TABLE 2: EFFECT OF ZJE ON SERUM LIPID LEVELS IN SUCROSE-INDUCED OBESE RATS

Treatment (mg/kg)	Serum lipid levels (mg/dL)				
	TC	HDL-C	LDL-C	VLDL-C	TGs
Normal control	88.63±1.15	12.35±0.58	57.10±1.04	17.18±0.64	85.90±0.72
Sucrose control	111.7±1.23	10.70±0.41	76.72±0.98	24.24±0.64	121.21±0.38
ZJE (200)	103.2±0.70	13.15±0.75	67.29±0.86	22.72±0.2	113.63±0.35
ZJE (400)	93.63±1.22*	15.61±1.31	60.13±1.12*	19.89±0.57	99.48±0.31*
ZJE (600)	86.83±0.65*	17.30±0.72*	50.89±1.04*	18.64±0.5	93.21±0.27*
Fluoxetine (10)	86.92±0.84*	17.63±0.58*	51.43±0.96*	17.86±0.39	89.30±0.39*

Values are mean±SEM for 6 rats. *p<0.05 significant when compared to sucrose control.

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Hepatoprotective activity of the *Trikatu Churna* – an Ayurvedic formulation

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Accepted 19 April 2004

Revised 4 February 2004

Received 17 September 2003

Ethanol extract of *Trikatu Churna* an Ayurvedic formulation was evaluated for hepatoprotective activity in rats by inducing liver damage with carbon tetrachloride. The ethanol extract at an oral dose of 150 mg/kg exhibited a significant protective effect by lowering serum levels of glutamic oxaloacetic transaminase, glutamic pyruvic transaminase, alkaline phosphatase and total bilirubin. Liv 52 syrup was used as positive control.

Trikatu Churna, an important Ayurvedic formulation, is official in Ayurvedic formulary of India, Part II[1]. It is therapeutically useful in the treatment of anorexia, dyspepsia, throat infections and tuberculosis[1]. It contains one part of each of *pippali* (fruits of *Piper longum*), *marica* (fruits of *Piper*

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nigrum) and *sunthi* (dried rhizomes of *Zingiber officinale*). Of all the three ingredients *pippali* and *marica* contain an alkaloid piperine as chief constituent[2,3]. Literature survey revealed that the piperine isolated form *piper nigrum* possess significant hepatoprotective activity[4]. *Piper longum* contains piperine, piper longumine, volatile oil, resins, gums and fatty oil. The fruits of *piper longum* are useful in spleen disorders, bronchitis, tuberculosis and jaundice[5]. Since piperine forms the major constituent of two of three ingredients of *Trikatu Churna*, the present study was under taken to evaluate the hepatoprotective effect of this formulation in experimental animals against carbon tetrachloride-induced hepatotoxicity.

Pippali, marica and *sunthi* were purchased form local market in Tirupati and their identity was confirmed at the Srinivasa Ayurvedic Pharmacy, Tirumala Tirupati Devasthanams, Narasingapuram, Tirupati. Liv 52 syrup of Himalaya Drug Company, Bangalore was purchased from a local pharmacy. The crude drugs are powdered and sieved through sieve No. 80 individually and stored in air tight containers. The laboratory sample was prepared as per the formula given in Ayurvedic Formulary of India part II. The *Trikatu Churna* was extracted with petroleum ether (60-80°) to remove the lipids and the marc was dried and again extracted with ethanol in a Soxhlet extractor. The solvent was distilled off under vacuum to concentrate the extract and the resultant ethanolic extract was dried in a desiccator. The test suspension was prepared in the vehicle i.e., 5% w/v acacia mucilage and was administered in the dose of 150 mg/kg orally.

Wistar rats weighing 175-225 g of either sex, maintained under standard husbandry conditions (Temp 23±2°, relative humidity 55±10% and 12 h light dark cycle) were used for all sets of experiments in groups of six animals. Animals were allowed to take standard laboratory feed and tap water. The experiments were performed after getting the experimental protocols approved by the Institutional Animal Ethics Committee, M. S. University of Baroda, Vadodara. All chemicals used for serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), alkaline phosphatase (AP) and total bilirubin (TB) determination were of analytical grade and procured from either E. Merck, Mumbai or Qualigens, Mumbai. The extract was administered to different groups of rats in doses ranging from 150-1500 mg/kg. There was no lethality in any of the groups. One tenth of the maximum dose of the extract tested for acute toxicity was selected for evaluation of hepatoprotective activity i.e., 150 mg/kg[6]. The ethanol extract was also evaluated for its effect on normal liver function by studying serum biochemical parameters.

Rats were divided into control and test groups each comprising of six animals. To the control group, vehicle (5% acacia mucilage 1ml/kg p.o) was given at 0, 24 and 48 h intervals and to the test group the test sample (150 mg/kg p.o) at 0, 24 and 48 h intervals, After 72 h of first dose administration blood was collected by puncturing retro orbital plexus and was allowed to clot at room temperature for 30 min. Serum was separated by centrifuging at 2500 rpm. The serum obtained was used for the determination of SGOT[7], SGPT[7]. Serum AP was assayed by phenyl phosphate method[8] and TB assay was carried out according to a reported method[9].

Rats were divided into four groups of six each, control, hepatotoxin, positive control and test groups. To the control group vehicle was given at 0, 24 and 48 h orally. To the hepatotoxin group at 0 h , vehicle followed by CCl_4 (in liquid paraffin 1:1 ratio, intraperitoneal) at a dose of 1 ml/kg and at 24 and 48 h only vehicle was given. The test group has received the first dose of extracts at 0 h , second dose of extract at 24 h followed by a dose of CCl_4 and the third dose at 48 h. The positive control group has received the first dose of Liv-52 syrup in acacia mucilage at 0 h, second dose at 24 h followed by a dose of CCl_4 and the third dose at 48 h. After 72 h blood was collected from all the groups and was allowed to clot, for the separation of serum[10,11]. Serum was utilized for estimation of SGOT, SGPT, AP and TB by reported methods to assess liver function. The mean values±SEM are calculated for each parameter. For determining the significant inter group difference each parameter was analyzed separately and one-way analysis of variance (ANOVA)[12] was carried out. Then the individual comparisons of the group mean values were done using Dunnet's procedure[13].

In the study of the effect of the ethanolic extract of *Trikatu Churna* on normal liver functions, it was found to be non-toxic at the selected dose (150 mg/kg p.o) since the parameters SGOT, SGPT, AP and TB are with in the limits similar to that of control (Table 1). CCl_4 Treatment in normal rats elevated the levels of SGOT, SGPT, AP and TB significantly indicating acute hepatocellular damage. The rats treated with ethanolic extract of *Trikatu Churna* and positive control showed a significant reduction in all four biochemical parameters elevated by CCl_4 (Table 2). The reduction in biochemical parameters exhibited by ethanolic extract is similar when compared to that of positive control.

TABLE 1: EFFECT OF ETHANOLIC EXTRACT OF *TRIKATU CHURNA* ON NORMAL LIVER FUNCTION

Group	SGOT U/ml	SGPT U/ml	AP KA units/100 ml	TB mg/dl
Control	119.2±5.35	91.0±4.48	86.3±5.20	0.9± 0.09
Ethanol Extract	114.2±6.72	95.2±4.71	90.3± 6.26	1.0±0.12
$F_{calculated}$	0.3	0.4	0.2	0.1
5% Allowance	22.1	16.7	20.9	0.4

$F_{critical}$=4.96 (P<0.05)

TABLE 2 : EFFECT OF ETHANOLIC EXTRACT OF *TRIKATU CHURNA* ON CCL_4-INDUCED HEPATOTOXICITY

Group	SGOT U/ml	SGPT U/ml	AP KA units / 100 ml	TB mg/dl
Control	119.2±5.35	91.0±4.48	86.3±5.20	0.9±0.09
CCl_4	188.3±8.18	177.9±7.26	112.2±6.16	1.8±0.10
Ethanolic Extract	113.2±9.89*	113.9±5.03*	92.3±5.71*	1.3±0.06*
Positive Control (Liv-52)	126.8±8.16*	102.6±51.12*	80.2±4.72*	1.1±0.12*
$F_{calculated}$	15.3	48.3	8.4	13.9
5% Allowance	29.5	20.4	18.6	0.3

$F_{critical}$=3.10(P<0.05), *Significant reduction compared to CCl_4.

The hepatotoxicity of CCl_4 is due to the formation of highly reactive metabolite, the trichloro free radical, which attacks the poly unsaturated fatty acids[14]. CCl_4 -induced liver peroxidation was inhibited significantly by the extract of *Trikatu Churna*, which confirms the protective action of the extract against experimentally induced liver damage in rats. SGOT, SGPT, AP and TB are the tests usually employed in the diagnosis of hepatic disease. The elevated levels of these parameters were significantly reduced by the treatment with ethanolic extract of *Trikatu Churna*. It can be concluded from this investigation that *Trikatu Churna* possesses hepatoprotective activity.

ACKNOLWEDGEMENTS

The authors are thankful to the authorities of Sri Padmavathi School of Pharmacy, Tirupati, and M. S. University of Baroda, Vadodara, for providing necessary facilities.

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