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Research Article

Study the Effect of Phytochemical Constituents of *Piper betel* Leaves Extracts on Liver Disorders by *in vivo Model*

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ABSTRACT

An *in vivo* study was conducted to evaluate the hepatoprotective activity of ethanolic extracts of *Piper betel* leaves. Liver damage was induced in male Wistar rats weighed 150-200 gm by administering CCl_4 (2ml/kg i.p) regularly for 10 days and tissue damage was noted by various biochemical parameters like serum glutamate oxaloacetate transaminase (SGOT), serum glutamate Pyruvate transaminase (SGPT), Alkaline Phosphatase (ALP), Acid Phosphatase (ACP), Lipid peroxidation, Level of antioxidant enzyme like catalase (CAT), Superoxide dismutase (SOD), Glutathione (GSH) in liver. Ethanolic extract of *piper betel* 100 mg/kg and 200 mg/kg i.p administered regularly for 10 days and compared with a control group silymarin (25 mg/kg i.p.). A significant reduction in serum SGOT, SGPT, ALP, ACP, lipid peroxidation and improvement in CAT, SOD, GSH, MDA level in the liver were observed when compared with CCl_4 administered rats. *Piper betel* leaves extract (PBLE) was able to protect the liver against the oxidative damage produced by CCl_4.

Key words: Phytochemical, *Piper betel*, hepatotoxicity, biochemical, antioxidant

INTRODUCTION

Plant sources play an important role in modern and traditional system of medicine. The noteworthy contribution made by plant medicines to human health has lead to increased popular, official and commercial interest. *Piper betel* plant is one among the great herbals used in folk and tribal medicines in India. It belongs to the family Piperaceae used as ingredients in a chew commonly known as pan. Chemically *piper betel* has light yellow aromatic essential oil with sharp burning taste. Aromatic odor containing chavi betel, chavicol, eugenol, cadinene, alkaloids, sugar, tannin, diastase, betel oil, terpene, sesquiterpene. Biologically *Piper betel* is aromatic, stimulant, carminative, astringent and antiseptic [1,2]. Leaf possesses activities like antifungal, hypotensive, respiratory depressant, anthelmintic, cardiotonic [3], antiplatelet [4], antifertility [5], antitumour [6], antiulcer [7] and antibacterial [8].

Liver plays a central role in metabolizing a large number of organic, inorganic chemicals and drugs [9]. The greater susceptibility of the liver to damage by chemical agents appears to be a consequence of its primary role in the metabolism and deposition of foreign substances. The diverse aspects include the nature of the hepatotoxic agents, the character of the injury, the mechanism of the hepatotoxic effects, circumstances of exposure and medico-social importance. CCl_4 is one of the most commonly used hepatotoxin in the experimental study of liver disease. The lipid peroxidative degeneration of biomembranes is one of the major cause hepatotoxicity of CCl_4. The increase in the levels of serum bilirubin reflects the depth of jaundice and the increase in serum transaminases (SGOT and SGPT). Acid and alkaline Phosphatase was a clear indication of cellular leakage and loss of functional integrity of cell membrane [10, 11]. An attempt has been made in the present work to develop a new hepatoprotective drug from the natural plant source.

MATERIALS AND METHODS

Plant materials

Piper betel leaves (Local name - Karpoora vettillai) was purchased in the local market, Coimbatore. The herbarium (Herbarium No.BSI/SC/5/21/04-05/Tech-1374) of this plant was identified and authenticated by the taxonomist, Botanical Survey of India, TNAU, Coimbatore, India.

Preparation of plant material

Fresh leaves were collected and air-dried in shade at room temperature. Dried leaves were powdered mechanically through mesh sieve. 100 g of freshly powdered leaves were evenly packed in soxhlet apparatus and the extraction

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was done with 70% alcohol. Then solvent was evaporated at low temperature under reduced pressure.

Drugs and Chemicals

Carbon Tetrachloride was purchased from E. Merck, Mumbai, Thiobarbituric acid, 5, 5, 1-dithiobis-2 nitrobenzoic acid (DTNB), Glutathione and Silymarin, were purchased from Sigma Chemical Company, USA. All other chemicals used in this study were of analytical grade.

Experimental animals

Male Wistar rats weighing between 150 – 200 gm were procured from the Department of Animal house, SASTRA Deemed University, Thanjavur, India. The animals were housed in metallic cages maintained in a controlled room temperature (28 ± 2ºC; RH: 45–55%) with 12 hours light – dark day cycle and fed with commercial rat feed pellets and given drinking water *ad libitum*. The experimental study was conducted after obtaining ethical committee approval from the Institutional Animal Ethical Committee bearing the Ethical Committee number 817/04/ac/cpcsea.

Selection of dose

CCl_4 (2 ml/kg 50% in olive oil) was administered to animals for the induction of hepatotoxicity. For the hepatoprotective studies, the amount of dose administered was adjusted on the basis of observation during the toxicity studies. *Piper betel* extracts in two doses 100 mg/kg and 200 mg/kg with the reference drug silymarin (25.0 mg/kg i.p)were administered.

Experimental design

After the hepatotoxicity induction, the rats were randomly divided in to five groups of six rats in each. Drug dose administered to different groups were listed (Table 1). The entire group was administered with appropriate dose once daily up to 10 days. Acute toxicity was noted on the basis of normal behavioral pattern and mortality. All the animals were sacrificed on 11th day, blood was drawn from the cardiac puncture and serum was collected for biochemical estimations.

Biochemical estimations

Serum glutamate pyruvate transaminase (SGPT) and Serum glutamate oxaloacetate transaminase (SGOT) was estimated by the method of Reitman *et al* [12]. Measurement of tissue lipid peroxidation levels performed by the method of Draper *et al* [13]. Assay of superoxide dismutase (SOD) was done by the method of Beavchamp *et al* [14]. Assay of glutathione peroxidases (GSH) was performed by the method of Flohe *et al* [15]. Assay of catalase (CAT) was performed by the method of Aebi *et al* [16]. Alkaline and Acid Phosphatase was estimated by the method as mentioned in the committee on enzymes of the Scandinavian society [17].

Statistical analysis

Statistical analysis was done by one-way analysis of variance (ANOVA) followed by Student's't' test. Results are expressed as means ± SD from six rats in each group. P values <0.05 were considered significant [18].

RESULTS

Phytochemical study

All extracts subjected for phytochemical study presence of alkaloids, carbohydrates, proteins, amino phenolic compounds, glycosides and flavonoids.

Acute toxicity studies

Ethanolic extract of *Piper betel* leaves evaluated for its hepatoprotective effect on hepatocellular damaged CCl_4 intoxicated rats studies showed no acute toxicity of the plant drug and the animals were safe up to a maximum dose of 2000 mg/kg body weight. There were no changes in normal behavioral pattern, no signs and symptoms of toxicity and no mortality were observed.

Effects of extracts on SGOT, SGPT, ACP and ALP

There was a significant ($P< 0.05$) increase in serum hepatic enzyme levels like SGOT, SGPT, ALP and ACP after CCl_4 treatment when compared to control group. The toxic effect of CCl_4 was significantly ($P<0.05$) prevented in the animals treated with PBLE by the way of restoration of levels in the liver function biochemistry similar to that of the standard drug silymarin (Table 2). Among the extract treated groups significant hepatoprotective activity was highly observed in those treated with a dose of 200 mg/kg. On the comparison to control group, malondialdehyde level was found to be elevated after the administration of CCl_4. This was significantly ($P<0.05$) prevented by PBLE treated groups and these levels were similar to the standard drug silymarin (Table 2).

Effect of PBLE on liver SOD, GSH, CAT and MDA

The activity of the antioxidant enzymes viz. SOD, GSH and CAT were measured in the liver homogenates. Significant ($P<0.05$) decrease in the antioxidant enzyme levels were observed in the CCl_4 treated group which was significantly prevented with the treatment of PBLE and silymarin (Table 3).

DISCUSSION

CCl_4 is one of the most commonly used hepatotoxin in the experimental study of liver disease, which shows lipid peroxidative degeneration of biomembranes and leads to hepatotoxicity [19]. Administration of PBLE showed significant

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Table 1. Descriptions of Study model for dose administration in rats

Group	Dose administration in animals	Type
Group I	(Normal saline 2ml/kg i.p.)	Control group
Group II	CCl_4 (2ml/kg)	Induced hepatotoxicity
Group III	CCl_4 (2ml/kg) and 100mg/kg PBLE	activity
Group IV	CCl_4 (2ml/kg) and 200mg/kg PBLE	activity
Group V	CCl_4 (2ml/kg) and Silymarin 25mg/kg i.p.	Standard

n = 6 in each group

Table 2. Effect of PBLE on various biochemical parameters in experimental animals

Treatment	SGOT (U/L)	SGPT (U/L)	ALP (U/L)	ACP (U/L)
Control (NaCl)	121 ± 15.3	29 ± 1.8	123.6 (17.3)	165.4 (17.3)
CCl_4 - Olive oil (3ml/kg)	383 ± 18.2	82 ± 3.3	404.9 (19.8)	291.3 (19.3)
Silymarin (25 mg/kg)	191 ± 13.1*	43 ± 3.8*	131.6* (16.5)	183.5* (17.1)
PBLE (100 mg/kg)	203 ± 19.3*	47 ± 2.2*	158.7* (13.2)	206.3* (16.3)
PBLE (200 mg/kg)	188 ± 12.3*	38 ± 2.7*	135.6* (11.4)	191.4* (11.6)

Data are expressed in mean ± S.E., n = 6 in each group,*P < 0.001 vs CCl_4 – treated rats by student's 't' test.

Table 3. Effect of PBLE on liver SOD, GSH, CAT and MDA in experimental animals

Treatment	Enzyme activity (units mg/liver protein)			TBARS
	Superoxide dismutase	Glutathione peroxidase	Catalase	
Control (NaCl)	77.91 ± 6.21	0.96 ± 0.030	297.07 ± 1.81	1.27 ± 0.38
CCl_4-olive oil (3 ml/kg)	51.57 ± 4.50	0.70 ± 0.041	177.83 ± 6.51	1.79 ± 0.14
Silymarin (25 mg/kg)	69.13 ± 2.51*	0.84 ± 0.047*	258.17 ± 6.31*	1.23 ± 0.13*
Plant extract (100 mg/kg)	68.10 ± 3.73*	0.91 ± 0.032*	251.73 ± 4.91*	1.24 ± 0.11*
Plant extract (200 mg/kg)	72.53 ± 3.93*	0.97 ± 0.068*	264.28 ± 4.61*	1.21 ± 0.08*

Specific activities of enzymes and levels of thiobarbituric acid-reactive substances (TBARS) in the liver are expressed as the mean ± SD, n=6 in each group.

reduction in the serum enzyme levels, which was comparable with the standard drug silymarin. The effect was more distinct with PBLE at a dose of 200 mg/kg. In the study, elevation in the level of end products of lipid peroxidation (MDA) in CCl_4 treated animals was observed. The increase in MDA level in liver suggests enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanisms [20]. Treatment with PBLE significantly prevented these changes. Hence, the mechanism of hepatoprotection of *Piper betel* leaves may be due to its antioxidant effect. Since PBLE has significantly increased the level of SOD, GSH and CAT contents of the liver. The antioxidant enzyme levels of the CCl_4 treated group were decreased whereas that of PBLE treated are almost similar to the control and silymarin treated groups.

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