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ORIGINAL RESEARCH ARTICLE

Effect of Antioxidant Activity of Ethanol Extract of Leaf of *Cajanus indicus* Spreng as Hepatoprotective Agent: A Novel Role of Free Radical Scavenging Action

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ABSTRACT

Phytochemicals are present in various plants and herbs, are now becoming important candidates for development of drugs. Wide range of medicinal plants (plants from which potential phytochemicals are isolated for development of drugs for treatment of diseases) present in South Asian countries have now been increasingly utilized for development of phytomedicines. Treatment with ethanol extract of leaf of *Cajanus indicus* Spreng at a dose of 50 mg/kg body weight for 20 days, after induction of hepatotoxic damage by carbon tetrachloride (CCl_4), produce significant elevation of the hepatic injury. The liver marker enzymes like AST (aspartate transaminase), GGT (gamma glutamyl transferase), ALT (alanine transferase) and ALP (alkaline phosphatase) decreased significantly at the above dose showing the optimum effect against hepatic damage. The liver antioxidant enzymes: superoxide dismutase (SOD), catalase, glutathione peroxidase, glutathione reductase and glutathione transferase and the membrane damaging indicators TBARS (thiobarbituric acid reactive species), conjugated diene and marker of glutathione status indicate the mechanism of healing action to be due to scavenging of free radicals or reactive oxygen species (ROS). The results thus gives a confirmatory proof that the healing action of ethanol extract of leaf of *Cajanus indicus* Spreng is for shifting of equilibrium from the peroxidant to antioxidant side and the leaf acts as a natural antioxidant and healer of CCl_4 induced hepatotoxicity.

Key words: *Cajanus indicus* Spreng; CCl_4; Hepatotoxicity, Antioxidant.

INTRODUCTION

The leaf of *Cajanus indicus* Spreng (Leguminosae family) is commonly known as *Arhar pata* in Bengali is widely grown plant in large parts of India and South-West Asian countries, particularly in the regions of subtropical humid climate. In the Indian traditional system of medicine, extract of leaf of *Cajanus indicus* Spreng has been reported to have prominent healing effects against trauma, significant hepatoprotective and anti-inflammatory activity [1-3]. However, no systematic scientific investigations have been done to understand the biochemical mechanism of action. As the extract of the leaf has been found to give relief against liver diseases particularly; hepatitis A while the liver injury has been proven to be due to damage by free radicals or reactive oxygen species (ROS) [4, 5]. Hence the present study was undertaken to evaluate the curative effect of leaf extract against CCl_4 induced hepatic injury and to elucidate any relation with its antioxidant activity.

MATERIALS AND METHODS

Plant material

Leaves of *Cajanus indicus* Spreng were collected from the local market in Kolkata in the months of November –December. Authenticity was established by experts of Botanical Survey of India, Shibpur, Howrah, West Bengal. The leaves were thoroughly washed in water and then soaked in 900 ml of 95% ethanol for 7 days with intermittent shaking. On the 8th day, the whole material was filtered through nylon mesh. The filtrate was collected and concentrated under reduced pressure. The residual solvent was removed under vacuum in a rotary evaporator and the solid brownish red mass obtained (8.7% w/w) was kept in a vacuum desiccators at 4^{0}C until used for further studies.

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Animals and treatment

Healthy, pathogen free, colony bred, adult male Charles Foster rats (150-200g) were used in this experiments. The animals were housed in environmentally controlled rooms (25 ± 1^0C) with 12hr light/dark schedule and fed with pellet food (Hindustan Lever, India) *ad libitum* and had free access to water. All the chemicals used were of analytical grade. Chemicals were purchased either from Sigma Chemicals Co. (St Louis, MO, USA) or E-Merck (Germany) and SRL (India).

Animals were divided into following 3 groups of 6 animals each.

Group 1: Controls, which received subcutaneously liquid paraffin (lp) twice a week at the dose of 3 ml/ kg body weight.

Group II: The animals in which liver damage was indicated by subcutaneous administration of CCl_4 (0.1 ml/100 g body weight) + lp twice a week.

Group III: Animals were the same as in Group II above. Additionally, they received the ethanol extract daily at the dose of 50 mg/Kg body weight as suspension in 1 ml water orally.

The animals were kept for 20 days. Animals were fasted overnight on the 19th day and were sacrificed on day 20th by decapitation. Blood was collected from incision of jugular vein and serum was prepared from the collected blood. The liver was dissected out, rinsed in phosphate saline buffer (pH 7.4) and immediately proceeded for biochemical estimations.

The measurement of thiobarbituric acid reactive substances (TBARS) was done as an index of lipid peroxide (LPO), conjugated diene (CD) content was found out by the method of Klein [6]. The activity of superoxide dismutase (SOD), one of the most prominent antioxidant enzymes in the eukaryotic defense machinery against ROS damage was determined by the method of McCord and Fridovich [7]. The assay procedure involved the inhibition of epinephrine auto oxidation in an alkaline medium (pH 10.2).

The enzyme activity was expressed in arbitrary units considering 50% inhibition in the reaction mixture under the experimental condition as one unit of SOD. Catalase activity was determined according to the method of Luck *et al* [8]. Reduced glutathione (GSH) or the total sulphhydryl group was measured according to the method of Ellman [9] with minor modifications. Glutathione peroxidase (GPX) was assayed by the method of Rotruck *et al* [10]. Glutathione transferase (GTS) and glutathione reductase (GRD) were assayed according to the methods of Habig *et al* [11] and Racker [12] respectively.

Statistical analysis

Statistical analysis was carried out using Analysis of Variance (ANOVA) test followed by using Student 't'- tests to estimate the level of significance among the mean ± SE values in different groups of animals.

RESULTS AND DISCUSSION

The study reveals the hepatoprotective action of the medicinal plant and that phytochemicals present in the plant are responsible for the above action, which can well be utilized for development of antihepatotoxic drug. The action of the phytochemicals is due to the antioxidant action, as now free radicals (species with unpaired electrons produced by various biochemical reactions within the body) have been found to be the underlying cause of various diseases from hepatic injury, gastric ulcer, heart diseases and immunological reactions. The ethanol extract of leaf of *Cajanus indicus* Spreng exhibited significant protection against CCl_4-induced hepatic damage, as is evident from the various biochemical parameters related to the hepatic conditions in eukaryotic system.

Treatment with CCl_4-induced significant damage and the effect of CCl_4 has now been established to be due to the reactive oxygen species (ROS) as it is evident from the decrease in the level of the antioxidant enzymes. The level of the liver marker enzymes decreased within the range of 25-100 mg/kg body weight in case of treated animals as compared to untreated control.

The prooxidant antioxidant balance is essential in free radical mediated injuries in eukaryotes is now being emerged as a vital alternative [13,14,15] to the long termed and established medical theories. Oxygen toxicity and resultant generation of most aggressive reactive oxygen species (ROS) result in consequent tissue damage and necrosis. CCl_4 mediated hepatotoxic injury, have taken as a model for liver injury as established by the mechanism of CCl_4 accumulated in hepatic parenchymal cells and metabolically activated by cytochrome P-450 dependent mono oxygenases to form a trichloromethyl free radical (CCl_3). Thus, alkylating cellular proteins and other related biological macromolecules, with a simultaneous attack on polyunsaturated fatty acids and with a consequent formation of lipid peroxides leading to hepatic damage.

The study reveals that leaf of *Cajanus indicus* Spreng at a dose of 50 mg/kg body weight maximally protects hepatic damage caused by CCl_4 and the mechanism is believed to be due to free radical scavenging one. The results involving AST, ALT, ALP and GGT confirm the proposal of protective mechanism. The SOD, CAT, GPX and GRD, which maintains the intricate balance of prooxidant antioxidant ratio, are increased showing the utility of balance towards antioxidant side is a major healing indication. Elevated level of TBARS and CD observed in CCl_4-treated animals indicates excessive formation of free radicals and activation of LPO system resulting in hepatic damage. TBARS produced as byproducts of LPO that occurs in hydrophobic core of biomembranes. The significant decline in these concentrations of these constituents in the liver tissue by extract of leaf of *Cajanus indicus* Spreng administration rats indicates anti-lipid peroxidative effect of the leaf extract. GSH is a major non-protein thiol in living organisms, which plays a central role in coordinating the body's antioxidant defense processes. Perturbation of GSH status of a biological system has been reported to lead to serious consequences. Decline in GSH content in the liver of CCl_4-intoxicated rats, and its subsequent return towards near normalcy in extract of leaf of *Cajanus indicus* Spreng treated rats reveal antioxidant effect of the leaf extract.

Explanations of the possible mechanism underlying the hepatoprotective properties of drugs include the prevention of GSH depletion and destruction of free radicals. GTS also plays an essential role in liver by eliminating toxic compounds by conjugating them with glutathione. GRD is concerned with the maintenance of cellular level of GSH (especially in the reduced state) by effecting fast reduction of oxidized glutathione to reduced glutathione. Thus it can be concluded that the extract of leaf of *Cajanus indicus* Spreng has a possible healing action on hepatic tissue and the mechanism is by free radicals scavenging pathway of the plant extract.

Table 1: Effects of ethanol extract of leaf of *Cajanus indicus* Spreng on the antioxidant status

Parameters	Group I	Group II	Group III
Thiobarbituric acid reactive species [μM /mg protein]	1.36±0.04	0.76±0.04	1.14±0.26
Diene Conjugate (μM /100g tissue)	0.28±0.05	0.84±0.08	0.33±0.09
Reduced glutathione (μM /100g tissue)	366.2±19.83	206.1±5.57	324.0±34.94

Values are mean ±SEM of 6 animals in each group.
P< 0.01as compared to groups.

Table 2: Effect of ethanol extract of leaf of *Cajanus indicus* Spreng on activity of ROS scavenging enzymes

Parameters	Group I	Group II	Group III
Superoxide dismutase (U/mg protein)	15.98±1.59	7.64±0.94	11.55±0.77
Catalase (U/mg protein)	7.56±0.78	4.40±0.85	7.08±0.88
Glutathione Peroxidase (U/mg protein)	0.85±0.09	0.50±0.01	0.73±0.05
Glutathione Reductase (U/mg protein)	6.71±0.39	3.02±0.15	6.01±0.36
Glutathione transferase (μM/mg protein)	8.50±0.34	16.05±1.04	8.63±0.48

Values are mean ±SEM of 6 animals in each group.
P< 0.01as compared to groups.

Table 3: Percentage of hepatoprotection offered byethanol extract of leaf of *Cajanus indicus* spreng in respect of liver marker enzymes

Tratmnt (mg/kg body weight)	Percentage of relative decrease of enzyme markers AST	GGT	ALT	ALP
25	20.76±1.6	17.90±1.20	20.21±1.17	15.73±1.48
50	9.82±0.87	8.10±0.62	8.83±1.06	7.48±0.74
75	27.07±3.3	18.37±1.36	15.57±1.97	18.28±1.12
100	69.50±5.4	62.84±2.27	74.15±1.77	66.78±3.03

Values are mean ±SEM of 6 animals in each group.
P< 0.01as compared to groups.

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