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Research Article

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Hepato protective activity of Vedi Annabethi Chenduram against Paracetamol induced liver damage in Wistar rats

Sundari S *[1], Thiruthani M [2], Essakky Pandian G[3]

[1] PG Scholar, Department of Nanju Noolum Marthuva Neethi Noolum (Siddha Toxicology) Govt Siddha Medical College, Palayamkottai, Tamilnadu, India.
[2] HOD Department of Nanju Noolum Maruthuva Neethi Noolum (Siddha Toxicology) Govt Siddha Medical College, Palayamkottai, Tamilnadu, India.
[3]Lecturer Department of Gunapadam (Siddha Pharmacology) Govt Siddha Medical College, Palayamkottai, Tamilnadu,India.
Corresponding Author: **Dr.S.Sundari,** PG Scholar, Department of Nanju Noolum Marthuva Neethi Noolum (Siddha Toxicology) Govt Siddha Medical College, Palayamkottai, Tamilnadu, India.
E-mail: 22mardrsundari@gmail.com

Abstract

Hepatitis is one of the major health problems in human which sometimes may even lead to death. Natural products are the best source of remedies for the treatment of liver diseases. Our studies identified a Siddha formulation with potential hepato protective activity. The Siddha metallic mineral formulation Vediannabethi Chenduram was screened for its hepato protective activity in paracetamol induced liver damage in wistar albino rats at a dose of 25mg/kg. The Siddha metallic mineral formulation VABC exhibited a significant protective effect by SGOT, SGPT, ALP total protein and total bilirubin. Silymarin was used as a positive control. The effect of the drug was judged by changes in serum marker SGOT, SGPT, ALP, total Protein and total bilirubin levels.

Keywords: Vediannabethi, Chenduram, Paracetamol, Hepato protective activity, Siddha formulation.

Introduction

Siddha system is one of the oldest conventional medical system in the world. The usage of heavy metals in siddha system of medicine having some queries regarding the threating effects of those metals which in use though metallic Siddha medical formulations. Vediannabethi Chenduram is a metallic mineral formulation cited in Gunapadam Thathu Jeevam prepared through the special oxidation procedure involving purified form of minerals processed under herbal juice. Numerous medicinal plants and their formulations are used for liver disorders in medical practice as well as traditional system of medicine in India.

Materials and Methods

Animals

Male Wistar albino rats weighing 180 – 200 gms were used for the study. The animals were obtained from animal house, Nandha College of Pharmacy, Erode. The animals were placed at random and allocated to treatment groups in polypropylene cages with paddy husk as bedding. Animals were housed at a standard environmental condition (Humidity of 30 – 70 % and 12:12 light: dark cycle at 24±2^{o}C). All animals were allowed to free access to water and fed with standard commercial pelleted rat chaw (M/s. Hindustan Lever Ltd, Mumbai). All the experimental procedures and

protocols used in this study were reviewed by the Institutional Animal Ethics Committee (688/PO/Re/S/02/CPCSEA) and were in accordance with the Institutional ethical guidelines.

Hepatoprotective Activity (Araya *et al.,* 1987)

The animals were divided into four groups of five animals in each and fasted for 48 hours. After 48 hours of fasting, group I served as controls received distilled water (1ml/kg) for 7 days and group II, served as induced control was treated similar as group I. Group III served as reference control, received silymarin 50 mg/kg once daily for 7 days and group IV received Vedi Annabethi Chenduram (25mg/kg) once daily for 7 days. On 7^{th} day, Paracetamol (3mg/kg) was administered orally to all the animals except group I. 48 hours after hepatic injury by Paracetamol, blood was collected through retro orbital sinus puncture under Pentobarbitone (45mg/kg, ip) anaesthesia.

Biochemical Studies

The blood samples were allowed to clot for 45 min at room temperature. Serum was separated by centrifugation at 2500rpm at 30^{o}C for 15 min and utilized for the estimation of various biochemical parameters namely SGOT, SGPT, SALP, serum Bilirubin and total Protein.

Serum Hepatospecific Markers

Serum Glutamate Oxaloacetate Transaminase (SGOT) &Serum Glutamate Pyruvate Transaminase (SGPT) (Reitman and Frankel, 1957)

0.05 ml of serum with 0.25 ml of substrate (aspartate and α-ketoglutarate for SGOT; alanine and α -ketoglutarate for SGPT, in phosphate buffer at pH 7.4) was incubated for an hour in case of SGOT and 30 min. for SGPT. 0.25 ml of DNPH solution was added to arrest the reaction and kept for 20 min in room temperature. After incubation 1 ml of 0.4N NaOH was added and absorbance was read at 505 nm in UV spectrophotometer. Activities were expressed as IU/L.

Alkaline Phosphate (ALP) (King and Armstrong, 1934)

Alkaline phosphatase activity was assayed using disodium phenyl phosphate as substrate. The colour developed was read at 510 nm in UV spectrophotometer after 10 min. Activities of ALP was expressed as IU/L.

Serum Bilirubin (Malloy and Evelyn, 1937)

Diazotisedsulphonilic acid (0.25 ml) reacts with bilirubin in diluted serum (0.1 ml serum + 0.9 ml distilled water) and forms purple colored azobilirubin, which was measured at 540 nm in UV spectrophotometer. Activities of total bilirubin were expressed as mg/dl.

Total Protein (Gornall *et al*. 1949)

Biuret reagent (1.0 ml) reacts with serum (10 µL) and the colour developed was read at 578 nm in *uv-vis* spectrophotometer. Activities of total protein were expressed as mg/dl.

Statistical Analysis

The values were expressed as mean ± SEM. The statistical analysis was carried out by one way analysis of variance (ANOVA) followed by Dunnett's '*t*' – test using graphpad version I. P values <0.05 were considered significant.

Results

The results of hepatoprotective activity of *Vedi Annabethi Chenduram* (25mg/kg) on Paracetamol treated rats are shown in Table 1. The hepatic enzymes SGOT, SGPT, and total bilirubin were increased and total protein was decreased in paracetamol treated animals when compared to control groups. The reference control silymarinreversed the levels of serum enzymes, total bilurubin and total protein on the paracetamol induced hepatic injury by significantly (P <0.001) reduced the serum hepatic enzymes, total bilirubin and decreasing the total protein. The *Vedi Annabethi Chenduram* also significantly (P<0.001) reduced serum hepatic enzymes, total bilirubin and decreased the total protein compared to Paracetamol control.

Table: 1. The table shows the effect of VediAnnabethiChenduram on paracetamol induced liver damage in rats.

Groups	Drug Treatment	Liver Function Test				
		SGOT (IU/L) AST	SGPT(IU/L) ALT	SALP(IU/L)	Total Bilirubin (mg/dl)	Total Protein (mg/dl)
I	Vehicle Control Distilled Water (1ml/kg)	143.44±3.72	88.14±3.97	241.90±2.67	0.58±0.03	18.40±0.39
II	Induced Control Paracetmol (750 mg /kg)	297.63±6.78	153.39±5.79	390.21 ±5.42	6.36±0.38	34.03±0.25
III	Reference Control Silymarin (50mg/kg)	147.21±1.66***	90.52±2.68***	253.07±2.24***	1.32±0.09***	20.84±0.35***
IV	VediAnnabethiChenduram (25mg/kg)	150.51±2.32***	97.33±3.04***	261.28±1.98***	1.65±0.16***	23.94±0.27***

Values are in mean ± SEM (n=6),
*P<0.05 , **P<0.01, ***P<0.001 Vs Paracetamol Control

Discussion

In living system liver is considered as highly sensitive to toxic agents. Paracetamol (Acetaminophen) is a commonly and widely used analgesic and antipyretic agent. Hepatotoxic doses of paracetamol deplete the normal levels of heaptotic glutathione. The study of different enzyme activities such as SGOT,SGPT,SALP ,Total protein and Total Biliubin have been found to be great value in the assessment of experimental liver damage. In the present investigation it was observed that the animals treated with Acetaminophen resulted in significant Hepatic damage as shown by the elevated levels of serum markers in table 1. These changes in the marker levels will reflect in hepatic structural integrity. The rise in SGOT is usually accompanied by an elevation in the levels of SGPT which play a vital role in the conversion of aminoacids to ketoacids. The results of hepato protective activity of Vediannabethi Chenduram (25mg/kg) on paracetamol treated rats shown in table 1.The reference control silymarin reversed the levels of serum enzymes total protein and total bilirubin on Paracetamol induced hepatic injury by significantly ($p<0.001$) reduced the serum hepatic enzymes, total bilirubin and decreasing the total protein. The Vediannabethi Chenduram also significantly ($p<0.001$) reduced serum hepatic enzymes, total bilirubin and decreased the total protein compared to paracetamol control.

Conclusion

The study of hepato protective activity of Vediannabethi Chenduram (Trail drug) and Silymarin (Reference control) in male wistar albino rat having hepatic injury induced by paracetamol, proved that the hepato protective activity of Vediannabethi Chenduram has similar effect as that of silymarin (Reference control). VediannabethiChenduram has no adverse effect or drug dependency. The heavy metals were present in VediannabethiChenduram with in permissible limits as per norms of World Health Organization (WHO).Further the Vediannabethi Chenduram is traditionally used by the Siddha practitioners since thousands of years which proves its safety and efficacy also the drug is more affordable. Hence it can be used as an alternate drug of choice in the treatment of hepatic injury.

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