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Preliminary evaluation of the antihepatotoxic activity of Kamilari, a polyherbal formulation

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Abstract

Hepatoprotective efficacy of Kamilari, a polyherbal preparation was studied in carbon tetrachloride (CCl_4)-induced liver dysfunction in albino rats by determining different biochemical parameters in serum and tissues. In serum, the activities of enzymes like aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and the concentrations of protein and bilirubin were evaluated. The concentrations of total lipids, cholesterol, triglycerides (TG) and phospholipids were studied in serum and different tissues. Here, a dose-dependent study was conducted and oral administration of Kamilari at a dose of 750 mg/kg body weight significantly reduced the toxic effects of CCl_4. From the observations, the conclusion drawn is that Kamilari stabilized the hepatic frame against the toxicity of CCl_4.

Keywords: Carbon tetrachloride; Hepatoprotection; Herbal formulation; Medicinal plant; Rats

1. Introduction

Liver disease is a worldwide problem. Liver is an organ of paramount importance as it plays an essential role in maintaining the biological equilibrium of vertebrates. The spectrum of its functions include: metabolism and disposition of chemicals (xenobiotics) to which the organ is exposed directly or indirectly; metabolism of lipids, carbohydrates and proteins; blood coagulation and immunomodulation.

Conventional or synthetic drugs used in the treatment of liver diseases are sometimes inadequate and can have serious adverse effects. So there is a worldwide trend to go back to traditional medicinal plants. Many natural products of herbal origin are in use for the treatment of liver ailments (Venkateswaran et al., 1997; Latha et al., 1999; Mitra et al., 2000; Dhuley and Naik, 1997).

In the present investigation Kamilari, a polyherbal formulation consisting of medicinal plants derived from the traditional system of medicine in India, Ayurveda, has been evaluated for its hepatoprotective action. The hepatotoxin used was CCl_4 because CCl_4-induced liver dysfunction in rats simulates liver cirrhosis in man (Pérez-Tamayo, 1983; Wensing et al., 1990). The herbal formulation consists of a mixture of dried powders of the following medicinal plants (Table 1).

2. Materials and methods

2.1. Animals

Male albino rats of Sprague-Dawley strain weighing 120–150 g were used for the study. They were housed in polypropylene cages under standard conditions (23 ± 2 °C, humidity 60–70%, 12 h light/dark cycles). They were given standard pellet diet (M/s Hindustan Lever Ltd., Bombay, India) and water ad libitum.

2.2. Drug treatment and experimental design

Kamilari tablets (M/s Nupal Remedies Private Limited, Cochin, Kerala, India) were received as gift from the manufacturers.

The following groups of animals were studied:

Group 1	Pairfed control
Group 2	Received CCl_4
Group 3	Received CCl_4 + Kamilari at a dose of 750 mg/kg body weight
Group 4	Received CCl_4 + Kamilari at a dose of 1000 mg/kg body weight

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Table 1
Composition and concentration of Kamilari

Sample number	Botanical name	Family	Part used	Concentration (mg%)
1	*Berberis aristata*	Berberidaceae	Leaf	10
2	*Cordia myxa*	Boranginaceae	Fruit	10
3	*Curculigo orchioides*	Hypoxidaceae	Stem	15
4	*Elettaria cardamomum*	Zingiberaceae	Seed	10
5	*Glycyrrhiza glabra*	Fabaceae	Root	15
6	*Piper longum*	Piperaceae	Fruit	10
7	*Thespesia populnea*	Malvaceae	Leaf	20
8	*Zingiber officinale*	Zingiberaceae	Rhizome	10

Group 5 Received CCl_4 + Kamilari at a dose of 2000 mg/kg body weight

Group 6 Received CCl_4 + Kamilari at a dose of 3000 mg/kg body weight

The rats of all groups except group 1 received CCl_4 at a dose of 0.1 ml of CCl_4 in groundnut oil (1:1, v/v) per 100 g body weight through an intragastric tube twice a week for a period of two months. The herbal formulation was suspended in distilled water and given orally through an intragastric tube daily in the morning for two months.

2.3. Collection of serum and tissue samples

At the end of the experimental period, rats were deprived of food overnight and sacrificed by decapitation. Blood was collected by excising the jugular vein. It was allowed to clot and then centrifuged at 3000 rpm for 15 min. The serum samples were collected and left standing on ice until required. Tissues (liver and kidney) were excised and transferred into ice cold containers for biochemical estimations.

2.4. Biochemical evaluation

Activities of serum enzymes such as AST, ALT (Mohun and Cook, 1957) and ALP (Kind and King, 1954) were determined in serum. The protein content of serum was determined according to the procedure described by Lowry et al. (1951). The concentration of serum bilirubin was also estimated (Malloy and Evelyn, 1937).

The concentrations of total lipids (Frings and Dunn, 1970), phospholipids (Varley, 1988) cholesterol (Zlatkis et al., 1953) and triglycerides (Van Handel and Zilversmit, 1957) were determined in serum and tissues.

2.5. Statistical analysis

The difference among means has been analysed by Student's *t*-test. Results of biochemical estimations are expressed as mean ± S.E.M.

3. Results

Rats administered CCl_4 for two months showed lower weight gain (36.6 ± 2 g) compared to that of the pairfed control rats (80.0 ± 3.5 g). Co-administration of the herbal preparation resulted in normal increase in their body weight compared to CCl_4-treated group (60.8 ± 2.3 g).

Rats treated with CCl_4 developed significant liver damage as observed from elevated serum levels of hepatospecific enzymes as well as severe alterations in other biochemical parameters (Tables 2 and 3). Activities of AST, ALT and

Table 2
Effect of Kamilari on the activities of enzymes and the concentrations of bilirubin and protein in serum

Group	AST (IU/l)	ALT (IU/l)	ALP (IU/l)	Bilirubin (mg/dl)	Protein (g/dl)
(1) Pairfed control	17.37 ± 0.43	26.39 ± 0.66	93.19 ± 2.35	1.50 ± 0.04	5.15 ± 0.13
(2) CCl_4-treated	28.48 ± 1.05*	66.67 ± 2.33*	381.63 ± 13.85*	1.97 ± 0.06*	4.01 ± 0.14*
(3) CCl_4 + Kamilari (750 mg/kg)	17.42 ± 0.78†††††	27.16 ± 1.21†††††	119.82 ± 5.39†††††	1.56 ± 0.07††††	5.12 ± 0.23††††
(4) CCl_4 + Kamilari (1000 mg/kg)	20.70 ± 0.80†††††	48.41 ± 1.98†††††	210.52 ± 8.42†††††	1.64 ± 0.07†††	4.80 ± 0.22†††
(5) CCl_4 + Kamilari (2000 mg/kg)	25.69 ± 0.90†	57.63 ± 2.25††	240.34 ± 10.09†††	1.65 ± 0.08†††	4.55 ± 0.21††
(6) CCl_4 + Kamilari (3000 mg/kg)	26.40 ± 0.91†	63.32 ± 2.53†	320.81 ± 12.83†††	1.69 ± 0.08†††	4.26 ± 0.19†

Values are mean ± S.E.M. of six animals in each group.
* $P < 0.01$ group 2 compared with group 1.
† $P > 0.10$ drug treated groups compared with group 2.
†† $P < 0.10$ drug treated groups compared with group 2.
††† $P < 0.05$ drug treated groups compared with group 2.
†††† $P < 0.02$ drug treated groups compared with group 2.
††††† $P < 0.01$ drug treated groups compared with group 2.

Table 3
Effect of Kamilari on the concentrations of total lipids, phospholipids, triglycerides and cholesterol in serum

Group	Total lipids (mg/dl)	Phospholipids (mg/dl)	Triglycerides (mg/dl)	Cholesterol (mg/dl)
(1) Pairfed control	220.54 ± 5.50	142.81 ± 3.57	7.80 ± 0.19	63.72 ± 1.62
(2) CCl_4-treated	294.04 ± 10.42*	178.31 ± 6.24*	9.85 ± 0.37*	109.52 ± 3.83*
(3) CCl_4 + Kamilari (750 mg/kg)	234.01 ± 10.12†††	145.97 ± 6.36††	8.01 ± 0.34††	71.65 ± 3.07††††
(4) CCl_4 + Kamilari (1000 mg/kg)	248.84 ± 10.45††	155.63 ± 6.94†	8.66 ± 0.36†	81.33 ± 3.62††††
(5) CCl_4 + Kamilari (2000 mg/kg)	252.88 ± 11.76†	163.28 ± 7.60†	8.95 ± 0.39†	87.52 ± 4.03†††
(6) CCl_4 + Kamilari (3000 mg/kg)	270.52 ± 12.12†	170.62 ± 7.26†	9.28 ± 0.43†	95.02 ± 4.26†

Values are mean ± S.E.M. of six animals in each group.
* $P < 0.01$ group 2 compared with group 1.
† $P < 0.1$ drug treated groups compared with group 2.
†† $P < 0.05$ drug treated groups compared with group 2.
††† $P < 0.02$ drug treated groups compared with group 2.
†††† $P < 0.01$ drug treated groups compared with group 2.

Table 4
Effect of Kamilari on the concentrations of total lipids, phospholipids, triglycerides and cholesterol in liver

Group	Total lipids (mg/dl)	Phospholipids (mg/dl)	Triglycerides (mg/dl)	Cholesterol (mg/dl)
(1) Pairfed control	4576.41 ± 113.93	2347.28 ± 59.22	508.39 ± 13.44	589.27 ± 14.81
(2) CCl_4-treated	5898.72 ± 204.37*	3027.91 ± 103.65*	621.93 ± 21.84*	796.10 ± 77.58*
(3) CCl_4 + Kamilari (750 mg/kg)	4677.36 ± 202.06††††	2254.71 ± 97.52††††	523.63 ± 23.88†††	627.66 ± 26.99††††
(4) CCl_4 + Kamilari (1000 mg/kg)	4927.81 ± 217.41††††	2150.31 ± 90.59†††	544.29 ± 23.46††	643.87 ± 28.33††††
(5) CCl_4 + Kamilari (2000 mg/kg)	5233.42 ± 220.33†	2022.06 ± 87.25†††	556.44 ± 24.66†	676.42 ± 28.41†††
(6) CCl_4 + Kamilari (3000 mg/kg)	5351.28 ± 227.43†	1948.36 ± 84.75††	566.56 ± 24.14†	689.83 ± 31.04†††

Values are mean ± S.E.M. of six animals in each group.
* $P < 0.01$ group 2 compared with group 1.
† $P > 0.10$ drug treated groups compared with group 2.
†† $P < 0.05$ drug treated groups compared with group 2.
††† $P < 0.02$ drug treated groups compared with group 2.
†††† $P < 0.01$ drug treated groups compared with group 2.

ALP in serum were increased in CCl_4-intoxicated rats. A marked elevation in the concentration of bilirubin, total lipids, phospholipids, triglycerides and decrease in protein content in serum were observed in the hepatotoxin-treated rats. Treatment with Kamilari showed a significant protection against CCl_4-induced alterations in the serum enzyme levels, protein, bilirubin and lipid profile. The degree of protection was observed maximally with the lowest dose of the herbal preparation (i.e., 750 mg/kg body weight).

Tables 4 and 5 depict the concentration of total lipids, cholesterol, phospholipids and triglycerides in liver and kidney respectively. Significant enhancement in the concentrations of total lipids, cholesterol and triglycerides was observed in the tissues of group 2 rats which received CCl_4-alone. Remarkable increase in the concentration of phospholipids was noticed in the liver of group 2 rats. However, the concentration of phospholipids in kidney was found to decrease in CCl_4-treated rats. The altered biochemical

Table 5
Effect of Kamilari on the concentrations of total lipids, phospholipids, triglycerides and cholesterol in kidney

Group	Total lipids (mg/dl)	Phospholipids (mg/dl)	Triglycerides (mg/dl)	Cholesterol (mg/dl)
(1) Pairfed control	4685.28 ± 124.53	2893.17 ± 72.73	285.48 ± 7.31	589.91 ± 17.40
(2) CCl_4-treated	6207.98 ± 217.27*	2160.62 ± 72.59*	397.39 ± 14.10*	790.48 ± 28.92*
(3) CCl_4 + Kamilari (750 mg/kg)	4750.76 ± 223.82†††	2771.94 ± 127.79††††	295.16 ± 12.54††††	605.73 ± 27.26††††
(4) CCl_4 + Kamilari (1000 mg/kg)	5012.61 ± 225.02†††	2698.69 ± 120.71†††	313.44 ± 13.98†††	633.03 ± 28.35†††
(5) CCl_4 + Kamilari (2000 mg/kg)	5292.46 ± 228.91††	2534.40 ± 114.00††	327.89 ± 14.42††	661.51 ± 29.90††
(6) CCl_4 + Kamilari (3000 mg/kg)	5573.23 ± 249.12†	2462.50 ± 110.81†	333.12 ± 14.04††	668.04 ± 30.46††

Values are mean ± S.E.M. of six animals in each group.
* $P < 0.01$ group 2 compared with group 1.
† $P < 0.10$ drug treated groups compared with group 2.
†† $P < 0.05$ drug treated groups compared with group 2.
††† $P < 0.02$ drug treated groups compared with group 2.
†††† $P < 0.01$ drug treated groups compared with group 2.

parameters in different tissues were significantly brought towards normalization by co-administration of Kamilari. The maximum protection against hepatic damage was achieved with the lowest dose of the drug (i.e., 750 mg/kg body weight).

4. Discussion

In order to efficiently metabolize drugs, during the process of evolution, the liver has developed "drug metabolizing enzymes" which are different from the enzymes of intermediate metabolism (Rao, 1973). Most of these enzymes are largely located in the hepatic microsomes. Biotransformation of a drug or xenobiotic compound following its exposure can alter its distribution and action leading to its detoxification and excretion or enhance its toxicity due to the activation of the compound or due to the biochemical disruption caused by reactive metabolites arising from biotransformation (Athar et al., 1997; Plaa, 1991). Biotransformation of xenobiotics usually occurs in two phases. Phase I metabolism (detoxification) involves oxidative, reductive and/or hydrolytic reactions that cleave substrate molecules to produce a more polar moiety. Phase II reactions (synthetic reactions) involve conjugation of certain endogenous molecules to the products of phase I reaction (Remmer, 1970). Cytochrome P_{450} (Cyt. P_{450}) enzymes are responsible for the metabolic conversion of many drugs to the polar metabolites via Phase I and Phase II reactions to earlier excretion.

CCl_4-induced hepatotoxicity in rats represents an adequate experimental model of cirrhosis in man and it is used for the screening of hepatoprotective drugs (Al-Shabanah et al., 2000; Pérez-Tamayo, 1983; López-Novoa et al., 1977). The liver represents the principal site of toxicity, although it induces sublethal proximal tubular injury in the kidney and focal alterations in granular pneumatocytes (Striker et al., 1968).

The biotransformation of CCl_4 occurs in the ER and is mediated by Cyt. P_{450} (Castro et al., 1968); the principal isoform implicated as the catalyst being CYP2E 1 (Al-Shabanah et al., 2000). Cyt. P_{450} is inhibited suicidally by the reactive metabolites of CCl_4 (Athar et al., 1997). $CCl_3^{\bullet}$ radical initially formed being relatively unreactive reacts very rapidly with oxygen to yield a highly reactive trichloromethyl peroxy radical ($CCl_3OO^{\bullet}$), which is the probable initiator of lipid peroxidation (Bhat and Madyastha, 2000).

Hepatic cells participate in a variety of metabolic activities and contain a host of enzymes. In tissues, AST and ALT are found in higher concentrations in cytoplasm and AST in particular also exists in mitochondria (Wells, 1988). In liver injury, the transport function of the hepatocytes is disturbed, resulting in the leakage of plasma membrane (Zimmerman and Seef, 1970), thereby causing an increased enzyme level in serum. If injury involves organelles such as mitochondria, soluble enzymes like AST normally located there, will also be similarly released. The elevated activities of AST and ALT in serum are indicative of cellular leakage and loss of the functional integrity of cell membranes in liver (Drotman and Lawhorn, 1978). Administration of CCl_4 significantly raises the serum level of enzymes like AST and ALT in rats (Naziroglu et al., 1999) as observed in our results. Oral administration of Kamilari at a dose of 750 mg/kg body weight to rats caused a decrease in the activity of the above enzymes, which may be a consequence of the stabilization of plasma membrane as well as repair of hepatic tissue damage caused by CCl_4. This is supported by the view that serum levels of transaminases return to normal with the healing of hepatic parenchyma and regeneration of hepatocytes (Thabrew et al., 1987).

The activity of serum alkaline phosphatase was also elevated during CCl_4 administration. Alkaline phosphatase is excreted normally via bile by the liver. In liver injury due to hepatotoxin, there is a defective excretion of bile by the liver which is reflected in their increased levels in serum (Rao, 1973). Hyperbilirubinaemia is a very sensitive test to substantiate the functional integrity of the liver and severity of necrosis which increases the binding, conjugating and excretory capacity of hepatocytes that is proportional to the erythrocyte degeneration rate (Singh et al., 1998).

Depletion of elevated bilirubin level together with the suppression of the activity of ALP in the serum of rats treated with Kamilari, suggests the possibility of the herbal product being able to stabilize biliary dysfunction of rat liver during chronic injury with CCl_4.

In hepatotoxicity, a depression in total protein is observed due to the defect in protein biosynthesis (Clawson, 1989; Dubey et al., 1994) similar to our results. This is due to the disruption and disassociation of polyribosomes from endoplasmic reticulum following CCl_4 administration (Clawson, 1989). Administration of Kamilari at a dose of 750 mg/kg body weight prevented this change. This may be due to the promotion of the assembly of ribosomes on endoplasmic reticulum to facilitate uninterrupted protein biosynthesis.

Treatment of rats with CCl_4 causes centrilobular necrosis, which results in the accumulation of fat in liver and kidney. Fat from the peripheral adipose tissue is translocated to the liver and kidney leading to its accumulation during toxicity (Devarshi et al., 1986). Hepatotoxin treatment produces an increase in the level of phospholipids in liver which may be due to the decrease in mitochondrial fat oxidation. The decrease in the level of phospholipids in kidney may be the result of CCl_4-induced lipid peroxidation. Kidney phospholipids is concerned with the regulation of blood and body acidity by renal excretion of acid phosphate (Weissberger, 1940). The decrease in the level of phospholipids in kidney which may be the consequence of CCl_4-induced lipid peroxidation, can also contribute to the pathogenesis in CCl_4-induced hepatotoxicity.

Previous reports indicate that total cholesterol and triglycerides (TG) increase in CCl_4-induced fatty liver (Seakins and Robinson, 1963; Torres-Durán et al., 1998). It is well

known that CCl_4 administration induces an increased synthesis of fatty acids as well as decreased release of hepatic lipoproteins (Maling et al., 1962). According to Recknagel and Lombardi (1961), the accumulation of TG in liver of CCl_4-treated rats is not due to the interference with the formation TG by the liver, but due to the inhibition or destruction of TG secreting mechanism. The observed restoration of the CCl_4-evoked changes in the lipid profile of serum and tissues shows the protective nature of Kamilari.

On the basis of our results it can be concluded that Kamilari has antihepatotoxic activity. Further investigations are necessary to isolate the active principles of Kamilari and establish their chemical nature.

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