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Research Article

Dawa-Ul-Kurkum, A Unani Polyherbal Preparation as a Hepatoprotective in D-Galactosamine Induced Liver Cirrhosis in Rats and its Possible Mechanisms

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Abstract

Background: Hepatotoxicity is commonly associated with necrosis, an increase in oxidative stress markers such as NOx, Malondialdehyde levels, GSH depletion, and increased liver markers.

Objectives: The hepatoprotective effect of Unani preparation was tested in rats that had been given Galactosamine to produce liver cirrhosis.

Material and Methods: In rats, liver toxicity was created by giving them a thrice weekly dose of Galactosamine, which caused hepatic derangement and an increase in various liver indicators when compared to a control group. Different markers of liver damage were used to examine the effects of pharmacological treatments. Hydropic degeneration, fatty alterations, necrosis, and septal cirrhosis were also found on histopathological investigation in some locations.

Results: Both DK and HA treatment revealed hepatoprotective effects that were comparable to those seen following normal medication treatment. When compared to controls, D-galactosamine-induced liver damage was associated with higher levels of MDA and NOx, but lower levels of GSH. Different degrees of attenuation in various oxidative stress markers were elicited by DK and HA treatments.

Conclusion: It finds that both treatment DK and its extract were helpful in preventing D-Galactosamine-induced liver cirrhosis in rats, as they greatly reduced hepatotoxic damage indicators.

Keywords: *Hepatotoxicity; D-Galactosamine; Dawa-Ul-Kurkum; Histopathology*

Abbreviations

DK: Dawa-Ul Kurkum; HA: Hydroalcoholic Extract; SGOT: Serum Glutamic Oxaloacetic Transaminase; SGPT: Serum Glutamic-Pyruvic Transaminase; ALP: Alkaline Phosphatase; GSH: Glutathione; NOx: Nitrates and Nitrites; MDA: Malondialdehyde; UTP: Uridine-5'-Triphosphate

Introduction

The liver is a pivotal organ in the human body and its strategic site and multidimensional functions support remaining other organ in the body [1]. One of the crucial parts in the human body is liver, which controls secretion, storage, detoxification and metabolism in

our body [2]. Therefore, the injury that is caused by hepatotoxic agents is deleterious to the whole body as it prevents the liver of its major functions [3]. Liver toxicity is a common problem, which produces to serious complication ranging from metabolic disorders to even death [4]. Hepatocyte injury is usually linked with various disorders necrosis, rise in MDA and depletion in the GSH levels. In addition there is also elevated many other biochemical parameters. Treatment with other Conventional/synthetic drugs used for the liver diseases has given disagreeable results and occasionally lead many adverse effects. The D-Galactosamine induced liver toxicity is the best designated model of xenobiotics induced hepatic damage and regularly used for the screening of hepatoprotective effects of medicines. So D-Galactosamine induced was chosen as experimental model in rats [5]. Galactosamine is a derivative of galactose. It is an ingredient of specific glycoprotein hormones, such as follicle-stimulating hormone or luteinizing hormone under physiological conditions [6]. Galactosamine is a potent hepatotoxic substance that can leads hepatocyte death both by necrosis and apoptosis. It hinders the synthesis of liver RNA through the production of uridine diphosphate hexosamines that inhibit the transcription of genetic material [7]. In experimental animals short-term administration of GAL leads liver damage and ALF [8]. In this respect, various toxic agents may lead hepatic injury. D-Galactosamine is a well-known hepatotoxic agent which leads hepatic damage with close similarity to human viral hepatitis [9].

Herbal medications are useful supplements to contemporary medical treatment. Traditional treatments offer fewer adverse effects, and regulatory difficulties arising from the TRIPPS agreement have sparked increased interest in traditional treatments. [10]. Various types of herbal medications have been utilised for immunomodulation and hepatoprotection in the past, thus their effects should be evaluated using modern scientific terminology. In the Unani system of medicine, a polyherbal preparation called Dawa-Ul-Kurkum is used to treat liver problems. The purpose of this study was to assess the hepatoprotective effects of Dawa-Ul-Kurkum in a D-Galactosamine-induced experimental paradigm. [11,12].

Materials and Methods

Drugs and chemicals

The drug and chemicals are taken from different suppliers like Dawa-Ul- Kurkum provided by Central Research Institute of Unani Medicine, Hyderabad, Silymarin and D-Galactosamine were purchased from Sigma and other chemicals were taken from SRL, New Delhi. Biochemical kits were purchased from ERBA.

Animals

The study employed either a male or female Wistar strain. Animals were seized from the VPCI Animal House and kept in a controlled environment. They were provided with unlimited food and drink. Animals were cared for according to CPCSEA criteria for animal usage, which were approved by the Institutional Animal Ethics Committee (IAEC) (Registration number 170/GO/ReBi/S/99/CPCSEA).

The investigational drug

Dawa-ul-Kurkum, was provided by Central Research Institute of Unani Medicine (CRIUM), Ministry of AYUSH, Govt. of India with a batch no. 3-1/2018-19/CRIUM. This preparation is composed of Sunbul-ut-Teeb, Mur Makki, Saleekha, Qust, Shagufa-eIzkhir, Darcheeni, Zafran with Sharab-e-musallas and QandSafaid Q.S. The formulation is well documented in standard Unani literature [13] and is certified to have been prepared as per traditional classical Unani text by CRIUM.

Experimental procedure

D-Galactosamine induced liver cirrhosis in rats

The experimental model of liver cirrhosis was induced in wistar rats by administration of three doses weekly of D-Galactosamine (500 mg/kg, i.p.) till cirrhosis induced over a period of one to 3 months [14]. Animals were divided into different groups. Group 1 as normal control given as normal saline; In Group 2 as experimental control administered with D-Galactosamine; In Group 3 as positive control given Silymarin (50 mg/kg, orally) [15] + D-Galactosamine; In Group 4 and 5 animals were given Dawa-Ul-kurkum at two different doses (250 or 500 mg/kg, orally) respectively + D-Galactosamine; In Group 6 and 7 animals were administered with hydroalcoholic extract

(HA) at two different doses (500 or 1000 mg/kg, orally) + D-Galactosamine. All drugs were administered for 45 days and Galactosamine was administered thrice weekly dose in all groups except normal control group 1. On 46th day, animals were anesthetized and blood was collected by cardiac puncture, centrifuged and stored at -80ºC. After blood collection, animals were sacrificed and liver was collected for histopathological studies and estimation of biochemical and oxidative stress parameters. As per approval of the IAEC, total 39 animals were included in the experimental study.

Biochemical estimations

Serum alanine aminotransferase (ALT), serum aspartate aminotransferase (AST) and serum alkaline phosphatase (ALP) were estimated by Kinetic method of International Federation of Clinical Chemistry (IFCC), serum bilirubin and total protein were estimated by End Point assay as per the instruction of the Kit Manufacture's manual.

Estimation of MDA levels

Malondialdehyde (MDA) is widely used as oxidative stress biomarker in biomedical research. Lipid peroxidation is measured spectrophotometrically as 2-thiobarbituric acid-reactive substance (TBARS) in supernatant of liver homogenate [8]. 0.1 ml of supernatant was mixed with 0.2 ml of sodium dodecyl sulfate (8.1%), 1.5 ml of 20% acetic acid and 1.5 ml of 2-thiobarbituric acid (0.8%). The reaction mixture was finally made up to 4.0 ml with distilled water. After vortexing, samples were incubated for 1 h in 95° C and after cooling with tap water; 1.0 ml of distilled water and 5.0 ml of mixture of butanol–pyridine 15:1 (v/v) were added. The mixture was shaken for 10 min. and then centrifuged at 4000 rpm for 10 min. Then Butanol–pyridine layer is taken and measured spectrophotometrically at 532 nm. TBARS values are expressed as MDA equivalents. 1, 1, 3, 3-tetramethoxypropane (TMP) was used as the standard [16].

Assay of reduced glutathione (GSH) levels

Glutathione (GSH) levels were estimated by the method of Ellman [17]. This assay is based on the enzymatic recycling procedure in which glutathione was sequentially oxidized by the DTNB and reduced by NADPH in the presence of glutathione reductase. For assay, an equal quantity of sample was mixed with 10% trichloroacetic acid and centrifuged to separate the proteins. To 0.1 ml of this supernatant, 2 ml of phosphate buffer (pH 8.4), 0.5 ml of 5'5-dithiobis (2-nitrobenzoic acid) and 0.4 ml of double distilled water was added. The mixture was vortexes and absorbance was read at 412 nm within 15 min. The concentration of 2-nitro-5-benzoic acid formation was measured and reduced glutathione is expressed as µmol/mg protein.

Nitrates and Nnitrites (NOx) assay

NOx concentrations were determined by using the Griess reaction described by Tracey., *et al.* [18]. 6µl of supernatant was mixed with 44µl of distilled water, 20µl of 310 mM phosphate buffer (pH 7.5) and 10µl each of 0.86 mM NADPH, 0.11 mM flavin adenine dinucleotide (FAD) and 10µl Nitrate reductase (1 U/ml) in individual wells of a 96-well plate. Plate was thereafter incubated for 1 h at room temperature in the dark. 200µl 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 an additional 10 min at room temperature. Absorbance was measured at 540 nm using a microplate reader. Total protein was estimated by method of Lowry., *et al.* [19]. Concentration of total nitrate and nitrite (NOx) in liver homogenates was calculated from the standard curve and expressed as nM/mg protein.

Histopathological examination

All groups were subjected to histological examination. Microscopic examination was done by a qualified pathologist using hemotoxylin and eosin staining in a blinded fashion.

Statistical analysis

The values were expressed as mean ± standard error of the mean. One-way analysis of variance (ANOVA) followed by appropriate post hoc test (Tukey test) were used for analysis. $P < 0.05$ was considered as statistically significant.

Results

Effects of Dawa-Ul-Kurkum and its hydro-alcoholic extract on Liver Function test (LFT) in D-Galactosamine induced liver cirrhosis in rats.

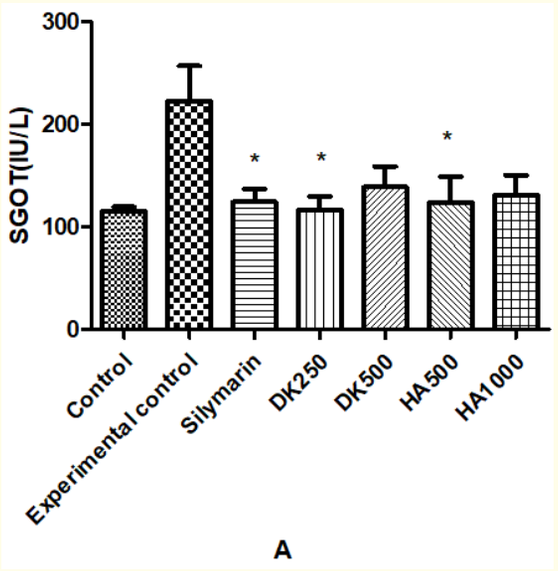
In experimental control group, D-Galactosamine given thrice weekly 45days days resulted in significant increase in serum levels of SGPT ($p < 0.05$), ALP ($p > 0.05$), direct bilirubin ($p < 0.05$), non-significant increase in SGOT, total bilirubin and reduction in total protein as compared to normal control rats. This suggests that notable degree of hepatotoxicity and tissue injury in the rat liver and validated our model of D-Galactosamine induced liver cirrhosis. In Group 4 and 5, treatment with Dawa-Ul-Kurkum at two different doses 250 and 500mg/kg for 45 days significantly attenuated the effects of D-Galactosamine and reduced level of serum SGOT ($p < 0.05$ at 250 dose), SGPT ($p < 0.05$ at 500 mg/kg dose), ALP ($p < 0.05$ and $p < 0.01$ at both doses), total bilirubin and direct bilirubin ($p < 0.05$ at 500 mg/kg dose) and increased level of serum total protein as compared to that in Experimental control group (treated with D-Galactosamine alone). Similarly, in Group 6 and 7 treatment with two different doses of 50% hydro-alcoholic (500 and 1000 mg/kg) produced hepatoprotective effect as it reduced significantly the levels of serum SGOT ($p < 0.05$ at 500 dose), SGPT ($p < 0.05$ at 1000 dose), ALP ($p < 0.05$ and $p < 0.01$ at both doses), total bilirubin and direct bilirubin ($p < 0.05$ at 500 dose) as compared to that in Experimental control. However, non-significant change was observed in the levels of total protein. Pretreatment with silymarin also significant reduced the hepatotoxic effects of D-Galactosamine and reduced the levels of serum SGOT ($p < 0.05$), SGPT ($p < 0.005$), ALP ($p < 0.01$), Total bilirubin, but non-significantly Direct Bilirubin and increase in total protein as compared to that in Experimental control. The results of Dawa-Ul-Kurkum and its hydro-alcoholic extract are comparable to that of Silymarin. The results are shown in (Table 1 and 2 and Figure 1 and 2).

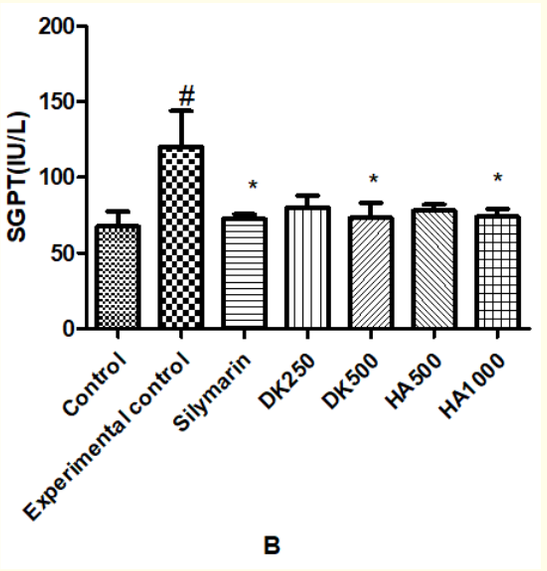
Treatment	SGOT(IU/L)	SGPT (IU/L)	ALP(IU/L)
Control	115.7 ± 3.653	67.80 ± 9.571	116.2 ± 4.071
Experimental control	222.7 ± 33.93	120.0 ± 23.92#	213.9 ± 53.94#
Silymarin	124.4 ± 12.53*	72.58 ± 2.937*	104.2 ± 3.315**
DK250	116.9 ± 12.80*	80.04 ± 7.935	119.8 ± 11.74*
DK500	138.6 ± 20.37	73.19 ± 10.06*	116.9 ± 5.477**
HA500	123.9 ± 24.99*	78.05 ± 4.009	121.5 ± 11.44*
HA1000	131.2 ± 19.18	74.07 ± 4.934*	111.2 ± 10.67**

Table 1: *Effects of Dawa-Ul-Kurkum and its hydro-alcoholic extract on SGOT, SGPT and ALP in D-Galactosamine induced liver cirrhosis in rats.*

Treatment	Total bilirubin (mg/dl)	Direct bilirubin (mg/dl)	Total protein (g/dl)
Control	0.5200 ± 0.06429	0.4033 ± 0.006667	6.267 ± 0.9837
Experimental control	1.227 ± 0.3718	0.8467 ± 0.03756#	4.537 ± 0.4497
Silymarin	0.5080 ± 0.1461*	0.4600 ± 0.03362	6.020 ± 0.4191
DK250	0.5680 ± 0.1238	0.5480 ± 0.1414	5.738 ± 0.4554
DK500	0.4020 ± 0.09982*	0.4380 ± 0.04831*	5.936 ± 0.4287
HA500	0.5120 ± 0.1001*	0.4460 ± 0.06976*	5.586 ± 0.4113
HA1000	0.5560 ± 0.1069	0.4560 ± 0.07215	6.100 ± 0.6541

Table 2: *Effects of Dawa-Ul-Kurkum and its hydro-alcoholic extract on total bilirubin, direct bilirubin and total protein in D-Galactosamine induced liver cirrhosis in rats.*





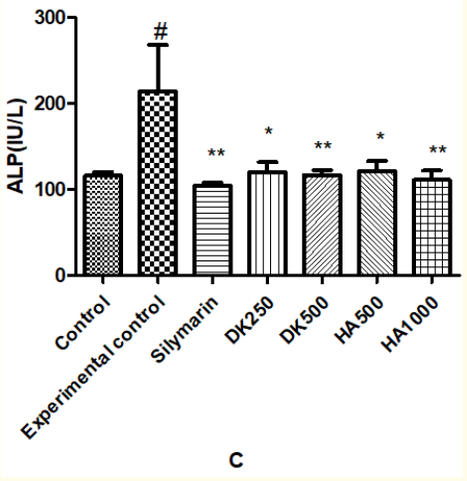
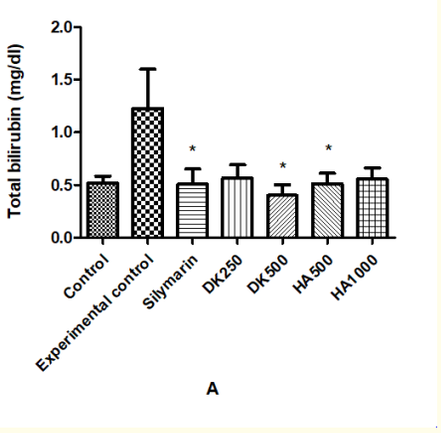


Figure 1: (A) SGOT (B): SGPT (C): ALP. The values are expressed as mean ± SEM; DK-Dawa-Ul-kurkum; HA-Hydroalcholic extract of DK. All groups except control group were treated with D-Galactosamine.

($p < 0.05$) when compared with control group; * ($p < 0.05$) and ** ($p < 0.01$) when compared with experimental control. The data were analyzed using one way ANOVA followed by Tukey test.



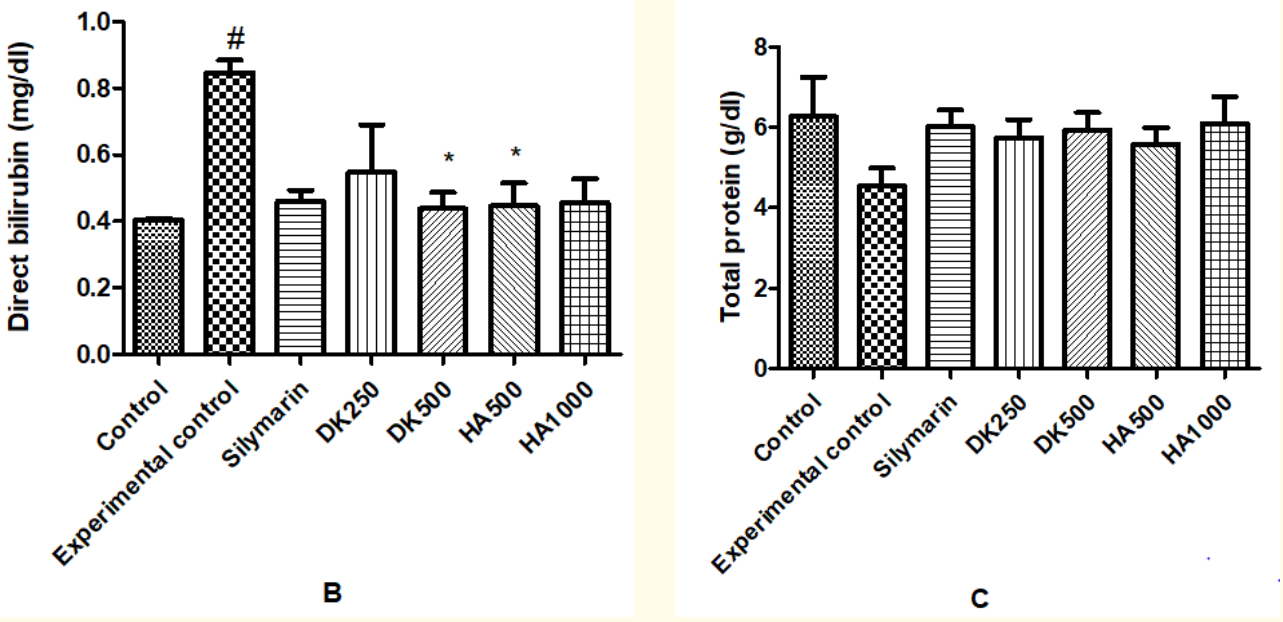


Figure 2: (A) Total bilirubin (B): Direct bilirubin (C): Total protein.
The values are expressed as mean ± SEM; DK-Dawa-Ul-kurkum; HA-Hydroalcholic extract of DK. All groups except control group were treated with D-Galactosamine. # ($p<0.05$) vs control group; * ($p < 0.05$) vs Experimental control. The data were analyzed using one way ANOVA followed by Tukey test.

Effects of Dawa-Ul-Kurkum and its hydro-alcoholic extract on body weight and liver weight in D-Galactosamine induced liver cirrhosis in rats

The mean body weight was measured in all groups at 0 and 46th day and liver weight was also measured on 46th day after various drug treatments. The results showed that dose of D-Galactosamine (500mg/kg) thrice weekly dose caused reduction in the body weight and change in the liver weight when compared to that control rats. Interestingly, treatment with Dawa-Ul-Kurkum with two different doses (250 and 500 mg/kg), 50% hydro-alcoholic extract of two different doses (500 and 1000mg/kg) and silymarin blocked the effects of D-Galactosamine and resulted in increase in the body weight with no significant changes in the liver weight. The increase in body weight can be due to improvement in appetite which may have due to hepatoprotective effect of Dawa-Ul-Kurkum. The results are shown in (Table 3).

Treatment	Initial body weight (g)	Final body weight (g)	% change in body weight	Liver weight (g)	Liver index (%)
Control	241.7 ± 4.410	258.3 ± 8.333	6.426	6.867 ± 0.4672	2.658
Experimental control	176.1 ± 6.089	173.3 ± 7.265	-1.615	7.130 ± 0.2007	4.114
Silymarin	184.6 ± 5.157	215.0 ± 10.49	14.139	6.750 ± 0.3734	3.139
DK 250	135.6 ± 3.087	156.0 ± 5.339	13.076	6.828 ± 0.4638	4.376
DK500	147.7 ± 4.869	170.4 ± 6.202	13.321	6.854 ± 0.4600	4.022
HA500	137.7 ± 5.343	168.2 ± 12.42	18.133	6.368 ± 0.7129	3.785
HA1000	137.1 ± 2.619	163 ± 6.442	15.883	6.572 ± 0.4177	4.031

Table 3: *Effects of Dawa-Ul-Kurkum and its hydro-alcoholic extract on body and liver weight in D-Galactosamine induced liver cirrhosis in rats.*

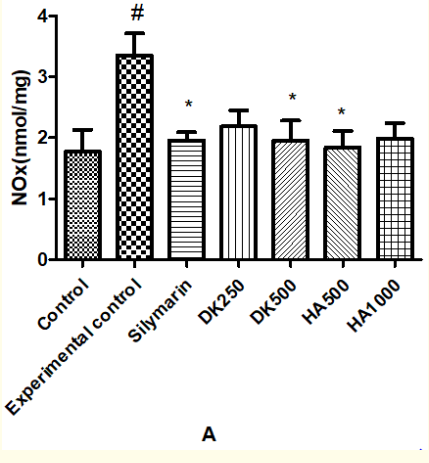
The values are expressed as mean ± SEM; DK-Dawa-Ul-kurkum; HA-Hydroalcholic extract of DK. Initial and final body weight was measured on 0 and 46th day of treatment. All groups except control group were treated with D-Galactosamine. Liver index was calculated as (liver weight/body weight×100%).

Effects of Dawa-Ul-Kurkum and its hydro-alcoholic extract on oxidative stress parameters in D-Galactosamine induced liver cirrhosis in rats

In experimental control group, D-Galactosamine given thrice weekly 45days resulted in increase in stable metabolites of nitric oxide (NOx) ($P < 0.05$) and MDA in supernatant of liver homogenates and significant reduction in GSH ($P < 0.05$) as compared to control rats. This suggests a notable degree of hepatotoxicity and tissue injury in the rat liver and corroborated to validate this model of hepatotoxicity. In Group 4 and 5, treatment with Dawa-Ul-Kurkum at two different doses 250 and 500mg/kg for 45 days significantly attenuated the effects of D-Galactosamine and reduced level of homogenate supernatant NOx ($p < 0.05$ at 500 mg/kg doses), MDA ($p < 0.05$ at both doses) and significantly increased GSH ($p < 0.05$ at 500 mg/kg, dose) as compared to that in Experimental control group (treated with D-Galactosamine). Similarly, in Group 6 and 7 treatment with 50% hydro-alcoholic extract of two different doses (500 and 1000 mg/kg) produced hepatoprotective effect as it significantly reduced the levels of NOx in homogenate supernatant ($p < 0.05$ at dose 500 mg/kg), MDA ($p < 0.05$ at both doses) and significant increased GSH ($p < 0.05$ at dose 1000 mg/kg) as compared to that in Experimental control group. Pretreatment with silymarin also significantly reduced the hepatotoxic effects of D-Galactosamine and reduced the levels of NOx ($p < 0.05$), MDA ($p > 0.05$) and increased GSH ($p < 0.05$) as compared to that in Experimental control group. The results of Dawa-Ul-Kurkum and its hydro-alcoholic extract are comparable to that of Silymarin [20]. The results are shown in (Table 4 and Figure 3).

Treatment	NOx (nmol/mg) protein	MDA (nmol/mg)protein	GSH (μmol/mg)protein
Control	1.773 ± 0.3584	0.3133 ± 0.02174	2.184 ± 0.3628
Experimental control	3.352 ± 0.3640#	0.6207 ± 0.2294	0.7810 ± 0.2442#
Silymarin	1.954 ± 0.1378*	0.3062 ± 0.01279*	2.025 ± 0.2182*
DK250	2.186 ± 0.2610	0.3312 ± 0.01991*	1.931 ± 0.2391
DK500	1.960 ± 0.3232*	0.3236 ± 0.02238*	2.009 ± 0.1768*
HA 500	1.840 ± 0.2685*	0.3224 ± 0.02154*	1.868 ± 0.3057
HA 1000	1.985 ± 0.2602	0.3040 ± 0.01284*	2.016 ± 0.1822*

Table 4: *Effects of Dawa-Ul-Kurkum and its hydro-alcoholic extract on oxidative stress parameters in D-Galactosamine induced liver cirrhosis in rats.*



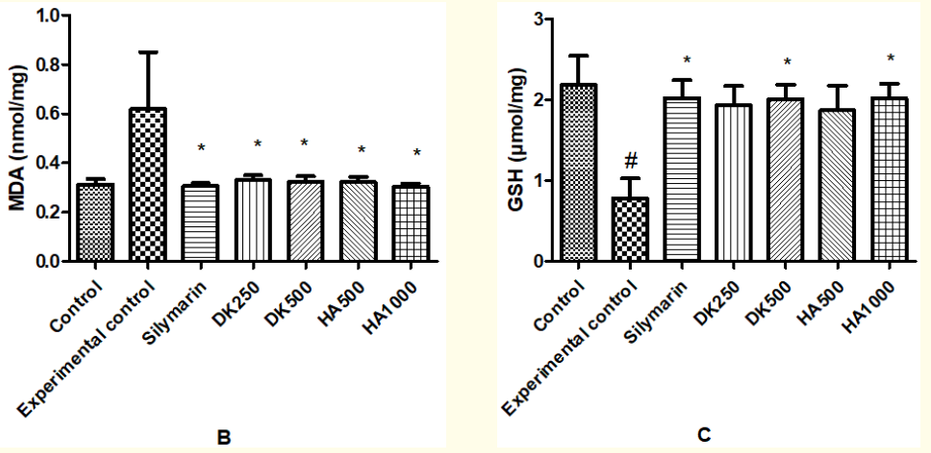
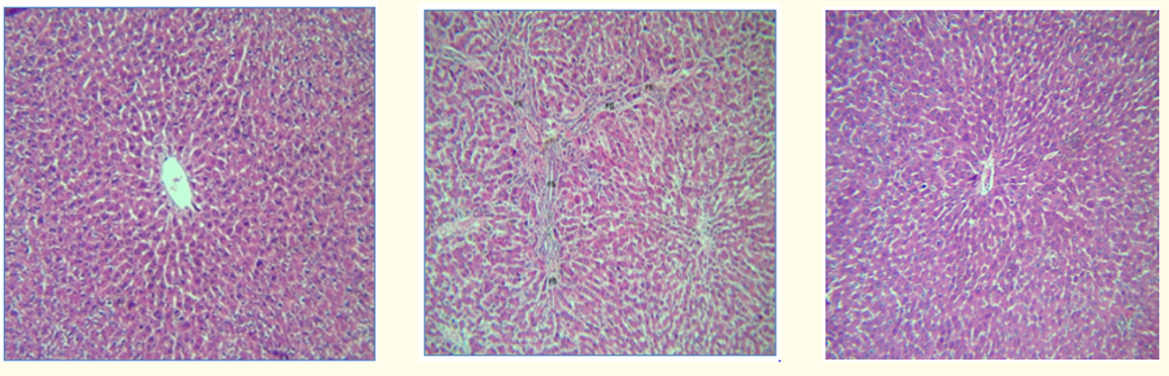


Figure 3: (A) NOx (B): MDA (C): GSH. The values are expressed as mean ± SEM; DK-Dawa-Ul-kurkum; HA-Hydroalcholic extract of DK; # ($p < 0.05$) vs control group; * ($p < 0.05$) vs Experimental control. The data were analyzed using one way ANOVA followed by Tukey test. All groups except control group were treated with D-Galactosamine.

Effects of Dawa-Ul-Kurkum and its hydro-alcoholic extract on histopathological changes in liver in D-Galactosamine induced liver cirrhosis in rats

Histopathological examination of the liver sections of vehicle treated (control) rats showed most of the hepatic parenchymal cells appeared normal. No any significant degenerative changes seen. The lobular architecture is discernible with normal central vein, hepatocyte arranged in radiating pattern and sinusoids. No sinusoidal dilation, congestion or inflammatory cell infiltrate seen. In experimental control group, administration of D-Galactosamine (500 mg/kg, i.p) given thrice wekly for 45 days showed focal areas of hydropic degeneration, fatty changes and necrosis in hepatocytes. Incomplete septal cirrhosis is seen indicated by slender fibrous connective tissue bands containing few chronic inflammatory cells. Silymarin treated group showed most of the hepatic parenchymal cells appeared normal. No any significant degenerative changes seen. The lobular architecture is discernible with normal central vein, hepatocyte arranged in radiating pattern and sinusoids. Increased hepatocytes regeneration is seen as indicated by increase in binucleated hepatocytes. Short slender fibrous septa are seen. Mild periportal inflammatory cell infiltrate is seen focally. In Group 4 and 5, treatment with Dawa-Ul-Kurkum at doses 250 and 500 mg/kg respectively for 45 days showed most of the hepatic tissue appeared normal. No any degenerative lesion seen. No significant inflammatory lesion seen however mild inflammatory cell infiltrate is seen focally. The lobular architecture of the hepatocytes is well preserved. No congestion seen. Sinusoids appeared normal. A large hemorrhagic area is seen which an unexpected finding was as most of the hepatic is normal and regenerative nodules are seen. Short slender fibrous bands are seen. In Group 6 and 7 treatment with 50% hydro-alcoholic extract of Dawa-Ul-Kurkum (500 and 1000 mg/kg) also showed most of the hepatic tissue appeared normal. No any degenerative lesion seen. No significant inflammatory lesion seen however mild inflammatory cell infiltrate is seen focally. The lobular architecture of the hepatocytes is well preserved. No congestion seen. Sinusoids appeared normal. Mild fibrosis is seen around central vein. Increased bile ducts are also seen. Mild degenerative changes are seen in hepatocytes in some areas. The lobular architecture of the hepatocytes is fairly preserved. The results are shown in (Figure 4).



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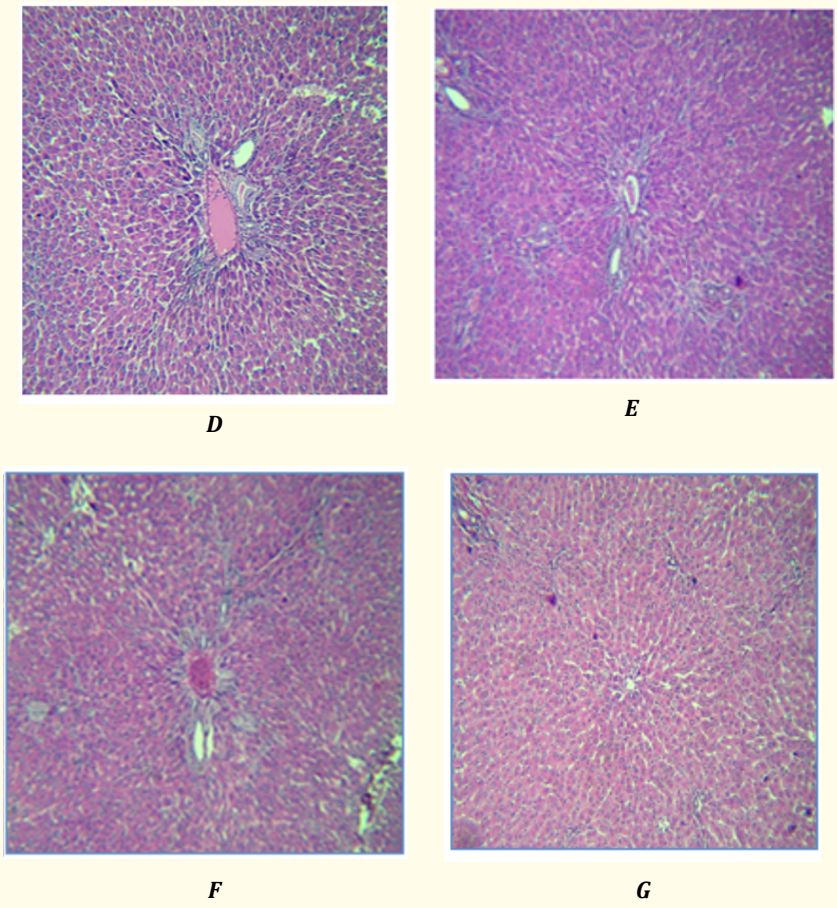

Figure 4: Histopathological picture of liver sections after various drug treatment in rats. (A): Control (B): Experimental control (C): Silymarin (D) DK250 (E) DK500 (F) HA500 (G) HA1000. All groups except control group were treated with D-Galactosamine (500mg/kg, orally). DK-Dawa-Ul-kurkum; HA-Hydroalcholic extract of DK.

Discussion

D-Galactosamine-induced hepatic injury is a well-known approach for simulating xenobiotic-induced hepatotoxicity and is commonly used to screen for hepatoprotective drugs. It is critical to create a safe and effective plant-based hepatoprotective drug with anti-inflammatory and antioxidant properties to reduce tissue damage in order to effectively manage hepatitis [16, 17]. As a result, D-Galactosamine-stimulated liver injury was chosen as the experimental paradigm for assessing DK's hepatoprotective properties. Previous research has shown that D-Galactosamine produces a shift in hepatic biomarker enzymes as well as a significant increase in hepatic markers [17, 18]. D-Galactosamine is also used to prevent endotoxemia, which causes fulminant hepatitis, by inhibiting transcription and translation [19]. Another mechanism of D-Galactosamine-induced liver injury is reactive oxygen species (ROS) produced by activated hepatic macrophages [21]. D- Galactosamine injection has also been shown to cause hepatic damage by stimulating processes that produce ROS or oxidative stress [22]. Nitric oxide (NO), a ubiquitous free radical moiety, was first detected in the vascular endothelium and is now recognised to be present in a wide range of tissue/organ systems, including the gastrointestinal and hepatobiliary systems, with higher amounts reported in inflammatory conditions [20].

The current study found that liver damage was characterised by an increase in serum SGOT, SGPT, ALP, total bilirubin, and direct bilirubin levels, but a decrease in serum protein level in the experimental control group. Oxidative stress measures such as tissue MDA and NOx levels were similarly elevated, accompanied by a reduction in GSH levels. Increased lipid peroxidation and tissue damage were associated with an increase in MDA, a thiobarbituric acid reactive molecule (TBARS). This suggested oxidative damage caused by the anti-oxidant defence system's failure to prevent the creation of excess free radicals. The decrease in protein levels in the D-Galactosamine-treated group could have been due to liver dysfunction caused by a drop in reduced glutathione levels in the tissues, which inhibited many SH-containing enzymes and inhibited protein synthesis [23], which is a sign of severe liver damage [24,25]. Histopatological examination of liver tissue confirmed the biochemical findings, which revealed hydropic degeneration, fatty alterations, necrosis, partial septal cirrhosis, and chronic inflammatory cell changes in rat hepatic tissue.

The current findings revealed that Dawa-Ul-Kurkum and 50 percent Hydro-alcoholic extract, in combination with D-Galactosamine, considerably reduced the rise in serum SGOT, SGPT, ALP, total bilirubin, and direct bilirubin levels. Furthermore, measurements of oxidative stress parameters in liver homogenates revealed that Unani polyherbal preparations and hydroalcoholic extracts protected against increased levels of reactive oxygen and nitrogen species in response to D-Galactosamine, as evidenced by lower levels of MDA and NOx and significantly higher levels of GSH. On the oxidative stress measure, the DK had the same effects as the HA extract. Dawa-Ul-Kurkum and HA extract both showed that most of the hepatic tissue appeared normal, with no degenerative lesions or significant inflammatory lesions, but mild inflammatory cell infiltrates were seen focally, reiterating the protective effect of this polyherbal formulation against D-Galactosamine-induced hepatotoxin. Both Dawa-Ul-Kurkum and HA extract, on the other hand, had a protective effect. These findings demonstrated that Dawa-Ul-Kurkum and its HA formulation are both potent hepatoprotective agents, preventing the development of liver cirrhosis.

Conclusion

This study concluded that D-Galactosamine is possibly hepatotoxic in rats when given for an extended period of time, as evidenced by changes in oxidative stress, biochemical indicators, and histological evaluation. Both doses of DK were found to be effective against drug-induced liver cirrhosis, as they considerably reduced the incidence of the disease.

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