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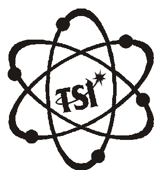

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Antioxidant Potentials Of Roots Of *Desmodium Gangeticum*

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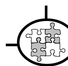

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ABSTRACT

In the present study the aqueous and ethanol extracts of roots of *Desmodium gangeticum* was screened for antioxidant activity against CCl_4 induced hepato toxicity in rats. Hepatotoxicity in rats was induced using carbon tetrachloride: Olive oil (1:1). DG (100 and 250mg/kg, p.o.) significantly increased glutathione s-transferase, glutathione reductase, super oxide dismutase, glutathione peroxidase, catalase and decreased lipid peroxidation levels. Hence *D.gangeticum* can be of enormous use in the treatment and management of hepatic disorders.

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KEYWORDS

Desmodium gangeticum;
Antioxidant activity.

INTRODUCTION

Oxygen free radicalinduced cellular damages have been implicated in many pathobiological conditions, viz. malignancy, ageing process and degenerating diseases, etc[1]. Increased generation of oxidative free radicals (or) impaired antioxidant defense mechanisms, have been implicated in the ageing process, neurodegenerative conditions, including Parkinsonism and Alzheimer's disease, chronic stress induced/perturbed homeostasis, including immuno depression, inflammation, diabetes mellitus, peptic ulcer and other diseased conditions[2]. Major natural defence system comprises oxidative free radical scavenging enzymes such as glutathione S-transferase (GST), glutathione reductase(GRD), gluthione peroxidase (GPX), superoxide dismutase (SOD) and lipid peroxidase (LPO). Deficient functioning of these enzymes leads to accumulation of toxic oxidative free radicals and consequent degenerative changes.

Desmodium gangeticum DC. (Leguminosae) is commonly known as salpan, Salpani in hindi and shalparni in sanskrit. It is abundantly found throughout India and is one of the important plants used in

indigenous system of medicine as bitter tonic, febrifuge, digestive, anticatarrhal, antiemetic, in inflammatory conditions of chest and in various other inflammatory conditions which are due to vata disorder[3]. It is used in ayurvedic preparations like 'Dashmoolarishta' and 'Dashmoolakwaath' for the post-natal care to avoid secondary complications and also in nervous debility[4]. The sterols, N,N-dimethyltryptamine,5-methoxy-N,N-dimethyltryptamine, their oxides and other derivatives have been isolated from aerial parts, three pterocarpenoids, gangetin, gangetinin and desmodin, are the major chemical constituents of the roots[5]. Alkaloid isolated from aerial part comprises indol-3-alkyl-aminesand β-carbolines and has anticholinesterase, smooth muscle stimulant, CNS stimulant response[6]. Gangetin, a pterocarpan, shows anti-fertility activity by affecting alkaline phosphatase activity in uterine fluids[7]. It is reported to possess antiulcer[8], antioxidant[9], cardiotonic[10], anti-inflammatory, anti-nociceptive[11] activities and useful in neurological disorders[12]. *D.gangeticum* was also reported for antiamnesic[13] and antidiabetic[14] activity. In the present study, the ethanolic and aqueous extracts of roots of *D.gangeticum* were assessed for antioxidant activity against CCl_4 indued hepatotoxicity in rats.

MATERIALS AND METHODS

Plant material

The roots of *Desmodium gangeticum* were collected from Robber's cave, Dehradun, Uttaranchal during its flowering season in December and were authenticated at botanical survey of India, Dehradun, India. The voucher specimen has been deposited in the department of pharmacology, Dept. of pharmaceutical sciences, Guru Jambheshwar University of Sciences and Technology, Hisar, Haryana, India.

Preparation of ethanol and aqueous extracts

The freshly collected roots were shade dried and coarsely powdered. The powder was successively extracted with ethanol and distilled water using soxhlet extractor. The aqueous (DAE) and ethanol extracts (DEE) were concentrated under reduced pressure using rotary flash evaporator. A suspension of the extracts was prepared using propylene glycol.

Acute toxicity studies

Ethanol and aqueous extracts of *Desmodium gangeticum* at various doses (50-2000mg/kg,) were administered orally to normal mice. During the first four hours after the drug administration, the animals were observed for gross behavioral changes, if any for 7 days. The parameters such as hyper activity, grooming, convulsions, sedation, hypothermia and mortality were observed and the doses selected were 100 and 250mg/kg.

Chemicals

Reduced glutathione (GSH), 1-chloro-2, 4-dinitrobenzene (SD Fine Chem.Ltd, Mumbai), 5,5-dithiobis-2-nitro benzoic acid, nicotine adenine dinucleotide phosphate, epinephrine (Sigma, USA), ethylene diamine-tetra-acetic acid, hydrogen peroxide (Qualigen Free chemicals, Mumbai), thiobarbituric acid (Rolex Laboratory, Mumbai) and silymarin (Monteloeder, Spain) were used in the present study. All other reagents used were of analytical grade.

Animals

Adult isogenized Wister rats (150-200g) of either sex were used. They were acclimatized to the laboratory conditions for 5 days before studies. The animals had free access to food and water and were provided with alternate light and dark cycles of 12h each. All experiments were carried out during the day time from 0900 to 1400h. The experimental protocol was approved by IAEC (Institutional Animal Ethics Committee of CPCSEA, Dept. of Animal Welfare, Govt. of India)

Experimental design

The adult wistar rats were divided into seven groups of six animals each. The experimental protocol was as follows.

Group I: Represented control group propylene glycol(5 ml/kg) was administered p.o. for 7 days. Group II: Received single dose of equal mixture of carbon tetra chloride and olive oil (50% v/v, 5ml/kg, i.p.) on the 7th day. Group III and IV: DAE (10mg/kg and 250mg/kg) was administered p.o respectively for 7 days. On the 7th day, a single dose of equal mixture

TABLE 1: Antioxidant activity of *Desmodium gangeticum* against CCl_4 induced liver damage

Parameters	Control	CCl_4 treated group	*D.gangeticum* extracts+CCl_4				Silymarin 25 mg/kg+CCl_4
			Aqueous Extract		Ethanolic extract		
			100 mg/kg	250 mg/kg	100 mg/kg	250mg/kg	
GPX (nmol of GSH oxidized/min/mg protin	315±22.8	190.12±1.5^{b,a}	240.2±12.2^{c,d}	272±13.1^{b,c}	292±4.2^{c,e}	304.2±9.8^{b,c}	292 ±17.2^{b,c}
GST (nmol of CDNB conjugate formed/min /mg protin)	293.5±26.2	165.9±16.4^{a,b}	208.2±5.2^{c,f}	242.2±10.9^{b,c}	250±8.9^{b,c}	281.2±12^{b,c}	251.4± 5.1^{b,c}
GRD (nmol of GSSG utilized/min/mg protin)	22.4±1.5	11.2±0.9^{a,b}	15.2±1.2^{c,d}	17.3±0.9^{b,c}	19.2±2.3^{c,e}	22.4 ±0.8^{b,c}	19.42± 0.9^{b,c}
SOD (Kat/g protin)	84.2±4.5	42±2.3^{a,b}	57±3.1^{c,e}	65.9±3.5^{b,c}	70.2±4.9^{b,c}	78.2± 3.5^{b,c}	74± 4.3^{b,c}
CAT (nmol of H_2O_2 decomposed/min/mg protin)	196±6.8	49±4.8^{a,b}	68.4±2.1^{c,f}	142±9.9^{b,c}	150.4±3.7^{c,e}	167± 4.7^{b,c}	153.4± 3.9^{b,c}
LPO (nmol of Mda/mg protin)	4.5±0.9	14.2±1.3^{a,b}	9.3±0.6^{c,e}	7.5±1.5^{b,c}	7.9± 0.9^{c,e}	5.2 ±0.7^{b,c}	6.8 ±s0.8^{b,c}

a-Control compared with CCl_4 treated animals, b-P<0.001, c-CCl4 treated animals compared with DAE and DEE, d-P<0.02, e-P<0.01. f P<0.05

of CCl_4 and olive oil was given(50% v/v, 5ml/kg, i.p.). Group V and VI:DEE(100 and 250mg/kg p.o.) respectively for 7 days. On the 7th day, a single dose of equal mixture of CCl_4 and olive oil was administered (50% v/v, 5ml/kg, i.p.). Group VII:Silymarin (25mg/kg, p.o) was administered for 7 days. On the 7th day, a single dose of equal mixture of CCl_4 and olive oil (50% v/v, 5ml/kg, i.p.) was administered.

Biochemical analysis

All animals were sacrificed by cervical decapitation under light ether anesthesia on the eighth day. Immediately after sacrifice, the livers were dissected out, washed in the ice cold saline and the homogenate was prepared in 0.1M Tris-HCl buffer (pH 7.4). The homogenate was centrifuged and the supernatant was used for the assay of marker enzymes, glutathione S-transferase(GST), glutathione reductase(GRD)[13] and gluthione peroxidase(GPX)[14]. The activities of super oxide dismutase(SOD)[15], catalase(CAT)[16] were also determined. The absorbance was measured in a UV-visible spectrophotometer (Uv-1601; Shimadzu, Kyoto, Japan). Lipid per oxidation level (LPO) was estimated[17]. The total protein content was estimated by biuret method.

Statistical analysis

All results are expressed as mean ± standard error. Data was analyzed by one way ANOVA followed by Dunnet's t-test, P<0.05 were considered as statistically significant.

RESULTS

Glutathione S-transferase (GST)

In the present study, GST levels in livers of CCl_4 treated animals were significantly reduced (p<0.001). GST compared to CCl_4 treated group. Whereas, DAE and DEE (100mg/kg, p.o.) showed less significant activity of GST in liver homogenate (p<0.05 and p<0.01). Silymarin (25mg/kg, i.p.) significantly elevated (p<0.001) GST in the liver homogenates.

Glutathione reductase (GRD)

Significant GRD activity(p<0.001) was observed in CCl_4 treated animals when compared to control. A significant increase(p<0.001) in the GRD levels was exhibited by DEE(250 mg/kg) compared to CCl_4 treated groups. DAE and DEE(100 mg/kg) showed less significant(p<0.02 and p<0.01) increase in the GRD when compared with CCl_4 treated rats. Silymarin exhibited significant(p<0.001) elevation in GRD levels when compared to CCl_4 treated groups.

Glutathione peroxidase

GPX levels in the liver homogenate of CCl_4 treated rats were significantly reduced (p<0.001) compared to control group. Pretreatment with DAE and DEE (250 mg/kg) for seven days profoundly increased the GPX levels (p<0.001) DAE and DEE (100 mg/kg, p.o.) significantly decreased GPX levels. Silymarin treated animals also showed significant (p<0.001) elevations in

GPX levels compared to CCl_4 treated animals.

Super oxide dismutase (SOD)

SOD levels were significantly reduced ($p<0.01$) in CCl_4 treated animals when compared with normal rats. The DAE (250mg/kg) and DEE (250mg/kg) exhibited prominent increase ($p<0.01$) in SOD as compared with CCl_4 treated rats.

Catalase (CAT)

CAT activity was significantly ($p<0.01$) reduced in CCl_4 treated group when compared to control. DAE and DEE at 100mg/kg dose significantly increased ($p<0.01$) CAT in liver homogenate when compared to CCl_4 treated animals. DAE and DEE at 250 mg/kg dose levels showed significant ($p<0.02$ and $p<0.01$) increase of CAT when compared with CCl_4 challenged rats.

Lipid peroxidation

LPO levels of CCl_4 treated rats were significantly high ($p<0.01$) when compared to control rats. Treatment with DAE and DEE at 100mg/kg dose levels showed significant ($p<0.01$) decrease in LPO when compared with CCl_4 treated animals. DAE and DEE at 250mg/kg dose also showed less significant ($p<0.01$) decrease in LPO in liver homogenate when compared with CCl_4 treated animals. The silymarin treated group showed a significant ($p<0.001$) decline in the LPO when compared to CCl_4 treated animals.

DISCUSSION

Free radicals oxidative stress has been implicated in the pathogenesis of variety of diseases, resulting usually from defective natural antioxidants, hence therapy should include either enzymes or agents natural antioxidant enzymes or agents, which are capable of augmenting the function of these oxidative free radical scavenging enzymes[19]. GPX plays pivotal role in H_2O_2 catabolism and detoxification of the endogenous metabolic peroxides and hydroperoxide which catalyses GSH. In the present study, GPX activity was significantly reduced after CCl_4 treatment when compared to control. Many investigations have suggested that GST offers protection against LPO by promoting the conjugation of toxic electrophiles with GSH. GST plays a physiological role initiating the detoxification of potential alkylating agents. Chemicals like chloroform and CCl_4 alter the hepatic GST activity. GST levels were significantly reduced in CCl_4 treated animals and upward reversal was observed after the treatment of DAE and DEE. This may be attributed to a direct action on the hepatic GST activation; however, the mechanism of action needs to be explored. An increase in GRD activity suggests that DAE and DEE protect the liver tissue from oxidative damage by GSH regenerated from its oxidized from (GSSG). In the present study, the SOD activity was significantly reduced CCl_4 intoxicated rats. The SOD activity was brought to near normal after treatments with the extracts in CCl_4 intoxicated rats. Decreased activity of a CAT was observed in animals treated with CCl_4 presumably a decrease in CAT activity could be attributed to the cross linking and inactivation of the enzyme protein in the lipid peroxides. Decreased CAT activity is linked upto exhaustion of the enzyme as a result of oxidative stress caused by CCl_4. The levels of thiobarbituric acid relative substance (TBARS) is an indirect measurement of lipid preoxidation. Lipid peroxide levels in tissues were found to be significantly elevated in CCl_4 challenged rats. These free radicals trigger cell damage through a mechanism namely covalent binding to cellular macromolecules and lipid preoxidation which affects the ions permeability of the membrane preventing the disintegration and solubilization of membrane structures. The free radical reactions are implicated in the progression of cancer, inflammation, atherosclerosis, hepatocellular damage and biological process of aging. The hepatoprotective action combined with antioxidant activity has a synergistic action to prevent the processes of initiation and progress of hepatocellular diseases. Hence *D.gangeticum* roots can be safely employed for treatment and management of hepatic disorders. However, further investigations using more experimental paradigms are warranted for the confirmation of liver protective potential of *D.gangeticum.*

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