



Hepatoprotective Activity of Kadukkai Maathirai (A Siddha Polyherbal Formulation) Against Carbon Tetrachloride Induced Liver Damage in Rat

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Abstract

Kadukkai maathirai (KM) is a polyherbal formulation used in traditional Siddha Medicine for the treatment of liver diseases and iron deficiency anemia. This study was aimed to create preclinical scientific evidence on hepatoprotective activity of KM. Five groups, with six rats in each group were used in this study. Group 1 (normal rats) did not receive any drug. Group 2, 3, 4 and 5 received carbon tetrachloride (CCl₄) 1mg/kg twice a week (on third day and seventh days of each week) for four weeks to induce hepatic damage. Group 2 (vehicle control) received only vehicle. Group 3 and group 4 were treated with two doses of KM, 36mg/kg and 72mg/kg respectively for 28 days. Group 5 (standard) received silymarin 25mg/Kg for 28 days. On 29th day, blood was collected for estimation of aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP) and total bilirubin (TB). Liver was collected for histopathological examination. Group 2 showed significant ($P < 0.001$) increase in levels of AST, ALT, ALP and TB compared to group 1 indicating the liver damage caused by CCl₄. KM treated Group 3 and group 4 showed a dose dependent reduction of all enzyme levels. Group 3 and group 4 showed significant ($P < 0.001$) reduction in AST, ALT, ALP and TB levels when compared to vehicle control indicate the recovery of hepatic cells. Histology examination revealed that KM treatment reduced the hepatocellular necrosis in a dose dependent manner. Our study demonstrated the dose dependent hepatoprotective activity of KM against CCl₄ induced liver injury. However, future study will be directed on molecular mechanism of individual ingredients, toxicity profile and clinical studies in various liver diseases.

Keywords: Siddha medicine, Ayurveda, hepatoprotective, jaundice, iron deficiency anaemia.

Introduction

Global health system is stuck at a bottleneck regarding equity of providing modern medicine among the world population. Despite availability of resources, newer innovative drug discoveries are not pouring in at the expected rates or paradigms with benefit of cost ratio. Conventional drug discovery from traditional medical knowledge has been overtaken by modern approaches, but the outcome is not as per the expectation. The situation might need rejuvenation in Traditional Medicine (TM) since people in developing countries, particularly in rural areas, strongly depend on their own traditional medical system as a primary or major health care provider. For example, the usage of TM is estimated as 90% in Ethiopia, 70% in Benin, India, Rwanda and Tanzania. In such situation, modern medicine is possibly preferred as an alternative need¹.

This is the new age where modern medicine and TM will blend into a holistic synergy in tune with dynamical complex systems to provide affordable, available and accessible health to every citizen of global human society. Broadly, the deficiencies in TM can be categorized as; lack of validation, lack of standardization, lack of delivery infrastructure, lack of integration-intra or interdisciplinary, lack of professional training and lack of

government policies². These situations lead to make new approaches such as Reverse Pharmacology which provides innovative opportunities that is based on experimental wisdom and holistic viewpoint of TM. At times, the experimental results in TM could become innovative original idea to the modern medicine drug discovery. Thus, scientific information based on research at all levels of preclinical and clinical stages should be strengthened in TM.

Siddha medicine is one of the ancient medical systems which is practiced in India, Srilanka, Malaysia and other Tamil speaking countries. Kadukkai maathirai (KM) is a polyherbal formulation consists of *Terminalia chebula*, *Piper nigrum*, *Eclipta alba*, *Citrus limon* and ferrous sulphate. This drug is indicated for liver diseases, anaemia and jaundice³. Although this drug is claimed as a hepatoprotective agent, there is no supportive scientific data available. In order to create scientific evidence for the traditional medicine, this study was conducted.

The aim of the present study was to evaluate the protective effect of Kadukkai maathirai against CCl₄ induced liver damage in rats.

Material and Methods

Drug: Kadukkai maathirai (KM) was prepared with strict adherence to the traditional methods mentioned in the Siddha Hospital Pharmacopeia – Government of Tamil Nadu, India. Ferrous sulphate was soaked in lemon juice (*Citrus limon*) for 24h and dried. After that, dried ferrous sulphate, epicarp of *Terminalia chebula* and *Piper nigrum* seed were ground with *Eclipta alba* leaf juice for three hours. Finally, the tablets were made by compression method which was known as Kadukkai maathirai⁴.

Animals: Wistar albino rats of either sex (150 – 200 g) were obtained from the inbred colony of department of Pharmacology, A.J College of Pharmacy, Chennai. The animals were kept in polypropylene cages at 25 ± 2°C with relative humidity 45 - 55% under 12 h light and 12 h dark cycles. They were fed with standard laboratory animal feed (Poultry Research Station, Tamil Nadu Veterinary and Animal Sciences University, Chennai, India) and water *ad libitum* throughout the study. The experimental protocol was approved (IAEC - AJ/IAEC/11/42) by the Institutional ethics committee.

Acute toxicity study: Acute oral toxicity study was performed as per OECD guideline number 423 (acute toxic class method)⁵. Female Wistar rats (n = 3) were kept fasting for overnight providing only water, after which the Kadukkai maathirai dissolved in normal saline was administered orally at the dose level of 5 mg/kg body weight by intragastric tube. The toxic symptoms and death of animal were observed for 14 days. Since no toxic symptom was observed in all three animals, 50mg/kg dose was administered in another three rats. Similarly, the procedure was repeated for further with higher doses such as 300 and 2000 mg / kg body weight. Even at 2000 mg/kg dose, there was no toxic symptom observed.

Since KM is administered in human being in the dose of 400mg/day in two divided doses, the corresponding dose for rat was calculated based on body surface area. The corresponding dose (36 mg/kg) and double (72 mg/kg) the dose were chosen for the pharmacological study in rats.

Hepatoprotective activity: Five groups with six rats in each group were used in this study. Group 1 (normal rats) did not receive any drug. Liver damage was induced in group 2, 3, 4 and 5 by injecting subcutaneous carbon tetrachloride (CCl₄) 1ml/kg twice a week (on third day and seventh days of each week) for four weeks⁶. Group 2 (vehicle control) received only normal saline. Group 3 and group 4 were treated with two doses of KM, 36mg/kg and 72mg/kg respectively for 28 days. Group 5 (standard) received silymarin 25 mg/Kg for 28 days. On 29th day, blood was collected by retro-orbital puncture and kept one hour at room temperature, then centrifuged at 3000 rpm for 10 minutes to obtain the serum. Biochemical estimation of aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP) and total bilirubin (TB) were done by standard procedures^{7,8}.

Liver tissue was collected and fixed in 10% phosphate buffered formaldehyde solution. After embedding in paraffin wax, thin sections of 5 µm thickness were cut and stained with hematoxylin-eosin.

Statistics: Results were expressed in mean ± standard deviation. The data was analyzed by one-way ANOVA followed by Tukey test using SPSS 11.5. A p value of < 0.05 was considered as significant.

Results and Discussion

Biochemical parameters: The biochemical parameters showed a significant (p < 0.001) rise in the enzyme levels due to CCl₄ administration in vehicle group compared to normal rats indicate the liver tissue damage. The KM treatment showed a dose dependent reduction in AST, ALT, ALP and total bilirubin. Two doses of KM and standard drug treatment significantly reduced the level of AST, ALT, ALP and total bilirubin (p < 0.001). Since there was no significant difference in the biochemical parameters between the standard treatment and KM treatment, the effect of KM to reverse the hepatic function could be comparable with standard treatment (table-1).

Table-1
Estimation of serum enzymes after different drug treatments
in carbon tetrachloride induced liver damaged rats

Parameter, unit	Group 1 (normal rats)	Liver damage was induced by CCl ₄			
		Group 2 (Vehicle control)	Group 3 (KM, 36mg/kg)	Group 4 (KM, 72mg/kg)	Group 5 (Silymarin 25 mg/Kg)
AST (Aspartate transaminase), IU/L	41.13 ± 0.92	88.35 ± 0.52*	62.0 ± 0.92 [§]	54.52 ± 1.59 [§]	48.43 ± 1.52 [§]
ALT (Alanine transaminase), IU/L	33.09 ± 1.42	79.24 ± 1.25*	60.5 ± 1.59 [§]	51.46 ± 2.16 [§]	45.3 ± 2.416 [§]
AST : ALT ratio	0.01	1.11	1.02	1.06	1.07
ALP (Alkaline phosphatase), KA unit	130.26 ± 8.51	251.32 ± 8.89*	205.51 ± 9.32 [§]	185.59 ± 6.34 [§]	50.56 ± 8.316 [§]
Total bilirubin, mg/dl	0.48 ± 0.9	2.05 ± 0.12*	0.82 ± 0.29 [§]	0.71 ± 0.02 [§]	0.57 ± 0.01 [§]

Values are expressed in Mean ± SD (n=6), KM – Kadukkai maathirai, *p < 0.001 vs. normal rats, [§]p < 0.001 vs. vehicle control group

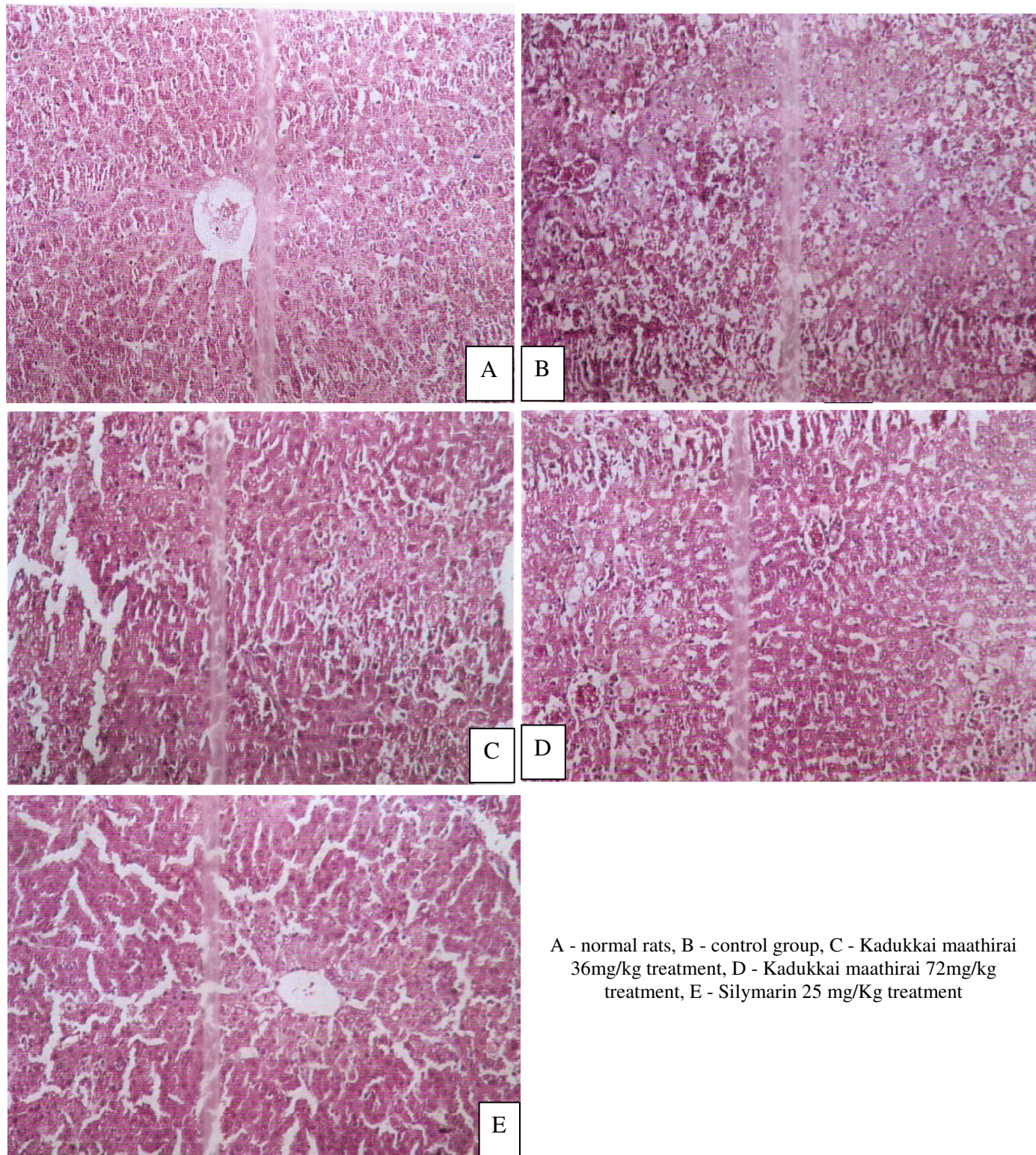


Figure-1
Histopathological appearance of liver after different drug treatments
in carbon tetrachloride induced liver damaged rats (Hematoxylin & Eosin staining)

Carbon tetrachloride-induced hepatic injury is commonly used as an experimental method for the study of hepatoprotective effect of drugs or medicinal plant extracts. Toxicity begins with the change in endoplasmic reticulum, which results in the loss of metabolic enzymes located in the intracellular structures⁹. The toxic metabolite trichloromethyl radical (CCl₃) is produced by microsomal oxidase system binds covalently to the macromolecule and causes peroxidative degradation of lipid membranes. CCl₄ produces an experimental damage that histologically resembles viral hepatitis in which liver necrosis is evident¹⁰.

Elevated serum transaminases (AST and ALT) indicate the liver necrosis. Clinically, these enzyme levels are elevated in acute hepatitis, chronic hepatitis, chronic alcoholic hepatitis, diffuse intrahepatic cholestasis, extra hepatic obstruction and focal intrahepatic disease. AST: ALT ratio of 1 to 2 indicates the clinical picture of chronic alcoholic liver disease¹¹. Serum level of transaminases returns to normal once the healing of hepatic parenchyma and regeneration of hepatocytes occurs¹². Our finding suggests the ability of KM to regenerate hepatocytes after CCl₄ intoxication.

Alkaline phosphatase (ALP) is the prototype enzyme that reflects the pathological alteration in biliary flow¹³. Clinically, this enzyme indicates diffuse intrahepatic cholestasis, extra hepatic obstruction and focal intrahepatic disease. CCl₄ induced elevation of this enzyme in the serum is in line with high level of serum bilirubin content. Elevated total bilirubin is a sign for acute hepatitis, chronic hepatitis, chronic alcoholic hepatitis, diffuse intrahepatic cholestasis and extra hepatic obstruction cholestasis¹¹. The KM mediated reduction in the increased serum ALP level with the concurrent depletion of raised bilirubin suggests the possibility of the test drug to stabilize biliary dysfunction in rat liver during hepatic injury. The KM reversed the elevated levels of transaminases, ALP and total bilirubin which indicate the healing of damaged hepatic cells. Biochemical studies revealed that hepatic damage due to CCl₄ was recovering after KM treatment which supports the claim of hepatoprotective activity of KM.

Histopathology: Group 1 (normal rats) animals showed a normal hepatic architecture, where the central vein surrounded by cords of hepatocytes, portal tracts and sinusoids appear normal (figure 1.A). In group 2 (control group), the normal liver architecture was disturbed by CCl₄ intoxication. It showed the damaged liver cells with extensive necrosis and fatty changes (Figure 1.B). The liver section of rats treated with KM and silymarin after CCl₄ intoxication showed cellular regeneration that is the sign of hepatoprotection as it was evident by the reduction in necrosis and vacuoles (figure 1.C, D and E).

The hepatoprotective activity of *Terminalia chebula*¹⁴, *Piper nigrum*^{15,16}, *Eclipta alba*^{17,18,19,20} and *Citrus limon*²¹ were proved in different hepatotoxic agents induced liver damage in various animal studies. No wonder being these herbals as ingredients of KM for the management of liver diseases. The combination

might be having certain scientific advantage through exhibiting synergistic pharmacological action.

Ferrous sulphate in this formulation has a specific role to do. Liver diseases are frequently associated with hematological abnormalities. Anaemia of diverse etiology occurs in about 75% of patients with chronic liver disease. In patient with alcoholic liver disease, different effects of alcohol also contribute anemia such as malabsorption, malnutrition or direct toxic effect. Anaemia might be caused secondary to hepatic infections or side effects of antiviral drugs used in the treatment of viral hepatitis. Thus anaemia of different pathogenesis should be also considered while treating liver diseases²². Ferrous sulphate is one of the ingredients of KM and the previous study showed the presence of 18.03% iron in KM³, which is a good iron supplement during the KM administration for liver diseases. By having lemon juice as one of the ingredient, a rich source of ascorbic acid, it might contribute for higher oral iron absorption²³ from KM. Iron preparations causes constipation in 60% of cases which often associated with poor patient compliance²⁴.

In addition to hepatoprotective activity, *Terminalia chebula* also acts as a prokinetic agent and in one animal study, it increased gastric emptying²⁵. Previous clinical study on triphala (in which *Terminalia chebula* is one among three ingredient) in patients with constipated bowel habit showed a good laxative effect²⁶. This activity could alleviate the constipation caused by iron content of KM. Thus, KM could be considered as a balanced polyherbal formulation for liver diseases associated with iron deficiency where iron needs to be orally administered. The KM could be a better choice for the management of chronic liver diseases of various etiology, since its multiple target pharmacotherapy in single formulation.

In this study, only two doses of KM were used, so that the maximum efficacy of KM could not be evaluated, which is the limitation of this study. The possibilities of using KM in pregnant women as an iron supplement, in iron deficiency anemia, in liver diseases and as adjuvant along with antiviral drugs, anti-cancer drugs and other hepatotoxic drugs have to be studied in detail.

Conclusion

Our study showed the hepatoprotective activity of Kadukkai maathirai against carbon tetrachloride induced liver damage in rats which provides a valuable scientific evidence for the practicing of kadukkai maathirai in the treatment of liver diseases and iron deficiency anaemia by Traditional Siddha physicians. However, further scientific studies on the dose which produces maximum therapeutic efficacy with tolerable adverse effect, molecular mechanism of individual ingredients, toxicity profile and clinical studies on different liver diseases should be carried out to strengthen the evidence for kadukkai maathirai in the treatment of liver diseases and iron deficiency anemia.

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