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SHORT COMMUNICATION

COMPARISON OF THE PROTECTIVE EFFECT OF WITHAFERIN-'A' AND HYDROCORTISONE AGAINST CCL_4 INDUCED HEPATOTOXICITY IN RATS

S. SUDHIR* AND R. D. BUDHIRAJA

Department of Pharmacology
Medical College, Rohtak - 124 001

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Abstract : Protective effect of Withaferin 'A' against CCl_4 induced hepatotoxicity has been assessed and the compound at 10 mg/kg dose was found to possess significant protective effect. A comparison of this protective effect with that of hydrocortisone showed it to be as effective as hydrocortisone dose per dose.

Key words : Withaferin 'A' hepatotoxicity

INTRODUCTION

Leaves of *Withania somnifera* (N.O. *Solanaceae*) have been found to possess significant anti-inflammatory activity and protective effect against CCl_4-induced hepatotoxicity in rats (1). Withaferin A is a major Withanolide isolated from these leaves and has been found to possess remarkable antibacterial (2, 3), antitumour (4), immunosuppressive (5, 6, 7), and anti-arthritic (8, 9) properties. Further, a Withanolide isolated from the fruits of *Withania coagulans* has been found to possess significant protective effect against CCl_4-induced hepatotoxicity (10). In view of these biological effects of the Withanolides, especially of Withaferin 'A', it has now been assessed and compared with hydrocortisone for protective effects against CCl_4-induced hepatotoxicity.

METHODS

Withaferin 'A' was obtained from Prof. D. Lavie of Israel. One percent suspension of Withaferin 'A' was made in 1% solution of sodium carboxymethylcellulose (CMC) and sterilized in boiling water for 15 minutes.

Hepatotoxicity was induced in albino rats of either sex (150-170 g), using 6 rats for each experiment, with a fresh mixture of equal volumes of CCl_4 and olive oil, given as 3 injections at 3 days interval, i.e. on Ist, 4th and 7th day, in doses of 2 ml/kg of CCl_4 ip (1, 10). Test drugs were administered ip, once daily beginning one day prior to CCl_4 administration (o day), and continued for 10 consecutive days. Control group received CMC solution only. On the 10th day, the rats were anaesthetised with ether, thoracoabdominal incision made and blood was withdrawn by cardiac puncture. Total hepatectomy was then performed. Protective effect was assessed by determinations of :

(a) SGOT/SGPT levels (11), (b) Serum alkaline phosphatase (12), (c) Serum proteins (13) and (d) histopathological examination of hepatic tissue after staining with haemotoxylin and eosin solution.

RESULTS

Rats treated with 3 doses of CCl_4 alone developed significant hepatic damage as was observed from significant rise in the levels of SGOT/SGPT and serum alkaline phosphatase, and histopathological examination of the hepatic tissue. Concomitant treatment of the rats with 10 mg/kg dose of Withaferin 'A' or Hydrocortisone (10 mg/kg) protected the liver significantly ($P<0.05$), as deduced from the reduction in SGOT/SGPT and serum alkaline phosphatase levels (Table I). There was no change in serum proteins.

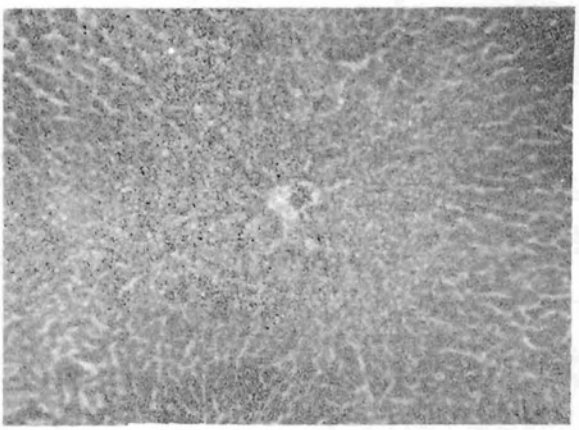
*Corresponding Author

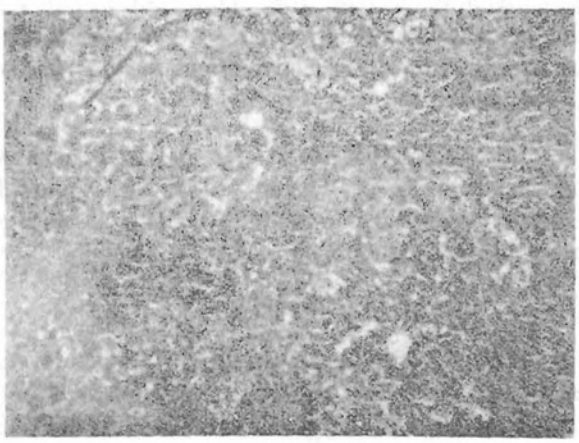
TABLE I : Protective effect of withaferin A (10 mg/kg) and hydrocortisone (10 mg/kg) against CCl_4 (2 ml/kg) induced hepatotoxicity (n=6).

Parameter	*Control*	*CCl_4*	*CCl_4 + withaferin A*	*CCl_4 + hydrocortisone*
SGPT (IU/L)	16.0±0.58	72.0±5.5*	26.25±1.7a**	32.2±1.3**
SGOT (IU/L)	29.0± 0.60	65.0±1.5*	42.0±1.6a**	48.0±1.9**
Serum Alkaline phosphatase (KU/100 ml)	18.0±1.3	30.6±1.6*	22.6± 0.7**	22.4±0.53**
Serum Protein (g/dl)	7.2±0.62	6.3±1.30	6.8± 0.56	6.7±0.43

Values are Mean ± SEM; *P<0.01 compared to control; **P<0.05 compared to CCl_4 treated; aP<0.05 compared to CCl_4 + hydrocortisone treated.

Results were further confirmed by histopatholgical studies. Rats treated with CCl_4 alone showed cirrhosis of liver with central vein pushed towards one side and areas of fatty changes. In Withaferin 'A' treated rats, the liver showed central vein congesion and patchy areas of fatty changes, whereas in hydrocortisone treated rats, the fatty change was seen all around the central vein; changes being almost identical. Moreover, identical results were observed in multiple sections prepared from different sites of the hepatic tissue (Fig. IA, B & C).





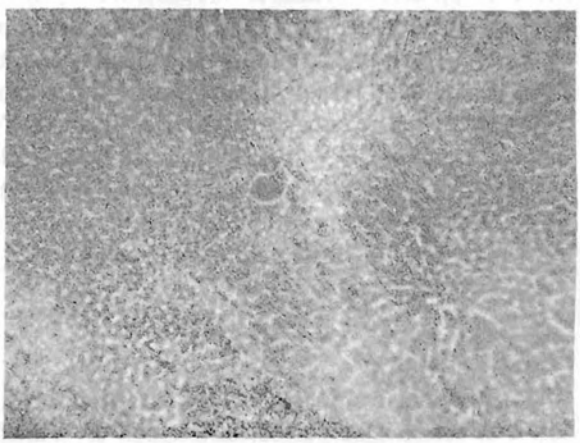


Fig. 1 : Histopathological changes in rat liver; (a) : treated with CCl_4 ; (b) Treated with CCl_4 and withaferin A; (c) : Treated with CCl_4 and hydrocortisone : (a) shows cirrhotic changes, (b) and (c) show central vein dilatation and mild fatty change.

DISCUSSION

Since CCl_4 is known to cause marked elevation of serum transaminases, and produces histological abnormalities (14), this model of hepatotoxicity has been widely used to assess the protective effect of drugs on liver. The results given in Table I and histopathological examination of liver tissue (Fig.1) show that Withaferin 'A' has significant protective effect against CCl_4 induced hepatotoxicity at dose level of 10 mg/kg. Its protective effect is almost as active as resulting from hydrocortisone, on dose per dose basis. Since Withaferin 'A' is a major Withanolide from the leaves of *Withania somnifera*, the protective effect observed with alcoholic extract of the leaves can be attributed to this Withanolide, although other Withanolides may also be contributing to the protective effect observed with alcoholic extract.

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