ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2000 Sep; 21 (9): 777 - 781
http://www.chinaphar.com

Inhibition of experimental cirrhosis in rats by HD-03

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KEY WORDS herbal medicine; HD-03; liver cirrhosis

ABSTRACT

AIM: To investigate the protective effect of HD-03 in experimental cirrhosis following chronic intoxication with thioacetamide (TAA). **METHODS**: The effect of HD-03 (750 mg/kg *po*) was studied in rats following TAA-induced intoxication (50 mg/kg *po*) for a period of 90 d. HD-03 was administered as an aqueous suspension. Levels of biochemical markers indicative of hepatotoxicity were assessed in serum and liver. Histopathological evaluation of liver was also carried out to find out the protective effect of HD-03 following TAA-induced chronic intoxication. **RESULTS**: Administration of TAA at a dose of 50 mg/kg *po* for 90 d resulted in a significant derangement of serum [serum glutamic pyruvate transaminase (SGPT), serum glutamic oxaloacetate transaminase (SGOT), alkaline phosphatase (ALP), albumin and bilirubin] and hepatic (triglycerides, protein, hydroxyproline, collagen and glycogen) biochemical parameters. Histopathological evaluation of liver sections following TAA-intoxication showed necrosis and proliferative changes characteristic of cirrhosis. Simultaneous treatment of TAA-intoxicated rats with HD-03 at a dose of 750 mg/kg *po* for the same duration significantly prevented the changes in both serum and hepatic biochemical parameters. The reversal of serum and hepatic biochemical parameters also correlated with the preservation of liver histoarchitecture in HD-03 treated rats. **CONCLUSION**: The responses such as membrane stabilization, hepatocellular regeneration, and inhibition of collagen formation are the contributing factors in the correction of TAA-induced cirrhosis by HD-03.

INTRODUCTION

Cirrhosis denotes a diffuse proliferation of fibrous connective tissue of the liver with areas of both necrosis and regeneration of parenchymal cells imparting a nodular or glandular texture. This condition leads to hepatic failure and portal hypertension with attended complications such as jaundice, encephalopathy, ascites, and digestive tract hemorrhage.

Liver cirrhosis is one of the major public health problems worldwide and has a very high mortality rate in people of the age groups of 40 to 50 years. It is well known that despite extensive investigation of liver function and pathology, there is no effective therapy for many liver diseases excepting at best through symptomatic management of the complications of chronic liver disease[1].

Herbal resources are known to be coming up in a promising way especially in the context of liver diseases and one such preparation is HD-03. It is a multi-herbal formulation consisting of *Solanum nigrum* L, *Cichorium intybus* L, *Picrorrhiza kurroa* Benth, *Tephrosia purpurea* L, and *Andrographis paniculata* Nees. It has been reported to possess hepatoprotective effects against chemical and drug-induced liver damage in various experimental models[2-5]. The present study was aimed at evaluating the effect of HD-03 against thioacetamide-induced cirrhosis in rats.

MATERIALS AND METHODS

Preparation of HD-03 HD-03 comprises *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 %). The herbs were individually powdered, weighed, and mixed in appropriate proportions. The constituents of the plant material were procured from local supplier and identified by Dr R KANNAN, Botanist, R&D Centre, the Himalaya Drug Company, and voucher specimens were preserved at R&D Centre. Two or more such batches of preparations from raw materials of different origin were standardized by finger print analysis for characterization using high performance thin layer chro-

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E-mail skmitra@bgl.vsnl.net.in
Received 2000-02-14 Accepted 2000-06-05

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2000 Sep; 21 (9)
E-mail aps@mail.shcnc.ac.cn Phn/Fax 86-21-6474-2629

matography (HPTLC).

HPTLC analysis One gram of HD-03 was weighed and extracted by refluxing on a water bath with 15 mL of dichloromethane. Extract was filtered and concentrated to 2 mL. Ten microlitre of concentrate was spotted on pre-coated silica gel plate. Plate was developed using dichloromethane : methanol (97 : 3). Plate was scanned using densitometer at 366 nm. Fingerprint of HD-03 is shown in Fig 1.

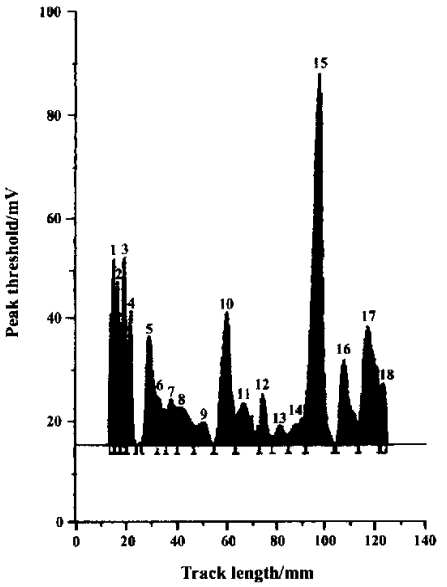

Fig 1. HPTLC finger print pattern of HD-03. Wavelength: 366 nm.

Animals Thirty inbred male Wistar rats weighing 220 – 250 g were used for the study. The animals were maintained on a 12-h light and dark cycle, at (22 ± 3) ℃, fed *ad libitum* with standard pellet diet (Hindustan Lever Ltd, Mumbai) and had free access to water.

Study protocol The rats were randomized into three groups comprising of 10 animals each. Group Ⅰ, serving as normal control, received 10 mL/kg of water as a vehicle once a day orally for 90 d. Rats of group Ⅱ received thioacetamide (TAA) at a dose of 50 mg/kg *po* for the same duration. Group Ⅲ rats were administered TAA 50 mg/kg + HD-03 750 mg/kg *po* for the same duration. HD-03 was administered in the form of an aqueous suspension and TAA was administered as an aqueous solution. On d 91, the overnight fasted animals were euthanised by decapitation, under ether anesthesia. Blood was collected for the estimation of biochemical parameters such as SGPT, SGOT, alkaline phosphatase, protein, albumin, and bilirubin (Boehringer Mannheim kits, Germany). The liver was dissected immediately, rinsed with cold saline and subjected to estimation of protein, hydroxyproline[6], collagen[7], triglycerides[8], and glycogen[9]. Pieces of liver were also fixed in 10 % neutral buffered formalin and processed by the paraffin technique. Sections of 5 μm thickness were cut and stained by routine H&E method for histopathological evaluation.

Statistical analysis The values are expressed as $\bar{x} \pm s$. The results were statistically analyzed using unpaired t-test to find out the level of significance. The minimum level of significance was fixed at $P < 0.05$.

RESULTS

The findings of SGPT, SGOT, ALP, total bilirubin, albumin, globulin, and A/G ratio have been summarized in Tab 1. The parameters in Group Ⅱ showed significant elevation of SGPT, SGOT, ALP, total bilirubin, and globulin compared with Group Ⅰ and Group Ⅲ, while albumin and A/G ratio were observed to be lowered. Simultaneous treatment with HD-03 at 750 mg/kg significantly prevented the changes in serum parameters induced by thioacetamide intoxication as compared with Group Ⅱ.

The parameters with respect to evaluation of liver tissue following TAA intoxication showed a significant increase in liver weight, triglycerides, hydroxyproline, collagen, and decrease in liver glycogen and protein (Tab 2). HD-03 treatment significantly prevented the changes in hepatic biochemical parameters as compared to Group Ⅱ (Tab 2).

Histopathological evaluations of liver sections from Group Ⅰ showed a normal histological picture without any apparent damages or disruptions (Fig 2A). All the liver sections from Group Ⅱ showed changes of necrosis within the lobules and also proliferative changes involving connective tissue component as well as the biliary component in the periportal and interlobular areas. The extent of connective tissue proliferates varied from dense patches to strands and bands (Fig 2B,2C). Liver sections from Group Ⅲ showed distinct preservation of structural and architectural frame of the hepatic parenchyma indicating membrane stability (Fig 2D).

Tab 1. Effect of HD-03 on serum biochemical parameters in TAA-induced liver cirrhosis in rats.

Parameters	Group Ⅰ (Control)	Group Ⅱ (TAA)	Group Ⅲ (TAA + HD-03)
SGPT (IU/L)	31.9 ± 2.4	44 ± 13^c	33.7 ± 7.1^e
SGOT (IU/L)	92 ± 14	111 ± 22^b	91 ± 16^e
ALP (IU/L)	302 ± 82	796 ± 212^c	471 ± 147^f
Protein (g/L)	70.5 ± 2.8	71.5 ± 4.4	69.8 ± 3.2
Albumin (g/L)	38.1 ± 4.1	35.3 ± 2.5^b	38.0 ± 2.8^e
Globulin (g/L)	32.4 ± 1.9	36.2 ± 1.9^c	31.8 ± 3.8^f
A/G ratio	1.18 ± 0.22	0.98 ± 0.06^b	1.22 ± 0.22^e
Bilirubin (mg %)	0.20 ± 0.03	0.51 ± 0.13^c	0.33 ± 0.09^g

$^bP < 0.05$, $^cP < 0.01$, *vs* Group Ⅰ.
$^eP < 0.05$, $^fP < 0.01$, *vs* Group Ⅱ.

Tab 2. Effect of HD-03 on hepatic biochemical parameters in TAA-induced liver cirrhosis in rats. $\bar{x} \pm s$. $^cP < 0.01$ *vs* Group Ⅰ. $^fP < 0.01$ *vs* Group Ⅱ.

Parameters mg/g tissue	Group Ⅰ (Control)	Group Ⅱ (TAA)	Group Ⅲ (TAA + HD-03)
Protein	38.2 ± 3.3	32.7 ± 1.90^c	36.24 ± 0.35^f
Triglycerides	4.8 ± 0.9	6.73 ± 1.93^c	3.91 ± 1.90^f
Hydroxyproline	0.54 ± 0.95	0.97 ± 0.13^c	0.60 ± 0.09^f
Collagen	4.02 ± 0.63	7.16 ± 0.95^c	4.44 ± 0.73^f
Glycogen	94 ± 19	3.48 ± 0.92^c	5.60 ± 1.36^f

DISCUSSION

The experimental induction of thioacetamide toxicity over a period of 90 days affected the hepatic and serum biochemical parameters in rats. The histopathological changes in the liver showed features of well-defined hepatopathic changes comprising of focal to patchy necrosis within the lobules and proliferative changes characteristic of cirrhosis. The proliferative changes also involved the biliary components. Similar features in histoarchitecture of the liver were reported by Torres *et al*, in the context of the appearance of cirrhosis following chronic exposure to thioacetamide in rats[10]. Muller *et al*, has laid particular emphasis on bile duct proliferation and peribiliary inflammation in thioacetamide intoxicated rats[11].

The increase in SGPT and SGOT levels on TAA treatment was further substantiated by necrosis of the hepatocytes. It is reported that TAA caused cell membrane damage with a resultant loss of semipermeable character, which resulted in the leakage of the enzymes, disturbances in electrolyte mobilization and failure of cell metabolism[12]. The reduction in the levels of transaminases in the HD-03 treated group could be due to the membrane-stabilizing effect of the formulation whereby the leakage of the enzymes was prevented as indicated by minimal necrosis and structural intactness of hepatocytes. Judah *et al*, reported that strophanthin-G inhibited cell necrosis by acting on the ion transport mechanism[13]. Our results following HD-03 treatment are also suggestive of a similar mechanism of action.

Chronic intoxication with TAA also resulted in hyperglobulinemia, a feature observable in most forms of chronic liver disease. The possible mechanism for this increase in serum antibody (viz, globulin) levels in cirrhosis can be due to the fact that protein antigens from the gut bypass the reticulo-endothelial cells (Kupffer cells) in the liver and produce antigenic stimulus to other organs, particularly the spleen, thereby increasing serum antibodies[14,15]. HD-03 treatment significantly prevented the elevation of globulin levels possibly by inhibiting this mechanism. The A/G ratio was also lowered significantly in the TAA-intoxicated group, which was hindered in the HD-03 treated group. The elevation of total bilirubin and ALP levels in thioacetamide treated group was also restored in the HD-03 treated group.

Several reports have recorded fatty degeneration in

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2000 Sep; 21 (9)
E-mail aps@mail.shcnc.ac.cn Phn/Fax 86-21-6474-2629

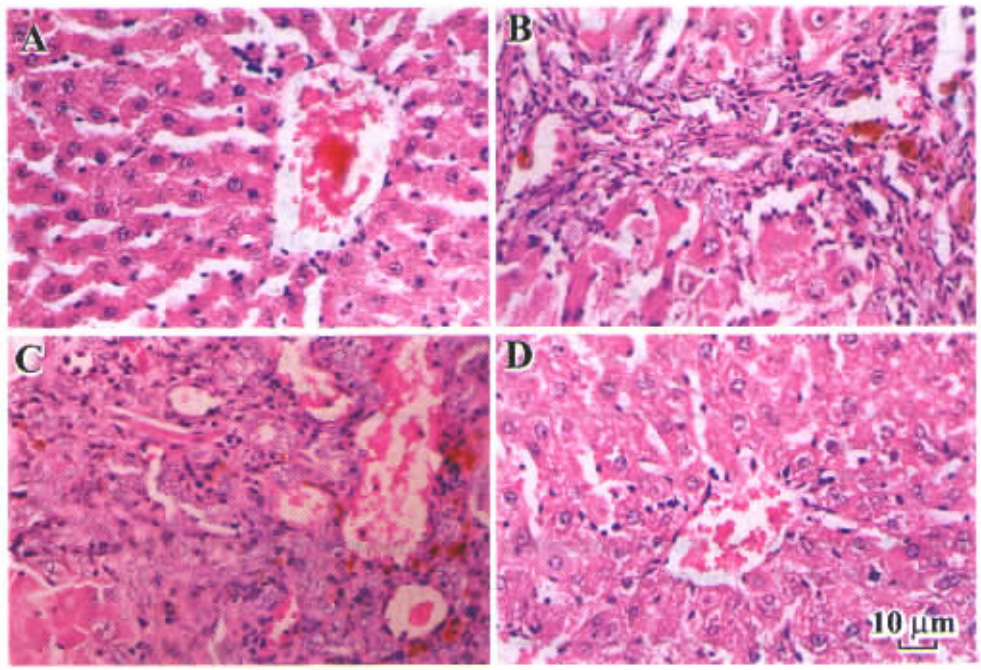

Fig 2A. Photomicrograph of normal rat liver. Fig 2B. Periportal connective tissue proliferation and focal areas of necrosis in rats treated with thioacetamide. Fig 2C. Photomicrograph of liver showing hyperemia, connective tissue proliferation and bile duct proliferation in rats treated with thioacetamide. Fig 2D. Photomicrograph of liver showing minimal damage and intactness of hepatocytes in rats treated with combination of thioacetamide and HD-03. H&E, × 1000.

the TAA toxicity though the magnitude is much less as compared with CCl_4 toxicity[16]. There was an increase in hepatic triglyceride levels, which could be due to the fatty degeneration following TAA intoxication. Triglyceride levels were diminished in rats treated with HD-03. Liver glycogen levels were significantly depleted in TAA-treated group, which was prevented with HD-03. treatment.

Rats intoxicated with thioacetamide showed lower protein synthesis on account of cell necrosis. The normalization of hepatic protein levels were further supported by minimal necrosis of hepatocytes in the HD-03 treated group. Collagen, a heterogenous class of extracellular proteins, contains hydroxyproline as one of its major component. Thioacetamide intoxication increases collagen levels, which disrupts the hepatic architecture and converts sinusoids to capillaries, thereby impede metabolic exchanges through basement membranes between liver cells and the circulation. An increase in the hydroxyproline and collagen levels was noted in TAA treated group as evidenced by the increased connective tissue proliferates in the liver section. HD-03 treatment reduced the hepatic levels of hydroxyproline and collagen, which correlated well with the restricted connective tissue proliferates in the liver sections.

It can be concluded from the biochemical and histopathological findings that membrane stabilization, hepatocellular regeneration, and inhibition of collagen formation are the contributing factors in the correction of HD-03 mediated thioacetamide-induced cirrhosis.

REFERENCES

1 Kradjan WA. Cirrhosis. In: Herfindal ET, Gourley DR, Hart LL, editors. Clinical pharmacy and therapeutics. Baltimore, USA: Williams and Wilkins; 1992. p 473.

2 Mitra SK, Venkataranganna MV, Sundaram R, Gopumadhavan S. Effect of HD-03, a herbal formulation, on the antioxidant defense system in rats. Phytother Res 1998; 12: 114 – 7.

3 Mitra SK, Venkataranganna MV, Sundaram R, Gopumadhavan S. Protective effect of HD-03, a herbal formulation, against various hepatotoxic agents in rats. J Ethnopharmacol 1998; 63: 181 – 6.

4 Mitra SK, Venkataranganna MV, Sundaram R, Gopumadhavan S. Anti-cholestatic activity of HD-03, a herbal formulation, in thioacetamide (TAA)-induced experimental cholestasis. Indian J Exp Biol 1999; 37: 409 – 10.

5 Mitra SK, Seshadri SJ, Venkataranganna MV, Gopumadhavan S, Venkatesh Uḍupa U, Sarma DNK. Effect of HD-03, a herbal formulation, in galactosamine-induced hepatopathy in rats. Indian J Physiol Pharmacol 2000; 44: 82 – 6.

6 Varley H, Gowenlock AH, Bell M. Amino acid nitrogen. Practical clinical biochemistry; v 1. London: William Heinemann Medical Book Limited; 1980. p 525.
7 Man M, Nagle RB, Adams E. Chemical estimation of interstitial and basement membrane collagen in obstructive nephropathy. Exp Mol Pathol 1978; 29: 144 – 8.
8 Gottfried SP, Rosenburg B. Improved manual spectrophotometric procedure for determination of serum triglycerides. Clin Chem 1973; 19: 1077 – 8.
9 Dwivedi Y, Rastogi R, Garg NK, Dhawan BN. Picroliv and its components kutkoside and picroside I protect liver against galactosamine-induced damage in rats. Pharmacol Toxicol 1992; 71: 383 – 7.
10 Torres MJ, Fernandex MI, Gilatid Rios A. Effect of dietary nucleotides on degree of fibrosis and steatosis induced by oral intake of TAA. Dig Dis Sci 1997; 42: 1322 – 8.
11 Muller D, Enderle GJ, Low O, Dietz E, Kreu H. Bile duct proliferation and altered leukotrine elimination in TAA induced fibrosis of rat liver. J Hepatol 1996; 25: 547 – 53.
12 Singh A, Handa SS. Hepatoprotective activity of *Apium graveolens* and *Hygrophila auriculata* against paracetamol and thioacetamide intoxication in rats. J Ethnopharmacol 1995; 49: 119 – 26.
13 Judah JD, Ahmed K, McLean AEM. Protection provided against carbon tetrachloride and thioacetamide poisoning by strophanthin-G. J Pathol Bacteriol 1965; 89: 619 – 23.
14 Jacob AI, Goldberg PK, Bloom N. Endotoxin and bacteria on portal blood. Gastroenterology 1977; 72: 1268 – 70.
15 Thomas HC, Ryan CJ, Benjamin IS. The immune response in cirrhotic rats. The induction of tolerance to orally administered protein antigens. Gastroenterology 1976; 71: 114 – 7.
16 Moreira E., Luis F, Jose LP, Fermin SDM, Angel G. Changes in fatty acid composition of plasma, liver microsomes and erythrocytes in liver cirrhosis induced by oral intake of TAA in rats. Hepatology 1995; 21: 199 – 206.

HD-03 对实验性肝硬化的抑制

关键词 草药医学; HD-03; 肝硬化

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