondria thus prepared[10] were suspended in 0.25 M sucrose-containing 10 mM Tris-HCl and 1 mM EDTA to a known volume of 3 mL.

Acute toxicity studies

Wister Albino rats (150-250 g) maintained under standard laboratory condition were used. A total of five animals were used which received a single dose (2000 mg/kg, b.wt.) of DG. Animals were kept overnight fasting prior to drug administration. After the administration of DG, the food was with-held for 3-4 hours. Animals were observed individually at least once during the first 30 minutes after dosing, periodically during the first 24 hours. (with special attention during the first 4 hours.) and daily thereafter for a period of 14 days. Daily cage side observation included changes in skin and fur, eyes and mucous membrane (nasal) and also respiratory rate, circulatory (heart and blood pressure), autonomic (salvation, lacrimation, perspiration, pilorection urinary incontinence and defecation) changes.

Biochemical assays

Thiobarbituric acid reactive substances (TBARS)[11] were measured as a marker of lipid peroxidation and endogenous antioxidants, such as superoxide dismutase (SOD): Cu-Zn SOD and Mn SOD,[12,13] catalase[14] and glutathione peroxidase (GPx),[15] were determined colorimetrically using an UV-1601 Shimadzu spectrophotometer. Protein concentration was measured with Folin phenol reagent, following the procedure described by Lowry.[16]

***In vitro* Anti-oxidant Activity**

Determination of the scavenging activity of super oxide radical

Superoxide scavenging capacity was determined by the nitroblue tetrazolium (NBT) reduction method.[17] The reaction mixture contained EDTA (6 μM) containing NaCN (3 μg), riboflavin (2 μM), NBT (50 μM), various concentrations of extracts (5-50 μg/mL) and phosphate buffer (67 mM, pH 7.8) in a final volume of 3 mL. The tubes were uniformly illuminated with an incandescent visible light for 15 minutes and the optical density was measured at 530 nm before and after the illumination. The percentage inhibition of super oxide generation was evaluated by comparing the absorbance values of the control and experimental tubes. Vitamin C used as standard.

Determination of the scavenging activity of hydroxyl radical

The scavenging capacity for hydroxyl radical was measured according to the modified method of Halliwell.[18] Stock solutions of EDTA (1 mM), $FeCl_3$ (10 mM), ascorbic acid (1 mM), H_2O_2 (10 mM) and deoxyribose (10 mM) were prepared in distilled deionized water. The assay was performed by adding 0.1 mL EDTA, 0.01 mL of $FeCl_3$, 0.1 mL of H_2O_2, 0.36 mL of deoxyribose, 1.0 mL of the extract (10-100 μg/mL) dissolved in distilled water, 0.33 mL of phosphate buffer (50 mM, pH 7.4) and 0.1 mL of ascorbic acid in sequence. The mixture was then incubated at 37°C for 1 hour. A 1.0 mL portion of the incubated mixture was mixed with 1.0 mL of 10 g/100 g TCA and 1.0 mL of 0.5 g/100 g TBA (in 0.025 M NaOH containing 0.025 g/100 g TBA) to develop the pink chromogen measured at 532 nm. The hydroxyl radical scavenging activity of the extract is reported as percentage inhibition of deoxyribose degradation. Vitamin C used as standard.

Lipid per-oxidation inhibition effect

Reaction mixture (0.5 mL) containing rat liver homogenate (0.1 mL, 25 g/100 mL) in Tris-HCl buffer (40 mM, pH 7.0), KCl (30 mM), ferrous iron (0.16 mM) and ascorbic acid (0.06 mM) was incubated for 1 hour at 37°C in the presence and absence of extract (20-180 μg/mL). The lipid peroxidation (LP) was measured by TBARS formation.[11] For this incubation mixture, 0.4 mL was treated with sodium dodecyl sulphate (8.1 g/100 mL, 0.2 mL), TBA (0.8 g/100 g,1.5 mL) and acetic acid (20 mL/100 mL, 1.5 mL, pH 3.5). The total volume was then made up to 4 mL by adding distilled water and kept in a water bath at 100°C for 1 hour. After cooling, 1 mL distilled water and 5 mL of a mixture of *n*-butanol and pyridine (15:1 v/v) was added and shaken vigorously after centrifugation, the absorbance of the organic layer was measured at 532 nm. The percentage inhibition of LP was determined by comparing results of the test compounds with those of controls and not treated with the extracts. Vitamin E used as standard.

DPPH radical scavenging activity

The free radical scavenging activity of the DG extract and butylated hydroxyl toluene (BHT) was measured in terms of hydrogen donating or radical-scavenging ability using the stable radical DPPH.[19] 0.1 mM solution of DPPH in ethanol was prepared and 1.0 mL of this solution was added to 3.0 mL of extract solution in water at different concentrations (10-100 μg/mL). Thirty minutes later, the absorbance was measured at 517 nm. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The antioxidant activity of the extract was expressed as IC_{50}. The IC_{50} value was defined as the concentration (in μg/mL) of extracts that inhibits the formation of DPPH radicals by 50%. Vitamin C and BHT were used as standard.

Nitric oxide scavenging

Nitric oxide was generated from sodium nitroprusside and measured by the Greiss reaction. Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide,[20] which interacts with oxygen to produce nitrite ions that can be estimated by use of Greiss reagent. Scavengers of nitric oxide compete with oxygen leading to reduced production of nitric oxide. Sodium nitroprusside (5 mM) in phosphate-buffered saline (PBS) was mixed with 3.0 mL of different concentrations (10-320 μg/mL) of the drugs dissolved in the suitable solvent systems and incubated at 25°C for 150 minutes. The samples from the above were reacted with Greiss reagent (1 g/100 mL, sulphanilamide, 2 mL/100 mL H_3PO_4 and 0.1 g/100 mL napthylethylenediamine dihydrochloride). The absorbance of the chromophore formed during the diazotization of nitrite with sulphanilamide and subsequent coupling with napthylethylenediamine was read at 546 nm and referred to the absorbance of standard solutions of potassium nitrite, treated in the same way with Griess reagent. Vitamin C and curcumin were used as standard.

Gs-Ms Analysis

All analyses were conducted with a Perkin Elmer Clarus 500 GS-MS equipped with mass spectrometry. The chromatographic conditions were as follows: Column: Elite-1 (100 g/100 mL dimethyl polysiloxane). Helium was used as the carrier gas with a flow rate of 1 mL/min. The 1 μL DG aqueous root extract was injected into the GS-MS in split less mode at 250°C. The column oven temperature was held at 110°C for 2 minutes, then programmed at 75°C/min to 200°C for 1 minute, 5°C/min to 280°C and held for 9 minutes.

Statistics

All dates are reported as mean ± SD. The results were statistically analysed by a one-way analysis of variance (ANOVA) by the SPSS software 12.00, followed by Duncan's multiple range test (DMRT). $P < 0.05$ was considered to be significant. Linear regression analysis was used to calculate IC_{50} values whenever needed.

RESULTS

The initial value of mean arterial pressure (MAP) and heart rate (HR) in the control IR group was 99 ± 6 mmHg and 260 ± 17.1 beats/min, respectively. Both the hemodynamic variables in the control IR group remained depressed throughout the I-R duration as compared to control baseline values. Because heart rate (HR) and left ventricular developed pressure (LVDP) may recover to different degrees, rate pressure product (RPP) was calculated via multiplying heart rate by LVDP and presented as reliable left ventricular function parameter for the isolated heart [Table 1]. No differences were obtained between the

Table 1: Hemodynamic characteristic

Group	n	LVDP (mmHg)	CF (ml/min)	HR (b.p/min)	RPP × 10^3 (mmHg.bt /min)	MAP (mmHg)
2.6.1	6	105.22 ± 4.3	9.2 ± 1.00	342 ± 20.1	35.98 ± 7.1	122 ± 7
2.6.2.1	6	90.39 ± 4.3	9.1 ± 0.91	260 ± 17.1*	23.68 ± 6.5*	98 ± 6*
2.6.2.2	6	94.2 ± 4.4	9.1 ± 1.02	241 ± 17.5*	22.60 ± 6.3*	97 ± 6*
2.6.3.1	6	107.2 ± 4.2	9.2 ± 1.08	339 ± 34.1	36.34 ± 8.2	115 ± 8
2.6.3.2	6	107.3 ± 4.5	9.3 ± 1.10	338 ± 31.3	36.26 ± 5.1	114 ± 7
2.6.3.3	6	106.4 ± 4.6	9.2 ± 0.94	323 ± 33.2	32.94 ± 6.8	104 ± 5
2.6.3.4	6	106.2 ± 4.0	9.4 ± 1.05	329 ± 33.5	34.94 ± 7.4	103 ± 6
2.6.3.5	6	106.1 ± 4.1	9.3 ± 1.00	319 ± 32.8	33.84 ± 5.9	107 ± 7
2.6.3.6	6	105.6 ± 4.2	9.2 ± 1.02	321 ± 33.5	33.89 ± 7.3	105 ± 6

Values are expressed as 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; *$P < 0.05$, compared with control

experimental groups for RPP at the end of 30 minutes adaptation before starting treatments and global ischemia. During 30 minutes of global ischemia there was a reduction in RPP to zero which started to recover gradually by continuation of the reperfusion. Pretreated DG increased the recovery of the RPP in the drug group (69% basal value) compared with the IR group (38% basal value, $P < 0.05$).

GS-MS analysis resulted in the identification of 22 volatile compounds. The major compounds comprised phenol,4-[2-(dimethylamino)ethyl]-(Cactine), tri-tetracontane,docosanoic acid docosyl ester, 5 hydroxy -7-(4- methoxyphenyl)-2, 2-dimethyl-10-(3-methyl-2-butenyl)-2H,6H-pyrano [3,2-g]chromen-6-one and 15-isobutyl-(13αH)-isocopalane. It represents around 35%. Minor compounds such as 2,2-dimethyl *N*-phenethyl propinamide, 5 hydroxymethyldihydrofuran-2-one and 3-methyl-2-(2-oxopropyl)furan were also present.

Several concentrations ranging from 2 to 500 μg/mL of aqueous extract of DG were tested for their antioxidant activity in different *in vitro* models. It was observed that free radicals were scavenged by the test compounds in a concentration-dependent manner up to the given concentration in all the models. The maximum inhibitory concentration (IC_{50}) in all models, viz, DPPH, superoxide scavenging activity, hydroxide scavenging activity, nitric oxide scavenging activity and lipid peroxidation were found to be 51.3, 24.6, 52.7, 53.2 and 415 μg/mL, respectively.

The *in vivo* antioxidant effect of the extract was determined by administering the rats with DG orally for about 30 days and then sacrificing the animal for reperfusion induces ischemic injury. The observations in the present study suggested a potent *in vivo* antioxidant capacity for DG as compared to standard drug namely verapamil against revascularization injury.

DISCUSSION

The volatile chemical composition of aqueous extract of DG was analyzed by employing GS-MS, leading to a comparison of the relative retention times and the mass spectra of different components with those of authentic samples and mass spectra from the data library. As shown in Table 2, GS-MS analysis resulted in the identification of 22 compounds. Some pterocarpanoids such as gangetin, gangetinin and desmodin, the isoflavanoid phytoalexin desmocarpin[21] and the flavone glycosides 4′,5,7-trihydroxy-8-prenyl-flavone, 4′O-α-L-rhamnopyranosyl-(1®6)-β-D-glucopyranoside have been isolated from the stems of DG. However, there has been little study of the volatile components of the roots. Many studies have shown the significance of volatile compounds

Table 2: Volatile compound present in aqueous extract of *Desmodium gangeticum* root by GS-MS analysis

R.T	Name	Molecular formula
1.66	Hexane	C_6H_{14}
1.77	1-Pentanol, 4-methyl-	$C_6H_{14}O$
2.27	Butanoic acid	$C_4H_8O_2$
3.04	Pentanoic acid	$C_5H_{10}O_2$
3.12	Hexanoic acid	$C_6H_{12}O_2$
4.45	Phenol	C_6H_6O
7.03	Maltol	$C_6H_6O_3$
8.38	5-Hydroxymethyldihydrofuran-2-one	$C_5H_8O_3$
15.42	Phenol, 4-[2-(dimethylamino) ethyl]-(Cactine)	$C_{10}H_{15}NO$
18.63	á-Phenylethyl butyrate	$C_{12}H_{16}O_2$
20.65	2,2-Dimethyl-*N*-phenethylpropionamide	$C_{13}H_{19}NO$
20.92	Pentanoic acid, 2-phenylethyl ester	$C_{13}H_{18}O_2$
21.73	3-Methyl-2-(2-oxopropyl) furan	$C_8H_{10}O_2$
26.45	Tridecane, 4-cyclohexyl-	$C_{19}H_{38}$
26.74	Tridecanol, 2-ethyl-2-methyl-	$C_{16}H_{34}O$
30.56	Eicosane, 2-methyl-	$C_{21}H_{44}$
32.16	Tritetracontane	$C_{43}H_{88}$
36.64	Hexanedioic acid, bis (2-ethylhexyl) ester	$C_{22}H_{42}O_4$
35.58	Docosanoic acid, docosyl ester	$C_{44}H_{88}O_2$
38.57	2H,6H-Benzo[1,2-b5,4-b'] dipyran-6-one, 5-hydroxy-7-(p-methoxyphenyl)-2,2-dimethyl-10-(3-methyl-2-butenyl)-	$C_{26}H_{26}O_5$
41.84	Hexatriacontane	$C_{36}H_{74}$
44.96	15-Isobutyl-(13àH)-isocopalane	$C_{24}H_{44}$

like isoflurane, enflurane and halothane in cardioprotection against IR injury.[22]

DG root extract caused a dose-dependent protection against lipid peroxidation with 91% protection at 1 mg/mL concentration. The values of TBARS upon incubation with the extract are represented in Table 3. The inhibition could be caused by the absence of ferryl–perferryl complex or by scavenging of.OH radical or superoxide radical or by changing the Fe^{3+}/Fe^{2+} ratio or by reducing the rate of conversion of ferrous to ferric ion or by chelating the iron itself.[23]

The effect of DG root extracts on inhibition of hydroxyl radical production was assessed by the iron(II)-dependent deoxyribose damage assay. According to Fenton reaction, the generated hydroxyl radicals ($OH^{\cdot}$) degraded deoxyribose using Fe^{2+} salts as an important catalytic component. Oxygen radicals may attack the sugar, which leads to sugar fragmentation. Addition of transition metal ions such as iron at low concentrations to deoxyribose causes degradation of the sugar into malondialdehyde and other related compounds that form a chromogen with thiobarbituric acid (TBA). Table 4 presents the results of the effects of the examined DG root extracts on hydroxyl scavenging activity. The DG was capable of reducing hydroxyl radical production at all concentration (IC_{50} = 52.7 μg/mL). Ascorbic acid, used as a standard was highly effective in inhibiting the oxidative DNA damage, showing an IC_{50} = 15 μg/mL.

DPPH assay is based on the measurement of the scavenging ability of antioxidants towards the stable radical DPPH. From the present result, it may be postulated that DG root extract reduces the radical to corresponding hydrazine when it reacts with the hydrogen donors in the antioxidant principles. DPPH radicals react with suitable reducing agents, the electrons become paired off and the solution loses colour stoichiometrically depending on the number of electrons taken up.[24] A 100 μg/mL of DG and BHT exhibited 87.44 and 92.27% inhibition, respectively, and the IC_{50} values were found to be 38.5 μg and 15 μg/mL for DG and BHT, respectively.

Nitric oxide (NO) plays protective roles in the ischemic heart by several mechanisms such as stimulating soluble guanylate cyclase and thus reducing $[Ca^{2+}]i$ partly through activation of cGMP-dependent protein kinase, terminating chain propagating lipid radical reactions caused by oxidative stress, and by inhibiting the activity of platelets and neutrophils and their adhesion to the endothelial surface. NO is necessary for normal cardiac physiology, but it is potentially toxic in excess concentrations. NO can be converted to various other reactive nitrogen species (RNS) such as nitrosonium cation (NO^+), nitroxyl anion (NO^-) or peroxynitrite ($ONOO^-$). Some of the physiological effects may be mediated through the intermediate formation

Table 3: Effect of aqueous extract of *Desmodium gangeticum* on ferrous sulphate-induced lipid peroxidation in rat liver homogenate

Extract concentration (μg/mL)	TBARS (nmol/mg protein)[a]	Inhibition (%)[a]
Control	2.35 ± 0.23	
1000	0.20 ± 0.02	91.11 ± 1.1
800	0.60 ± 0.04	74.21 ± 2.5
600	0.88 ± 0.12	62.27 ± 2.3
400	1.21 ± 0.14	48.39 ± 3.3
200	1.32 ± 0.23	43.54 ± 4.7
Tocopherol (10 μmol/L)	0.06 ± 0.02	97.26 ± 4.5

a = Mean ± SEM, 6 independent analysis

of *S*-nitroso-cysteine or *S*-nitroso-glutathione. In the present study, the extract competes with oxygen to react with nitric oxide and thus inhibits the generation of the anions. Curcumin was used as a reference compound. The concentration of DG needed for 50% inhibition was found to be 50.0 μg/mL, whereas 32.5 μg/mL for curcumin.

Super oxides are produced from molecular oxygen due to oxidative enzymes of body as well as via non-enzymatic reactions such as auto-oxidation by catecholamine. In the present study, super oxide radicals reduce NBT to a blue coloured formazan that is measured at 560 nm. The effect of DG in this regard is shown in Table 3. The probable mechanism of scavenging the super-oxide anion may be due to the inhibitory effect of DG towards generation of super-oxides in the *in vitro* reaction mixture.

Since possible antioxidant properties have been correlated with cardioprotective effects, we examined the antioxidant properties DG that has been extensively studied for its cardioprotective effects in isolated ischemic reperfused rat heart. Table 5 shows the concentration of TBARS in the myocardium of ischemic reperfused rat heart. Massive reactive oxygen species production was identified as an important causative factor for ischemia and reperfusion that leads to tissue injury. Multiple sources of oxidant generation could function during ischemia, although a likely source is the mitochondria. The mitochondrial electron transport system consumes approximately 85% of the oxygen utilized by the cell, and about 5% of the oxygen is converted to reactive oxygen intermediates. The generation of reactive oxygen intermediates in mitochondria has been reported to impair various cellular functions during ischemic reperfusion.[25] Thus the protection against ischemia reperfusion-induced oxidative stress in DG-treated rat hearts was evidenced by preservation of endogenous antioxidants and prevention in rise of TBARS [Table 5].

The cardio-protective activity of the examined extracts could be mainly due to the presence of compounds such as phenol, 4-[2-(dimethylamino) ethyl]-(Cactine) and other secondary

Table 4: Free radical scavenging activity of *Desmodium gangeticum* extract

Extract concentration (μg/mL)	Inhibition (%)			
	DPPH	Nitric oxide	Super-oxide	Hydroxyl radical
1000	94.16 ± 3.61	92.44 ± 3.82	96.45 ± 2.15	91.38 ± 4.95
500	92.32 ± 4.75	88.65 ± 5.40	93.46 ± 4.24	88.54 ± 5.57
250	87.54 ± 3.62	80.11 ± 3.23	85.42 ± 4.23	84.37 ± 5.53
125	83.22 ± 2.55	70.77 ± 4.71	72.99 ± 3.34	75.43 ± 3.21
62	54.84 ± 3.73	54.23 ± 5.34	68.38 ± 2.74	61.76 ± 4.33
32	42.33 ± 4.32	46.48 ± 4.50	55.57 ± 3.85	40.54 ± 3.83
16	13.62 ± 3.64	27.64 ± 3.15	44.65 ± 3.81	31.55 ± 2.43
10	3.46 ± 1.42	5.78 ± 1.52	32.63 ± 1.39	7.13 ± 1.68
7	1.02 ± 0.31	3.11 ± 0.50	16.04 ± 1.26	4.34 ± 1.25
5	0.30 ± 0.01	1.36 ± 0.10	5.21 ± 1.05	1.23 ± 0.33
Ascorbic acid (100 μg)	95.11 ± 4.22	85.34 ± 4.11	87.32 ± 5.87	94.44 ± 4.71
BHT (20 μg)	92.27 ± 3.31	-	-	-
Curcumin	-	91.7 ± 3.11	-	-
IC_{50}	51.3 ± 1.52	53.2 ± 2.14	24.6 ± 1.34	52.7 ± 2.66

Values are expressed as mean ± S.E.M of 3 replicates; NT = Not tested

Table 5: Effect of aqueous root extract of *Desmodium gangeticum* on Thiobarbituric acid reactive substances, catalase, superoxide dismutase, and glutathione peroxidase in the tissue homogenate of isolated rat heart

Group	TBARS (μM/g wet tissue)	Catalase (μM of H_2O_2 consumed/min/g protein)	SOD (U#/mg protein)		GPx (μg of GSH consumed/min/g protein)
			Mn SOD	Cu-Zn SOD	
1	6.0 ± 0.4	7610 ± 440	8.2 ± 0.61	52.2 ± 4.2	1850 ± 180
2.1	7.8 ± 0.6*	4080 ± 240*	5.2 ± 0.52*	32.4 ± 3.2*	1220 ± 140*
2.2	7.4 ± 0.5*	5170 ± 370*	6.0 ± 0.55*	36.1 ± 3.3*	1110 ± 110*
2.3	7.0 ± 0.5*	5200 ± 310*	5.7 ± 0.58*	35.2 ± 3.4*	1210 ± 110*
3.1	5.9 ± 0.4	7850 ± 440	8.1 ± 0.73	51.6 ± 4.1	1850 ± 171
3.2	5.8 ± 0.4	7570 ± 430	8.1 ± 0.71	51.0 ± 4.1	1800 ± 186
3.3	4.7 ± 0.3*	6170 ± 450*	7.2 ± 0.65*	47.3 ± 4.0	1570 ± 172*
3.4	4.4 ± 0.3*	5860 ± 370*	7.4 ± 0.62	44.5 ± 3.8	1510 ± 167*
3.5	5.8 ± 0.4	5510 ± 350*	6.9 ± 0.62*	41.1 ± 3.6*	1430 ± 164*
3.6	5.5 ± 0.5	5230 ± 400*	6.8 ± 0.68*	35.6 ± 3.3*	1390 ± 168*
3.7	5.9 ± 0.5	5110 ± 380*	6.5 ± 0.62*	39.7 ± 3.1*	1480 ± 152*
3.8	6.4 ± 0.6	5020 ± 330*	6.1 ± 0.71*	34.1 ± 3.2*	1400 ± 163*

#SOD unit: One unit is defined as the enzyme concentration required to inhibit the OD (at 560 nm) produced by 50% of chromogen 50% in 1 minute. Values are mean ± SD for six rats in each group. Significantly differing values (from normal control group) are expressed as $*P < 0.05$

biomolecules present in the extracts [Table 2]. The alkaloid cactine has a similar effect to digitalis, and when used as a tincture it gives relief from angina pectoris, palpitations, weak heart beat, rapid heartbeat accompanied by loss of bodily strength, coronary disease, low blood pressure, numbness of the left arm (often associated with angina), dropsy, incipient apoplexy and hemoptysis (bloody sputum).

CONCLUSION

This study provide the evidence that free radical scavenging potential possessed by aqueous extract of DG in both *in vitro* and *in vivo* model and may be due to the presence of phenolic compounds and biologically active alkaloids like cactine.

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