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Hepatoprotective Activity of Ethyl acetate Extract of aerial part of *Clerodendrum phlomidis Linn* in Paracetamol induced Hepato-toxic in Albino Rats

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Abstract: The plant *clerodendrum phlomidis* (Linn). Belonging to family Verbaneceae traditionally used in Ayurvedic system as antidiabetic, anthelminitic, antibacterial, antitumor and also used in liver disorders. The present study was conducted to evaluate the hepato-protective activity of ethyl acetate extract of aerial parts of *clerodendrum phlomidis* are evaluated in paracetamol-induced hepato toxicity in albino rats. Silymarin (200mg/kg) was given as reference standard. The ethyl acetate extract of aerial parts of *clerodendrum phlomidis* have shown very significant against paracetamol-induced hepatotoxicity in albino rats in reducing serum total bilirubin, SALP, SGPT , SGOT levels and liver homogenates LPO, SOD, CAT, GPX, GST and GSH levels. The ethyl acetate extract of aerial parts of *clerodendrum phlomidis* showed significant hepatoprotective activity.
Keywords : *Clerodendrum phlomidis* (Linn), hepatotoxicity, Paracetamol and Silymarin.

Introduction

The liver regulates many important metabolic functions. Hepatic injury is associated with distortion of these metabolic functions[1]. Additionally, it is the key organ of metabolism and excretion, thus it is continuously and variedly exposed to xenobiotics because of its strategic placement in the body .The toxins absorbed from the intestinal tract go first to the liver resulting in a variety of liver ailments. Thus liver ailments remain one of the serious health problems. Modern medicines have little to offer for alleviation of hepatic diseases and it is chiefly the plant based preparations which are employed for their treatment of liver disorders[2]. But there are not much drugs available for the treatment of liver disorders. Therefore, many folk remedies from plant origin are evaluated for its possible antioxidant and hepatoprotective effects against different chemical-induced liver damage in experimental animals. Paracetamol-induced hepatotoxicity model is frequently used for the investigation of hepatoprotective effects of drugs and plant extracts[3, 4].

Siddha system of medicine is one of the ancient system of Indian medicine most popularly practicing in Southern part of India especially Tamilnadu. In Tamil and Siddha medicine *Clerodendrum phlomidis* (Linn). Is known as Thazhuthazhai, Thalangi, Thakkari and Vathamatakki. It is a plant from Verbaneceae family and it is leafy medium sized tree up to 6 meter in height. Throughout India it is present in plains often by fencings. It is distributed in India, Sri Lanka, Pakistan, Burma and Baluchistan. As per Siddha medicine it is useful for inflammation, nasal congestion, fever, joint disorders and tumors. The root, leaf, bark of this plant

traditionally used as a bitter tonic, cure for diarrhea and worms, analgesic, aromatic, demulcent in gonorrhea etc. In Ayurveda it is used for inflammation, piles and tumors. The present work was under taken to evaluate hepatoprotective effects of the extract of the aerial part[5, 6].

Experimental

Plant Material

The aerial parts of the *Clerodendrum phlomidis* were collected from the foothill of Annavasal, Pudukkottai (DT), and Tamilnadu in the month of June2010. The collected plant was identified and authenticated by a botanist Dr. P.Jayaraman, Director, Plant Anatomy Research Centre, Chennai. A voucher specimen (PARC/2010/574.) The collected aerial part were washed, and dried in the sun for about a Week. After drying the plant materials were kept in an oven at 40ºC to ensure completes drying. The dried plant parts were finally ground into coarse powder (2.5kg) and preserved in an airtight container for future use.

Preparation of the Extracts

The shade dried aerial part of *Clerodendrum phlomidis* was extracted with ethyl acetate successively by soxhlation method. The extract was filtered, evaporated to dryness (40 ^{0}C). The yield of extract was calculated.

Animals

Albino rats (either sex) of Sprague dawley strain, weighing 150-200g were used. The animals were acclimatized to laboratory conditions (RT-25°C) for 4 days and given pelleted animal feed (Hindustan Lever) and drinking water, Diagnostic reagent kits (Enzopak) were used for the estimation of serum SALP, SGPT and SGOT levels[7] and assay procedure was used for the estimation of liver homogenates LPO, SOD, CAT, GPX, GST and GSH. All the experimental procedures and protocols used in this study were reviewed by the Institutional Animal Ethics Committee ((JKKMMRFCP/IAEC2010/012) and were in accordance with the guidelines of the IAEC.

Toxicity studies

Toxicity studies were performed according to OECD-423 guidelines (acute toxic class method). Albino mice (n=3) of either sex selected by random sampling technique were employed in this study. The animals were fasted for 4hrs with free access to water only. The plant extract of *Clerodendrum phlomidis* was administered orally with an initial dose of 1000 mg.kg-1 body weight. The mortality was observed for three days. If mortality was observed in 2/3 or 3/3 of animals, then the dose administered was considered as a toxic dose. However, if the mortality was observed only one mouse out of three animals then the same dose was repeated again to confirm the toxic effect. If mortality was not observed, the procedure was then repeated with higher dose such as 50,300 and 2000 mg.kg-1[8].

Hepatoprotective Activity

The animals were divided into four groups comprising of six albino rats in each group using randomization technique and treated with the extract for sixteen days to assess the hepato-protective potential of the plant. The first group (vehicle control) received vehicle for all the days. The second group was kept as toxin control and given only the Paracetamol treatment. The third group received ethyl acetate extract in the dose of 200mg/kg p.o. and the forth group received the Silymarin in the dose of 200mg/kg p.o. as a reference material for the study. All the animals except the vehicle control received Paracetamol all 16th day of the treatment. The animals were sacrificed by cervical dislocation after 48 hours of Paracetamol administration. The blood samples were collected by cardiac puncture in heparinized microfuge tubes. The blood samples thus collected were immediately centrifuged at 2200rpm for 15 minutes. When serum clearly separated out, the serum was analyzed for SGPT, SGOT and SALP levels using enzopak reagent kits by the method proposed by Reitman and Frankel. The results thus obtained were subjected to statistical analysis using *student t-test* and analysis of variance the livers were dissected out immediately, washed with ice cold saline and 10% homogenates in 1.15% (w/v) KCl were prepared. The homogenates were centrifuged at 7000rpm for 10 min at 4oC and the supernatants were used for the assays of LPO, SOD, CAT, GPX, GST and GSH. The results thus obtained were subjected to statistical analysis using *Student t-test* and analysis of variance[9, 10].

Table 1: Effect of ethyl acetate extract of *Clerodendrum phlomidis* aerial parts on Paracetamol-induced hepatotoxicity (Serum parameters).

S.No	Groups	Total Bilirubin(mg/dl)	SALP (Units/ml)	SGPT (Units/ml)	SGOT (Units/ml)
1	Control(propylene glycol) 1 ml	0.87 ± 0.09	236.41 ± 2.33	84.4 ± 1.45	196.23 ± 1.32
2	Paracetamol (1000mg/kg)	2.04 ± 0.23	337.23±22.12	212.12±15.06	354.21±23.32
3	Ethyl acetate Extract (200mg/kg)	0.84 ± 0.05	228.34 ± 2.12	84.6 ± 2.15	195.12 ± 1.00
4	Silymarin (200mg/kg)	0.8 ± 0.04	230.56±23.08	81.53 ± 26.62	197.38 ±1.53

A Values of mean ± S.E.M. (n=6) P<0.001 Vs Control group P<0.05;** P<0.01;*** P<0.001 Vs Paracetamol group.

Table 2: Effect of ethyl acetate extract of *Clerodendrum phlomidis aerial* parts on Paracetamol-induced hepatotoxicity (Liver homogenates).

S.No	Groups	LPO (nmoles/mg of protein)	SOD (units/mg of protein)	CAT (units/mg of protein)	GPX (µg/mg)	GST (units/mg of protein)	GSH (units/mg of protein)
1	Control Propylene glycol(1 ml)	0.46± 0.09	107.1±6.3	24.64±1.4	2.96±0.02	1.26±0.13	0.34±0.03
2	Paracetamol (1000mg/kg)	1.39± 0.04	37.19±1.42	6.05± 0.35	0.9±0.06	0.47± 0.04	0.16± 0.05
3	Ethyl acetate extract (200mg/kg)	0.47± 0.89	108.49±1.31	23.43±1.3	3.14±0.03	1.27± 0.08	0.35± 0.01
4	Silymarin (200mg/kg)	0.45± 0.02	109.35±1.57	24.53± 1.2	3.16±0.04	1.25± 0.34	0.37± 0.01

A Values of mean ± S.E.M. (n=6) P<0.001 Vs Control group P<0.05;** P<0.01;*** P<0.001 Vs Paracetamol group.

Figure: 1

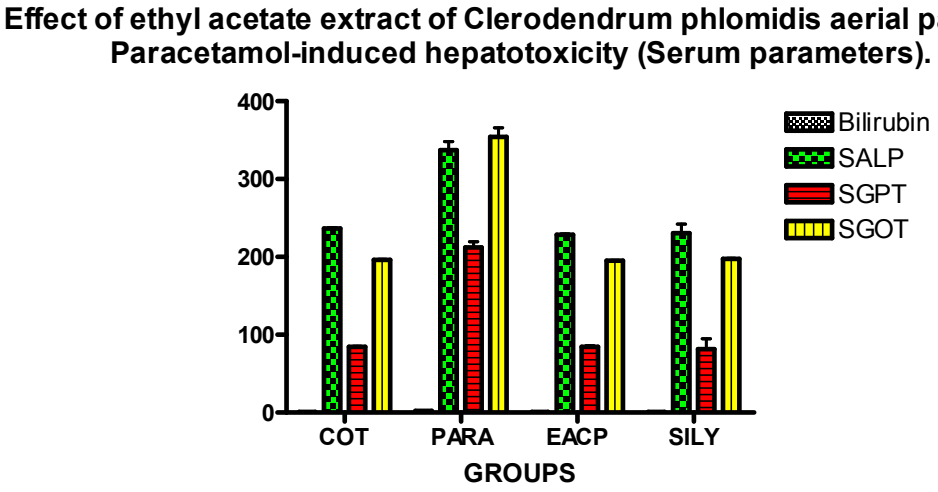
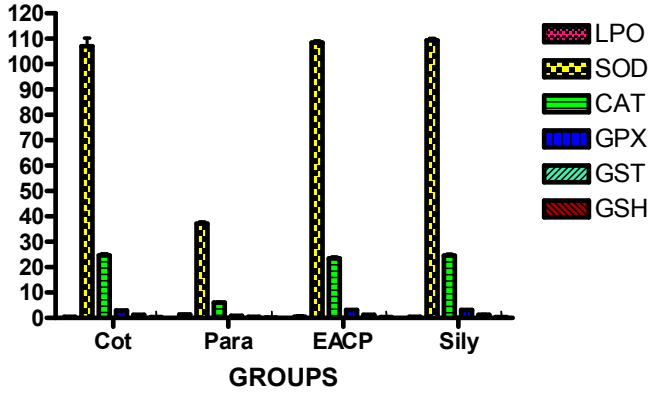


Figure: 2

Effect of ethyl acetate extract of Clerodendrum phlomidis aerial parts on Paracetamol-induced hepatotoxicity (Liver homogenates).



Result and Discussion

The present studies were performed to assess the hepatoprotective activity in rats against Paracetamol as hepatotoxin to prove its claims in folklore practice against liver disorders. Paracetamol-induced hepatic injury is commonly used as an experimental method for the study of hepatoprotective effects of medicinal plants extracts and drugs.

The extent of hepatic damage is assessed by histological evaluation and the level of various biochemical parameters in circulation. Highly reactive trichloro free radical formation, which attacks polyunsaturated fatty acids of the endoplasmic reticulum, is responsible for the hepatotoxicity of Paracetamol. It produces hepatotoxicity by altering liver microsomal membranes in experimental animals. From the Table 1 it was evident that extract was able to reduce all the elevated biochemical parameters due to the hepatotoxin intoxication. The levels of total proteins and albumin were reduced due to the Paracetamol induced hepatotoxicity. The reduction is attributed to the initial damage produced and localised in the endoplasmic reticulum which results in the loss of P450 leading to its functional failure with a decrease in protein synthesis and accumulation of triglycerides leading to fatty liver. Reduction in the levels of SB, SALP, SGOT and SGPT towards the normal value is an indication of regeneration process.

The protein and albumin levels were also rose suggesting the stabilization of endoplasmic reticulum leading to protein synthesis. The protective effect exhibited by extracted at dose level of 200 mg/kg was comparable with the standard drug silymarin. The histological examination of the liver sections reveals that the normal liver architecture was disturbed by hepatotoxin intoxication. In the liver sections of the rats treated with extracted and intoxicated with Paracetamol; rats treated with ethyl acetate extract and intoxicated with Paracetamol the normal cellular architecture was retained as compared to silymarin, thereby confirming the protective effect of the extract. In accordance with these results, it may be hypothesized that tannin, saponins and flavonoids, which are present in extracts, could be considered responsible for the hepatoprotective activity.

The ethyl acetate extract of aerial parts of *Clerodendrum phlomidis* have shown very significant hepatoprotection against Paracetamol-induced hepatotoxicity in albino rats in reducing serum total bilirubin, SALP, SGPT and SGOT levels. It is also found that treatment with ethyl acetate extract of plant have brought down the elevated level of LPO and also significantly enhanced the reduced levels of SOD, CAT, GPX, GST and GSH. Liver section of *Clerodendrum phlomidis* treated animal group clearly showed normal hepatic cells and central vein thereby confirming hepatoprotective activity. In conclusion the ethyl acetate extract of *Clerodendrum phlomidis* could be an important source of hepatoprotective compounds.

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