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EFFECT OF HD-03 – A HERBAL FORMULATION IN GALACTOSAMINE-INDUCED HEPATOPATHY IN RATS

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Abstract : The effect of HD-03 a herbal preparation was studied on galactosamine (400 mg/kg b.wt., i.p.) induced hepatotoxicity in rats. Animals were pre-treated for 14 days with HD-03 and compared against untreated group for SGPT, SGOT, serum bilirubin and liver glycogen. Histopathology of liver lobes was considered to evaluate the extent of hepatic injury induced by galactosamine. These were reversed by HD-03 pre-treatment. HD-03 provided convincing evidence of hepatoprotection against galactosamine induced hapatotoxicity.

Key words: HD-03 herbal formulation hepatoprotective galactosamine

INTRODUCTION

HD-03 is a multi-herbal formulation consisting of *Solanum nigrum* L. (whole plant, 30%), *Cichorium intybus* L. (seeds, 20%), *Picrorrhiza kurroa* Benth. (roots, 20%), *Tephrosia purpurea* L. (whole plant, 20%) and *Andrographis paniculata* Nees. (leaves, 10%). Many of the individual ingredients of the formulation were earlier investigated for their protective effect against different models of experimental hepatotoxicity (1–5). HD-03 is proved to be an useful hepatoprotective agent against paracetamol, thioacetamide and isoniazid-induced hepatic damage and 750 mg/kg b.wt. was found to be a optimum dose (6). It is also found to posses free radical scavenging activity in CCl_4-C induced hepatotoxicity in rats (7) and anti-cholestatic activity in thioacetamide-induced cholestasis in Guinea pigs (8). In the present study, HD-03 was investigated for its effect against galactosamine-induced hepatopathy in rats at a dose of 750 mg/kg b. wt.

METHODS

Galactosamine-induced hepatopathy in rats :

Twenty-four inbred Wistar rats of either sex weighing between 220–250 g were used for the study. The animals were maintained on a 12–hour light and dark cycle, fed *ad libitum* with standard pellet diet (Lipton India Ltd., Mumbai) and had free

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access to water. The animals were classified into groups of eight animals each as follows.

Group I : Represented control that received 10 ml/kg b. wt., of water (vehicle) orally for 15 days.

Group II : Rats received 10 ml/kg b. wt., i.p. water (vehicle) orally for 15 days and galactosamine 400 mg/kg b. wt., i.p. on day 15th.

Group III : Rats received HD-03 at a dose of 750 mg/kg b. wt. as aqueous suspension, once daily, p.o. for a period of 15 days and galactosamine 400 mg/kg b. wt., i.p. on day 15th.

After 24 hours of galactosamine injection, blood was collected by excising the jugular vein under ether anaesthesia. Serum was separated and SGPT, SGOT (9) and bilirubin levels were estimated (10). Portions of liver was excised carefully and washed in normal saline. Portions of liver was collected for glycogen estimation (11) and for histopathology rest fixed in 10% neutral buffered formalin and processed by paraffin technique sections of 5 µ thickness, cut and stained routinely by H & E method.

Statistical analysis :

Results of biochemical estimations have been indicated in terms of mean ± SEM. The difference among means has been analysed by Student's *t*-test. Minimum level of significance was fixed at $P<0.05$ (95%).

RESULTS

Effect of HD-03 on biochemical parameters :

Administration of galactosamine at a dose of 400 mg/kg b. wt. i.p. to rats resulted in significant elevation in SGPT, SGOT and serum bilirubin levels and significant depletion in liver glycogen levels (Table I). Pre-treatment with HD-03, 750 mg/kg b. wt. for 15 days prevented elevation of above mentioned serum and liver parameters.

TABLE I: Effect of HD-03 on galactosamine-induced hepatopathy in rats.

Parameters	*Group I (Water 10 ml/kg)*	*Group II (Water 10 ml/kg+ Galactosamine 400 mg/kg on day 15th*	*Group III (HD-03 500 mg/kg+ Galactosamine 400 mg/kg on day 15th*
SGPT (IU/L)	38.50±1.38*	427.57±79.58	71.33± 7.99*
SGOT (IU/L)	111.33±3.66*	423.71±42.52	128.00± 13.20*
Liver glycogen (mg%)	13.97±0.147*	1.225±0.117	3.817± 0.284*
Total bilirubin (mg/dl)	0.333±0.03*	0.680±0.07	0.416± 0.04

*P<0.001 and *P<0.01 as compared to galactosamine group.

Fig. 1: Bile duct proliferations and periportal inflammatory cells collections (H & E, x450).

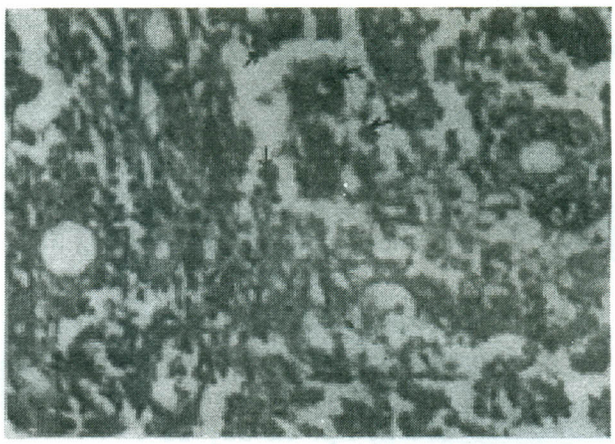

Fig. 2: Patches of necrotic areas characterized by degeneration and lysis of the hepatic parenchyma with also appearance of inclusion like structures in the nuclear components (H & E, x450).

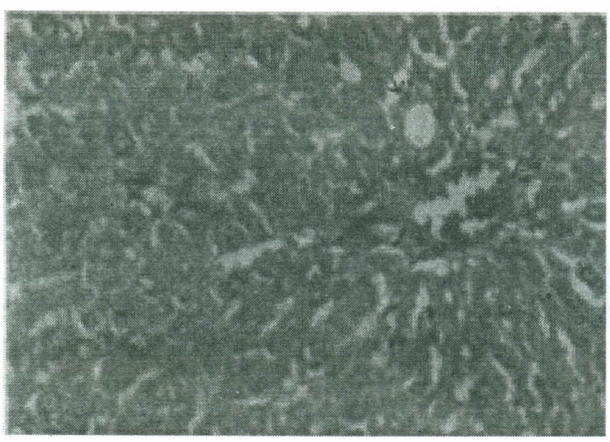

Fig. 3: Membrane stability as reflected by intactness of hepatic structure and architecture; and stray collections of inflammatory cells (H & E, x450).

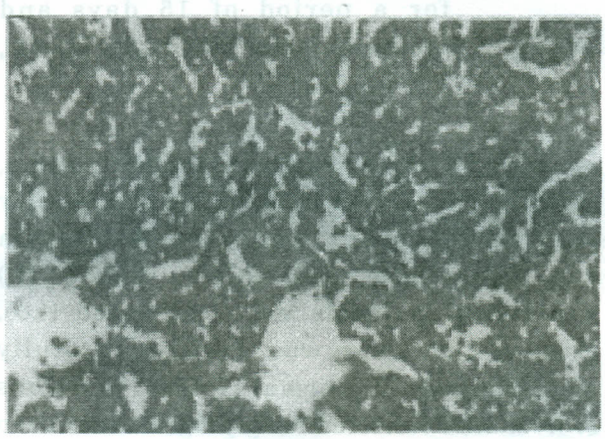

Fig. 4: Minimal areas of necrosis marked by focal condensates and stray demonstrability of nuclear inclusions (H & E, x450).

Histopathology :

The histological changes observed in the liver of the rats belonging to the three groups are as follows:

In group I, the structural and architectural frame of the different lobes of liver of all the eight rats showed normal histological picture. In group II, the pathological changes noticed consisted of hyperaemia, oedema, and bile duct proliferation with collections of inflammatory cells around the portal areas and necrotic areas with degeneration and lysis in the parenchyma. There were

also inclusion like structures in the nuclei of the degenerate hepatocytes (Figures 1 and 3). In group III, minimal changes were noticed as compared to the galactosamine damage described under group II. The histological picture showed near normal hepatic architecture with stray collections of inflammatory cells. Areas of necrosis were minimal with stray demonstrability of the nuclear inclusions (Figures 3 and 4).

DISCUSSION

The result of the present study indicate that pre-treatment with HD-03 reversed the serum GOT, GPT and bilirubin levels to normal, when compared to galactosamine intoxication, which resulted in increased levels. In addition, intoxicated group also showed hepatic glycogen depletion from sub-cellular injury and disturbances of intercellular Ca^{-} pool with resultant glycogenolysis (12). Pre-treatment with HD-03 significantly prevented liver glycogen depletion, which indicated membrane-stabilising activity. It is known that, galactosamine disrupts the permeability of plasma membrane causing the leakage of enzyme from the cell, which leads to elevation in serum enzymes (13). Since galactosamine produces toxicity via its intermediate metabolic products, it is likely that HD-03 inhibited the metabolism of galactosamine leading to reduced generation of toxic metabolites.

The characteristic changes of necrosis and eosinophilic condensates resembling inclusions in the hepatic parenchyma in association with biliary and periportal changes matched with the toxic changes ascribed to galactosamine and simulating human viral hepatitis (14). The biochemical changes in relation to serum enzymes and bilirubin levels were convincingly proportionate to the extent of damage to the hepatic and biliary components.

The HD-03 pre-treated group provided substantial evidence of hepatoprotectiveness in which membrane stability of hepatocytes, near normal manifestations of structure and architecture, highly restricted biliary changes were observed. The levels of serum transaminases and liver glycogen matched with the histological features recorded in this group as compared to the control. The outcome of these observations provided evidence that HD-03 stabilised the hepatic frame against the toxic impact of galactosamine.

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