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Research Article

Effect of Ethanol Extract of Leaf of *Piper betle* Linn in Carbon Tetrachloride Induced Hepatopathy in Rates in Relation to Free Radical Scavenging Action

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ABSTRACT

Phytochemicals, that is, chemicals present in various plants and herbs , are now becoming important candidates for development of drugs.Wide range of medicinal plants {Plants from which potential photochemicals 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 Piper betle Linn at a dose of 150 mg /kg body weight for 20 days, after induction of hepatotoxic damage by CCl_4, produce significant elevation of the hepatic injury. The liver marker enzymes like (Aspartate Transaminate) AST, GGT (Gamma Glutamyl Transferase), ALT (Alanine Transaminase) and ALP (Alkaline Phosphatase) decreased significantly at the above dose showing the optimum effect against hepatic damage. The liver antioxidant enzymes SOD, catalase, glutathione peroxidase, glutathione reductase and glutathione transferase and the membrane damaging indicators TBARS (Thiobarbituric Acis Reactive Species), conjugate diene and marker of glutathione status indicate the mechanism of healing action to be due to scavenging of free radicals or 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.

INTRODUCTION:

The leaf of *Piper betle Linn* which is commonly known as *Tambul or paan* in Bengali is a 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 *Piper betle Linn* has been reported to have prominent healing effects against trauma, and significant hepatoprotective and anti-inflammatory activity[1] .

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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)[2-4] 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 *Piper betle Linn* 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 , Hawrah. 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 desiccator at 4^0C until used for further studies.

Animal 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^{0}C) with 12hr: 12 hr light: dark schedule and fed with pellet food (Hindustan Lever, India) ad *libitum* and had free access to water. All 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. Additonally they received the ethanol extract daily at the dose of 150 mg/Kg body weight as suspension in 1 ml water orally.

The animals were kept for 20 days. Animals were fasted over night on the 19th day and were sacrificed on day 20 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 preceded 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 by the methods of Habig *et al* [11]and Racker[12].

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:

Table 1: Effects of Ethanol Extract of Leaf of *Piper Betle* Linn on the Antioxidant Status.

Parameters	Group I	Group II	Group III
Thiobarbituric acid reactive species [mM /mg protein],	1.34 ±0.04	0.86 ±0.04 ,	1.24 ±0.26
Diene Conjugate (mM /100g tissue),.	0.30 ±0.05,	74 ±0.08,	0.43 ±0.09
Reduced glutathione (mM /100g tissue),	356.2 ±19.83,	196.1 ± 17.24 ±5.5 7	318.0 ±34.2 94

Values are mean ± SEM of 6 animals in each group. **P<0.001as compared to group I

*P< 0.01as compared to group.

Table 2: Effect of Ethanol Extract of Leaf of *Piper Betle* Linn on Activity of ROS (Reactive Oxygen Species) Scavenging Enzymes

Parameters	I	2	3
Superoxide dismutase (U/mg protein)	16.98 ±1.5 9	6.64 ±0.94	10.55 ±0.77
Catalase (U/mg protein)	8.56 ±0.78	3.40 ±0.85	7.28 ±0.88
Glutathione Peroxidase (U/mg protein)	1.85 ±0.09	0.80 ±0.01	1.23 ±0.05
Glutathione Reductase (U/mg protein)	5.71 ±0.39	2.02 ±0.15	4.01 ±0.36
Glutathione transferase (mM/mg protein)	9.50 ±0.34	15.05 ±1.04	11.63 ±0.48

Values are mean ± SEM of 6 animals in each group. *P< 0.01as compared to group **P<0.001as compared to group I

Table 3: Percentage of Hepatoprotection Offered by Ethanol Extract of Leaf of *Cajanus Indicus* Spreng in Respect of Liver Marker Enzymes.

Treatment (mg/ Kg Body weight)	Percentage of relative decrease of enzyme markers			
	AST (Aspartate Transaminase)	GGT (Gamma Glutamyl Transferase)	ALT (Alanine Transferase)	ALP (Alkaline Phosphatase)

Values are mean ± SEM of 6 animals in each group.

*P< 0.01as compared to group

**P<0.001as compared to group I

The study reveals the hepatoprotective action of ethanolic extract of leaf of the medicinal plant Piper betle Linn 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 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 ethanolic extract of leaf of *Piper betle Linn* 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 hepatic damage and the effect of CCl_4 is now established to be due to the reactive oxygen species (ROS) as is evident from the decrease in level of the antioxidant enzymes.The level of the liver marker enzymes decreased within the range of 125-200 mg/Kg body weight in case of treated animals as compared to untreated control.

That 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 been taken as a model for liver injury is 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 *Piper betle Linn* at a dose of 150 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 aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and gamma glutamyl transpeptidase (GGT) confirms the proposal of protective mechanism.[16] 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[17]. 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 of extract of leaf of *Piper betle* Linn 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 co-ordinating the body's antioxidant defense processes[18]. Perturbation of GSH status of a biological system has been reported to lead to serious consequences[19,20,21]. Decline in GSH content in the liver of CCl_4-intoxicated rats, and its subsequent return towards near normalcy in extract of leaf of *Piper betle* Linn 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[22,23] also plays an essential role in liver by eliminating toxic compounds by conjugating them with glutathione. GRD is concerned with the maintainance of cellular level of GSH (especially in the reduced state) by effecting fast reduction of oxidized glutathione to reduced glutathione[24,25].Free radicals had always been the underlying cause of increase of both chronic and acute diseases[26,27,28,29] and phytochemicals with free radical scavenging ingredients are likely to act as good drugs without major side effects. Thus it can be concluded that a possible healing action of extract of leaf of *Piper betle* Linn and the mechanism is

by free radicals scavenging pathway of the plant extract.

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