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Original Research Article

Immunomodulation During Hepatoprotective Effects of Dawa-Ul-Kurkum in D-Galactosamine- Induced Liver Cirrhosis in Rats

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Abstract

Background: The immunomodulatory and hepatoprotective effects of Unani preparation were evaluated in an experimental model of liver cirrhosis induced by administration of the D-galactosamine in rats

Methods: Rats were administered with D-galactosamine, three times in a week (on alternate days) to induce hepatic derangement for about 06 weeks. The model of liver cirrhosis was confirmed by observation of alterations in various cytokines and markers of immunity as compared to that in vehicle control rats. The effects of Dawa-ul-kurkum and its hydro-alcoholic extract were evaluated on markers of immunomodulation and liver injury in the above model of liver cirrhosis in rats.

Results: D-galactosamine-induced liver damage was associated with higher levels of inflammatory cytokines, IgG, MDA, and NOx, as well as lower levels of GSH and a delayed-type hypersensitivity response, as compared to that in normal controls. Histopathological examination of liver tissue also revealed hydropic degeneration, chronic inflammatory cells, and partial septal cirrhosis in this experimental group. Treatment with Dawa-ul-kurkum and its hydroalcoholic extract evoked varying degrees of modulation of several cytokines, immunoglobulins, delayed-type hypersensitivity, and oxidative stress indicators and provided protection against deranged liver functions. The treatment with Unani polyherbal preparation and its extract had immunomodulatory effects similar to those seen with positive control (silymarin).

Conclusion: The results showed that both Dawa-ul-kurkum and its extract had hepatoprotective and immunomodulatory effects against liver cirrhosis induced by D-galactosamine in rats and the effects were comparable to those seen with silymarin.

Keywords: Cytokines, Delayed type hypersensitivity, Histopathology, Immunomodulatory, Liver, Oxidative stress

Introduction

Liver is a vital organ in humans that is involved in a variety of processes including detoxification, metabolism, immunity, digestion, vitamin storage, etc [1]. Hepatotoxic chemical harm the entire body because they stop the liver from performing essential tasks [2]. Common liver toxicity can result in major problems like metabolic abnormalities and even death [3]. Hepatocyte necrosis is identified with an increase in MDA, and a decrease in GSH levels.

A lot of other biochemical markers are abnormally elevated as well. Other conventional/synthetic medications used to treat liver problems have had unfavorable outcomes and occasionally resulted in a slew of side effects. The best-known model of xenobiotic-induced hepatic injury is galactosamine-induced hepatic toxicity, which is commonly used to screen medicines for hepatoprotective effects. Therefore, rats were administered D-galactosamine to induce experimental model of liver cirrhosis [4]. Galactosamine is a derivative of galactose a component of several glycoprotein hormones, such as Follicle-stimulating hormone or Luteinizing hormone [5].

Galactosamine is a powerful hepatotoxic chemical that can cause both necrosis and apoptosis of hepatocytes. It prevents the transcription of genetic information by creating uridine diphosphate hexosamines, which hinder the synthesis of hepatic RNA [6]. Galactosamine single injection results in necrosis of hepatocytes, and repeated administration over a short period of time results in liver cirrhosis in test animals [7]. The well-known hepatotoxic chemical, D-galactosamine is reported to damage the liver in a manner that is strikingly similar to that of viral hepatitis in humans [8].

Herbal medicines can be adjuvants to modern medical care as traditional medicines have fewer side effects. The TRIPS agreement's regulatory issues have revived interest in traditional therapies [9]. since there are no suitable therapies for the management of liver cirrhosis. The effects of herbal medicines are being evaluated using current scientific terminology. Many different kinds of herbal preparations have been utilized historically for hepatoprotection and immunomodulation in traditional medicine viz Unani, Ayurveda.

A combination of nine herbs known as Dawa-ul-kurkum is used in Unani medicine to treat hepatic disorders. The purpose of this study was to investigate the potential hepatoprotective and immunomodulatory effects of Dawa-ul-kurkum in an experimental model of liver cirrhosis induced by D-galactosamine [10,11].

Materials and Procedures

Chemicals and Drugs

Central Research Institute of Unani Medicine (CRIUM), Hyderabad, prepared and provided the Unani medicine Dawa-ul-kurkum. Silymarin and D-galactosamine were procured from Sigma-Aldrich, the rest of the chemicals were purchased from SRL in New Delhi. The ELISA kits used were from Diaclone and QAYEE-Bio.

Animals

The animals were used after the approval by the Institutional Animal Ethics Committee vide registration number 170/GO/ReBi/S/99/CPCSEA. For the study, inbred Wistar rats of either sex (180-250 g) was obtained from animal house of Vallabhbhai Patel Chest Institute, University of Delhi. Under standard laboratory conditions, the rats were maintained and administered various drugs as per protocol.

The Experimental Drug

Batch number (3-1/2018-19/CRIUM), the standardized Unani polyherbal medicine, Dawa-ul-kurkum was provided by the Central Research Institute of Unani Medicine, Hyderabad, India. It was made up Sunbul-ut-Teeb, Mur Makki, Saleekha, Qust, Shagufa-eIzkhir, Darcheeni and Zafran. Phytochemical profile/fingerprint analysis by advanced Unani methods (HPTLC and TLC etc.) was performed on crude herbal extracts and the preparation was standardized [12]. This Unani medicine is widely documented in standard Unani literature [13] and CRIUM has validated that it was made in accordance with old classical Unani text.

Experimental procedure

In order to develop a liver cirrhosis model, Wistar rats were given three doses of D-galactosamine (500 mg/kg, i.p.) per week for 6 weeks [14]. There were following seven groups of animals; Group 1: normal control; Group 2: galactosamine induced liver cirrhosis; Group 3: positive control Silymarin (50 mg/kg) [15] + galactosamine; Group 4 and 5: administered Dawa-ul-kurkum (DK) at doses of 250 and 500 mg/kg respectively, together with galactosamine; Groups 6 and 7: received hydro-alcoholic extract at doses of 500 and 1000 mg/kg respectively, along with galactosamine. The human dose prescribed by Unani physicians was used to compute the Dawa-ul-kurkum dose.

All of the medicines were given for 06 weeks. All groups except group 1 received a dosage of D-galactosamine (500 mg/kg, i.p.) three times each week. Animals were anesthetized after 24 hours of the last drug administration, and blood was withdrawn through heart puncture, centrifuged, and kept at -80^0 C. Animals were sacrificed and the liver was removed for histological examinations and the evaluation of immunomodulatory and oxidative stress markers.

Cytokine and Immunoglobulin levels

Interleukin (IL-4), Tumor Necrosis Factor (TNF-α), and Interferon (IFN-γ) were estimated as instructions given in the Kit Manufacture's manual (Diaclone), serum Immunoglobulin (IgE), Immunoglobulin (IgG), Immunoglobulin (IgM), Immunoglobulin (IgA) and Interleukin (IL-13) were estimated as instruction given in the Kit Manufacture's manual (QAYEE-BIO).

Delayed type hypersensitivity (DTH) reaction

The cell-mediated immune response of treated rats was assessed using the DTH test. On day 0, the animals were given 1 mg KLH (0.4 ml, s.c.) antigen preparation (PBS and FCA were added in equal volumes to prepare antigen suspension) at the base of the tail. All the animals were challenged in 14th day by injecting 100 mg of KLH (0.08 ml) sterile PBS into the left paw and an equal volume of PBS into the right paw (served as a control) after various medications from day 0 to 14 in separate groups. Using UGO basile plethysmometer, both the paw volumes were measured at times 0 (basal), and 24 hours later, and specific paw swelling (D percent) [16].

MDA level test

The chemical molecule malondialdehyde, a byproduct of the lipid peroxidation is commonly used as a marker of oxidative stress. As 2-thiobarbituric acid-reactive substance was measured spectrophotometrically in the supernatant of liver homogenate (TBARS). 1.5 ml of 20 percent glacial acetic acid, 0.2 ml of sodium lauryl sulphate (8.1%), 1.5 ml of 2-thiobarbituric acid (heated till dissolved), and 0.1 ml of supernatant were mixed together. The reaction mixture was

ultimately increased to 4.0 mL with distilled water. After vortexing, samples were incubated for 1 hour at 95°C and then cooled with tap water before adding 1.0 mL distilled water and 5.0 mL butanol–pyridine 15:1 (v/v) combination. The mixture was mixed for 10 minutes before being centrifuged for 10 minutes at 4000 rpm. At 532 nm wavelength, the butanol–pyridine layer is spectrophotometrically quantified. MDA equivalents are used to express TBARS levels. The standard was 1, 1, 3, 3-tetramethoxypropane (TMP) [17].

Assay of reduced glutathione (GSH) levels

Ellman's approach was used to calculate Glutathione (GSH) levels. In the presence of glutathione reductase, glutathione was successively oxidized by DTNB and reduced by NADPH in an enzymatic recycling cycle. To separate the proteins for the experiment, 10 percent trichloroacetic acid was added to an equivalent amount of material and centrifuged. To 0.1 ml of this supernatant, 2 ml phosphate buffer (pH 8.4), 0.5 ml 5'5-dithiobis (2-nitrobenzoic acid) and 0.4 ml distilled water were mixed. The mixture was vortexed after 15 minutes, and the reading was taken at 412 nm wavelength. The concentration of 2-nitro-5-benzoic acid synthesis was evaluated and GSH was expressed as µmol/mg protein [17].

Nitrates and Nitrites assay

This test is done using Griess reagent. In 96-well Elisa plate, 6 µl of sample is mixed with 44 µl distilled water, 20 µl of phosphate buffer (pH 7.5), and 10 µl each NADPH, FAD and 10 µl Nitrate reductase (1 U/ml). After that, the plate was incubated in the dark for one hour at room temperature. 200 µl of Griess reagent [1:1 mixture of 1% sulfanilamide (1% solution with 5% orthophosphoric acid) and 0.1% N(1-naphthyl) ethylenediamine (NEDA) (1% solution with distilled water)] was added to each well and the plate was incubated for 10 minutes at normal room temperature. A microplate reader was used to measure absorbance at 540 nm wavelength. The concentrations of total nitrate and nitrite (NOx) in homogenates were calculated using the standard curve, which was expressed as nM/mg protein [2].

Histopathological studies

Histological examinations were performed on liver tissue from all the animal groups and structural changes compared. A trained pathologist performed a blinded microscopic examination using hemotoxylin eosin staining of tissue.

Statistical Analysis

Graph Pad software was used to analyze the results using the mean and standard error mean. One-way analysis of variance was performed, followed by the Tukey's post hoc test. Statistical significance was defined as $p < 0.05$.

Results

Effect of Dawa-ul-kurkum and its extract on the body and liver organ weight of rats with D-galactosamine- induced liver cirrhosis

After various pharmacological treatments for 6 weeks, the mean body and liver weights of all groups were measured and compared with basal values. The results demonstrated that three times weekly (alternate day) administration of D-galactosamine (500mg/kg) caused reduction in body weight gain over the period of 06 weeks as compared to the control rats. Although, D-galactosamine caused a reduction in % change in body weight but no discernible change in the size of the liver was observed. Treatment with the Unani preparation at two different doses (250 and 500 mg/kg), its hydro-alcoholic extract (500 and 1000 mg/kg), and silymarin treated group reversed the effect. The increase in

body weight could be attributed to an increase in appetite, which could be linked to Dawa-ul-kurkum hepatoprotective effect. The results are shown in (Table 1)

Effect of Dawa-ul-kurkum and its extract on humoral immunity: Antibody Response and Immunoglobulin levels in rats with D-galactosamine- induced liver cirrhosis

The administration of galactosamine (500mg/kg, i.p.) for 06 weeks in experimental control group resulted in significant rise of Immunoglobulin levels i.e., IgM, IgG, IgE and IgA, a marker for humoral immunity in serum. Similarly significant rises in cytokine levels of IL-13, Interferon- γ, TNF- α and IL-4 were observed compared to that in control rats. This significant rise in immunoglobulin and cytokine levels could have led to liver tissue injury and toxicity that confirmed present rat hepatotoxicity model. In Groups 4 and 5, treated with Unani preparation at two different doses for 06 weeks significant reversal of the effect of galactosamine and significantly decreased level of IgM ($p<0.05$ for 500mg/kg dose), IgG ($p < 0.05$ for 500mg/kg doses), IgA ($p < 0.01$ and $p< 0.05$ for both dose) as well as in IgE ($p < 0.05$ and $p< 0.01$ for both dose) were observed. The levels of IL-13($p < 0.01$ for both doses), TNF- α ($p < 0.05$ for both doses), IL-4 ($p < 0.05$ for both doses) and Interferon- γ ($p < 0.05$ for dose 500mg/kg) as compared to that in drug-induced control group. Similar to treatment Groups 6 and 7, when its extract was administered orally at two different doses, it reversed the effects of galactosamine and significantly decreased the levels of IgM ($p < 0.05$ for both doses), IgG ($p < 0.05$ for dose 1000mg/kg), IgA($p < 0.05$ for both doses) and IgE ($p<0.05$ and $p<0.01$ for both doses), similarly cytokine levels also reduced significantly IL-13 ($p<0.01$ for both doses), Interferon-γ (**(**$p<0.05$ for both doses), TNF-α ($p < 0.05$ for both doses) and IL-4 ($p < 0.05$ for dose 1000mg/kg) as compared to that in drug-induced experiment group. Pretreatment with silymarin (positive control), also significantly decreased the levels of IgM ($p<0.05$), IgA ($p<0.01$), IgE ($p<0.01$), similarly Interferon-γ, TNF-α and IL-4 ($p<0.05$) & IL-13($p<0.01$) levels were reduced as compared to D-galactosamine-induced experimental group, indicating that polyherbal Unani preparation and its extract has immunomodulatory effects in this model. Results from Dawa-ul-kurkum and its extract were comparable to those of silymarin. The results are depicted in Table 2 and Figure 1.

Effect of Dawa-ul-kurkum and its extract on oxidative stress in rats with D-galactosamine- induced liver cirrhosis

When compared to normal control rats, D-galactosamine (500mg/kg, i.p.) given three times weekly for 6 weeks resulted in a rise in metabolites of nitric oxide ($p<0.05$) and MDA in the supernatant, as well as a substantial reduction in GSH ($p<0.05$). This supports the hepatotoxicity model and is consistent with severe tissue damage and hepatotoxicity in the rat liver. Treatment with the Unani preparation at dosages of 250 and 500 mg/kg for 06 weeks considerably decreased supernatant NOx ($p< 0.05$ at both doses), MDA ($p< 0.05$ at dose 500mg/kg), and enhanced GSH ($p <0.05$ at dose 500mg/kg) as compared to that of drug-induced experimental group (treated with D-galactosamine only). Similarly, treatment with hydro-alcoholic extract at two different doses (500 and 1000 mg/kg) had a protective effect against oxidative stress, as it markedly decreased NOx in the supernatant ($p<0.05$ at dose 500 mg/kg), MDA ($p<0.05$ at dose 500 mg/kg) and marked increased GSH ($p<0.05$ at dose 1000 mg/kg) in comparison to that of Experimental disease control group. Pretreatment with silymarin (positive

control) considerably reduced the hepatotoxic effects of D-galactosamine and lowered NOx, MDA ($p<0.05$), and elevated GSH ($p<0.05$) in contrast to the Experimental group. Both, Dawa-ul-kurkum and its hydro-alcoholic extract produced a beneficial effect similar effect to that of the Silymarin group. Results are shown in figure 2.

Effect of Dawa-ul-kurkum and its extract on Delayed type hypersensitivity response in rats with D-galactosamine- induced liver cirrhosis

When compared to the healthy control group, D-galactosamine (500mg/kg, i.p.) administration in Experimental control group resulted in a significant reduction in delayed-type hypersensitivity reaction. Treatment with Dawa-ul-kurkum at doses of 250 and 500 mg/kg for 06 weeks significantly increased the effects of dose (250 mg/kg) delayed-type hypersensitive reaction as compared to the experimental control group. Similarly, treatment with hydro-alcoholic extract (500 and 1000 mg/kg) considerably increased the effects at 500 mg/kg dose delayed-type hypersensitive reaction as compared to the experimental control group. Pretreatment with silymarin also increased the delayed-type hypersensitivity response when compared to the experimental control group. The results are depicted in Table 3.

Effect of Dawa-ul-kurkum and its extract on histopathology of liver tissue in rats with D-galactosamine- induced liver cirrhosis

Histopathological examination of liver tissue of normal (control) rats showed hepatic parenchymal cells with minimal degenerative changes. The liver tissue of rats of experimental control group (D-galactosamine, 500mg/kg, i.p; administered three times weekly for 06 weeks) showed focal areas of incomplete septal cirrhosis as thin bands of fibrous connective tissue with few chronic inflammatory cells. Focal areas of fatty change are also seen in hepatocytes. In the silymarin treated group, most of the hepatic parenchymal cells appeared normal; there were no visible degenerative changes; and the lobular architecture was observed with a normal central vein, hepatocytes arranged in a radiating pattern with normal sinusoids. Increased hepatocyte regeneration was seen as indicated by the increase in binucleated hepatocytes. A focally visible mild periportal inflammatory cell infiltrate was present. In treatment groups 4 and 5 that received polyherbal Unani preparations at doses of 250 and 500 mg/kg for 06 weeks showed most of the hepatic tissue appeared normal; no degenerative lesions were seen; however, there was a slight localized infiltration of inflammatory cells. The hepatocytes lobular architecture was well maintained with no congestion. There was evidence of hepatoprotection as regenerative nodules and short slender fibrous bands were observe. In groups 6 and 7, treatment with hydro-alcoholic extract (500 and 1000mg/kg) also showed that most of the hepatic tissue was normal with no degenerative lesion. The lobular architecture of the hepatocytes was well preserved, and sinusoids appeared normal. However, mild inflammatory cell infiltrate and focal Mild fibrosis around the central vein was seen. Increased bile ducts are also seen. Mild degenerative changes are seen in hepatocytes in some areas shown in (Figure 3).

Table 1: Effects of Dawa-ul-kurkum (DK) and its hydroalcoholic extract (HA) on body and liver weight in experimental model of liver cirrhosis in rats

Treatment mg/kg	Initial body weight (g)	Final body weight after 06 weeks (g)	% Change in body weight	Liver weight (g)	Liver index (%)
Control	146.2± 27.88	242.7± 33.84	39.76	9.070± 0.6755	3.73
Experimental control	178.3± 12.04	175.0± 14.43	-1.88	11.51± 1.200	6.57
Silymarin 50	166.2± 8.156	272.0± 15.70	38.89	11.45± 0.8566	4.20
DK 250	176.1± 9.453	308.0± 11.14	42.82	13.91± 0.8095	4.51
DK500	206.7± 13.95	323.0± 19.14	36.00	11.73±0.2490	3.63
HA500	187.1± 13.25	296.0± 21.99	36.79	11.70± 1.150	3.95
HA1000	159.6±18.73	237.0± 28.31	32.65	11.07±1.078	4.67

Liver index was calculated as (liver weight/body weight×100%). The values were expressed as mean ± SEM. As p >0.05, the data on body and liver weight is not significantly different among various groups. The Experimental group was treated with D-galactosamine three times in a week (on alternate days) for 6 weeks,

Table 2: Effects of Dawa-ul-kurkum (DK) and its hydroalcoholic extract (HA) on immunoglobulins in experimental model of liver cirrhosis in rats

Treatment mg/kg	IgM (µg/ml)	IgG (µg/ml)	IgA (µg/ml)	IgE (µg/ml)
Control	0.0266±0.0018	0.0756±0.0037	0.0660±0.0030	0.1753±0.0035
Experimental control	0.0426±0.0029#	0.0983±0.0107#	0.0880±0.0060#	0.2030±0.0066##
Silymarin	0.029±0.0015*	0.0814±0.002	0.0682±0.0013**	0.1794±0.0020**
DK 250	0.031±0.0019	0.0808±0.002	0.0674±0.0027**	0.1816±0.0018*
DK500	0.029±0.0021*	0.0770±0.003*	0.0702±0.0045*	0.1780±0.0045**
HA500	0.030±0.0025*	0.0836±0.003	0.0684±0.0032*	0.1840±0.0019*
HA1000	0.029±0.0031*	0.0766±0.003*	0.0702±0.0016*	0.1760±0.0051**

The values were expressed as mean ± SEM. ##p<0.01 and #p<0.05 as compared to control group; **p<0.01 and *p<0.05 as compared to experimental control group, the data was analyzed using one-way ANOVA followed by Tukey test.

Table 3: Effects of Dawa-ul-kurkum (DK) and its hydroalcoholic extract (HA) on Delayed type hypersensitivity (DTH) response in experimental model of liver cirrhosis in rats

Treatment	DTH%
Control	20.79 ± 19.55
Experimental control	8.930 ± 10.54#
Silymarin	15.55 ± 9.279
DK250	18.14 ± 1.15*
DK500	23.13 ± 15.32
HA500	18.82 ± 9.732*
HA1000	26.97 ± 4.403

#p<0.05 when compared with control group; *p<0.05 when compared with experimental control

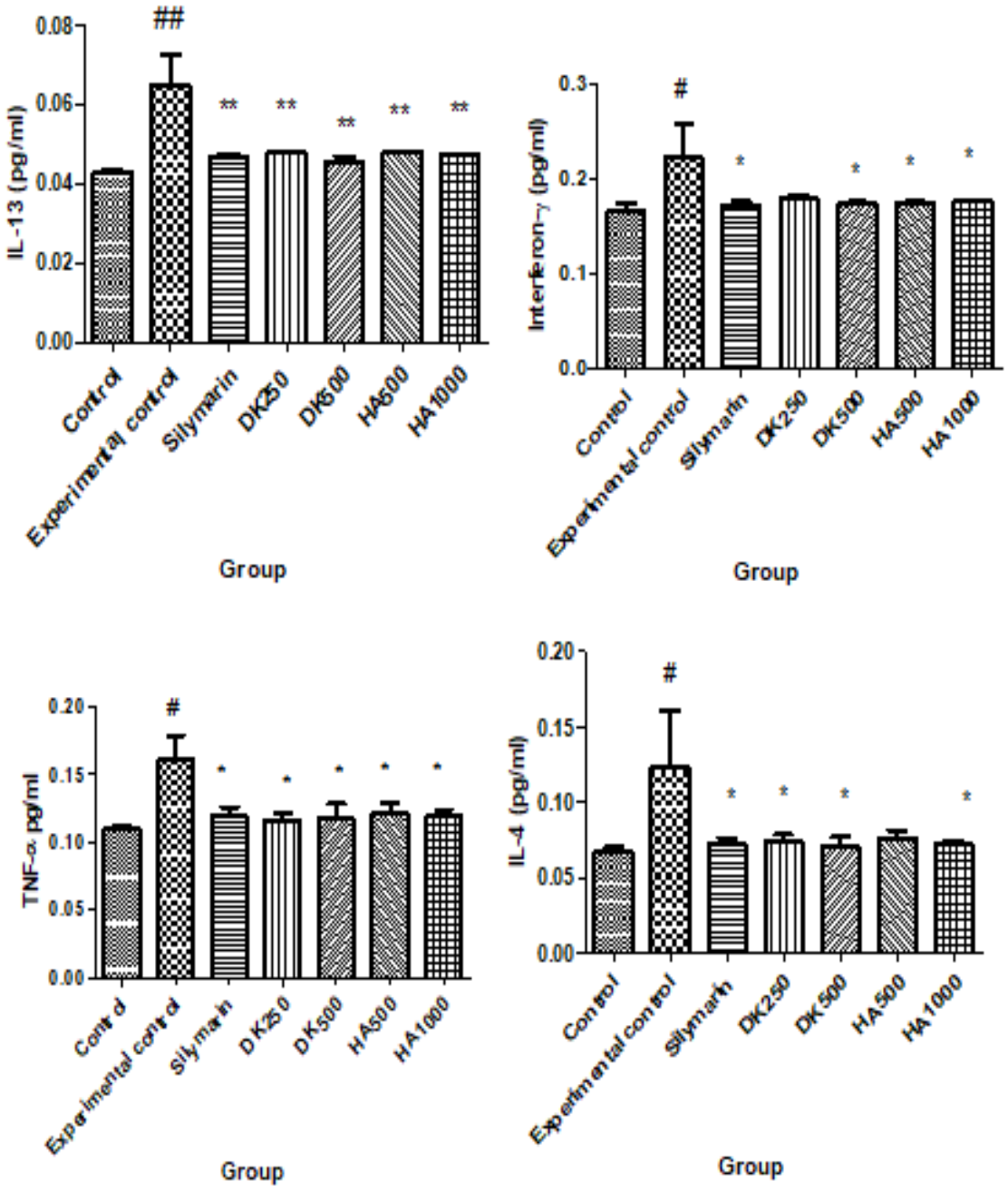

Figure 1: Effects of Dawa-ul-kurkum (DK) and its hydroalcoholic extract (HA) on Cytokine levels in experimental model of liver cirrhosis in rats

The values were expressed as mean ± SEM. #p<0.05 and ##p<0.01 as compared to control group; **p<0.01 and *p<0.01 as compared to experimental control group, the data was analyzed using one-way ANOVA followed by Tukey test.

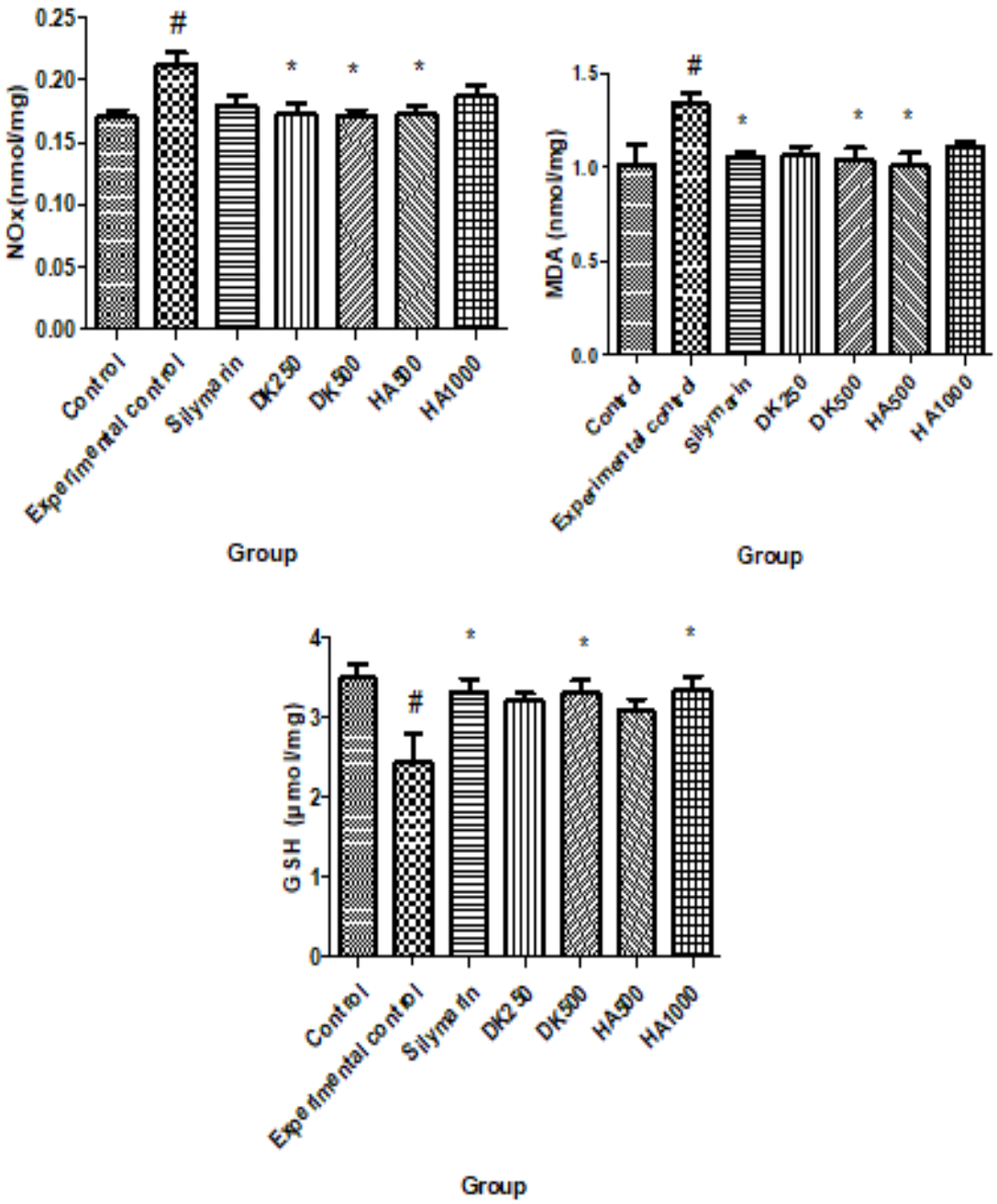

Figure 2: Effects of Dawa-ul-kurkum (DK) and its hydroalcoholic extract (HA) on oxidative stress parameters in experimental model of liver cirrhosis in rats

The values are expressed as mean ± SEM; DK-Dawa-ul-kurkum; HA-Hydroalcholic extract of DK; #p<0.05 vs control group; *p<0.05 vs Experimental control.

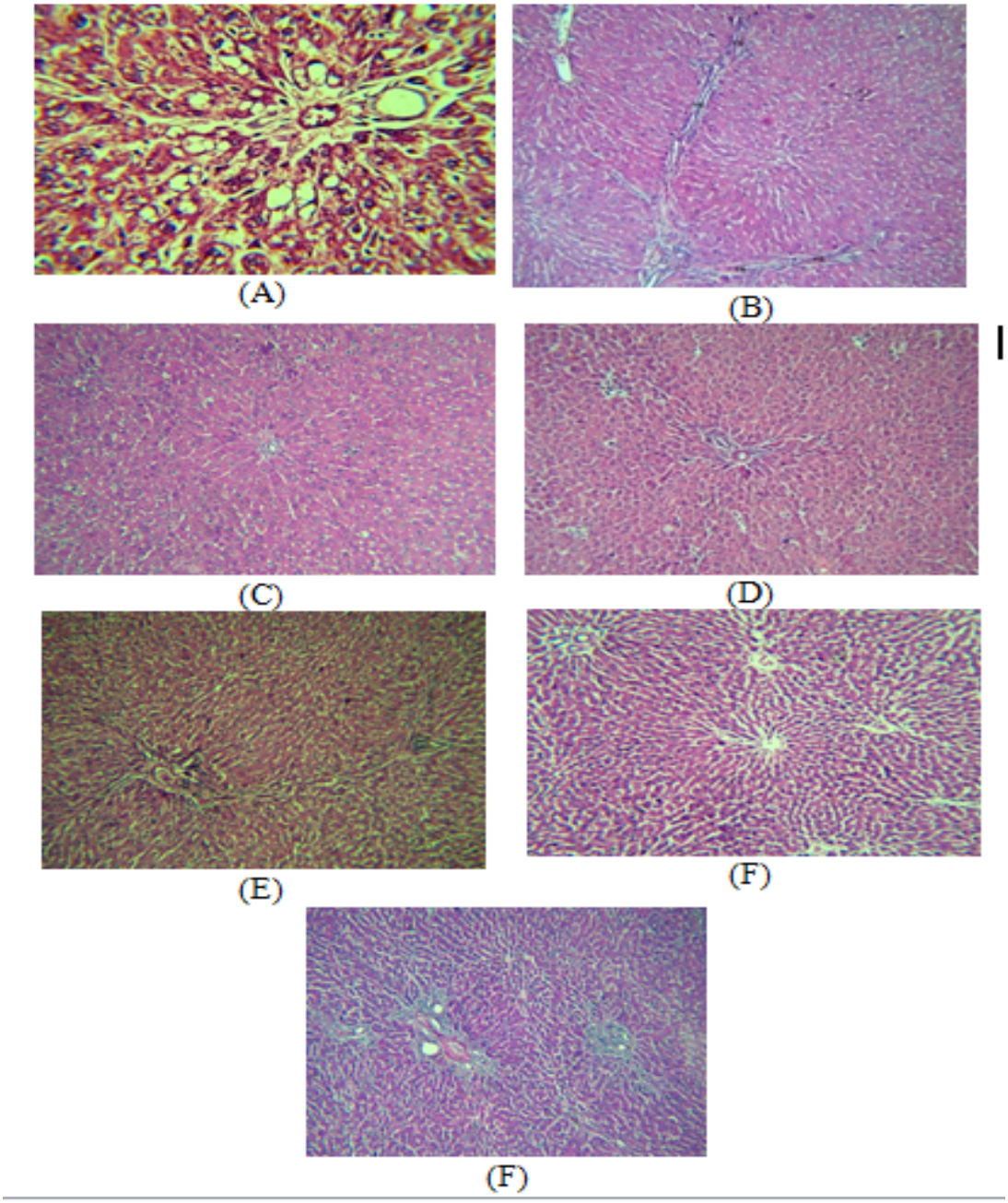

Figure 3: Histopathological picture of liver sections after various drug treatments in rats. (A): Control (B): Experimental control (C): Silymarin (D) DK250 (E) DK500 (F) HA500 (G) HA1000. All groups except control group were treated with D-Galactosamine (500mg/kg, orally). DK-Dawa-ul-kurkum; HA-Hydroalcholic extract of DK.

Discussion

D-galactosamine-induced liver damage is a well-known and universally accepted method for modeling xenobiotic-induced liver toxicity and is frequently utilized in drug screening for hepatoprotective agents. It is crucial to explore a safe and effective plant-based hepatoprotective drug with anti-inflammatory and antioxidant properties to reduce tissue damage in order to effectively manage hepatitis [18]. As a result, D-

galactosamine-stimulated liver injury was chosen as the experimental paradigm for assessing the hepatoprotective properties of Dawa-ul-kurkum. Previous research has shown that D-galactosamine produces a shift in hepatic biomarker enzymes as well as a significant increase in hepatic markers [19,20]. D-galactosamine is also used to prevent endotoxemia that causes fulminant hepatitis, by inhibiting transcription and translation [21].

Another mechanism of D-galactosamine-induced liver injury i.e. reactive oxygen species (ROS) produced by activated hepatic macrophages [22]. D- galactosamine injection has also been shown to cause hepatic damage by stimulating processes that produce ROS or oxidative stress [23]. The ubiquitous free radical molecule nitric oxide (NO) was first found in the vascular endothelium and is now known to exist in a variety of tissue/organ systems, including the gastrointestinal and hepatobiliary systems, with elevated amounts reported in inflammatory conditions [24].

Immunoglobulin (Ig) levels fluctuate regularly in hepatic disease and are widely employed as a marker for various types of hepatic damage. Cirrhosis, primary biliary cirrhosis, and chronic active hepatitis are all associated with an increase in serum IgA, IgM, and IgG concentrations. Immunoglobulin has been used to assess the severity of injury in hepatic disease, despite the lack of diagnostic specificity of any immunoglobulin composition in hepatic disease. Regardless, immunoglobulin levels are still recommended as a useful marker in the diagnosis and prevention of liver disorders [16].

Another study discovered that many patients with acute and chronic hepatic diseases were significantly higher serum IgE levels. It was postulated that a blocking factor could be inhibited in the serum of individuals with hepatic illness, causing a misleading elevation in IgE levels. Such inhibitors have been found in immunocompromised patients and cancer people [25].

Although the mechanism of elevated serum immunoglobulins in hepatic disease has been extensively studied, the reason is still unknown. Theoretically, increased immunoglobulin levels could be caused by increased immunoglobulin synthesis or decreased immunoglobulin catabolism. The decrease of the suppressor T-cell population in patients with hepatic illness may be the cause of elevated serum immunoglobulin [26].

The mechanism of drug-induced liver injury (DILI) has been described in terms of cell stress, mitochondrial damage, and specific immune response. The liver is the organ that detoxifies cells under a lot of strain. Inflammatory cytokines can be triggered by stress on the cell. As a result, liver cells are more sensitive to TNF-α and IFN-γ-induced apoptosis. Inhibitors of apoptosis proteins or Bcl-2 can block these effects [27]. One cytokine that has been connected to the development of immune-mediated DILI and the antibodies that go with it is IL-4. Variant IL-4 alleles have been linked to the development of immune-mediated DILI from diclofenac in a previous investigation [28].

According to the latest studies, liver injury is characterized by an increase in serum immunoglobulin levels (IgG, IgM, IgA, and IgE) and cytokines (TNF-α, IL-4, IL-13, and Interferon-γ), but a decrease in a delayed-type hypersensitivity reaction in the experimental induced group.

A decrease in GSH levels was seen coupled with an increase in oxidative stress indicators including tissue MDA and NOx levels. MDA, a thiobarbituric acid reactive molecule, has been connected to an increase in lipid peroxidation and tissue damage (TBARS). This demonstrated that the

antioxidant defense system had failed to prevent the creation of excess free radicals, resulting in oxidative damage. Low glutathione levels in the tissues may have induced liver failure, inhibiting many SH-containing enzymes and protein synthesis, resulting in a drop in protein levels in the D-galactosamine-treated group [29], which is a sign of severe liver damage [30,31]. Histopathological analysis of liver tissue confirmed the immunological and cytokine findings, which revealed focal areas of incomplete septal cirrhosis represented by slender fibrous connective tissue bands containing few chronic inflammatory cells in rat hepatic tissue.

Treatment with Dawa-ul-kurkum and its hydro-alcoholic extract of rats with D-galactosamine-induced liver cirrhosis, reduced serum immunoglobulin levels (IgG, IgM, IgA, and IgE) and increased delayed-type hypersensitivity response. In addition, inflammatory cytokines (TNF-α, IL-4, IL-13, and Interferon-γ) were also significantly reduced, thus indicating anti-inflammatory and immunomodulatory effects of the drug. Furthermore, measurements of oxidative stress parameters in liver homogenates revealed that Dawa-ul-kurkum, as well as the hydroalcoholic extract, significantly lowered levels of MDA and NOx, as well as increased GSH level, which further corroborated the protective effect of Dawa-ul-kurkum against increased levels of reactive oxygen and nitrogen species in response to D-galactosamine. Histopathological studies of liver tissue revealed that the majority of the hepatic tissue appeared normal, with no degenerative or inflammatory lesions, reiterating the protective effect of this polyherbal Unani formulation against D-galactosamine-induced liver cirrhosis. Both, Dawa-ul-kurkum and HA extract exhibited a hepatoprotective effect by their immunomodulatory and anti-inflammatory properties which may be mediated through maintaining a homeostatic balance between pro- and antioxidants during experimentally induced liver cirrhosis in rats.

Conclusion

Hepatotoxicity and liver cirrhosis in response to administration of D-galactosamine, was confirmed histopathologically and by alteration in immune function markers, cytokine levels, and oxidative stress. Although the effects on immunoglobulin, cytokine, and oxidative stress markers were differential by treatment with the polyherbal Unani formulation, Dawa-ul-kurkum and its hydroalcoholic extract, both were found to be effective in reducing D-galactosamine- induced liver cirrhosis in rats. Such reverse-pharmacology translational research, employing modern medical methodology, could help to validate the formulation being used in the traditional system of medicine and can bridge the gap between traditional and modern medicinal concepts for the benefit of patient.

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Authors Contributions

Mohd Rafi Reshi participated in the execution of the tests, the collection, analysis, and writing of the manuscript. Kavita Gulati contributed to the study's conceptualization, planning, and design. She also contributed to the data analysis and paper review. Arunabha Ray participated in the study's design, data interpretation, and manuscript review. The final draught of the work was accepted by all authors.

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