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ORIGINAL ARTICLE

Hepatoprotective and behavioral effects of jigrine in galactosamine-induced hepatopathy in rats

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Abstract

The hepatoprotective activity of jigrine, a polypharmaceutical herbal formulation, at a dose of 1 mL/kg/day p.o. was evaluated against galactosamine (400 mg/kg b.wt.)-induced hepatopathy in rats. Biochemical parameters such as alanine trasaminase (ALT), alkaline phosphatase (ALP), and bilirubin were estimated to assess liver function. Jigrine was also evaluated for its effect on the possible behavioral alterations secondary to liver damage produced by galactosamine (D-Gal) administration in rats. The D-Gal-induced elevation in serum levels of ALT, ALP, and bilirubin was significantly reduced (p values <0.01, <0.01, and <0.05, respectively) in jigrine- and silymarin-pretreated rats. Jigrine pretreatment also exhibited beneficial effects on D-Gal-induced behavioral abnormalities in rats. Silymarin (25 mg/kg/day p.o.) was used as reference standard. The biochemical observations were supplemented with histopathological examination of rat liver sections. Histopathological evaluation showed marked improvement in the livers of jigrine- and silymarin-treated animals.

Keywords: *Behavioral; galactosamine; hepatoprotective; jigrine; silymarin; Unani medicine*

Introduction

Jigrine [Hamdard (Wakf) Laboratories] is a polypharmaceutical herbal hepatoprotective syrup formulation containing aqueous extracts of 14 medicinal plants used in the Unani traditional Indian system of medicine for liver ailments (Table 1). A few studies are reported on its constituents (Najmi et al., 2002), safety evaluation (Valecha et al., 1990), mechanism of hepatoprotective action (Vivek et al., 1994; Aftab et al., 1999, 2002), and anti-inflammatory activity (Karunakar et al., 1997). In general, investigators have concentrated on behavioral study in hepatic damage only when the hepatic toxicant was administered in multiple and high doses to produce hepatic encephalopathy (Shimomura et al., 1992; Baraldi et al., 1995), although behavioral and psychiatric deficits are some of the earliest signs in cirrhotic patients (Watanabe et al., 1995). The present investigation was designed to evaluate the hepatoprotective effect of jigrine against D-galactosamine (D-Gal)-induced hepatotoxicity in rats. The aim of this investigation was also to determine whether a single dose of D-Gal could produce any behavioral impairment in rats. The effect of jigrine on the possible behavioral alterations secondary to liver damage produced by D-Gal in rats was also evaluated.

Materials and methods

Drugs and chemicals

Jigrine was provided by Hamdard (Wakf) Laboratories, Ghaziabad, India. Silymarin was purchased from Micro Laboratories, Holar, TN, India. Galactosamine was purchased from SRL, India. All the biochemicals and chemicals used were of analytical grade.

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Animals

Albino rats of Wistar strain weighing 150–200 g were used for the study. Animals were supplied by the Central Animal House Facility of Hamdard University, and were kept under standard laboratory conditions in a 12 h light/dark cycle at 25 ± 2°C. Animals were provided with a pellet diet (Lipton, India) and water *ad libitum*.

Experimental protocol

Rats were randomly divided into four groups of six animals each. Group I served as normal control and received normal saline for 21 days. Group II served as toxic control and received normal saline (1 ml/kg, p.o.) for 21 days. Groups III and IV were treated prophylactically with jigrine (1 ml/kg, p.o.) and silymarin (25 mg/kg, p.o.) for 21 days, respectively. Groups II, III, and IV also received D-Gal (400 mg/kg, i.p.) on the 21st day (Mitra et al., 2000). All the groups were observed in a number of behavioral tests 24 h after D-Gal administration. Behavioral observations were carried out from 7.00 to 11.00 h. Immediately after the behavioral study, blood was collected from the tail vein under light ether anesthesia for estimation of various marker enzymes. Liver samples were also collected for histopathological evaluation. The blood samples were allowed to clot at 37°C for 30–40 min. Serum was separated by centrifugation and used for the estimation of various biochemical parameters. All the procedures carried out on animals were approved by the institutional animal ethics committee (JHAEC).

Behavioral study

Each animal was subjected to a series of tests in the following order: first, the animal was observed inside a plus-maze, then in an open field arena, and finally in an "actophotometer." Between each observation the animal was returned to its home cage for 30 min (Vale et al., 1999).

Spontaneous alternation behavior

Spontaneous alternation testing was conducted by placing a rat on the central platform of the plus-maze and allowing 12 min of unimpeded exploration. The number and sequence of arm entries were recorded for calculation of a "percent alternation score." An alternation was defined as entry into four consecutive arms on overlapping quintuple sets. Five consecutive arm choices within the set of arm choices made up a quintuple set. A quintuple set consisting of arm choices ABDAC was considered an alternation. A quintuple set consisting of arm choices ABDAB was not considered an alternation. Using this procedure, possible alternation sequences are equal to the number of arm entries minus 4. The percent alternation score is equal to the ratio of (actual alternation/possible alternation) × 100.

The plus-maze was composed of four arms joined to a central platform. Each arm was 55 cm long and 10 cm wide, with 12 cm high walls. The central platform was 25 cm across. The floor and walls were made of gray wood. The maze floor was wiped with a cotton swab dipped in 70% alcohol between animals to prevent accumulation of odors (Ragozzino et al., 1996).

Table 1. Medicinal plant ingredients of jigrine (a phytopharmaceutical formulation).

Botanical name	Common name	Unani name	Family	Part used
Cichorium intybus L.	Chicory Kasni	Tukhme	Compositeae	Leaves
Tamarix dioica Roxb.	Tamarisk	Jhau	Tamaricaceae	Leaves
Solanum nigrum L.	Black nightshade	Makoh	Solanaceae	Fruit
Rheum emodii Wall.	Indian rhubarb	Revand Chini	Polygonaceae	Rhizome
Rubia cordifolia L.	Indian madder	Majeeth	Rubiaceae	Root
Vitex negundo L.	Nisinda	Sambhalu	Verbenaceae	Whole Shrub
Cassia occidentalis L.	Coffee senna	Kasaundi	Caesalpiniaceae	Leaves
Foeniculum vulgare Mill.	Fennel	Sonf	Umbellifereae	Fruit
Cuscuta reflexa Roxb.	Amarvella Kasoos	Tukhme	Convolvulaceae	Seed
Careya arborea Roxb.	Wild guava	Baokhamba	Barringtoniaceae	Fruit
Phyllanthus niruri L. & Hook	Jaramla	Bhui amla	Euphorbiaceae	Leaves
Plantago major L.	Isphagol	Bartang	Plantaginaceae	Leaves
Rosa damascena Mill.	Damask rose	Gul-e-surkh	Rosaceae	Flower
Solanum xanthocarpum Schrad. & H.Wendl.	Yellow berries Night shade	Katheli	Solanaceae	Root Fruit

Open field test

Rats were placed singly into the open field arena and the following behavioral parameters were scored: ambulation (number of areas entered with all four paws); frequency of rearing; and frequency of grooming. Fecal pellets were removed after every occupation and the floor wiped with clean damp tissues after every occupation. The open field consisted of a circular arena 85 cm in diameter, divided into 25 segments of approximately equal area by black painted lines on the white floor. The arena was bounded by a wall 30 cm high (Tricklebank et al., 1978).

Locomotor activity

The spontaneous motor activity of a rat was recorded by placing the animal in a photoelectric actimeter (actophotometer). This apparatus consisted of a square chamber, and the activity of the animal was measured by light beams connected to a photoelectric cell. The total number of beam breaks was measured for 6 min (Renault et al., 1999).

Biochemical estimations

Serum alanine trasaminase (ALT) (Reitman & Frankel, 1957), alkaline phosphatase (ALP) (King & King, 1954), and bilirubin (Varley, 1980) were estimated according to the reported procedures.

Histological studies

Livers were quickly removed and preserved in neutral buffered formalin. Histological liver sections were prepared as previously described (Luna, 1968).

Statistical analysis

Results are expressed as mean ± SEM. For biochemical results, the total variation present in a set of data was estimated by analysis of variance (ANOVA) followed by Dunnet's *post hoc* test. For behavioral study, the Mann–Whitney *U*-test was used. $p < 0.05$ was considered significant.

Results

A significant increase in ALT, ALP, and bilirubin was observed in animals treated with D-Gal (group II) as compared to the normal control animals (group I). The D-Gal-induced elevation in levels of the above indices was decreased significantly by prophylactic treatment of the rats with both jigrine and silymarin (Table 2). Spontaneous alternation behavior evaluation revealed that in D-Gal-treated animals (group II), the alternation score was decreased as compared to the normal group (group I). Jigrine pretreatment increased the percent alternation score significantly. Silymarin also increased the percent alternation score (Table 3). Motor activity was decreased by D-Gal administration. Jigrine and silymarin pretreatment restored the motor activity significantly (Table 4). Results for open field activity were not significant (Table 5).

Table 2. Effect of jigrine and silymarin on various serum biochemicals in D-Gal-induced hepatopathy in rats.

Group	Treatment	ALT (IU/mL)	ALP (IU/mL)	Bilirubin (mg%)
I	NS	39.83 ± 5.102	19.21 ± 3.28	0.504 ± 0.086
II	NS + D-Gal	385.0 ± 76.72*	47.10 ± 3.98*	1.070 ± 0.164*
III	Jig + D-Gal	87.16 ± 8.73*	19.97 ± 2.75*	0.595 ± 0.127**
IV	Sil + D-Gal	78.33 ± 9.46*	21.62 ± 2.08*	0.690 ± 0.115**
F ratio		22.97	16.71	4.52

Note. Data are expressed as mean ± SEM, $n = 6$. Significance of difference was evaluated with respect to group II by one-way ANOVA followed by Dunnet's *post hoc* test. *$p < 0.01$, **$p < 0.05$.

Table 3. Effect of jigrine and silymarin on spontaneous alternation score in D-Gal-induced hepatopathy in rats.

Group	Treatment	% Alternation score	No. of arm entries
I	NS	68.81 ± 4.10	20.83 ± 1.22
II	NS + D-Gal	57.51 ± 10.31*	17.83 ± 2.49
III	Jig + D-Gal	82.64 ± 6017**	16.0 ± 2.28
IV	Sil + D-Gal	65.20 ± 10.30*	15.83 ± 3.0

Note. Data are expressed as mean ± SEM, $n = 6$. Significance of difference was evaluated with respect to group II by Mann–Whitney *U*-test. *Non-significant, **$p < 0.05$.

Table 4. Effect of jigrine and silymarin on locomotor activity in D-Gal-induced hepatopathy in rats.

Group	Treatment	No. of beam breaks
I	NS	407 ± 36.83
II	NS + D-Gal	288.5 ± 15.78*
III	Jig + D-Gal	383.83 ± 31.29*
IV	Sil + D-Gal	365.67 ± 27.66*

Note. Data are expressed as mean ± SEM, $n = 6$. Significance of difference was evaluated with respect to group II by Mann–Whitney *U*-test. *$p < 0.05$.

Table 5. Effect of jigrine and silymarin on open field activity in D-Gal-induced hepatopathy in rats.

Group	Treatment	Ambulation	Grooming	Rearing
I	NS	89.33 ± 5.18	32.83 ± 3.09	37.66 ± 3.84
II	NS + D-Gal	70.66 ± 8.02*	13.16 ± 2.19*	29.33 ± 3.28*
III	Jig + D-Gal	77.5 ± 12.86*	16.83 ± 1.92*	22.16 ± 3.53*
IV	Sil + D-Gal	95.33 ± 11.49*	10.5 ± 1.99*	20.83 ± 3.08*

Note. Data are expressed as mean ± SEM, $n = 6$. Significance of difference was evaluated with respect to group II by Mann–Whitney *U*-test. *Non-significant.

Histopathological observations

Histology of the liver sections of normal control animals (group I) showed normal hepatic cells with normal liver architecture (Figure 1). The liver sections of D-Gal-treated animals (group II) showed hepatic cells with severe toxicity characterized by an inflammatory cell collection around the portal tract and central vein, scattered inflammation across the liver parenchyma, focal necrosis, and swelling of vascular endothelial cells (Figure 2). Jigrine pretreatment in group III appeared to significantly reverse D-Gal toxicity, as revealed by few scattered inflammatory cells with no periportal clustering and necrosis (Figure 3). Silymarin pretreatment (group IV) also exhibited protection from D-Gal-induced liver toxicity (Figure 4, Table 6).

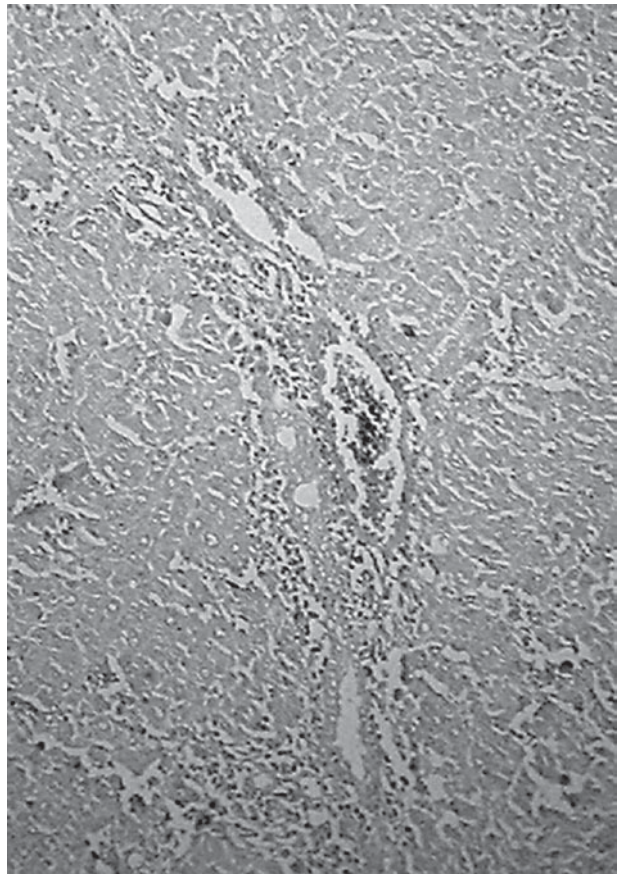

Figure 1. Group I: liver section of normal control rat showing normal liver architecture (H&E, ×100).

Discussion

D-Gal administration in rats disrupts the permeability of the plasma membrane, causing leakage of enzymes from the cell, which leads to an elevation in serum enzymes (Mitra et al., 2000). D-Gal-induced hepatotoxicity is considered to be an experimental model of acute hepatitis (Keppler & Decker, 1969), and resembles human viral hepatitis (Keppler et al., 1968). Elevated levels of serum enzymes are indicative of cellular leakage and loss of functional integrity of the cell membrane in the liver (Drotman & Lawhorn, 1978). Damage to liver cells causes leakage of cellular enzymes into the serum. A significant rise in the transaminase concentration can be taken as

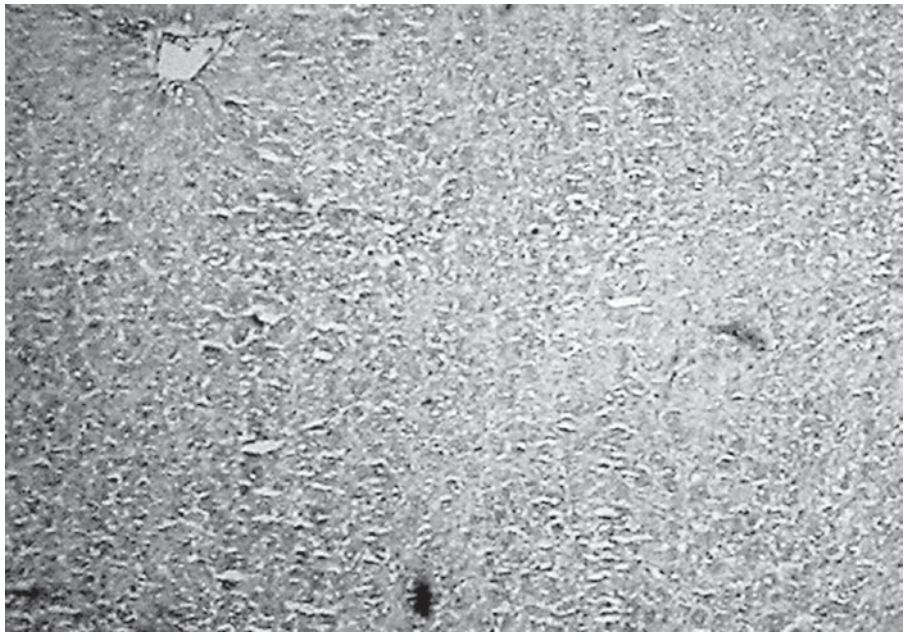

Figure 2. Group II: liver section of rat treated with D-Gal (400 mg/kg, i.p.) showing inflammatory cell collection around portal tract and central vein, scattered inflammation across liver parenchyma, and swelling of vascular endothelial cells (H&E, ×100).

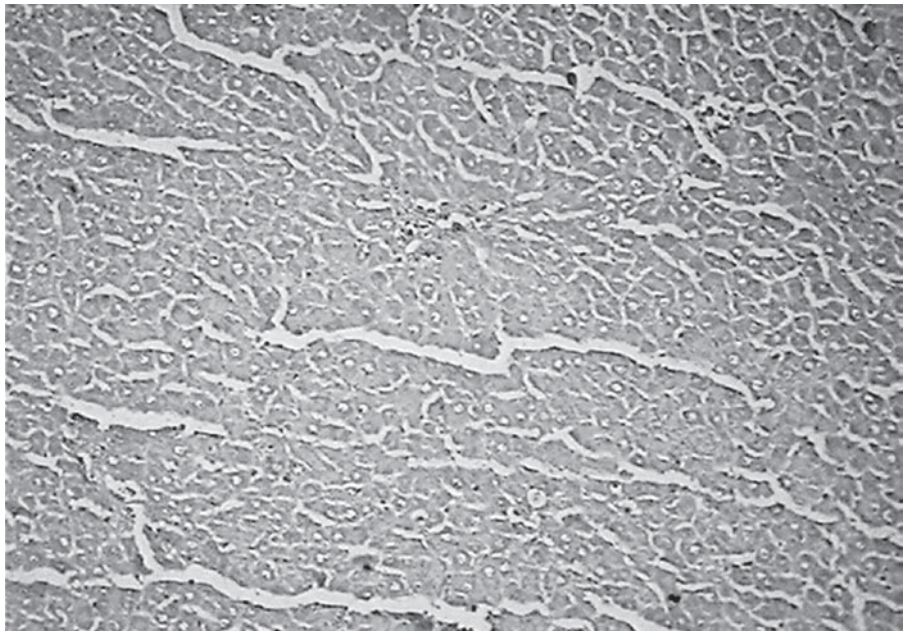

Figure 3. Group III: liver section of rat treated with jigrine (1 ml/kg, p.o.) + D-Gal (400 mg/kg, i.p.) showing only few scattered inflammatory cells (H&E, ×100).

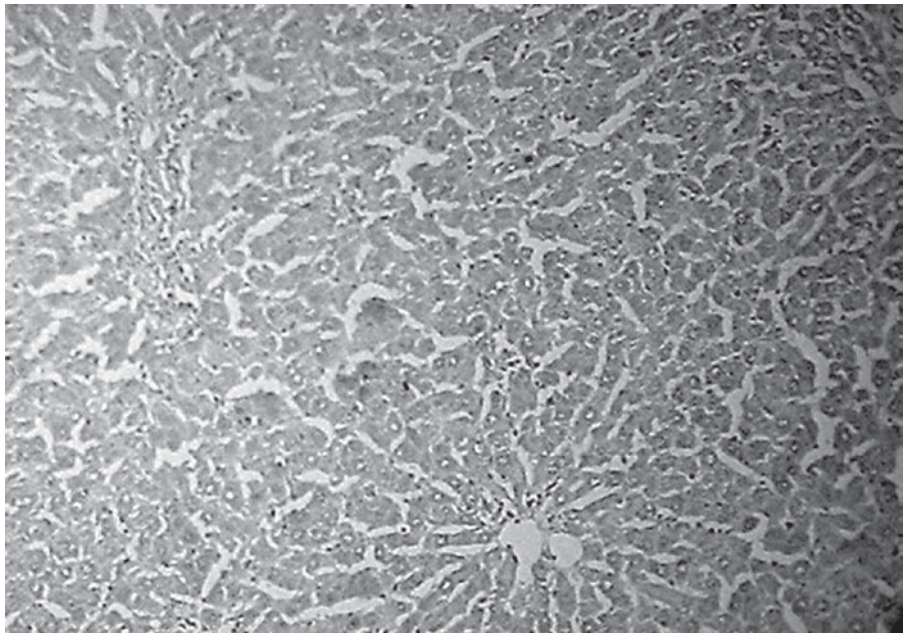

Figure 4. Group IV: liver section of rat treated with silymarin (25 mg/kg, p.o.) + D-Gal (400 mg/kg, i.p.) showing few scattered inflammatory cells and occasional periportal clustering (H&E, ×100).

an index of liver damage. In our study, the rise in ALT and ALP levels induced by D-Gal administration was significantly reduced by jigrine pretreatment, suggesting that its hepatoprotective activity might be due to its effect against cellular leakage and loss of functional integrity of the cell membrane in the liver. The membrane-stabilizing property of jigrine has already been reported (Karunakar et al., 1997). The D-Gal-induced elevation in

Table 6. Effect of jigrine and silymarin on the degree of liver damage induced by D-Gal administration.

Group	Degree of liver damage
I	0
II	3+
III	1+
IV	1.5+

bilirubin levels in serum was also significantly reduced by jigrine pretreatment. The biochemical results are also supported by histopathological examination of liver sections of various animals. Histopathological evaluation demonstrated significant protection of the liver cells of jigrine- and silymarin-pretreated animals against D-Gal-induced hepatic damage.

Central nervous system (CNS) functions are often affected secondary to liver damage (Gilberstadt et al., 1980). This effect is particularly observed on cognition and memory. D-Gal administration reduced the spontaneous alternation score (group II), indicating the spatial memory-impairing effect of this hepatotoxin. Pretreatment of these animals with jigrine increased the percent alternation score in these rats significantly. Silymarin treatment showed a statistically non-significant increase in the percent alternation score. D-Gal administration also reduced the motor activity (group II), and pretreatment of these animals with jigrine and silymarin increased the motor activity in these rats significantly. Open field results were not significant, although jigrine and silymarin both showed non-significant improvement. Jigrine might have produced these beneficial effects in D-Gal-treated animals due to an improvement of liver function, as observed by the reduction in elevated levels of serum enzymes. There are a few ingredients in jigrine, such as *Vitex negundo* Linn. (Verbenaceae) (Gupta et al., 1999) and *Phyllanthus niruri* Linn. & Hook (Euphorbiaceae) (Santos et al., 1995), which have strong CNS actions. The above ingredients might have acted directly on the CNS to produce these beneficial effects. The overall improvement in behavioral activity of the animals may also be because of an improvement in liver function.

It can be concluded that a single dose (400 mg/kg) of D-Gal used to induce hepatic damage (Mitra et al., 2000), which resembles human viral hepatitis (Keppler et al., 1968), also produces behavioral impairments in rats. Jigrine pretreatment not only protects from the hepatotoxic effects of D-Gal but also prevents the associated behavioral impairments in rats. Further studies are needed to establish the mechanisms of behavioral effects of these drugs.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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