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Evaluation of Hepatoprotective Activity of Arithiraadhi Chorine in Ccl_4 Induced Liver Damage in Rats

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ABSTRACT
Though symptoms of Liver disorders are challenging, Siddha system of medicine ameliorates them in a very effective way. This research aimed to evaluate the Hepatoprotective activity of Siddha herbal mineral preparation Arithiraadhi chooranam by CCl_4 induced hepatotoxicity in albino rats. Administration of Arithiraadhi chooranam significantly reduced the impact of CCl_4 toxicity. Protection of hepatocytes was evaluated by estimating the level of ALT, AST, ALP, serum bilirubin, and total protein. The results strongly indicate that Arithiraadhi chooranam has appreciable hepatoprotective potential.

Keywords: Siddha, Arithiraadhi Chooranam, CCl_4, Hepatoprotective Activity.

1. INTRODUCTION

The liver is the largest solid organ in the body. The liver plays astonishing vital functions in the maintenance, performance and regulating homeostasis of the body. It carries out a large number of critical functions, including the manufacture of essential proteins, and metabolism of fats and carbohydrates. It also serves to eliminate harmful biochemical waste products and detoxify alcohol, certain drugs, and environmental toxins. Maintenance of a healthy liver is essential for the overall wellbeing of an individual [1].

Liver diseases are becoming one of the significant worldwide health problems, with high endemicity in developing countries. Liver disease is a term for a collection of conditions, diseases, and infections that affect the cells, tissues, structures, or functions of the liver. The liver is continuously exposed to lots of toxins, carcinogenic agents, infections, alcohol, and many other harmful substances. This will lead to liver diseases. Jaundice is often seen in liver disease such as hepatitis or liver cancer [2].

Hence, there is an ever increasing need for a safe hepatoprotective agent. Hepatoprotective activity of Arithiraadhi chooranam has not been scientifically evaluated. In this study, Arithiraadhi chooranam was screened for hepatoprotective activity in CCl_4 induced hepatotoxicity in albino rats.

2. MATERIALS AND METHODS

2.1 Drugs and chemicals

Analytical Laboratory grade chemicals, solvents used for the studies, which were procured from S.D. fine and span diagnostic Ltd. Silymarin and CCl_4 from Sigma-aldrich Chemical Pvt Ltd, Bangalore.

2.2 Preparation of Arithiraadhi chooranam

Ingredients for the preparation of Arithiraadhi chooranam are Curcuma longa, Terminalia chebula, Terminalia bellerica, Phyllanthus emblica, Picrorhiza kurao[3] and Indhupu[4] (Sodium chloride) purchased from TAMPCOL,

Arumbakkam, Chennai. After purification of all ingredients, each material dried and was powdered separately by grinding method. Then they were mixed and those powders were sieved by white cloth [5].

2.3 Animals

Swiss albino mice (25-30g) and male Wistar rats (150-200 g) were procured from an in-house animal facility of School of Pharmaceutical Sciences, Vels University, and Chennai. They were maintained under standard laboratory conditions and were fed with rat pellet feed ad libitum. The Institutional Animal Ethics Committee (IAEC) Approved the protocol (Approval number) XIII/ VELS/ PCOL/ 12/2000/ CPCSEA/ IAEC/ 08.08.2012).

2.4 Acute toxicity studies

Acute toxicity of Arithiraadhi chooranam was done according to the OECD guidelines 425[6]. The overnight fasted mice were given in various doses (2000, 1000, 500, 250, 100, 50mg/kg b.w.), and observed continuously for the first two and at 24hrs to detect changes in behavioral responses, toxic symptoms, and mortality.

2.5 Hepatoprotective activity against CCl_4 induced hepatotoxicity

Animals were divided into five groups, each group containing six animals.
Group I (Normal) – received 2% CMC for 14 days.
Group II (Control) – received CCl_4 1ml/kg, i.p. 1:1 dilution on the 5th day.
Group III and IV – received AC (250mg and 500mg/kg orally) with CCl_4 for 14 days.
Group V – received standard drug Silymarin 100mg/kg orally for 14 days.
After 14 days of experimental period blood sample had been collected individually for all the animals by retro-orbital puncture method and the blood was allowed to clot for 30 min; serum was separated by centrifuging and was used for various parameter estimations. Later all, animals were sacrificed by cervical dislocation, liver samples were collected and the individual weights of the livers were estimated. For the histopathological study, liver tissue was fixed in 10% farming in saline. Blood samples were collected and analysed for biochemical parameters like Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Alkaline phosphatase (ALP), Total Protein and Serum bilirubin.

3. STATISTICAL ANALYSIS

The data was represented as mean ± S.E.M. Results were analyzed statistically by one-way ANOVA followed by Dunnet's multiple comparison tests using Prism software (Version 4). The minimum level of significance was set at $P < 0.05$.

4. RESULTS AND DISCUSSION

4.1 Acute toxicity study

The acute toxicity study revealed the absence of lethality among the tested animals when the Arithiraadhi Chooranam was administered as a single dose up to 5000 mg/kg. There were no signs of any gross behavioral changes except for mild tremors indicating the safe use of the Arithiraadhi Chooranam.

4.2 Hepatoprotective activity

The ability of a hepatoprotective drug to reduce the injurious effects or to preserve the normal hepatic physiological mechanisms, which have been disturbed by a hepatotoxin, is the index of its protective effects. The lowering of enzymes level is a definite indication of hepatoprotective action of the Arithirathi Chooranam.
The result of Table no:1 showed that CCl_4 administration caused elevation of ALT, AST, ALP, Total protein, and bilirubin. After treating with Arithiraadhi Chooranam (250mg/kg and 500mg/kg) significantly decreased the level of ALT, AST, ALP, Total protein, and bilirubin. Both the test groups of Arithiraadhi Chooranam decreased CCl_4 induced elevated enzyme levels, indicating the protection of the structural integrity of hepatocytic cell membrane or regeneration of damaged liver cells.

Table-1: Effect of Arithirathi Chooranam on serum constituents in CCl_4 induced hepatotoxic rats**.**

Group	Treatment	Dose	AST(i.u/l)***	ALT(i.u/l)***	ALP(i.u/l)***	T.P(g/dl)***	Bilirubin (mg/dl)***
Normal	---		49.50±0.99	7.33±0.33	69.33±0.49	5.23±0.02	0.25±0.02
Control	CCl_4	(3ml/kg)	63.17±0.48	13.0±0.73	86.33±0.97	5.77±0.05	0.80±0.02
Test I	CCl_4+Arithiraadhi Chooranam	250mg/kg	55.0±0.82	8.50±0.21	78.83±0.79	5.40±0.04	0.45±0.04
Test II	CCl_4+Arithiraadhi Chooranam	500mg/kg	53.83±0.48	8.0 ± 0.37	76.50±0.73	5.30±0.04	0.42±0.03
Standard	CCl_4+Silymarin	50mg/kg	50.17±0.70	7.67±0.21	70.33±0.76	5.25±0.03	0.28±0.03

Values are as mean ± SEM (n=6) Values are statistically significant at ***P<0.001

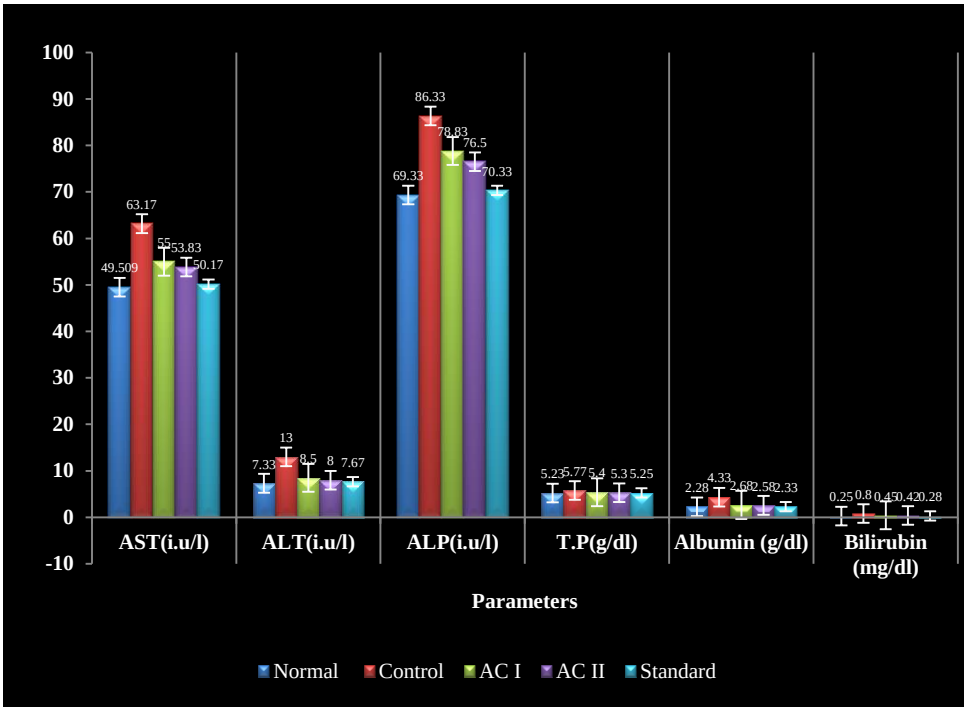

Chart -1: Effect of Arithiraadhi chooranam on serum constituents in CCl_4 induced hepatotoxic rats

5. CONCLUSION

Liver function test revealed the efficacy of the formulation Arithiraadhi chooranam as a hepatoprotective drug. Further studies can pave hope for a very standard drug for liver diseases.

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