Indian J Physiol Pharmacol 2001; 45(4) : 428–434

PROTECTIVE EFFECTS OF *INDIGOFERA TINCTORIA* L. AGAINST D–GALACTOSAMINE AND CARBON TETRACHLORIDE CHALLENGE ON 'IN SITU' PERFUSED RAT LIVER

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(Received on November 29, 2000)

Abstract : The effect of pre-treatment with *Indigofera tinctoria* (IT) extract against the toxicity of D–Galactosamine (D–GalN) and carbon tetrachloride (CCl_4) during '*in situ*' perfusion of the liver for 2 hr was studied in rats. Release of LDH and levels of urea in the liver effluent perfusate, was studied and the rate of bile flow was monitored. Perfusion with D–Galactosamine (5 mM) or carbon tetrachloride (0.5 mM) resulted in increased LDH leakage, decreased urea levels in the liver effluent and reduction in bile flow. IT pretreatment (500 mg/kg body weight) *in vivo* ameliorated D–GalN and CCl4 induced adverse changes towards near normalcy and thereby indicates its hepatoprotective effects in rats.

Key words : *Indigofera tinctoria* D-Galactosamine LDH carbon tetrachloride bile flow *In situ* perfusion

INTRODUCTION

Indigofera tinctoria Linn. (leguminosae-papilionatae) is an erect suffruticose pubescent shrub two to four feet of more high, cultivated extensively in Northern India, especially in Bengal, Bihar, Orissa, Sind and Southern India, Madras. Several medicinal properties are attributed to this plant (1) and the plant has been proved to possess anticancer (2) and antihepatotoxic activities (3).

D-Galactosamine (D-GalN) is a hepatotoxin which causes a transient period of UTP deficiency and results in the inhibition of protein synthesis. Administration of D-Galactosamine leads

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to liver injury with features closely resembling those observed in human viral hepatitis (4). Carbon tetrachloride (CCl_4) is a hepatotoxin that elicits its hepatotoxic action through the formation of highly reactive and unstable trichloromethyl radicals (CCl_3) and subsequent lipid peroxidation chain reaction (5). The aim of the present study is to examine if pre-treatment with IT extract in vivo is capable of reducing deleterious effects, in the liver of rats perfused with toxic doses of D-Galactosamine and carbon tetrachloride.

METHODS

Plant material

The plant (2 to 4 feet or more high) with a short stem and twiggy but firm woody terete branches light greenish brown to somewhat silvery grey in colour, bearing alternate pinnate leaves and small pale reddish brown to reddish yellow flowers are collected from the gardens of the Central Research Institute for Siddha, Arumbakkam, Chennai during October 1998. It was authenticated by Dr. S. Usman Ali (Drug Research Scheme – Multi Disciplinary), from the above institute where a voucher specimen of the plant was deposited.

Preparation of the extract

Shade dried and coarsely powdered plant material (whole plant 3 kg) was extracted with methanol in cold (48 hrs). The extract was filtered, concentrated on a water bath and then dried in vaccum (yield 10%) [During the preliminary studies, experiments were conducted with three different extracts of IT. viz acetone extract, methanolic extract and chloroform extract. As the best results were attained with the methanolic extract, all the further experiments were conducted with the methanolic extract].

Perfusion apparatus

The perfusion apparatus as described by Nayeem 1998 (6) with slight modifications to carry out liver perfusion experiments was used in our study. The perfusion buffer used is the Krebs Henseleit Buffer (KHB) (118.9 mM Nacl, 4.76 mM KCl, 1.19 mM KH_2PO_4, 1.2 mM $MgSo_4$, 2.55 mM CaCl2 and 24.8 mM $NaHCO_3$ gassed separately in 95% O_2 and 5% CO_2). 5.5 mM glucose is also added to the buffer to provide additional energy.

Chemicals

D-Galactosamine was obtained from Sigma Chemical Company, St. Louis, MO, USA.

Animals

Adult male albino rats of Wistar strain *(Rattus albinus)* weighing about 140–180 g obtained from the Fredrick Institute for Plant Protection and Toxicology, Padappai. Chennai were used for the study.

They were acclimatized to animal house conditions and were fed on commercial pelleted rat chow (Hindustan Lever Limited; Bangalore, India) and water ad *libitum*.

The animals were divided into six groups according to the following experimental regimen. Group 1 comprises rats whose livers were perfused with KHB alone for 2 hrs. Group 2 comprises rats whose livers were perfused with D-Galactosamine (5 mM). Group 3 comprises rats which were given pre-treatment with IT extract *in vivo* (500 mg/kg body wt/day orally for 21 days). [This particular dosage was fixed up after preliminary trials with five different dosages (100, 250, 500, 750 and 1000 mg/kg body wt for different time intervals viz 7, 14, 18 and 21 days). As the dosage of 500 mg/kg body wt for 21 days was found to exhibit maximum hepatoprotective effects (as judged by the activities of lactate dehydrogenase in the liver effluent perfusate after 2 hrs of perfusion with D-Galactosamine (Fig. 1) this particular dosage was fixed up as the optimum dosage for IT for all the subsequent experiments]. Group 4 comprises rats which were given IT extract *in vivo* prior to perfusion with D-Galactosamine. Group 5 comprise rats which were perfused with CCl_4.

Surgery and mode of perfusion

The rats were anesthetized with thiopentone sodium (40 mg/kg body wt i.p). Heparin (500 units) was given to prevent blood clothing. The abdomen was opened by a midline incision and the inferior vena cava was exposed and sutures were placed around the inferior vena cava by a thread, above and below the renal veins. The portal vein was exposed and cannulated with a polyethylene cannula (5 mm diameter). The diaphragm was incised (to facilitate easy and natural drainage from the inferior vena cava) carefully and the inferior vena cava was cannulated supra hepatically through the right atrium. Following the attachment of perfusion tubing to the cannulae, the liver was perfused *'in situ'* through the portal vein in an anterograde fashion (7).

Collection of Bile

For the collection of bile, the bile duct was exposed, secured with a thread and cannulated with a PE tubing (2mm diameter). The free end of the cannula was attached to a graduated tuberculin syringe for the collection of bile. The rate of bile flow was monitored for a period of 1 hr.

The pH of the perfusing buffer should be between 7.2 – 7.45. Temperature, perfusion pressure, flow rate and perfusate pH should be closely monitored during perfusion. The temperature in the perfusion cabinet and the perfusate should be maintained at 37°C. Perfusion pressure should not raise above 10–15 cm water with a flow rate of approximately 2–3.5 ml/min/g of liver. During perfusion, samples of liver effluent perfusate was collected from the inferior vena cava at 30 min intervals for a period of 2 hrs. Samples of effluent perfusate was used for the assay of lactate dehydrogenase (8) and for estimating the levels of urea (9).

Statistics

The values were expressed as mean ± SD. Statistical difference was analyzed by student's-t-test and P values were determined.

RESULT AND DISCUSSION

Fig. 1 illustrates the effect of different dosage of IT on D-Galactosamine perfusion *in situ* (after 2 hrs of perfusion). Figure shows that the maximum hepatoprotective effect was exhibited by the dose of 500 mg/kg body wt for 21 days. Fig. 2 illustrates the time course of the release of lactate dehydrogenase in the liver effluent perfusate of normal and experimental groups of rats. Results indicate that following perfusion with D-Galactosamine or CCl_4, leakage and ultimate release of lactate dehydrogenase into the perfusate was significantly increased ($P<0.001$) as compared with control rat livers perfused with KHB alone. Earlier studies have shown that the release of LDH in the liver effluent perfusate was increased following perfusion with D-Galactosamine or CCl_4 (10, 11). Maximum LDH leakage was observed in the liver effluent collected after 90 min of perfusion.

Table I shows the levels of urea in the liver effluent perfusate of normal and experimental groups of rats. The levels of

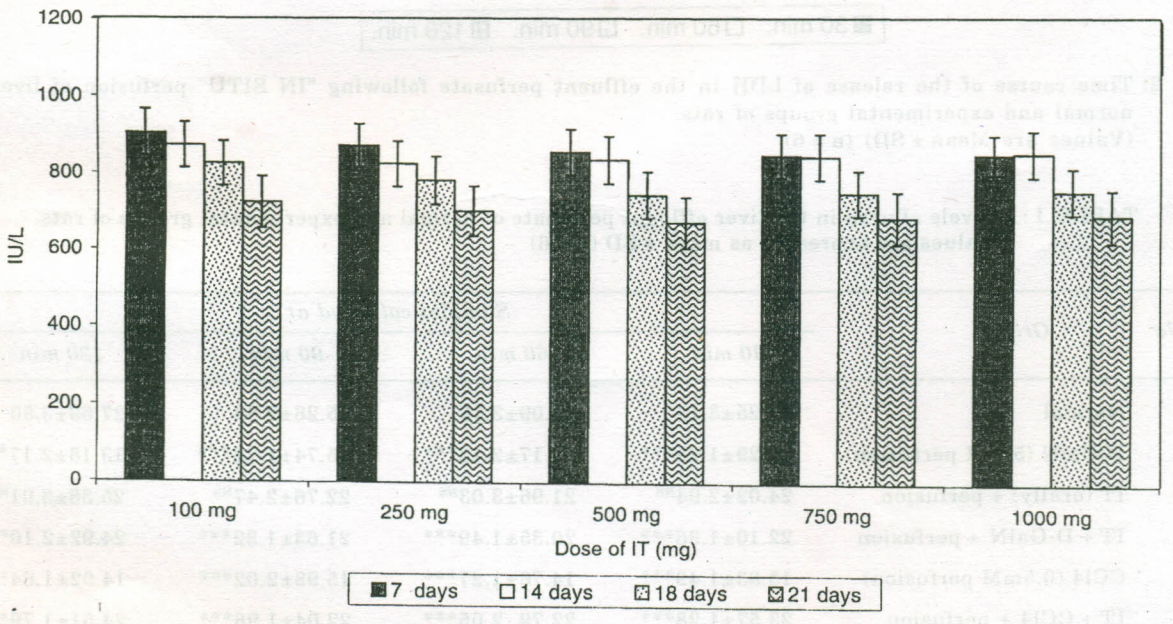

Fig. 1: Effect of different dosages of IT (for different time intervals) on LDH leakage following "In situ" perfusion of the liver (2 hrs)
(Values are Mean ± SD) (n = 6)

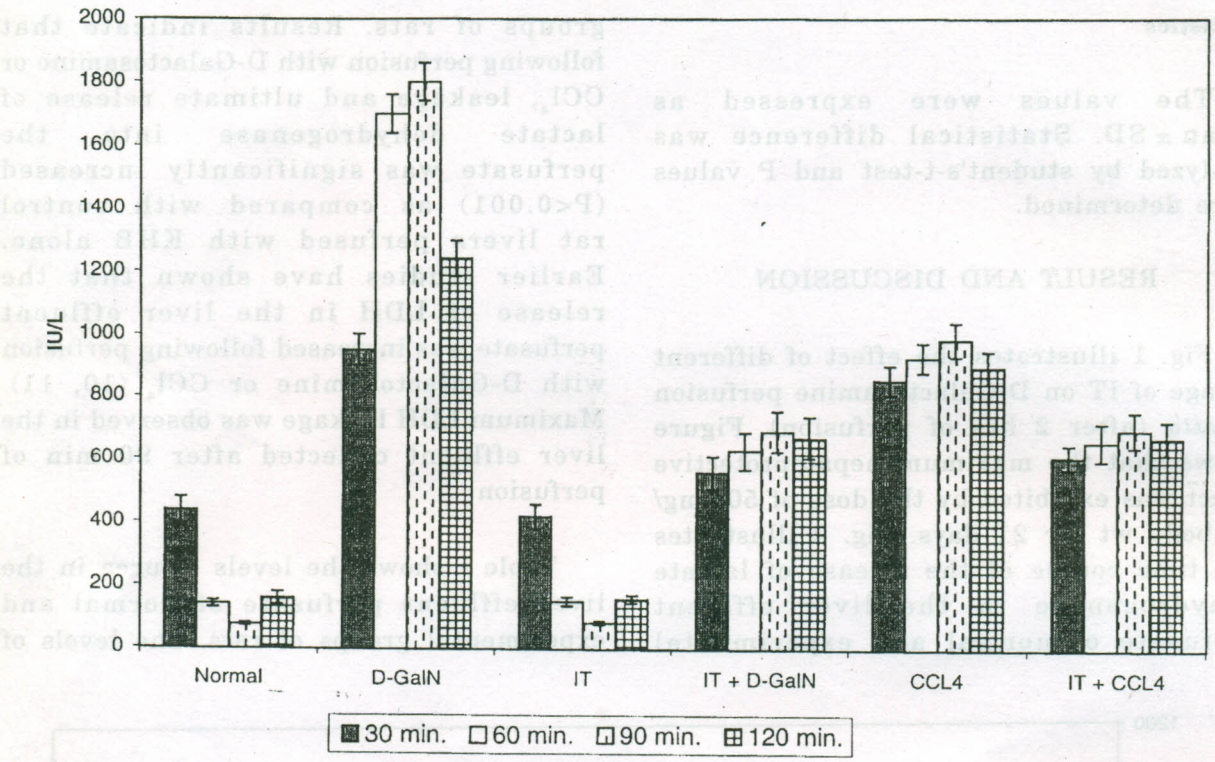

Fig. 2: Time course of the release of LDH in the effluent perfusate following "IN SITU" perfusion of liver in normal and experimental groups of rats.
(Values are Mean ± SD) (n = 6)

TABLE I : Levels of urea in the liver effluent perfusate of normal and experimental groups of rats. Values are expressed as mean ± SD (n = 6)

S. No.	*Groups*	*Samples collected at*			
		30 min	*60 min*	*90 min*	*120 min*
1.	Normal	26.25±3.13	24.09±3.16	25.28±2.80	27.59±3.50
2.	D-GalN (5 mM perfusion)	14.29±1.24***	12.17±2.13***	15.74±1.28***	13.18±2.17***
3.	IT (orally) + perfusion	24.02±2.94^{NS}	21.96±3.03^{NS}	22.76±2.47^{NS}	25.38±3.01^{NS}
4.	IT + D-GalN + perfusion	22.10±1.36***	20.35±1.49***	21.63±1.82***	24.92±2.10***
5.	CCl4 (0.5mM perfusion)	15.83±1.49***	14.76±1.27***	15.98±2.02***	14.92±1.64***
6.	IT + CCl4 + perfusion	23.57±1.28***	22.79 2.05***	22.04±1.96***	24.51±1.79***

Units – mg%

Statitical analysis by students-t-test

(Comparisons are made between Group 2 and Group 1; Group 3 and Group 1; Group 4 and Group 2; Group 5 and Group 1; Group 6 and Group 5).
***P<0.001, **P<0.01, *P<0.05, NS - Non Significant

urea showed a very significant decrease in the liver effluent perfusate after D-Galatosamine and CCl_4 challenge. Table II shows the rate of bile flow in the six groups of rats. Rat livers perfused with D-Galactosamine (Group 5) showed a significant reduction in bile flow, as compared with normal rats (Group 1). In the rats given pretreatment with IT extract in vivo prior to D-Galactosamine perfusion (Group 4) and CCl_4 perfusion (Group 6), the rate of bile flow was considerably increased as compared to Group 2 rats, and Group 5 rats respectively thereby giving an indication of its choleretic and anticholestatic effect. Rats treated with IT (Group 4) and (Group 6) prior to challenge with hepatotoxins also showed a significant reduction in LDH leakage and improved ureagenesis as observed by the levels of LDH and urea in the liver effluent.

TABLE II: Rate of bile flow in the normal and experimental groups of rats during *si situ* perfusion. Values are expressed as mean ± SD (n = 6)

S. No.	*Groups*	*Rate of bile flow (ml/100 g of liver/hr)*
1.	Normal	0.80±0.07
2.	D-GalN (5 mM perfusion)	0.25±0.03***
3.	IT (orally) + perfusion	0.84±0.09NS
4.	IT + D-GalN + perfusion	0.64±0.05***
5.	CCl_4 (0.5mM perfusion)	0.31±0.03***
6.	IT + CCl_4 + Perfusion	0.69±0.04***

Statistical analysis by students-t-test

(Comparisons are made between Group 2 and Group 1; Group 3 and Group 1; Group 4 and Group 2; Group 5 and Group 1; Group 6 and Group 5).

***P<0.001, **P<0.01, *P<0.05, NS - Non Significant

The release of lactate dehydrogenase reflects a non-specific alteration in the plasma membrane integrity / and or permeability as a response to D-Galactosamine and CCl_4 challenge (12). Any increase in lactate dehydrogenase activity during perfusion indicates an unacceptable level of liver cell necrosis (10). The decreased levels of urea in the liver effluent indicates decreased synthesis of urea which might be due to decreased activities of urea cycle enzymes in liver (13).

Thus the results of our study indicates that pretreatment with *Indigofera tinctoria* extract in vivo is capable of counteracting the toxic effects of D-Galactosamine and CCl_4 during *in situ* perfusion of the liver for 2 hr. Our study also suggests the hepatoprotective effects of *Indigofera tinctoria* in experimental animals.

ACKNOWLEDGEMENTS

The authors are thankful to Dr. R. Kathirvel, Dean, Madras Veterinary College, Vepery, Chennai for the encouragement provided to this work. The financial assistance provided to this work to one of the authors (M. Sreepriya) in the form of SRF, from the Council of Scientific and Industrial Research (CSIR), New Delhi, India is gratefully acknowledged.

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