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Prevention of galactosamine induced hepatic damage by Himoliv in rats

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Abstract

Objective: To study the role of Himoliv (HV), a polyherbal preparation on the prevention of liver damage caused by galactosamine. Materials and methods : The rats were pretreated with HV (1ml/kg), and silymarin (25 mg/kg) orally for 9 days. Liver damage was induced in all rats except control with galactosamine at the dose of 200 mg/kg, i.p. on the day 7. Two days later, glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT) and alkaline phosphatase (ALP) in serum were estimated. The concentration of malondialdehyde (MDA), superoxide dismutase (SOD) and protein in liver were also noted. Histopathological studies were also done to confirm the biochemical changes. Results : There was a significant elevation of hepatic enzyme (GOT, GPT and ALP) levels in serum after 48 h of galactosamine treatment, in comparison to normal control. These findings were reversed with HV pretreatment. Galactosamine treatment caused significant elevation in MDA concentration and reduction in SOD concentration when compared to control group. But, HV pretreatment showed significant reduction of MDA production and elevation of SOD concentration. These effects were very much similar to those of silymarin treated group. Histopathological studies also supported the biochemical findings. Conclusions: The results confirm the hepatoprotective activity of HV. It also provides a basis for the clinical use of HV in several clinical conditions involving liver diseases.

Key words : Hepatoprotective effect, galactosamine, free radicals, lipid peroxidation, liver.

1. Introduction

Himoliv (HV) is a polyherbal combination comprising 25 plants (Table 1) containing active parts of *Picrorrhiza kurroa, Boerhaavia diffusa, Tinospora cordifolia, Andrographis paniculata, Phyllanthus emblica* etc. These plants have been individually reported to possess hepato-protective and antioxidant properties [1]. HV was reported to possess marked activity against hepatotoxins, including carbon tetrachloride and paracetamol [2,3]. In addition, the main constituents of HV, *Picrorrhiza kurroa* showed marked anti-HBs-like activity on hepatitis B virus [4].

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Since galactosamine induced hepatotoxicity closely resembles acute human viral hepatitis [5,6], the present work was undertaken to evaluate the effect of HV against galactosamine induced damage.

In this study, the effect of HV was studied on serum enzyme levels (GOT, GPT and ALP) following galactosamine induced damage. In addition, antioxidant activity of HV was studied by analyzing the concentration of MDA, SOD and protein. The activity was compared with silymarin, a known hepatoprotective agent [7].

2. Materials and methods

2.1 Animals

Adult albino Wistar rats of either sex, weighing about150-175 g were used. They were housed in clean polypropylene cages and fed with commercial pelleted rat chow and water *ad libitum.* Permission from Institutional Animal Ethical Committee for laboratory use of animals was duly obtained.

2.2 Drugs and chemicals

Himoliv was prepared and supplied by M/s. Emami Limited, Kolkata, India in a liquid form (Table 1). Chemicals and reagents like, d-galactosamine hydrochloride, silymarin, superoxide dismutase, bovine serum albumin, epinephrine hydrochloride etc. were purchased from Sigma Chemical Co., USA and thiobarbituric acid, hydrogen peroxide, n-butanol, eosin, hematoxylin, paraffin, formaldehyde etc. were purchased from E. Merck, India. Biological kits used for serum glutamate oxaloacetate transaminase, serum glutamate pyruvate transaminase and serum alkaline phosphatase estimation were manufactured by Span Diagnostics Pvt. Ltd., India.

2.3 Experimental procedure

The rats were divided into four groups, containing six animals each, per dose schedule. The first group comprised of untreated animals, the second group animals received only galactosamine, while animals of group three and four were given both the test preparations. Himoliv, at the dose of 1 ml/kg (group 3) [2, 3] or silymarin at the dose of 25 mg/kg (group 4) [7] in aqueous suspension, administered orally for 9 consecutive days.

On day 7, galactosamine (200 mg/kg) dissolved in normal saline was administered intraperitoneally to all rats except normal control. After 48 h of galactosamine treatment, the animals were sacrificed under deep anaesthesia and blood was collected for the assay of GOT, GPT and ALP in serum. The livers were removed immediately, washed with ice-cold saline and a 10% homogenate was prepared in phosphate buffer at pH 7.0.

The homogenate was centrifuged at 3000 rpm for 15 min at 4°C and the supernatant was used for the estimation of MDA, SOD and protein. Simultaneously, a small portion of liver pieces were preserved in 10% formaldehyde solution for histopathological study.

The activities of hepatic marker enzymes – GOT, GPT and ALP were assayed in serum using standard biological kits [8, 9]. The quantitative measurement of lipid peroxidation in liver homogenate was done by the method of Yagi and Rastogi [10].

The results were expressed as nmol of MDA/ mg of protein. Protein was estimated in liver homogenate by the method of Lowry *et al.* [11]. SOD was estimated in the liver homogenate using epinephrine by the method of Mishra and Fridovich [12].

Formaldehyde fixation liver slices were processed, stained with hematoxylin-eosin, examined under microscope (Carl Zeiss, Germany) at 40x magnification and finally photographed.

2.4 Statistical analysis

The statistical analysis was carried out by Student's unpaired *t* - test and p values less than 0.05 was considered significant.

3. Results

3.1 Serum enzymes

The results are summarized in Table 2. The levels of serum GOT, GPT and ALP were significantly elevated ($p<0.001$) in galactosamine treated hepatotoxic rats in comparison to control group. However, pretreatment with HV or silymarin significantly reduced these enzyme concentrations (Table 2).

3.2 Histopathological studies in liver

In control animals, liver sections showed normal hepatic cells. In galactosamine treated animals the sections showed marked necrosis . Pretreatment with HV and silymarin showed mild fatty changes in hepatocytes.

3.3 Lipid peroxidation and SOD in liver

There was a significant elevation of MDA, and reduction of SOD concentration of liver tissue of galactosamine treated rats, when compared to the control. Whereas, pretreatment with HV further reversed these effects, similar to the standard hepatoprotective drug, silymarin (Table 2).

Table 1.
Composition of Himoliv (HV), Ayurvedic formulation (each 5 ml contains)

Picrorrhiza kurroa	60 mg
Cassia lanceolata	30 mg
Boerhaavia diffusa	30 mg
Trigonella foenumgraecum	15 mg
Tinospora cordifolia	30 mg
Oldenlandia corymbosa	15 mg
Andrographis paniculata	50 mg
Galega purpurea	50 mg
Phyllanthus emblica	80 mg
Cassia occidentalis	20 mg
Hygrophila spinosa	80 mg
Ptychotis ajowan	20 mg
Carica papaya	15 mg
Achyranthes aspera	30 mg
Terminalia chebula	30 mg
Holarrhena antidysenterica	20 mg
Amoora rohituka	100 mg
Berberis aristata	30 mg
Eclipta erecta	20 mg
Plumbago rosea	30 mg
Solanum nigrum	20 mg
Swertia chirata	30 mg
Cichorium intybus	80 mg
Pterocarpus santalinus	15 mg
Wrightia tinctoria	20 mg
Flavoured syrup base	q.s.

4. Discussion

Galactosamine has been widely used experimentally to induce hepatic damage, since the hepatic damage closely resembles that found in acute human viral hepatitis [6].

In the present investigation galactosamine induced hepatic damage was evidenced by the changes in various biochemical parameters. Pretreatment of the animals with HV showed prevention of galactosamine induced cellular lesions evidenced by the altered biochemical parameters towards the normal limit. These results suggest that HV may exert a stabilizing action on hepatic cell membrane and promote the repair of the injured tissues, as has been reported in case of silymarin [7].

The serum alkaline phosphatase and transaminases are considered to be sensitive indicators of liver injury. Intoxication with galactosamine increased the levels of GOT, GPT and ALT in serum [5, 6]. These changes result from the leakage of enzyme from the hepatic cells into the serum.

Table 2.
Effects of Himoliv (HV) on glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT) and alkaline phosphatase (ALP) in serum and malondialdehyde (MDA) and superoxide dismutase (SOD) in liver homogenate in galactosamine induced rats. (Data represents mean ± SE)

	Serum			Liver	
Groups (N=6)	**GOT (U/L)**	**GPT (U/L)**	**AL P (KA U)**	**MDA (nM/mg protein)**	**SOD (U)**
Control	42 ± 2.52	56 ± 1.25	35±2.25	3.2±0.019	1.5±0.01
Galactosamine	108±4.26[a]	103±2.75[a]	85±5.20[a]	5.2±0.097[a]	0.7±0.05[a]
HV + Galactosamine	48±5.90[b]	62±4.67[b]	38±4.27[b]	3.6±0.035[b]	1.6±0.02[b]
Silymarin + Galactosamine	47±4.05[b]	60±3.46[b]	36±2.98[b]	3.5±0.026[b]	1.7±0.02[b]

The test drugs were administered once daily for 9 days and galactosamine was given to all groups except control on day 7. [a] $p < 0.001$ as compared with control; [b] $p < 0.001$ as compared with galactosamine group.
Statistical analysis by Student's *t* - test.

In the present study, the enhanced level of serum GOT, GPT and ALP following galactosamine injection have been observed. Pretreatment with HV prior to galactosamine challenge significantly reduced GOT (55.5%), GPT (39.8%) and ALP (55.2%) concentrations in the serum. Restoration of the normal values of hepatic enzymes by HV has been reported *in vivo* following damage by carbon tetrachloride and paracetamol [2, 3]. The present work further corroborates these observations.

Silymarin, the known hepatoprotective compound has been found to possess a significant protective effect on the damaged tissues [7]. In the present study, silymarin showed similar protective effects.

Galactosamine increases lipid peroxidation in liver as assessed by MDA formation. The antioxidant activity or the inhibition of the generation of free radicals is important in the protection against galactosamine induced liver lesions [13]. In this work, elevation in the levels of end products of lipid peroxidation (MDA) in liver of rats treated with galactosamine was observed. Pretreatment with HV significantly reversed this change.

Further, HV also enhanced significantly, the levels of the protective enzyme, SOD in the liver. SOD acts as a protective enzyme against lipid peroxidation in tissues. These findings supported the role of HV in hepatic protection by modulation of the antioxidant pathway [14].

It was further supported by other workers elsewhere [15-19]. The major and active constituents of HV, *Picrorrhiza kurroa, Boerhaavia diffusa, Tinospora cordifolia, Andrographis paniculata, Phyllanthus emblica* have been reported to possess hepatoprotective activities and they have anti-oxidant properties [1].

Histopathological studies showed that galactosamine caused steatosis and degeneration of the liver tissue. HV pretreatment exhibited protection, which confirmed the results of biochemical studies. All the effects of HV were comparable with those of silymarin.

The present work provides conclusive evidence for the hepatoprotective effect of HV against galactosamine induced hepatitis, which is very similar to viral hepatitis. However, clinical

investigations are required to confirm these observations. It is possible that the mechanism of hepatoprotective action of HV might be at least, partly due to its anti-oxidant effects.

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