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# Hepatoprotective activity of Sarakonrai poo (flower of *Cassia fistula*) chooranum against $CCl_4$ induced liver damage in rats

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## Abstract

Hepatoprotective activity of Sarakonrai Poo Chooranum (SPC) was studied against $CCl_4$ induced hepatic damage in wistar albino rats. The test animals were divided into five groups. Each group contains six animals. Group I set as control received distilled water, Group II set as hepatic injury induced group by $CCl_4$.Group III and IV were treatment groups received 250mg/kg and 500mg/kg of SPC and $CCl_4$ respectively. Group V served as standard, treated with 100mg/kg silymarin and $CCl_4$. After 16 days of treatment, hepatocyte damage can be detected by assessment of serum glutamate pyruvate transaminase (SGPT), serum glutamate oxaloacetae transaminase (SGOT), alkaline phospatase(ALP) , bilirubin and histology of liver. It was found that SPC at doses of 250mg/kg and 500mg/kg exhibited significant hepatoprotective effect by lowering the serum levels of SGPT, SGOT, ALP and bilirubin. Histology of liver of SPC treated group showed normal hepatocytes and more nuclei compared with $CCl_4$ treated group. Silymarin treated group also lowering the liver enzymes levels and normal histology of liver cells. The present study conclude Sarakonrai Poo Chooranum possess hepatoprotective action.

**Keywords:** Sarakonrai Poo Chooranum(SPC), Siddha Medicine, $CCl_4$ induced hepatic damage, Hepatoprotective action.

## Introduction

Liver is the chief site for metabolism and excretion. The major function of the liver is carbohydrate, protein, fat metabolism, and detoxification, secretion of bile and storage of vitamins. Liver is continuously and variedly exposed to environmental toxins and abused by poor drug habits and alcohol which can eventually lead to various liver disorders like hepatitis, cirrhosis and alcoholic liver diseases[(1)] . Medicinal plants are great source of natural compounds such as phenolic acids and flavonoids which have been shown antioxidant properties and effective pharmacological actions [(2)]. Administration of synthetic drugs for the treatment of liver disorder produces serious side effects. Now a day's medicines derived from the natural sources are important to treat diseases. Sarakonrai Poo (flower of *Cassia fistula*) Chooranum have the property to treat liver disorder mentioned in Siddha literature[(3)]. *Cassia fistula* flower contains secondary metabolites like kaempferol, leucopelargomidin, tetramer, rhein,

fistulin, alkaloids, triterpenes, proanthocynadines, phenolics and flavonoids[4]. The present study was under taken to investigate the effect of Sarakonrai poo chooranum against $CCl_4$ induced liver damage in rats.

## Aim and Objective

The present study was under taken to identify the effect of Sarakonrai Poo Chooranum on $CCl_4$ induced liver damage in rats.

## Materials and Methods

All the experiments and protocols described in the present study were approved by the Institutional animal ethical committee.

### Sarakonrai Poo Chooranum preparation:

Sarakonrai poo were collected from Vandalur forest, Chennai. The flowers are purified by removing the stalk, sepals, dust particles and dried under shade. Then Sarakonrai Poo Chooranum was prepared as per siddha literature [5]. The prepared chooranum was stored in a clean, dry, air tight glass container.

### Animals:

Wistar albino rats were used for the study. The animals were housed in groups of six and maintained under standard conditions (27 ± 2°C, relative humidity 44-56% and light and dark cycles of 10 and 14 hrs respectively), fed with standard rat diet and purified drinking water ad libidum one week before and during the experiment.

### Acute toxicity study:

Acute toxicity study of Sarakonrai Poo Chooranum was carried out as per OECD guideline 423 as a single dose administration of 1000, 2000 and 5000mg/kg respectively. Each group contains 6 animals (3 female and 3 male).

### Hepatoprotective activity of Sarakonrai Poo Chooranum:

### Drugs and chemicals:

Silymarin was a gift sample from Micro Laboratories, Hosur, India. Aspartate amino transferase, Alanine amino transferase and Alkaline phosphatase kits were from RANDOX Laboratories Ltd. All other chemicals and reagents used were of analytical grade.

### Treatment protocol:

The animals were divided into five groups of 6 animals each (male). Group I served as normal control and received distilled water at dosage 1ml/kg b.wt. and Group II received equal volume of $CCl_4$ 10% and olive oil at a dose of 1ml/kg once daily for 16 days. Group III received equal mixture of $CCl_4$ and olive oil and Sarakonrai Poo Chooranum 250mg/kg once daily for 16 days. Group IV received equal mixture of $CCl_4$ and olive oil and Sarakonrai Poo Chooranum 500mg/kg once daily for 16 days. Group V received equal mixture of $CCl_4$ and olive oil silymarin (100 mg/kg) once daily for 16 days.

### Biochemical estimation:

At the end of the treatment period, all the animals were anaesthetized by application of light ether and blood samples were collected from a group of animals from retro orbital plexus. Plasma and serum samples were separated kept at – 20˚C for biochemical analysis. The activities of serum hepatic marker enzymes SGOT, SGPT and ALP, total bilirubin and direct bilirubin were analyzed using commercial kits.

### Histopathological studies:

After treatment, liver of all animals from each respective groups were dissected out and a portion of liver tissue section of nearly 5 µm thickness were fixed in Bowin's fixative, dehydrated by varying percentage of ethanol and stained with haemotoxylin and eosin. Microscopic evaluation of the thin section was undertaken and variations in histo architecture were photographed at a magnification of 10x.

## Statistical analysis:

The values were expressed as mean ± SEM. The statistical analysis was carried out by one way analysis of variance (ANOVA) followed by Dunnet's 't' - test. P values <0.05 were considered significant.

## Results

The acute toxicity study revealed the absence of lethality among the tested animals when the Sarakonrai Poo Chooranam was administered as a single dose (1000, 2000 and 5000mg/kg). There were no signs of any gross behavioral changes except tremor indicating the safe use of the Sarakonrai Poo Chooranam.

The results of hepatoprotective activity of Sarakonrai Poo Chooranum on $CCl_4$ treated rats are shown in Table. No: I. The liver enzymes SGOT, SGPT and ALP, total and direct bilirubin levels in serum significantly increased in $CCl_4$ treated animals when compare with control. The Sarakonrai Poo Chooranum treatment groups (250mg/kg and 500 mg/kg) significantly (P<0.01) reversed the level of liver enzymes and bilirubin when compared to $CCl_4$ treated animals. Silymarin treated animals also showed significantly (P<0.01) reverted the levels of hepatic enzymes when compared with $CCl_4$ treated animals.

**Table no: I. Effect of Saarakonrai Poo Chooranum on serum SGOT, SGPT, ALP and bilirubin in $CCl_4$ induced hepato toxicity in rats after 16 days treatment**

| Group | SGOT (U/L) | SGPT (U/L) | ALP (mg/dl) | T. Bilirubin (mg/dl) | D.Bilirubin (mg/dl) |
|---|---|---|---|---|---|
| Group I | 128.4±0.56$^{b}$ | 66.2±0.43$^{b}$ | 202.9±0.17$^{b}$ | 0.74±0.17$^{b}$ | 0.188±0.003$^{b}$ |
| Group II | 277.1±0.45$^{**}$ | 169.5±0.45$^{**}$ | 758.1±0.50$^{**}$ | 8.11±0.24$^{**}$ | 2.840±0.004$^{**}$ |
| Group III | 220.6±1.15$^{**,b}$ | 156.1±0.60$^{**,b}$ | 634.8±0.47$^{**,b}$ | 5.22±0.51$^{**,b}$ | 0.565±0.002$^{**,b}$ |
| Group IV | 188.7±0.44$^{**,b}$ | 124.2±1.27$^{**,b}$ | 600.2±0.45$^{**,b}$ | 4.00±0.18$^{**,b}$ | 0.333±0.002$^{**,b}$ |
| Group V | 175.4±0.38$^{**,b}$ | 80.7±0.48$^{**,b}$ | 408.3±1.31$^{**,b}$ | 3.15±0.12$^{**,b}$ | 0.196±0.002$^{b}$ |

Values are as mean ± SEM (n=6)

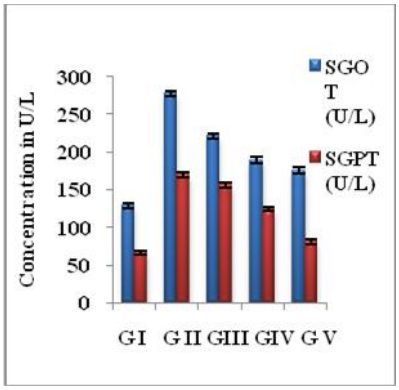
Values are statistically significant at *P<0.05, **P<0.01, ***P<0.001; Comparison made between Group II Vs Group I

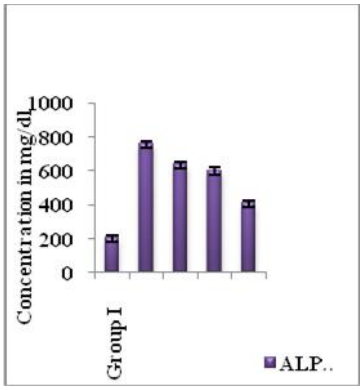
$^{a}$P<0.001, $^{b}$P<0.01, $^{c}$P<0.05 compared between Group III, IV, V Vs Group I.

Hepatocytes of the normal group (Group I) showed a normal lobular architecture of the liver (Fig.IV). A comparison of the liver section of animals treated with $CCl_4$ (Group II) showed the normal liver architecture was disturbed by hepatotoxin intoxication characterized by cell vacuolation, pyknotic and degenerated nuclei and wall of bile capillaries. The intralobular vein was badly damaged with wide spaces at some sinusoids (Fig:V). Sarakonraipoo Chooranam (250and 500 mg/kg b.w.) + $CCl_4$, livers showed (Fig:VI & VII) the nuclei are not very clear as in normal hepatocytes; however, when compared to the $CCl_4$ damaged group, the number of hepatocytes with normal nucleus was much more. The endothelium is disrupted in places. Pyknotic nucleus and vacuolation in cytoplasm are observed to be low, as compared to the $CCl_4$ group. Silymarin treated group (Fig:VIII) showed normal hepatocytes and their lobular architecture was normal.

**Fig: I, II & III: Effect of Saarakonrai Poo Chooranum on serum SGOT, SGPT, ALP and bilirubin in $CCl_4$ induced hepato toxicity in rats after 16 days treatment**

**Figure: I** **Figure: II** **Figure: III**



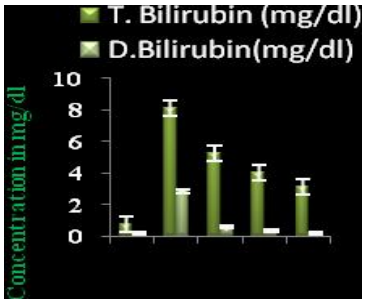




**Fig: IV to VIII: Stained microscopic images of liver tissues in the groups under study**

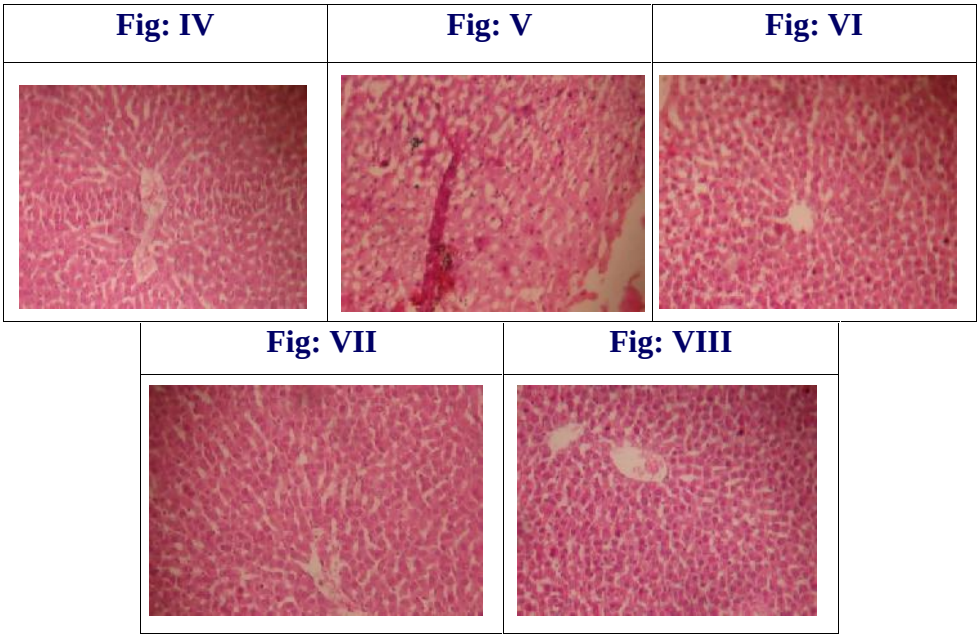



Fig:IV- Distilled water, Fig:V- hepatotoxic induced by CCl4 + olive oil, Fig: VI - $CCl_4$+ olive oil + SPC 250mg/kg.b.wt. , Fig: VII-: $CCl_4$+ olive oil + SPC 500mg/kg. b.wt and Fig: VIII- CCl4 + olive oil + Silymarin.

## Discussion

Hepatoprotective activity of Sarakonrai Poo Chooranum was examined against $CCl_4$ induced hepatic damage in rats. The extent of hepatic damage is assessed by the level of various biochemical parameters in circulation and histological evaluation of liver. The assessment of liver function can be made by estimating the serum enzymes such as SGOT, SGPT and ALP and bilirubin. Increase the level of these enzymes indicates liver pathology [6]. A number of reports indicates that overdose of carbon tetrachloride can produce centrizonal hemorrhagic hepatic necrosis in humans and experimental animals. Carbon tetrachloride -induced hepatic injury is commonly used as an experimental method for the study of hepatoprotective effects of medicinal plants

extracts and drugs [7]. Carbon tetrachloride is metabolically activated by cytochrome P450 in the endoplasmic reticulum to form a trichloro methyl free radical. It combines with cellular lipids and proteins in the presence of oxygen to induce lipid peroxidation, which leads to change in the structures of endoplasmic reticulum and other memberane, loss of metabolic enzyme activation, reduction in protein synthesis and elevation of serum transaminases leading to liver damage[8] .

Sarkonrai Poo Chooranum exhibited dose dependent (250 mg/kg and 500 mg/kg) hepato protective effect against $CCl_4$ induced hepatic damage in rats( Table no: I & Fig. I to VIII). Amino transferases contribute a group of enzymes that catalyze the interconversion of amino acids and α-keto acids by the transfer of amino groups. These are liver specific enzymes and are considered to be very sensitive and reliable indices for necessary hepatotoxic as well as hepatoprotective or curative effect. Both SGOT and SGPT levels increase due to toxic compounds affecting the integrity of the liver cells [9]. Alkaline phosphatase is a membrane bound glycoprotein. This enzyme reaches the liver mainly from the bone. It is excreted into the bile. ALP elevation in serum occurs in hepatobiliary diseases [10]. Reduction in the levels of SGOT, SGPT and ALP in Sarakonrai Poo Chooranam treated groups (Table.No: I) indicates the prevention of leakage of intracellular enzymes by its membrane stabilizing activity. Silymarin treated group (Table.No: I) also exhibited protective effect against CCl4 induced hepatic damage. Histology results revealed (Fig: VI and VII) regeneration of liver tissues due to the treatment of Sarkonrai Poo Chooranum. Silymarin treated group showed (Fig: VIII) normal liver histology.

## Conclusion

The present study demonstrates Sarakonrai Poo Chooranum have significant dose dependant hepatoprotective activity against liver injury induced by $CCl_4$. It can be concluded that the Sarakonrai Poo Chooranam is proved to be one of the useful Siddha drug for liver disorder.

**Conflict of interest:** None

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