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Hepatoprotective Potential of Ethanolic Leaf Extract of Plant *Piper attenuatum B. Ham and Caesalpinia crista Linn*

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Abstract

Piper attenuatum B. Ham and *Caesalpinia crista Linn* are traditional medicinal plants in India. It has been claimed in traditional Indian system of medicine that the phytochemical constituents present in both plants having hepatoprotective activity. So, the aim of the present study was to evaluate hepatoprotective potential of ethanolic leaf extract of plant *Piper attenuatum B. Ham* and *Caesalpinia crista Linn*. Hepatoprotective potential of ethanolic leaf extract of *Piper attenuatum B. Ham and Caesalpinia crista Linn* were evaluated by using D-Galactosamine (400 mg/kg) induced liver toxicity model in rats. 100 mg/kg and 200 mg/kg dose selected for evaluation of hepatoprotective potential for both plants. Various biochemical parameters like AST/SGOT, ALT/SGPT, ALP, Total protein and Serum bilirubin were analyzed for different groups. Liver histopathology was also carried out for various groups. Silymarin (50 mg/kg) used as standard drug. Both the plant ethanolic extracts showed significant hepatoprotective potential. *Piper attenuatum B. Ham* ethanolic extract 200 mg/kg showed best hepatoprotective activity among all other groups. From the above study it may be possible that Piperine and flavonoids present in ethanolic leaf extracts of *Piper attenuatum B. Ham and Caesalpinia crista Linn* respectively responsible for hepatoprotective potential.

Keywords: *Piper attenuatum (B. Ham)*; *Caesalpinia crista Linn*; Hepatoprotective activity; D-Galactosamine; Silymarin

Introduction

There are many traditional systems of medicine in the world each with different associated philosophies some of these like Tibetan traditional medicine remain localized in the country; while others like Ayurvedic and Chinese traditional medicines are continuously used in many different areas of the world [1]. Medicines that derived from various plants have been the first line of defense in maintaining health and combating diseases [2]. Liver is one of the largest organs in the human body that regulates metabolism, secretion, storage, and detoxification in our body in which the hepatic damage is often linked with alterations of these functions [3]. Most hepatotoxic chemicals damage liver cells mainly by inducing Lipid Peroxidation (LPO) or by oxidative damage [4]. Hepatotoxicity is a common disease which leads to serious consequences ranging from metabolic disorders to even death [5]. In this respect, different toxic agents may induce hepatic injury. D-Galactosamine is a well-known hepatotoxic agent which induces liver injury with close resemblance to human viral hepatitis showing necrosis, inflammation, and regeneration [6]. D-Galactosamine is mostly associated with the reduction of uridine pools that are linked to inadequate ribonucleic acid and protein synthesis, thus changing hepatocellular function [7]. Subsequently, organelle damage and necrosis of hepatocytes take place. Along this line, research findings indicated that D-Galactosamine directly triggers mast cells to release histamine and Kupffer cells to release tumor necrosis factor-alpha, which potentiates cell death in numerous ways, including elevation of oxidative stress and inflammatory procedure [8]. Modern lifestyle has isolated us from the natural way of life. Since we have ignoring the rich natural heritage inherited from our ancestors this ignorance has aggravated health issues. Many herbs are there which are of importance some of them are *Piper attenuatum B. Ham*, *Caesalpinia crista Linn* and many more.

Branches of *Piper attenuatum B. Ham* are flexuous and glabrous. Leaves are 6.3 to 15 cm, often as broad as long, from finely downy to glabrous beneath, membranous, orbicular-ovate or cordate, abruptly acuminate, upper more ovate glabrous or puberulous beneath, 7-nerved from the base; base usually equally rounded, truncate or cordate, of the upper usually acute; nerves are slender; petiole 2.5-7.5 cm., rarely shorter [9]. Various phytoconstituents reported in *Piper attenuatum B. Ham* are alkaloids like Cepharadione A, Cepharadione B, Cepharanone B, Guineensine, Norcepharadione B, Piperadione, Piperine, Piperlonguminine, Piperolactam A lignan like galbelgin, neolignans like Kadsurin A, Kadsurin B terpenes like β Bisabolene steroids like Sitosterol and miscellaneous compounds like Crotepoxide. Pipoxide chlorohydrin, Tetratriacontanoic acid [10]. There are more than 500 species of genus *Caesalpinia* (*Caesalpiniaceae*), many of them are not have been investigated yet for potential pharmacological activity [11].

Caesalpinia crista Linn plant is an extensive climber; branches finely grey-downy, armed with hooked and straight hard yellow prickles. Leaves 36-60 cm long; petioles prickly; stipules a pair of reduced pinnae at the base of leaf each furnished with a long mucronate point; pinnae 6-8 pairs, 5-7.5 cm. long, with a pair of

hook stipulary spines at the base. Leaflets 6-9 pairs, 2-3.8 by 1.3-2.2 cm., membranous, elliptic-oblong, obtuse, strongly mucronate, glabrous above, more or less puberulous beneath; petiolules very short; stipels of short hooked spines. Flowers in dense (usually spicate) long-peduncled terminal and supraaxillary racemes dense at the top, lax downwards, 15-25 cm. long; pedicles very short in bud, elongating to 5 mm. in flower and 8 mm. in fruit, brown-downy; bracts squarrose, linear, acute, reaching 1 cm. long, fulvous-hairy. Calyx 6-8 mm. long, fulvous-hairy; lobes obviate-oblong, obtuse. Petals oblancolate, yellow. Filaments declinate, flattened at the base, clothed with long white silky hairs. Pods shortly stalked oblong, 5-7.5 by 4.5 cm, densely armed on the faces with wiry prickles. Seeds 1-2, oblong, lead-colored, 1.3 cm long [12]. *Caesalpinia crista Linn* reported to contain flavonoids, tannins, alkaloids, triterpenoids and various proteins [13].

There is no scientific information on hepatoprotective potential of ethanolic leaf extract of the plant *Piper attenuatum B. Ham* and *Caesalpinia crista Linn*. The aim of present study is to evaluate scientifically hepatoprotective potential of ethanolic leaf extract of the plant *Piper attenuatum B. Ham* and *Caesalpinia crista Linn* to justify the traditional use of this plant.

Materials and Methods

Plant material

Fresh leaves of *Plant Piper attenuatum B. Ham and Caesalpinia crista Linn* were procured from Jawaharlal Nehru Tropical Botanic Garden and Research Institute (JNTBGRI) Palode, Thiruvananthapuram, Kerala, India. The voucher specimens were submitted and preserved as Institute Herbarium Nos: APC/2016/PCOG/9-10. Both the Plants were authenticated by Dr. Mathew Dan, Senior Scientist, Plant Genetic Resource division, JNTBGRI, Palode, Thiruvananthapuram, Kerala, India.

Animals

Healthy Wistar albino male rats weighing between 150-200 gm were taken for the study. They were housed under controlled conditions of temperature (22 ± 30ºC), the relative humidity should be at least 30% but not exceed 70% (other than during room cleaning) It was 55 ± 5%. Lighting was artificial it was 12 h light and 12 h dark cycles according to OECD Guideline 423. Standard pellet diet and water given to all animals. The present work was carried out with a prior permission by IAEC of Alwar Pharmacy College with CSPSEA registration number 963/c/06/CPCSEA.

Chemical

Liver toxicity inducing agent D-Galactosamine was purchased from Sigma-Aldrich. Silymarin purchased from Dr. Reddy's Laboratories Limited and used as standard drug for hepatoprotective activity. SGPT (ALT), SGOT (AST), Total Protein and Direct bilirubin assay kit were purchased by Anamol Lab. Pvt. Ltd. All other chemicals used in the study were of analytical grade and procured from local suppliers.

Ethanolic extract preparation and determination of % yield of *Piper attenuatum B. Ham and Caesalpinia crista Linn*

Authenticated leaves of both plants were washed with clean water before drying in oven at a temperature of 400ºC until the moisture content was below 14%. This reduces the chances of fungus infection in samples. The dried leaves were grinded and stored in airtight container [14]. Ethanolic extraction was carried out by Soxhlet extraction separately. Quantity of powdered material of both plants used for ethanolic extraction was 150 gm. These powdered materials of plant leaf were defatted with Petroleum Ether for 72 hours in a Soxhlet apparatus. Then after 72 hours these defatted material is subjected to extraction with ethanol (99.99%) in a Soxhlet apparatus for 48 hours. Make the extracts dry under reduced pressure and controlled temperature (40-50°C) using flash evaporator [15]. The ethanolic extracts obtained were concentrated under reduced pressure and % yield of both plants ethanolic extracts were measured by using formula.

Yield (%)=[(W2-W1)/W0] ×100

Where

W2-Weight of extract and container

W1-Container weight

W0-initial dried sample weight [16].

Hepatoprotective activity

All animals were divided into seven groups of six animals each. Group I received normal saline for 14 days and served as vehicle control group. Group II received normal saline (1 ml/kg, p.o.) for 14 days and served as toxic control. Groups III served as positive control and were treated with Silymarin (50 mg/kg, p.o.) for 14 days. Groups IV and V were treated with ethanolic leaf extract of Piper attenuatum B. Ham and Groups VI and VII treated with ethanolic leaf extract of Caesalpinia crista Linn. Hepatoprotective dose of both plant extract were 100 mg/kg and 200 mg/kg. On 15th day the groups II to VII received D-Galactosamine (400 mg/kg). After 24 hr of D-Galactosamine induced hepatotoxicity the blood was withdrawn from retro orbital plexus under anesthesia. Plasma was separated by centrifugation and transferred to pre-labeled eppendrof tubes for various biochemical parameters. After blood withdrawal all the groups' rats were sacrificed and liver tissue were collected for histopathological study [17].

Blood collection and biochemical parameter

Each animal was anaesthetized with diethyl ether. Blood was collected by retro-orbital method in a 5 ml disposable syringe and 2 ml blood was drawn very gently and slowly. The blood collected was immediately shifted to dried clean centrifugation tubes and serum was separated. Biochemical estimations were made the following day [18]. Different biochemical parameters like ALT (*Alanine Aminotransferases*), AST (*Aspartate Amino Transferases*), ALP (*Alkaline Phosphatase*), total protein and serum bilirubin were estimated in serum.

Statistical analysis

Results interpretation was done after subjecting the data obtained from various studies. Statistical analysis was performed using Graph pad Prism 9.0.2 version which included one way ANOVA followed by test like Dunnett and t-test. P<0.05 is considered as statistically significant.

Results

% Yield of ethanolic extract of *Piper attenuatum B. Ham and Caesalpinia crista Linn*

The % yield of ethanolic leaf extracts of plant *Piper attenuatum B. Ham and Caesalpinia crista Linn* were (28.33%), (32.25%) respectively.

Hepatoprotective activity of ethanolic leaf extracts of *Piper attenuatum B. Ham and Caesalpinia crista Linn* in D-galactosamine induced liver toxicity in rats

Sr. No	Groups	AST/ SGOT	ALT/ SGPT	ALP	T. P.	S.B.
1	Vehicle Control	13.43 ± 0.499	16.06 ± 0.861	43.33 ± 0.433	6.69 ± 0.051	0.67 ± 0.008
2	D-gal + Vehicle Control	80.31 ± 0.579#	92.49 ± 1.723#	131.8 ± 1.46#	2.33 ± 0.132#	2.72 ± 0.003#
3	D-gal + Silymarin (50 mg/kg)	19.71 ± 0.310***	23.12 ± 0.469***	50.38 ± 0.382***	6.29 ± 0.154***	0.85 ± 0.003***
4	D-gal + PAEE (100 mg/kg)	34.57 ± 0.746**	40.47 ± 0.861**	69.4 ± 1.345**	4.65 ± 0.093**	1.68 ± 0.003**
5	D-gal +PAEE (200 mg/kg)	27.64 ± 0.348***	32.12 ± 0.574***	58.73 ± 4.076***	5.82 ± 0.079***	1.32 ± 0.003***
6	D-gal + CCEE (100 mg/kg)	55.71 ± 0.809*	64.87 ± 0.861*	74.87 ± 6.418*	3.47 ± 0.062*	2.29 ± 0.036*
7	D-gal + CCEE (200 mg/kg)	36.5 ± 0.635***	42.39 ± 0.574***	62.1 ± 1.408***	3.61 ± 0.135***	1.79 ± 0.005***

Table 1: Hepatoprotective activity of ethanolic leaf extracts of *Piper attenuatum B. Ham and Caesalpinia crista Linn* in D-galactosamine induced liver toxicity in rats.

Values are expressed as mean ± SEM (n=6); ***p ≤ 0.001 when compared with D-Galactosamine control group, **p ≤ 0.01 when compared with D-Galactosamine control group, *p ≤ 0.05 when compared with D-Galactosamine control group, #p ≤ 0.001 when compared with vehicle control group (Figure 1).

PAEE (100 mg/kg))-*Piper attenuatum B. Ham* ethanolic extract (Dose-100 mg/kg)

PAEE (200 mg/kg)-*Piper attenuatum B. Ham* ethanolic extract (Dose-200 mg/kg)

CCEE (100 mg/kg)-*Caesalpinia crista Linn* ethanolic extract (Dose-100 mg/kg)

CCEE (200 mg/kg)-*Caesalpinia crista Linn* ethanolic extract (Dose-200 mg/kg)

D-Gal-*D Galactosamine*

AST (*Aspartate aminotransferases*), ALT (*Alanine aminotransferases*), ALP (*Alkaline phosphatase*), T.P. (*Total Protein*), S.B. (*Serum Bilirubin*), PA (*Piper attenuatum B. Ham*), CC (*Caesalpinia crista Linn*) (Table 1).

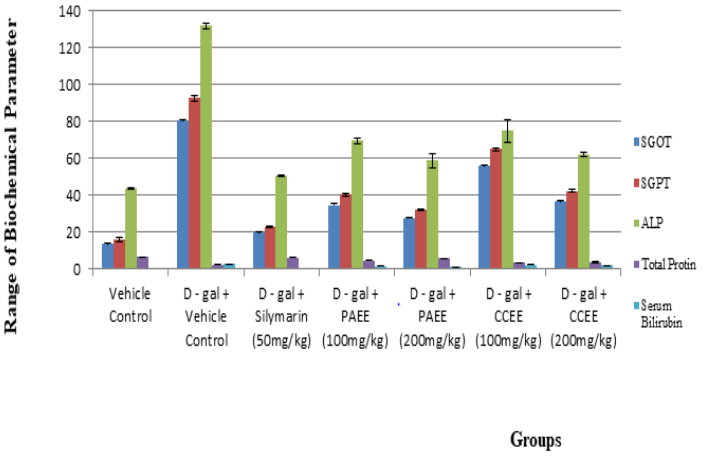

Figure 1: Effect of ethanolic leaf extract of *Piper attenuatum B. Ham and Caesalpinia crista Linn.* on D-Galactosamine induced liver toxicity in rats.

Histopathology of liver

Liver histopathology has been done for different groups like Vehicle control group, D-galactosamine treated group, Silymarin treated group and ethanolic extracts treated groups of plant *Piper attenuatum B.*

Ham and Caesalpinia crista Linn. Following pictures were taken (Figure 2).

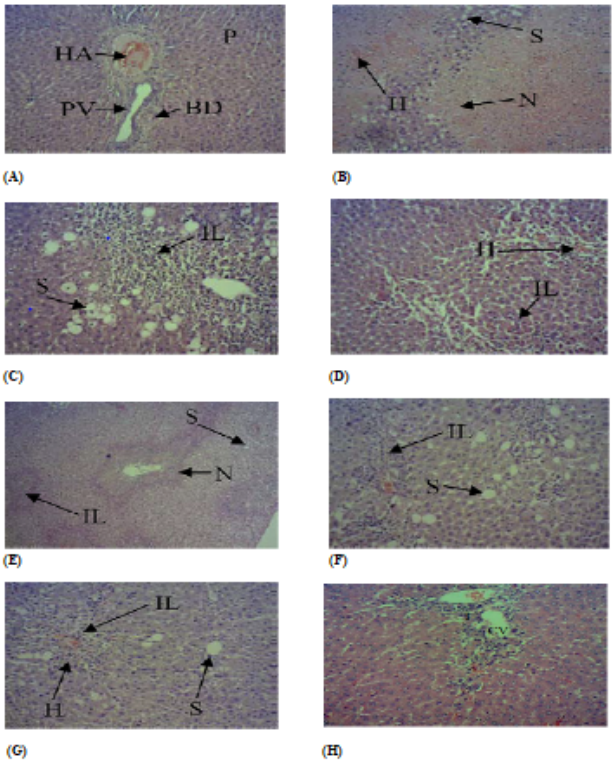

Figure 2: Histopathological view of different groups of animal screening models.

Discussion

Liver is a versatile organ of the body that regulates internal chemical environment. Liver injury induced by various hepatotoxins has been recognized as a major toxicological problem for years [19]. Single dose of D-galactosamine results in dose dependent hepatic damage resembling to viral hepatitis, with focal necrosis and periportal inflammation. It induces hepatitis by hindering the synthesis of RNA and protein via reduction in cellular UTP uptake that tips to the hepatic parenchyma necrosis [18]. Plants are important sources of medicines [20]. According to the literature survey it was found that piper species having hepatoprotective potential [21] and seeds of Caesalpinia crista Linn also having hepatoprotective potential against CCl4 induced hepatotoxicity in rats [18]. So in present protocol we used leaf of *Piper attenuatum B. Ham and Caesalpinia crista Linn* for their hepatoprotective potential against D-Galactosamine induced hepatotoxicity in rats. In the present protocol ethanolic leaf extract of *Piper attenuatum B. Ham and Caesalpinia crista Linn* were subjected to evaluate their % yield, it was found (28.33%), (32.25%) respectively. Both plants showing significant hepatoprotective activity in D-Galactosamine (400 mg/kg) induced liver toxicity in wistar albino rats. Silymarin (50 mg/kg, p.o.) was used as standard drug. Various biochemical parameters like AST, ALT, ALP, total proteins and serum bilirubin were analysed to determine hepatoprotective activity of both plants. Liver biopsy was also done for different experimental groups. AST/SGOT, ALT/SGPT, ALP and serum bilirubin were increased in group II while total protein was decreased in same group. All treated group with Silymarin and ethanolic extracts of *Piper attenuatum B. Ham Caesalpinia crista Linn*. at a dose of 50 mg/kg, 100 mg/kg and 200 mg/kg respectively showed significant results when compared with vehicle control group.

Histopathological study of different groups reveals following observations

(A) Normal liver architecture showing the Portal Vein (PV), Hepatic Artery (HA), and Bile Duct (BD), along with the surrounding hepatic parenchyma (P) directly abutting onto the portal tract as a limiting plate of continuous hepatocytes.

(B) Hepatotoxic liver after treatment with D-Galactosamine (D-Gal) showing severe necrosis (N) of the hepatocytes in parenchyma region, as well as steatosis (S) and hemorrhage (H).

(C) Hepatotoxic liver after treatment with D-Gal also showing steatosis (S) and infiltration of lymphocytes (IL).

(D) D-Gal induced hepatotoxic liver after pretreatment with 50 mg/kg Silymarin showing normal architecture of the hepatocytes and mild infiltration by lymphocytes (IL).

(E) D-Gal induced Hepatotoxic liver after pretreatment with 100 mg/kg ethanolic extract of Piper attenuatum B. Ham leaves (EEPA), showing focal multilobular steatosis (S), mild necrosis (N), and focal infiltration by lymphocytes (IL).

(F) D-Gal induced Hepatotoxic liver after pretreatment with 200 mg/kg ethanolic extract of Piper attenuatum B. Ham leaves (EEPA), showing normal architecture of the hepatocytes, mild steatosis (S), and mild infiltration by lymphocytes (IL).

(G) D-Gal induced hepatotoxic liver after pretreatment with 100 mg/kg ethanolic extract of Caesalpinia crista Linn showing infiltration of leukocytes (IL), mild hemorrhage (H) and macro vesicles of steatosis (S). (H) Section of pretreated 200 mg/kg ethanolic extract of Caesalpinia crista Linn showing normal histology with mild inflammation.

Conclusion

Present study revealed that ethanolic extract of authenticated leaf part of plant *Piper attenuatum B. Ham and Caesalpinia crista Linn* having hepatoprotective activity in D-Galactosamine induced liver toxicity in rats at 100 mg/kg and 200 mg/kg dose. Silymarin 50 mg/kg and plants extracts showed significant result when compared to vehicle control group and diseased group. From this study it may be possible that Piperine and flavonoids present in ethanolic leaf extracts of *Piper attenuatum B. Ham and Caesalpinia crista Linn* respectively responsible for hepatoprotective potential. To isolate bioactive compounds from both plants which are responsible for pharmacological activity further analytical measures should be adopted.

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