An *in vivo* and *in vitro* analysis of free radical scavenging potential possessed by *Desmodium gangeticum* chloroform root extract: Interpretation by gsms

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Abstract: In the present study, the effect of DG chloroform root extract was assessed on isolated rat heart and *in-vitro* antioxidant models. Ischemia reperfusion injury was experimentally induced by using Langendroff apparatus. The free radical scavenging potential was studied in vitro by using different antioxidant models such as DPPH, super oxide scavenging activity, hydroxide scavenging activity and nitric oxide scavenging activity. Both experimental approaches not only substantiate its antioxidant potential but also the cardio-protection imparted by the extract. The cardio-stimulatory effects were investigated for the extract by treating it as a pre-conditioning agent against myocardial ischemia reperfusion injury. The improved antioxidant status of the myocardium indirectly predicts reduced oxidative stress mediated by ischemic reperfusion with evident reduction of infarct size determined by cardiac marker protein. These findings indicate that DG chloroform root extract may possess therapeutic potential against ischemia reperfusion injury.

Keywords: *Desmodium gangeticum*, ischemic reperfusion, antioxidants, free radical scavenging, Gas spectroscopy/mass spectroscopy.

INTRODUCTION

Desmodium gangeticum (DG) belongs to the family namely Fabaceae and follow a terrestrial life cycle. It is widely used in indigenous system of medicine and is reported to have potent antioxidant activity (Govindarajan *et al*., 2003). It has a great therapeutic value in treating diseases such as, typhoid, piles, inflammation, asthma, bronchitis, and dysentery. DG root is prescribed in combination with other drugs for the treatment of snakebite and scorpion sting. The sterol N,N-dimethyl tryptamine, 5methoxy –N,N dimethyl tryptamine, their oxides and other derivatives have been isolated from aerial parts, three pterocarpinoids, gangetin, gangetinin and desmodin were explored from the roots. Alkaloids isolated from the aerial part comprise indole -3-alkylamines and β carbolines that has biological activities like anticholinesterase, smooth muscle stimulant and CNS stimulant response (Ghosal *et al*., 1972) Gangetin, a petrocarpin shows anti fertility activity. It also possesses antioxidant, anti ulcer and cardiotonic activity (Dharmani *et al*., 2005).

The main patho-physiological reason for ischemia reperfusion injury (IRI) lies in free radical release and calcium overload (Maxwell and Lip 1997). A reduction in cardiac infract size during IRI by long term administration of DG methanol root extract (Kurian *et al*., 2008) encourage us to explore more on DG root. In fact our unpublished data on different extracts of DG and its biological effect on myocardium emphasize the muscarinic receptor agonist like action for DG methanol root extract and calcium chloride like action for DG chloroform root extract. In the present study the cardio-stimulatory effect of DG chloroform root extracts was investigated in isolated rat heart model as preconditioning agent.

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MATERIALS AND METHODS

Preparation of chloroform root extracts of Desmodium gangeticum

The plant, after collection from the herbal garden was washed, cleaned and maintained in the department. The plant material was taxonomically identified by Prof. James Joseph, Head of the Department, Department of Botany, Saint Berchman's College, Mahatma Gandhi University, Kerala. The voucher specimen A/C no. 3908 was retained in our laboratory for future reference. The dried roots were then milled to fine powder (10 kg) and extracted with chloroform in Soxhlet's apparatus for 24 h and the extract was evaporated to dryness under vacuum and dried in vacuum desiccators (815.8 g).

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
Heart preparation follows the procedure describes by Neely 1972. Rats were anesthetized with 40 mg/kg sodium thiopentenone followed by an intravenous injection of 300 U heparin. The heart was rapidly excised following euthanasia with sodium thiopentenone via a mid-sternal thoracotomy and arrested in the ice cold Krebs-Henseleit buffer (KH) containing (m*M*/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 (*p*H 7.4). The heart was then saturated with a gas mixture of 95% O_2 and 5% CO_2. The coronary perfusion pressure was maintained at 80mm Hg. 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 3 groups.

Group 3.1: Rats (n=6) in this groups were pretreated orally with DG at a dose of 100mg/kg b. wt. for thirty 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 100mg/kg b. wt. for thirty days. Hearts were subjected to 30 minutes of global ischemia after equilibration and followed by 30 minutes of reperfusion

Group 3.3: Rats (n=6) in this groups were pretreated orally with DG at a dose of 100mg/kg b. wt. for thirty days. Hearts were subjected to 30 minutes of global ischemia after equilibration and followed by 45 minutes of reperfusion.

Biochemical assays
Thiobarbituric acid reactive substances (TBARS) was measured as a marker of lipid per-oxidation (Ohkawa *et al.,* 1979) and endogenous antioxidants, such as superoxide dismutase (SOD): Cu-Zn SOD and Mn SOD (Marklund, 1985 and Geller 1983), catalase (Aebi 1984) and glutathione peroxidase (GPx) (Wendel 1981) were estimated in a UV-1601 Shimadzu spectrophotometer. Protein concentration was measured with Folin phenol reagent, following the procedure described by Lowry (Lowry *et al.,* 1951).

In vitro anti oxidant activity
Determination of super oxide radical scavenging activity
Superoxide scavenging was determined by the nitroblue tetrazolium (NBT) reduction method (Mc Cord and Fridovich 1969). The reaction mixture contained EDTA (6uM) containing NaCN (3ug), riboflavin (2uM), NBT (50uM), various concentrations of extracts (5-500ug/mL) and phosphate buffer (67mM, pH 7.8) in a final volume of 3mL. The tubes were uniformly illuminated with an incandescent visible light for 15 min. 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.

Determination of hydroxyl radical scavenging activity
The scavenging capacity for hydroxyl radical was measured according to the modified method of Halliwell *et al.,* (1987). Stock solutions of EDTA (1mM), $FeCl_3$ (10mM), ascorbic acid (1mM), H_2O_2 (10mM) and deoxyribose (10mM) were prepared in distilled deionized water. The assay was performed by adding 0.1mL EDTA, 0.01mL of $FeCl_3$, 0.1mL of H_2O_2, 0.36mL of deoxyribose, 1.0mL of the extract (10–100µg/mL) dissolved in distilled water, 0.33ml of phosphate buffer (50mM, pH 7.4) and 0.1mL of ascorbic acid in sequence. The mixture was then incubated at 37°C for 1hr. A 1.0 ml

portion of the incubated mixture was mixed with 1.0mL of 10g/100g TCA and 1.0mL of 0.5g/100g TBA (in 0.025M NaOH containing 0.025g/100g 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.

Lipid peroxide scavenging activity

Reaction mixture (0.5mL) containing rat liver homogenate (0.1mL, 25g/100mL) in Tris–HCl buffer (40mM, pH 7.0), KCl (30mM), ferrous iron (0.16mM) and ascorbic acid (0.06mM) was incubated for 1hr.at 37°C in the presence and absence of extract (20-180 g/ml). The lipid peroxidation (LP) was measured by TBARS formation (Ohkawa *et al.,* 1979). For this incubation mixture 0.4mL was treated with sodium dodecyl sulphate (8.1g/100mL, 0.2ml), TBA (0.8g/100g, 1.5ml) and acetic acid (20ml/100ml, 1.5ml, pH 3.5). The total volume was then made up to 4mL by adding distilled water and kept in a water bath at 100 C for 1hr. After cooling, 1ml distilled water and 5mL 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.

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 (Bandoniene *et al.,* 2002). 0.1mM solution of DPPH in ethanol was prepared and 1.0ml of this solution was added to 3.0mL 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 percent.

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 (Green *et al*., 1982; Marcoci *et al*., 1994), 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 (5mM) in phosphate-buffered saline (PBS) was mixed with 3.0mL of different concentrations (10–320µg/ml) of the drugs dissolved in the suitable solvent systems and incubated at 25°C for 150 min. The samples from the above were reacted with Greiss reagent (1% sulphanilamide, 2mL/100ml H_3PO_4 and 0.1g/100ml napthylethylenediamine dihydrochloride). The absorbance of the chromophore formed during the diazotization of nitrite with sulphanilamide and subsequent coupling with napthylethylenediamine was read at 546nm and referred to the absorbance of standard solutions of potassium nitrite, treated in the same way with Griess reagent.

GS - MS Analysis

All analysis was conducted with a Perkin Elmer Clarus 500 GC equipped with mass spectrometry. The chromatographic conditions were as follows: Column: Elite -1 (100g/100mL dimethyl poly siloxane). Helium was used as the carrier gas with a flow rate of 1mL/minute. 1uL DG chloroform 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 min, 5°C/min to 280°C and held for 9 minutes.

Table 1: Chemical composition of chloroform extract of *Desmodium gangeticum* root by GSMS.

No.	RT	Name of the compound	Molecular formula	Molecular weight	Peak area (%)
1	12.84	2 methyl pentanal	$C_6H_{12}O$	100	0.89
2	13.11	1,14 Tetradecanediol	$C_{14}H_{30}O$	230	2.29
3	15.94	1,2 Benzenedicarboxylic acid bis(2 methylpropyl) ester	$C_{16}H_{22}O_4$	278	3.45
4	16.22	10 Undecenal	$C_{11}H_{20}O$	168	0.92
5	17.70	N hexadecanoic acid	$C_{16}H_{32}O_2$	256	36.42
6	19.80	9 Dodecenoic acid methyl ester	$C_{13}H_{24}O_2$	212	5.06
7	20.90	Oleic acid	$C_{18}H_{34}O_2$	282	36.63
8	21.18	9,9 Dimethoxybicyclo[3,3,1]nona 2,4 dione	$C_{11}H_{16}O_4$	212	7.32
9	23.22	4 dodecanol	$C_{12}H_{26}O$	186	0.75
10	26.46	1,2 Bis(trimethysilyl) benzene	$C_{12}H_{22}Si_2$	222	19.5
11	27.55	Didodecyl phthalate	$C_{32}H_{54}O_4$	502	4.31

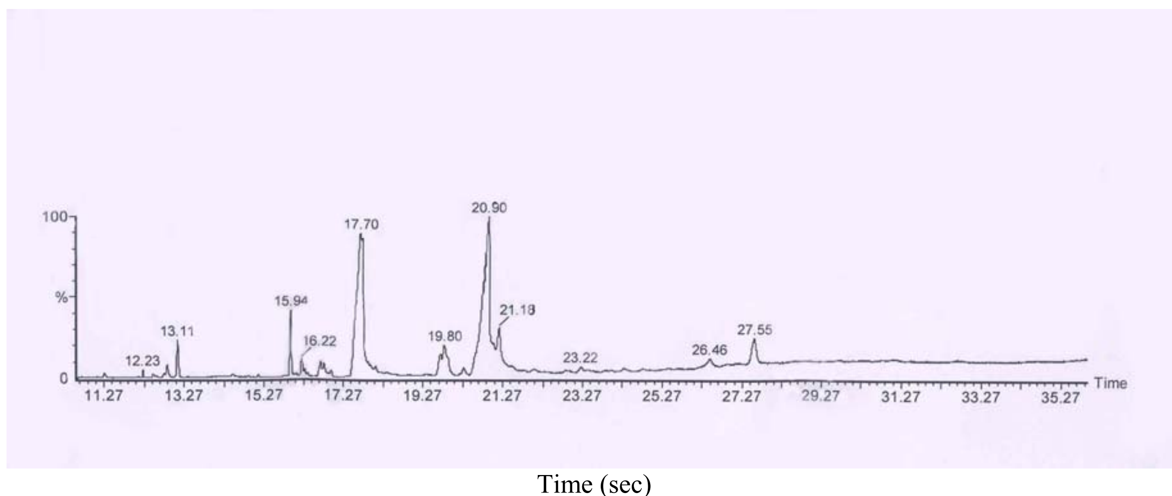
Statistics

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

RESULTS

GS/MS analysis resulted in the identification of 11 compounds (table 1). The major compounds comprises Oleic acid (RT: 20.90), N hexadecanoic acid (RT: 17.70), 1,2 Benzenedicarboxylic acid bis(2 methylpropyl)ester (RT:15.94), 9 Dodecenoic acid methyl ester (RT: 19.80), 9,9 Dimethoxybicyclo [3,3,1]nona 2,4 dione (RT: 21.18), 1,2 Bis(trimethysilyl) benzene (RT: 26.46), and Didodecyl phthalate (RT: 27.55). Minor compounds such as 2 methyl pentanal (RT: 12.84), 10 Undecenal (RT: 16.22), and 4 dodecanol (RT: 23.22), were also present (fig. 1).

Several concentrations ranging from 2-1000µg/ml of DG chloroform root extracts were tested for their antioxidant activity in different in-vitro models. It was observed that free radicals were scavenged by 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 36.0, 55.3, 43.7, 39.4 and 297 g/ml respectively (table 3 and fig. 2).



Time (sec)

Fig. 1: GSMS of chloroform extract of *Desmodium gangeticum.*

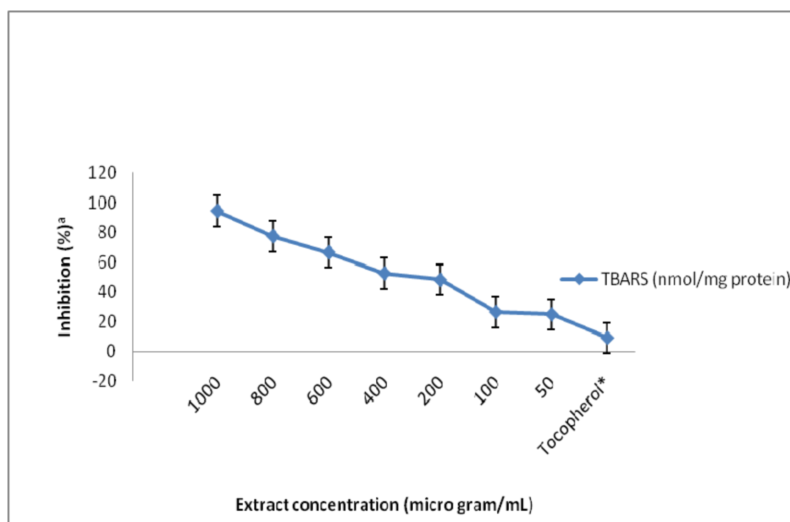


Fig. 2: Effect of chloroform root extract of DG on ferrous sulphate induced lipid peroxidation in rat liver homogenate.

"a" Mean ± SEM, 6 independent analysis: * Tocopherol (10µmol/L)

Table 2: Hemodynamic characteristic of rat hearts subjected to ischemia reperfusion

Group	N	LVDP(mmHg)	CF(ml/min)	HR (b.p/min.)	RPP × 10^3 (mmHg.bt min^{-1})	MAP (mmHg)
1	6	99.64±4.0	9.1±1.01	340 ± 19.1	33.88 ± 5.3	120± 7
2.1	6	50.53±4.0	9.0±0.99	260 ± 17.2*	13.14 ± 4.2*	97± 6*
2.2	6	52.22±4.5	9.0±1.03	238 ± 18.3*	12.43 ± 5.2*	96 ± 7*
2.3	6	40.29±4.3	9.1±1.09	237 ± 30.1	9.55 ± 7.4	113± 8
3.1	6	92.42±4.2	9.2±1.11	338 ± 27.3	31.24 ± 4.3	114± 7
3.2	6	75.45±4.2	9.1±0.95	321 ± 30.2	24.22 ± 5.6	104± 5
3.3	6	84.18±4.6	9.3±1.05	320 ± 30.5	26.94 ± 7.4	103± 6

Values are mean ± SD for 6 rats in each group. n, number of hearts in each group; LVDP, left ventricular developed pressure; CF, coronary flow; HR, heart rate; RPP, rate pressure product; MAP, mean arterial pressure. *$P < 0.05$, compared with control

Table 3: Free radical scavenging activity of DG extract

Extract concentration (ug/mL)	Inhibition (%)			
	DPPH	Nitric oxide	Super-oxide	Hydroxyl radical
1000	84.23± 2.31	81.32± 3.11	86.21± 2.63	81.27± 3.82
500	81.44± 3.45	76.43± 5.23	83.92± 3.51	78.63± 4.62
250	75.64± 2.62	67.23± 3.45	75.33± 3.65	74.41± 4.43
125	70.52± 3.25	70.63± 4.84	62.61± 2.78	65.52± 2.76
62	40.22± 2.83	41.61± 5.28	58.52± 3.51	51.62± 3.52
32	27.51± 3.12	32.53± 4.38	45.41± 2.54	30.61± 2.31
16	3.48± 2.67	17.71± 3.27	34.72± 2.89	21.42± 1.62
10	1.41± 1.48	5.22± 1.32	25.33± 1.12	4.21± 0.52
7	0.02± 0.31	3.11± 0.50	15.62± 1.44	3.34± 1.25
5	0.10± 0.01	1.36± 0.10	5.21± 1.05	1.23± 0.33
Ascorbic acid (100ug)	95.11± 4.22	85.34± 4.11	87.32± 5.87	94.44±4.71
BHT (20ug)	92.27± 3.31	-	-	-
Curcumin	-	91.7±3.11	-	-
IC_{50}	36.0±1.47	39.4± 2.33	55.3 ± 1.29	43.7± 2.43

Values are mean ± S.E.M of 3 replicates. NT: Not tested.

Table 2 shows the hemodynamic changes of rat heart during the experimental procedure. Mean arterial pressure (MAP) and heart rate (HR) in the IR control group remain depressed during ischemia and then early reperfusion stage of the experiment. 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 2). Not many differences were obtained between the 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 drug group (79% basal value) compared with the IR group (38% basal value, $P < 0.05$).

The *in vivo* antioxidant effect of the extract was determined by administering rats with DG orally for about 30 days and then sacrificing the animal for reperfusion induces ischemic injury. Elevated lipid per-oxidation level in ischemia reperfusion control rats were found to be declined in drug treated rat hearts (table 4). Similarly, antioxidant enzymes activity was preserved in drug treated rat hearts. The above observations were substantiated by the results of cardiac marker proteins in tissue homogenate (table 5) and coronary filtrate (fig. 3). Level of cardiac enzymes like CK and LDH were low in the coronary filtrate indicating the preserved myocardial architecture.

DISCUSSION

In the present study, free radical scavenging potential *of Desmodium gangeticum* (DG) chloroform root extract

was monitored through *in vitro* antioxidant model and *in-vivo* ischemia reperfusion model. Oxygen derived free radicals outburst immediately after the reperfusion of ischemic myocardium contribute to post ischemic dysfunction (Maxwell *et al*., 1997). The significant improvement in antioxidant status of the myocardium and the subsequent reduction in cardiac marker enzymes in the coronary perfusate were observed in rat hearts pretreated with DG chloroform root extracts. The above findings were complimented with the results of in-vitro antioxidant model.

The maximum inhibitory concentration (IC_{50}) in different antioxidant *in vitro* parameters like DPPH, superoxide scavenging activity, hydroxide scavenging activity, nitric oxide scavenging activity and lipid peroxidation were found to be 36.03, 55.3, 43.7, 39.4 and 377ug/ml respectively (table 3). DPPH stable free radical method is an easy, rapid and sensitive way to survey the antioxidant activity of a specific compound or plant extracts (Koleva *et al*., 2002). IC_{50} of the standard compounds, BHT and ascorbic acid were 10.83 mg/ml and 52.57 mg/ml respectively. Similarly hydroxyl radical scavenging activity was measured by studying the competition between deoxyribose and test compounds for hydroxyl radical generated by ferric^{3+} ascorbate-EDTA-H_2O_2 system (Fenton reaction).

Isolated rat heart perfusion model was used to study the *in vivo* antioxidant activity of DG root extracts. Administration of DG extract to rat for 30 days provide cardiac protection against ischemia-reperfusion-induced damages as evidenced by the reduction of infarct size, determined by cardiac marker protein (table 5). In addition to that, the extract mediates the significant recovery of post ischemic cardiac function, including CF, AF, and LVDP (table 2). The improved CK, LDH, AST and ALT levels suggested the efficacy of DG root extract in maintaining the integrity of myocytes. In fact, antioxidant potential possessed by DG (table 4) may be responsible for the prevention of cardiac enzyme leakage into the perfusate of DG pretreated Wistar rats.

ROS play an important role in the etiology of myocardial ischemia reperfusion (I/R) injury (Brown *et al*., 1988). As evidence from the *in-vitro* analysis, DG possesses antioxidant potential and is capable of reducing oxidative stress induced by ischemia reperfusion injury. Upon reperfusion of ischemic myocardium, oxygen interacts with the damaged mitochondrial respiratory chain to

Table 4: Effect of chloroform root extract of DG on TBARS, Catalase, Superoxide dismutase (SOD), Glutathione peroxidase (GPx) in the tissue homogenate of isolated rat heart

Group	TBARS (uM/g wet tissue)	Catalase (uM of H_2O_2 consumed/min/g protein)	SOD ($U^{\#}$/mg protein)		GPx (ug of GSH consumed/ min/g protein)
			Mn SOD	Cu-Zn SOD	
1	6.2 ± 0.3	7623 ± 426	8.1 ± 0.21	50.1 ± 4.2	1842 ± 180
2.1	7.9 ± 0.5*	4060 ± 241*	5.1 ± 0.32*	30.2 ± 3.1*	1231 ± 131*
2.2	7.6± 0.4*	5184 ± 364*	6.0 ± 0.35*	34.3 ± 3.2*	1121 ± 121*
2.3	7.2 ± 0.4*	5218 ± 323*	5.6 ± 0.24*	33.1 ± 3.5*	1220 ± 118*
3.1	5.8 ± 0.5	7864 ± 459	8.0 ± 0.41	50.4 ± 4.0	1842 ± 163
3.2	5.7 ± 0.6	5532 ± 342*	6.7 ± 0.50*	40.0 ± 3.4*	1435 ± 156*
3.3	5.3 ± 0.4	5254 ± 420*	6.6 ± 0.54*	34.4 ± 3.2*	1388 ± 152*

[#]SOD unit: One unit is defined as the enzyme concentration required to inhibit 50% of OD (at 560 nm) produced by chromogen in 1 min. Values are mean ± SD for 6 rats in each group. Significantly differing values (from normal control group) are expressed as (*) P< 0.05

Table 5: Activity of CK, LDH, SGOT &SGPT in the tissue homogenate of isolated rat heart

Group	CK	LDH	SGOT	SGPT
1	^{a}17.4± 1.5	^{a}104.2 ± 8.7	^{a}36.3 ± 3.9	^{a}25.1± 2.1
2.	^{b}8.2± 0.8	^{b}60.4± 4.6	^{b}18.8 ± 1.1	^{b}13.6± 1.2
2.2	^{a}8.1± 2.7	^{a}50.5± 7.2	^{c}17.1± 3.8	^{a}14.3± 2.2
2.3	^{a}7.4± 2.2	^{a}49.1± 7.8	^{a}14.6± 3.1	^{a}12.5± 2.7
3.1	^{a}18.77± 1.1	^{a}103.1± 5.6	^{a}34.6± 2.2	^{a}26.2± 1.5
3.2	^{a}15.88± 2.2	^{d}97.23± 6.3	^{a}39.8± 3.5	^{a}24.8± 2.2
3.3	^{c}15.43± 1.8	^{e}98.6± 4.2	^{a}35.4± 3.1	^{b}25.5± 1.5

Values are mean ± SD for 6 rats in each group. Significantly differ from normal control group are expressed as (*) P< 0.01. Values not sharing a common superscript (a, b, c, d, e,) differ significantly at P<0.05) when compared between the group

produce a burst of ROS leading to I/R injury (Brown *et al*., 1988). Apart from the mitochondrial respiratory chain, the activations of xanthine oxidase, arachidonic pathway, and NADPH oxidase have also been reported to contribute to the generation of ROS during I/R (Kloner *et al*., 1989). In the present study, significant decrease in TBARS, lipid peroxide marker was observed in DG pretreated rat heart. Moreover, depressed antioxidant status of the myocardium during ischemia reperfusion control was recovered by the administration of DG root extract. The protective effects of DG may lie within the ability of this compound to reduce oxyradical-related oxidant processes by either directly interfering with the oxidants, or up-regulating antioxidant systems such as superoxide dismutase or enhancing the catalytic activity of glutathione peroxidase (table 4).

The chemical composition of DG chloroform extract 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. The volatile components of the root extract have not been explored much. However, many studies have shown the significance of volatile compounds like isoflurane, enflurane and halothane in cardioprotection against IR injury (Sato *et al*., 2007). As shown in table 1, GS/MS analysis resulted in the identification of 11 compounds including oleic acid and 4-dodecanol, that possess antioxidant potential. Moreover the presence of hexadecanoic acid (36.42%), reported to has effect on analgesia and anti inflammation (Zhang *et al*., 2005), augmented the cardio-protection mediated by DG chloroform root extract.

In conclusion, our result indicates that DG chloroform root extract can protect the myocardium from oxidative stress mediated by ischemia reperfusion. Further studies are needed to address the selective efficacy and mechanism of action of this agent.

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