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## Hepatoprotective and antioxidant effects of *Sphaeranthus indicus* Linn against perchloroethylene – induced hepatotoxicity in rats

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### ABSTRACT

*Sphaeranthus indicus* is used as traditional herbal medicine in India to treat various disorders. In the present study, the hepatoprotective activity of ethanolic extract of the whole plant of *Sphaeranthus indicus* was evaluated against perchloroethylene 1000mg/kg bw induced hepatic damage in rats. The extract at dose of 500mg/kg bw was administered orally once daily for seven days. The elevated serum enzymatic levels of serum glutamate oxaloacetate transaminase (AST), serum glutamate pyruvate transaminase (ALT) and alkaline phosphatase(ALP) showed marked decrease as compared to normal control. Antioxidant enzymes like catalase, superoxide dismutase, glutathione peroxidase levels are increased towards the normal. The biochemical observations were supplemented with histopathological examinations of rat liver sections. The results of this study strongly indicate that *Sphaeranthus indicus* have potent hepatoprotective action against perchloroethylene- induced hepatic damage in rats.

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### KEYWORDS

*Sphaeranthus indicus*;  
Perchloroethylene;  
Antioxidant enzymes;  
Hepatoprotective activity;  
Histopathology.

### INTRODUCTION

*Sphaeranthus indicus* Linn (Asteraceae), a herb found mostly in South India, is found to be bitter, stomachic, stimulant, alterative, pectoral and demulcent and externally emollient<sup>[1]</sup>. All parts of the plant possess medicinal uses and have been reported to have beneficial effects on several ailments. The juice of the plant is styptic and diuretic and it is said to be used against liver and gastric disorders<sup>[2]</sup>. Roots and seeds are used as stomachic and anthelmintic<sup>[3]</sup>. It is reported that flowers are highly alternative, depurative, cooling and tonic. They are also used as blood purifiers in skin diseases<sup>[4]</sup>. Dried

and powdered leaves of *Sphaeranthus indicus* are used in the treatment of chronic skin diseases, urethral discharges and jaundice<sup>[1]</sup>. The wound healing effects of ethanolic extract of *Sphaeranthus indicus* have been investigated<sup>[5]</sup>. 7-hydroxy-3', 4', 5-6-tetramethoxy flavone 7-0-β-D-(1, 4)-di glucoside a new flavones glycoside from the stem of *Sphaeranthus indicus*<sup>[6]</sup>. Flavonoids have reported for various pharmacological properties including antioxidant activity, anticancer and immunomodulatory effects. No studies have to date focused on the hepatoprotective effect of ethanolic extract of *Sphaeranthus indicus*. Such knowledge is of great importance to better understand its contribution

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for treating liver damage. The principal aim of the study is to elucidate the potential hepatoprotective activity of ethanolic extract of *Sphaeranthus indicus* on perchloroethylene – induced liver damage in wistar rats.

### EXPERIMENTAL

#### Plant material

The whole plant of *Sphaeranthus indicus* was collected from Pollachi, Tamilnadu. The plant authenticated by Dr.R.Gopalan (Rtd Botanical Survey of India Coimbatore), Department of Biotechnology, Karpagam University, Coimbatore, India.

#### Preparation of extract

The whole plant was washed thoroughly in tap water, shade dried and powdered. The powder (100gm) was successively extracted with 500ml of ethanol overnight with constant stirring. The filtrate was then concentrated to dryness under reduced pressure at 45°C with a rotary evaporator and stored at 4°C for further use.

#### Animals

Wistar albino female rats (160-180g) obtained from the Institute's animal house were used for the study. They were housed under standard laboratory conditions and were fed with commercial rat feed (Hindustan Lever Ltd, Mumbai India) and *ad libitum*. All experiments were carried out according to the guidelines recommended by the Committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA) Government of India.

#### Acute toxicity studies

The acute toxicity study for ethanolic extract of *Sphaeranthus indicus* was performed on wistar strain of female albino rats. The animals were kept fasting overnight prior to the experiment and maintained under standard conditions. All the extracts were administered orally in increasing dosage and found safe up to dose of 2000mg/kg bw for all extracts.

#### Perchloroethylene induced hepatotoxicity

Perchloroethylene (E.Merk, Limited, Mumbai) at an oral dose of 1000mg/kg bw is known to cause liver

damage in rats. Rats were divided into four groups (Six per group). Group I serving as control. Group II received single dose of 1000mg/kg bw of perchloroethylene. Group III received a single dose of perchloroethylene and 500mg/kg bw of *Sphaeranthus indicus* for seven days. Group IV received a daily dose of 500mg/kg bw of *Sphaeranthus indicus*.

The animals were sacrificed after the last treatment. Blood was collected, allowed to clot and serum was separated at 2500rpm for 15min and biochemical investigations were carried out. Liver was dissected out and used for histopathological and biochemical studies.

#### Assessment of liver functions

Biochemical parameters like liver marker enzymes such as; Aspartate aminotransferase (AST), Alanine transaminase (ALT), alkaline phosphatase (ALP) were assayed according to standard methods<sup>[7]</sup>.

#### Antilipid peroxidation studies

The antilipid peroxidant effect of *Sphaeranthus indicus* was studied in vitro by following the modified method<sup>[8]</sup>.

0.5g of the rat liver tissue was sliced and homogenized with 10ml KCl- Tris HCl buffer (pH 7.2), 0.2ml of 10mM ferrous sulphate and sodium pyrophosphate. The tubes were incubated at 37°C with constant shaking for 20minutes. The reaction was stopped by the addition of 1.0ml of 10% TCA. The tubes were shaken well and 1.5ml of TBA reagent was added and was heated at 90°C for 20min. After cooling, the flocculent precipitate was removed by adding 5ml of n-butanol and they were centrifuged at 3000rpm for 20min. The absorbance of the supernatant was measured at 532nm.

#### Estimation of SOD, CAT, GPx

Grouping and dosing schedule in rats was followed similarly as mentioned in perchloroethylene induced hepatotoxicity. After seven days the animals were sacrificed by cervical dislocation. Liver samples were dissected out and washed immediately with ice cold saline remove as much blood as possible. Liver homogenates (5% w/v) were prepared in cold 0.01M phosphate buffer using a Remi homogenizer. The unbroken cells and cell debris were removed by centrifugation at 1000rpm for 1 min using a Remi refrigerated centrifuge.

The supernatant was used for the estimation of SOD, CAT, and GPx by the respective methods<sup>[9-11]</sup>.

### Histopathological studies

The liver specimens obtained from the control and treated groups were fixed in 10% formalin. The formalin-fixed liver samples were stained with haematoxylin – eosin for photomicroscopic observations of the liver histological architecture.

### Statistical analysis

All data are presented as mean  $\pm$  SD. Statistical significance was examined through one-way analysis of variance and Duncan's multiple range tests "p" values of  $<0.05$  were assumed to be statistical significant.

## RESULTS

Administration of perchloroethylene (1000mg/kg bw) induced a marked increase in the serum hepatic enzyme levels, AST, ALT and ALP as compared to normal controls indicating liver damage. Treatment of the rats with *Sphaeranthus indicus* (500mg/kg bw) after administration of perchloroethylene administration caused a significant reduction in the values of AST, ALT and ALP (TABLE 1).

**TABLE 1 : Effects of ethanolic extract of *Sphaeranthus indicus* on rat liver marker enzymes after oral administration of perchloroethylene.**

Group	AST (U/L)	ALT (U/L)	ALP (IU/L)
Normal control	46.80 $\pm$ 1.16	88.37 $\pm$ 1.84	119.73 $\pm$ 1.30
Perc Induced (1000mg/kg bw)	257.48 $\pm$ 6.21 <sup>#</sup>	236.32 $\pm$ 4.97 <sup>#</sup>	253.06 $\pm$ 5.68 <sup>#</sup>
Perc + Extract (500mg/kg bw)	129.32 $\pm$ 1.73*	123.46 $\pm$ 3.89*	132.62 $\pm$ 2.79*
Extract	48.23 $\pm$ 4.04	90.11 $\pm$ 2.64	123.01 $\pm$ 1.26

Values are expressed as Mean $\pm$ S.D., n = 6

<sup>#</sup>Significantly different from control group, p < 0.05

\*Significantly different from the group treated with Perc, p < 0.05

Lipid peroxidation is one of the major outcomes of free radical-mediated injury to tissue. Treatment with *Sphaeranthus indicus* significantly decreased the lipid peroxidation levels. The hepatic antioxidant enzymes SOD, CAT and GPx content had significantly increased in *Sphaeranthus indicus* treated groups. Whereas perchloroethylene intoxicated group had shown significant decrease in these parameters when compared to

control group (TABLE 2).

**TABLE 2 : Effects of Ethanolic Extract of *Sphaeranthus indicus* on Antioxidant Enzymes and lipid peroxidation levels on Perchloroethylene Induced Hepatocarcinogenesis**

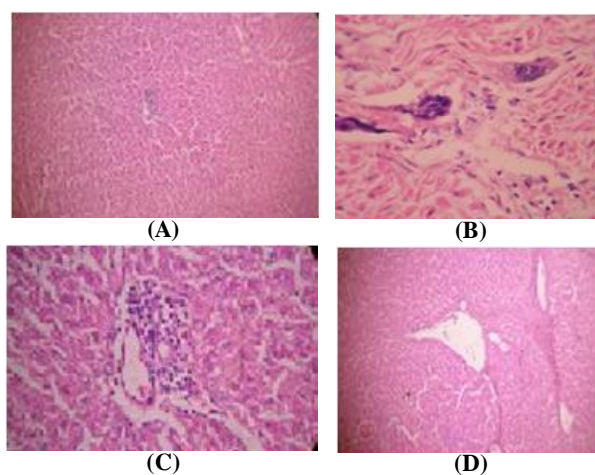
Group	SOD (U/mg protein)	CAT (U/mg protein)	GPx (U/mg protein)	LPO (MDA formed per mg protein)
Normal control	8.45 $\pm$ 0.31	65.08 $\pm$ 1.16	23.80 $\pm$ 1.04	0.90 $\pm$ 0.17
Perc Induced (1000mg/kg bw)	3.55 $\pm$ 0.55 <sup>#</sup>	31.24 $\pm$ 3.39 <sup>#</sup>	9.80 $\pm$ 0.71 <sup>#</sup>	5.90 $\pm$ 0.60 <sup>#</sup>
Perc + Extract (500mg/kg bw)	5.94 $\pm$ 0.32*	43.90 $\pm$ 1.98*	18.77 $\pm$ 0.47*	3.25 $\pm$ 0.41*
Extract	7.15 $\pm$ 0.47	61.19 $\pm$ 2.82	20.79 $\pm$ 0.95	1.75 $\pm$ 0.50

Values are expressed as Mean $\pm$ S.D., n = 6

<sup>#</sup>Significantly different from control group, p < 0.05

\*Significantly different from the group treated with Perc, p < 0.05

The hepatoprotective effect of *Sphaeranthus indicus* was confirmed by histopathological examinations of the liver tissue of control and treated animals. The histological architecture of perchloroethylene treated liver sections showed massive fatty changes, necrosis, ballooning degeneration and loss of cellular boundaries. However administration of *Sphaeranthus indicus* (500mg/kgbw) almost normalized these effects. Liver section of rat treated with extract alone showed normal hepatic cells (Figure 1)



**Figure 1 : (A) Liver of normal control rat showing hepatic cells with normal nuclei and cytoplasm. (B) Perchloroethylene (1000mg/kg bw) treated rat liver, showing loss of nuclei, kupffer cells and vacuolization. (C) Section of *Sphaeranthus indicus* (500mg/kg bw) + perchloroethylene treated rat liver, showing marked improvement over perchloroethylene control group. (D) Section of *Sphaeranthus indicus* (500mg/kg bw) treated rat liver showing normal architecture**

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### DISCUSSION

The present study reports the potential hepatoprotective activity of *Sphaeranthus indicus* against hepatic injury produced by perchloroethylene in rats. Perchloroethylene (PCE) is an unsaturated chlorinated hydrocarbon in the form of a colorless, volatile liquid that is used extensively as an industrial organic solvent for metal degreasing and for dry cleaning<sup>[12-14]</sup>. Perchloroethylene (PCE) is widely used in the textile industry for dry-cleaning, processing, and finishing fabrics<sup>[15]</sup>. It is also used in the degreasing of metals and as a chemical intermediate in the synthesis of fluorocarbons. Although chronic PCE intoxication by inhalation has been relatively well documented, severe acute PCE poisoning by ingestion is reported rarely<sup>[14]</sup>. PCE is an eye, skin, and respiratory irritant. The most sensitive endpoint of PCE toxicity is the central nervous system<sup>[16]</sup>.

Excessive absorption of PCE can produce depression of the central nervous system and hepatic and renal damage<sup>[17]</sup>. Acute, high-level perc exposures have been associated with blindness and child death<sup>[18]</sup>. Metabolism of perchloroethylene (Perc) occurs by cytochrome p450-dependent oxidation and glutathione (GSH) conjugation. The cytochrome p450 pathway generates tri- and dichloroacetate as metabolites of Perc, and these are associated with hepatic toxicity and carcinogenicity<sup>[19]</sup>. When liver cell plasma is damaged, a variety of enzymes located normally in cytosol is released into the blood, thereby causing increased enzyme levels in the serum. The estimation of enzymes in the serum is a useful quantitative marker of the extent and type of hepatocellular damage. In the present investigation, the dosage of Perchloroethylene by 1000mg/kg bw caused liver injury in rats. The ethanolic extract of *Sphaeranthus indicus* decreased the elevated level of serum marker enzymes.

The hepatoprotective effect of *Sphaeranthus indicus* was further confirmed by histopathological examinations of the liver. The histological observation basically supported the results from the serum assays as *Sphaeranthus indicus* administration reversed to a large extent, hepatic lesions produced by perchloroethylene.

Most of the hepatotoxic chemicals including perchloroethylene damaged liver mainly by inducing lipid peroxidation. In higher animals, lipid peroxidation was known to cause destabilization and disintegration of the cell membrane, leading to liver injury, atherosclerosis and kidney damage<sup>[20]</sup>. Peroxy radicals are important agents that mediate lipid peroxidation there by damaging cell membrane. Intracellular lipid peroxidation is regulated by antioxidant enzymes like catalase, superoxide dismutase and glutathione peroxidase. All these enzymes are found to be significantly decreased in perchloroethylene induced rats.

Phytochemical investigations on *Sphaeranthus indicus* revealed the presence of different types of compounds. Two new eudesmanolides along with a known eudesmanolide and two sesquiterpenoids, crytomerediol and 4-epicryptomerediol have been isolated from flower heads<sup>[21]</sup>. *Sphaeranthus indicus* has been shown to possess antibacterial and anti-inflammatory activity, besides having anthelmintic, blood purifier, aphrodisiac and anti-stomachache properties<sup>[22]</sup>. Three closely related new hydroxyls have been isolated from the chloroform extract of *Sphaeranthus indicus*<sup>[23]</sup>. Flavonoids have reported for various pharmacological properties including antioxidant activity, anticancer and immunomodulatory effects. The bioactive fraction of *Sphaeranthus indicus* offered protection against immunosuppression caused by cyclophamide<sup>[24]</sup>.

### CONCLUSION

In conclusion, the present results demonstrated that *Sphaeranthus indicus* has potent hepatoprotective action upon perchloroethylene induced hepatic damage in rats and possessed antilipid peroxidative and free radical scavenging activities. The present study recommend as the traditional use of *Sphaeranthus indicus* in the treatment of liver diseases.

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