Indian J Physiol Pharmacol 2003; 47 (4): 435–440

HEPATOPROTECTIVE EFFECT OF HIMOLIV®, A POLYHERBAL FORMULATION IN RATS

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(Received of November 10, 2002)

Abstract : The effect of Himoliv (HV) was evaluated in carbon tetrachloride or paracetamol induced hepatotoxicity in rats. Liver necrosis was produced by administering single dose of either carbon tetrachloride (CCl_4, 1 ml/kg, 50% v/v with olive oil, s.c.) or paracetamol (PC, 1 g/kg, p.o.). The liver damage was evidenced by elevated levels of serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT) and serum alkaline phosphatase (ALP) and hepatic thiobarbituric acid reacting substances (TBARS) and superoxide dismutase (SOD). HV pretreatment (0.5 and 1.0 ml/kg, p.o.) significantly ($P<0.001$) reduced CCl_4 or PC-induced elevations of the levels of SGOT, SGPT, ALP and TBARS, while the reduced concentration of SOD due to CCl_4 or PC was reversed. Silymarin (25 mg/kg, p.o.), a known hepatoprotective drug showed similar results.

Key words: hepatoprotection, carbon tetrachloride, paracetamol, lipid peroxidation, silymarin, anti-oxidant

INTRODUCTION

Himoliv® (HV) is a multi-herbal formulation, containing aqueous extracts of 25 indigenous medicinal plants. Many ingredients of the formulation (*Picorrhiza kurroa, Boerrhavia diffusa, Tinospora cordifolia, Andrographis paniculata, Phyllanthus emblica*) were earlier investigated for their protective effects against different models of experimental hepatotoxicity (1–5). HV is also claimed to be useful in the

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treatment of hepatitis, jaundice, biliary dysfunction and liver disorder. However, the pharmacological effects need experimental evidence for their actions. Oxidative damage through free radical generation (6–7) is among the various mechanisms involved in the hepatotoxic effect of carbon tetrachloride (CCl_4) and paracetamol (PC). An anti-oxidant property is claimed to be one of the mechanisms of hepatoprotective effect of the test drug. HV has been primarily proved to be an useful hepatoprotective agent against CCl_4 induced hepatic damage and 1 ml/kg body weight orally was found to be an optimum dose (8). In the present study, HV was investigated for its effect against CCl_4 or paracetamol-induced hepatotoxicity in rats at a dose of 0.5 ml/kg and 1.0 ml/kg.

METHODS

Carbon tetrachloride and paracetamol-induced hepatotoxicity in rats (9–10):

The test drug, Himoliv was prepared and supplied by M/s. Emami Limited, Kolkata, India in a liquid form. Inbred Wistar rats of either sex weighing 150–175 g were used. The animals were maintained on a 12 hour light and dark cycle, fed *ad libitum* with commercial pelleted chow (Amrut Laboratory Animal Feed, Pune) and having free access to water. Permission from institutional ethical committee for laboratory use of animals was duly obtained. The animals were classified into following groups for treatment. Group I consisted of the control animals which were six in numbers. All other groups had 8 animals in each.

Group I : Represented control that received 5 ml/kg of water per oral (p.o.) for 9 days.

Group II : 5 ml/kg of water p.o. for 9 days and carbon tetrachloride (CCl_4) 1 ml/kg, 50% v/v with olive oil, s.c. on day 7th.

Group III : HV at a dose of 0.5 ml/kg once daily, p.o. for 9 days and CCl_4 1 ml/kg, s.c., on day 7th.

Group IV : HV 1.0 ml/kg once daily, p.o. for 9 days and CCl_4 1 ml/kg, s.c., on day 7th.

Group V : Silymarin 25 mg/kg p.o., once daily for 9 days and CCl_4 1 ml/kg, s.c., on day 7th.

Group VI : 5 ml/kg of water p.o. for 9 days and paracetamol (PC) 1 g/kg, p.o. on day 7th.

Group VII : HV 0.5 ml/kg once daily, p.o. for 9 days and PC 1 g/kg, p.o. on day 7th.

Group VIII : HV 1.0 ml/kg once daily, p.o. for 9 days and PC 1 g/kg, p.o. on day 7th.

Group IX : Silymarin 25 mg/kg, p.o., once daily for 9 days and PC 1 g/kg, p.o. on day 7th.

After 48 hours of hepatotoxin administration, the animals were sacrificed under deep ether anesthesia and blood was collected directly from the carotid artery for the assay of SGOT, SGPT and ALP. The livers were removed immediately, washed with ice-cold saline and a 10% homogenate prepared in phosphate buffer (pH 7.0). The homogenate was centrifuged at 3000 rpm for

15 min at 4°C and the supernatant was used for the estimation of TBARS, SOD and protein.

Enzymes like, SGOT, SGPT and ALP were assayed using standard kits from Span Diagnostics Limited, India (11–12). Lipid peroxidation was quantitate by measuring the concentration of TBARS in liver homogenate using the method of Onkawa et al., 1979 (13). The results were expressed as nmol of MDA/mg of protein. SOD was estimated in the liver homogenate using epinephrine by the method of Mishra and Fridovich, 1972 (14) and protein was estimated by the method of Lowery et al., 1951 (15).

Statistical analysis : Result of biochemical estimations have been indicated in terms of mean ± SEM. The difference among means has been analysed by Student's unpaired t-test (16). Minimum level of significance was $P<0.05$.

RESULTS

Carbon tetrachloride induced hepatotoxicity: The results of SGOT, SGPT and ALP in control rats were 42±2.52, 56±1.25 and 35±2.25 respectively, whereas in carbon tetrachloride (CCl_4) treated rats, these levels were elevated to 99±5.62, 108±5.05 and 88±3.60 respectively. HV pretreatment at the dose 0.5 ml/kg, significantly ($P<0.001$) prevented the CCl_4 induced rise in the SGOT, SGPT and ALP to 72±4.08, 78±3.96 and 55±1.32 respectively being compared to CCl_4 treated group. With higher dose of HV (1.0 mg/kg) further reduction of SGOT, SGPT and ALP to 49±3.54, 63±3.25 and 48±2.92 respectively were noted. Silymarin (25 ml/kg) pretreatment also prevented the CCl_4 induced rise in SGOT, SGPT and ALP to 54.2±5.60, 60±5.80 and 40.3±4.62 respectively (Table I).

The liver SOD was 1.5±0.01 observed in control, whereas TBARS was 3.2±0.019. But

TABLE I: Effect of Himoliv (HV) pretreatment on carbon tetrachloride (CCl_4) induced hepatic toxicity in rats.

	Group I	*Group II*	*Group III*	*Group IV*	*Group V*
	(water 5 ml/kg	*(water 5 ml/kg +CCl_4 1 ml/kg on day 7th)*	*(HV 0.5 ml/kg +CCl_4 1 ml/kg on day 7th)*	*(HV 1.0 ml/kg CCl_4 1 ml/kg on day 7th)*	*(Silymarin 25 mg/kg +CCl_4 1/ml/kg on day 7th)*
	n=6	*n=8*	*n=8*	*n=8*	*n=8*
SGOT (U/L)	42±2.52	99±5.62#	72±4.08#	49±3.54#	54.2±5.60#
SGPT (U/L)	56±1.25	108±5.05#	78±3.96*	63±3.25*	60.7±5.80*
ALP (KAU)	35±2.25	88±3.60#	55±1.32*	48±2.92*	40.3±4.62*
Liver TBARS (MDA nM/mg protein)	3.2±0.019	5.9±0.007#	4.3±0.008*	39±0.002*	3.55±001*
Liver SOD (U)	1.5±0.01	0.3±0.04#	0.9±0.04*	1.5±0.02*	1.5±0.02*

Values are Mean ± SEM
#P<0.001 when compared with Group I (control)
*P<0.001 when compared with Group II (CC_4 treated).
Student's unpaird t-test.

TABLE II: Effect of Himoliv (HV) pretreatment on paracetamol (PC) induced hepatic toxicity in rats.

	Group I	*Group VI*	*Group VII*	*Group VIII*	*Group IX*
	(water 5 ml/kg	*(water 5 ml/kg +PC 1 g/kg on day 7th)*	*(HV 0.5 ml/kg +PC 1 g/kg on day 7th)*	*(HV 1.0 ml/kg +PC 1 g/kg on day 7th)*	*(Silymarin 25 mg/kg +PC 1 g/kg on day 7th)*
	n=6	*n=8*	*n=8*	*n=8*	*n=8*
SGOT (U/L)	42±2.52	108±4.06#	70±3.55*	47±5.85*	46±4.04*
SGPT (U/L)	56±1.25	102±2.62#	74±3.94*	62±4.62*	59±3.40*
ALP (KAU)	35±2.25	85±5.18#	55±3.62*	38±4.24*	35±2.96*
Liver TBARS (MDA nM/mg protein)	3.2±0.019	5.1±0.088#	4.7±0.010*	3.6±0.03*	3.5±0.024*
Liver SOD (U)	1.5±0.01	0.7±0.05#	1.4±0.02*	1.6±0.02*	1.7±0.02*

Values are Mean ± SEM
#P<0.001 when compared with Group I (control)
*P<0.001 when compared with Group VI (PC treated).
Student's unpaired t-test.

significant changes were noted in CCl_4 treated group, SOD was reduced to 0.3±0.04 and TBARS was enhanced to 5.9±0.007 (Table II). HV pretreatment (0.5 ml/kg) also significantly (P<0.001) reversed CCl_4 induced changes in SOD (10.7±0.05) and TBARS (5.0±0.085). Similar type of findings were observed with HV in the dose of 1.0 ml/kg (SOD 0.9±0.04 and TBARS 4.3±0.008). Silymarin corroborated these findings (SOD 1.5±0.02 and TBARS 3.9±0.002).

Paracetamol-induced hepatoxicity in rats: In paracetamol (PC) treated rats SGOT, SGPT and ALP levels were elevated significantly to (108±4.06, 102±2.65 nd 85±5.18 respectively) in comparison to control. But, HV (0.5 ml/kg) pretreatment prevented PC induced rises in SGOT, SGPT and ALP to 70±3.55, 74±3.94 and 55±3.62 respectively being compared to PC treated group. With higher dose of HV (1.0 ml/kg) further reduction of SGOT, SGPT and ALP to 47±5.85, 62±4.62 and 38±4.24 respectively were noted. Silymarin (25 mg/kg) pretreatment also prevented the PC induced rise in SGOT, SGPT and ALP to 46±4.04, 59±3.40 and 35±2.96 respectively (Table II).

After PC treatment it was noted that, liver SOD was reduced to 0.7±0.05 and TBARS was enhanced to 5.1±0.088 in comparison to the control (Table II). HV pretreatment (0.5 ml/kg) significantly (P<0.001) reversed PC induced changes in the level of SOD (14±0.02) and TBARS (4.7±0.010). Similar type of findings were observed rats were pretreated with HV in the dose of 1.0 ml/kg (SOD 1.6±0.02 and TBARS 3.6±0.033). Standard hepatoprotective drug, Silymarin showed similar results (SOD 1.7±0.02 and TBARS 3.5±0.024).

DISCUSSION

Large doses of carbon tetrachloride (CCl_4) and paracetamol (PC) induces hepatic necrosis in humans and experimental

animals. CCl_4 is metabolized in the liver to the highly reactive trichloromethyl radical. This free radical leads to auto-oxidation of the fatty acids present in the cytoplasmic membrane phospholipids and causes functional and morphological changes in the cell membrane (6, 17). Whereas, PC is primarily metabolized by sulphation and glucuronidation to unreactive metabolites, and then activated by cytochrome P_{450} system to induce hepatic injury (7, 18). Observation of the preventive effect to the liver damage, caused by CCl_4 and PC may give an indication of the hepatoprotective effect of drugs in general. This is evidenced by an elevation in the serum marker enzymes namely SGOT, SGPT and ALP by CCl_4 or PC and reversal of this effects by any hepatoprotective drug. HV significantly reduced these elevations of liver enzymes induced by CCl_4 and PC, dose dependently. Silymarin, a prototype hepatoprotective agent also showed similar changes.

The antioxidation activity or the inhibition of the generation of free radicals is important in the protection against CCl_4 as well as PC induced liver lesions (17, 19). In this work, elevation in the levels of end products of lipid peroxidation or MDA in liver of rats treated with CCl_4 and PC were observed. Pretreatment with HV (0.5 and 1.0 ml/kg) significantly reversed these changes. HV also significantly prevented the diminution in the level of the protective enzyme SOD, induced by CCl_4 or PC, when examined in the liver homogenate. It is well known that SOD plays an important role as a protective enzyme against lipid peroxidation in tissues (20–21). These findings support the hepatoprotection by HV by modulating the antioxidant pathway. Therefore, it may be conjectured that HV have preventive action both on CCl_4-induced and paracetamol induced hepatotoxicities in rats. It is possible that the mechanism of hepatoprotective action of HV might be due to its anti-oxidant effect.

ACKNOWLEDGEMENTS

The authors acknowledge M/s Emami Limited, Kolkata for their financial and material help to conduct this work.

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