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Research Article

Hepatoprotective Effects of Dawa-Ul-Kurkum, a Unani Polyherbal Preparation and the Possible Mechanisms in Experimental Model of D-Galactosamine Induced Liver Damage in Rats

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Abstract

The hepatoprotective effect of a polyherbal Unani formulation, Dawa-Ul-Kurkum was evaluated in the experimental model of D-Galactosamine induced liver damage in rats and its possible mechanisms were investigated. Liver damage was induced in Wistar rats by intra-peritoneal administration of D-Galactosamine and the effects of various drug treatments were assessed on morphological, biochemical and histological markers of liver toxicity. In the vehicle treated experimental group, administration of D-Galactosamine induced significant derangements in liver function as evidenced by increased levels of SGOT, SGPT, alkaline phosphatase and bilirubin, and reductions in body weight and increased liver weights as compared to controls. Histopathological examination showed multifocal areas of inflammatory cell infiltrate in hepatic parenchyma and mild haemorrhages, focal necrosis, and mild vasodilation. Pretreatment with Dawa-Ul-Kurkum (DK, 250 and 500 mg/kg) showed protective effects against the D-Galactosamine induced biochemical and histopathological derangements of liver function following D-Galactosamine. Similar effects were also seen after the hydroalcoholic extract of DK (HA, 500 and 1000 mg/kg) which showed marked protective effects on biochemical and histopathological parameters. The hepatoprotective effects of DK and HA were comparable to that seen after silymarin therapy. Liver damage induced by D-Galactosamine was associated with elevated levels of MDA and NOx whereas; GSH levels were reduced, as compared to controls. Pretreatments with DK and HA induced differential degrees of attenuations in these oxidative stress parameters. The results validate the hepatoprotective effects of Dawa-Ul-Kurkum in D-Galactosamine induced hepatotoxicity and suggest that attenuation of oxidative stress by the polyherbal may be the mechanism of action for such effects.

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 crucial organ and its multidimensional functions support almost every other organ in the body [1]. It is the main organ which manages diverse physiological metabolic processes in the body; it eliminates toxic substances/xenobiotics and synthesizes new

useful ones. Therefore, the injury which is produced by hepatotoxic agents is harmful to the whole body as it prevents liver to perform its important metabolic functions [2]. Liver damage is generally associated with cellular necrosis, increase in lipid peroxidation and reduction in the tissue GSH levels. In addition, serum levels of many biochemical markers like SGOT, SGPT, ALP and bilirubin levels are elevated. Conventional or synthetic drugs used for the treatment of liver diseases have not been satisfactory and sometimes cause many adverse effects. In the absence of an authentic hepatoprotective drug in modern medicine there is a need for search from alternative medicine. There are a good number of medicinal preparations in Ayurveda and Unani systems of traditional medicine which are recommended for the treatment of liver disorders. There is an increasing focus to validate these preparations following systematic research methodologies and evaluate scientific basis of their hepatoprotective effects. The experimental model of D-Galactosamine induced hepatotoxicity is the best designated system of xenobiotics induced liver damage and frequently used model for the screening of hepatotoxic and/or hepatoprotective effects of drugs. Hence, D-Galactosamine induced hepatotoxicity in rats was chosen as the experimental model [3]. Single dose of D-Galactosamine administration causes dose dependent hepatic injury resembling viral hepatitis, focal necrosis and periportal inflammation. It produces hepatitis by inhibiting the synthesis of RNA and protein via reduction in cellular UTP uptake that leads to the hepatic parenchyma necrosis [4,5].

Herbal drugs are emerging as strong alternatives or adjuncts to conventional modern medical therapy. The comparative lesser adverse effects of traditional combined with the regulatory issues arising out of the TRIPS agreement have generated a renewed interest in the traditional remedies. In recent years, complementary and alternative medicinal approach using medicinal plants for prevention and treatment of diseases is gaining popularity [6]. A huge number of medicinal plants are being used traditionally for immunomodulation and hepatoprotection and these effects need to be validated following modern scientific methodology. So that they can be a part of the main stream health care system for complex pathophysiological states. In Unani system of medicine, a polyherbal formulation Dawa-Ul-Kurkum is used in cases of liver dysfunction, anorexia, ascites and abdominal pain. The study has been designed to evaluate the hepatoprotective and immunomodulatory effects of Dawa-Ul-Kurkum and the possible mechanism in the experimental model of D-Galactosamine induced hepatic dysfunction. The polyherbal Unani preparation, Dawa-Ul-Kurkum is composed of 9 herbs namely Sunbul-ut-Teeb, Mur Makki, Saleekha, Qust, Shagufa-e-Izkhir, Darcheeni, Zafran, Sharab-e-musallas and Asal [7,8].

Materials and Methods

Drugs and chemicals

The drug Dawa-Ul- Kurkum has been prepared and provided by CRIUM, Hyderabad. Silymarin and D-Galactosamine hydrochloride were purchased from Sigma-Aldrich (USA); other routine chemicals were procured from SRL, New Delhi. Biochemical kits were purchased from ERBA Diagnostic Mannheim Gmbh.

Animals

Inbred male Wistar rats of either sex (180 - 250g) were used for the study. Animals were taken from the state-of the-art Animal House of Vallabhbhai Patel Chest Institute, University of Delhi. Animals were housed at a constant temperature (25 ± 2°C) under standard laboratory conditions. The animals had free access to food and water throughout the experiment. Care of animals was taken as per guidelines of CPCSEA for use of animals in Scientific Research with approval of Institutional Animal Ethics committee (IAEC) (CPCSEA Registration number 170/GO/ReBi/S/99/CPCSEA).

Preparation of 50% hydroalcoholic extract of Dawa-ul-Kurkum

The 50% Hydroalcoholic extract was prepared by mixing 100g of Dawa-Ul-kurkum with 100 ml ethanol (99% alcohol) + 100 ml distilled water. This solution was stirred for 9hrs by a magnetic stirrer. After 9 hours, the solution was filtered through muslin cloth for 3 - 4 times and finally filtered through filter paper. The filtrate is kept on heating mantle at a low temperature (25 - 30°C) till the volume was reduced to half (100 ml) which took around 5 hours. This extract was used for further comparative studies with the Dawa-Ul-Kurkum.

Experimental procedure

Galactosamine induced hepatotoxicity in rats

The study was approved by the Institutional animal Ethical Committee (IAEC) of V.P. Chest Institute, University of Delhi. The experimental model of liver necrosis was induced in wistar rats by administration of single dose of D-Galactosamine (400 mg/kg, i.p.) on 14th day [1,9].

Animals were divided into seven groups. Group 1 served as healthy control; Group 2 served as experimental control administered with D-Galactosamine; Group 3 served as positive control and received Silymarin (50 mg/kg, orally) [10] + D-Galactosamine; Group 4 and 5 animals were administered Dawa-Ul-kurkum (DK) at dose (250 or 500 mg/kg, orally) respectively + D-Galactosamine; Group 6 and 7 animals were administered with 50% hydroalcoholic extract of Dawa-Ul-Kurkum (HA) at dose (500 or 1000 mg/kg, orally) + D-Galactosamine. The dose of Dawa-Ul-Kurkum was calculated from the human dose being prescribed by the Unani physicians. All drugs were administered for 14 days. D-Galactosamine was administered (400 mg/kg, i.p.) in a single dose on 14th day in all groups except group 1. After 24 hours, i.e. on 15th 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 31 animals were included in the experimental study. Animals were divided into total seven groups, first two groups contain 3 rats/group and group number 3 to 7 contains 5 rats/group.

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) the organic compound [$CH_2(CHO)_2$] 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 1h 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 minutes and then centrifuged at 4000 rpm for 10 minutes. Butanol-pyridine layer is measured spectrophotometrically at 532 nm. TBARS values are expressed as MDA equivalents. 1, 1, 3, 3-tetramethoxypropane (TMP) was used as the standard [11].

Assay of reduced glutathione (GSH) levels

Glutathione (GSH) levels were estimated by the method of Ellman [12]. 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 vortexed and absorbance was read at 412 nm within 15 minutes. The concentration of 2-nitro-5-benzoic acid formation was measured and reduced glutathione is expressed as µmol/mg protein.

Nitrates and nitrites (NOx) assay

NOx concentrations were determined by using the Griess reaction as described previously by Tracey., *et al* [13]. 6 µl of sample/supernatant was mixed with 44 µl of distilled water, 20 µl of 310 mM phosphate buffer (pH 7.5) and 10 µl each of 0.86 mM NADPH, 0.11

mM flavin adenine dinucleotide (FAD) and 10 µl Nitrate reductase (1 U/ml) in individual wells of a 96-well plate. Plate was thereafter incubated for 1h at room temperature in the dark. 200 µl of Griess reagent [1:1 mixture of 1% sulfanilamide (1% solution with 5% ortho-phosphoric 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* [14]. 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 the groups were subjected to histological examination. Microscopic examination was done by a qualified pathologist using hematoxylin 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

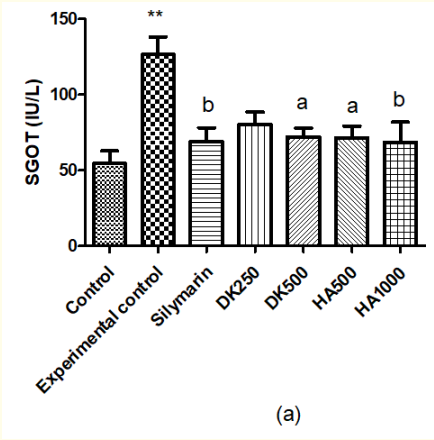
Effects of Dawa-Ul-Kurkum and its hydro-alcoholic extract on Liver Function test (LFT) in D-Galactosamine induced hepatotoxicity in rats

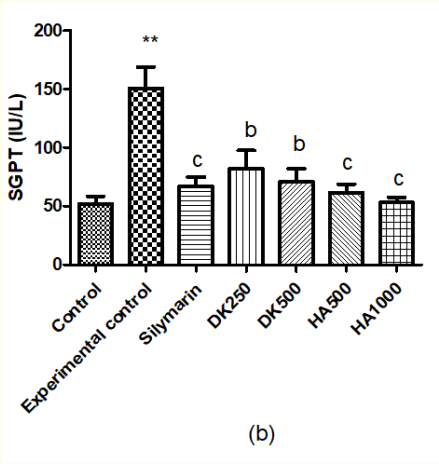
In experimental control group, single dose of D-Galactosamine (400 mg/kg) on 14th day resulted in significant increase in serum levels of SGOT ($P < 0.01$), SGPT ($p < 0.01$), ALP ($p > 0.05$), total bilirubin ($p < 0.01$), direct bilirubin ($p < 0.01$) and reduction in total protein as compared to control rats. This is suggestive of notable degree of hepatotoxicity and tissue injury in the rat liver and validated our model of D-Galactosamine induced hepatotoxicity. In Group 4 and 5, treatment with Dawa-Ul-Kurkum at doses 250 and 500 mg/kg respectively for 14 days significantly attenuated the effects of D-Galactosamine and reduced level of serum SGOT ($p < 0.05$ at 500 mg/kg dose), SGPT ($p < 0.01$ at each dose), ALP ($p < 0.05$), total bilirubin and direct bilirubin ($p < 0.05$ for 500 mg dose) as compared to that in Experimental control group (treated with D-Galactosamine alone). Similarly, in Group 6 and 7 treatment with 50% hydro-alcoholic extract of Dawa-Ul-Kurkum (500 and 1000mg/kg) produced hepatoprotective effect as it significantly reduced the levels of serum SGOT ($P < 0.05$ and < 0.01), SGPT ($p < 0.001$), total bilirubin and direct bilirubin ($p < 0.05$ and < 0.01) as compared to that in Experimental control. However, no significant change was observed in the levels of ALP and total protein. Pretreatment with silymarin also significant reduced the hepa-

Treatment	SGOT(IU/L)	SGPT (IU/L)	ALP(IU/L)
Control	54.27 ± 8.574	52.13 ± 6.207	104.8 ± 13.92
Experimental control	126.5 ± 11.64 **	150.7 ± 18.21**	207.2 ± 56.87 *
Silymarin	69.12 ± 8.918 b	67.16 ± 7.821 c	107.3 ± 4.329 a
DK250	80.04 ± 8.365	82.26 ± 15.25 b	116.6 ± 8.936 a
DK500	72.12 ± 5.705 a	71.16 ± 11.14 b	105.8 ± 9.875 a
HA500	71.62 ± 7.615 a	61.68 ± 7.314 c	135.3 ± 15.38
HA1000	68.58 ± 13.28 b	53.10 ± 4.423 c	147.9 ± 12.59

Table 1: *Effects of Dawa-Ul-Kurkum and its hydro-alcoholic extract on SGOT, SGPT and ALP in D-Galactosamine induced hepatotoxicity in ratsThe 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 400 mg/kg.*

** ($p < 0.05$) and ** ($p < 0.01$) when compared with control group; a ($p < 0.05$), b ($p < 0.01$) and c ($p < 0.001$) when compared with experimental control. The data were analyzed using one way ANOVA followed by Tukey test.*





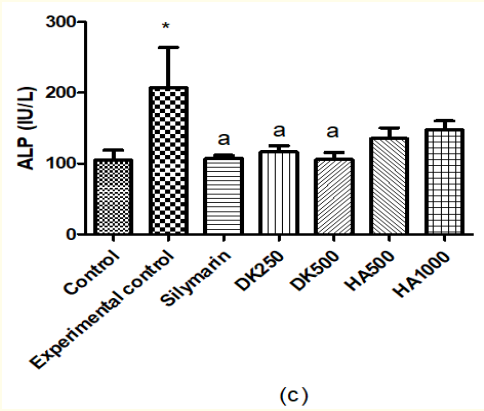


Figure 1: *Effects of Dawa-Ul-Kurkum and its hydro-alcoholic extract on (a) SGOT (b) SGPT and (c) ALP in experimental model of D-Galactosamine induced hepatotoxicity in rats. DK-Dawa-ul-kurkum; HA-Hydroalcoholic extract of DK.*

Treatment	Total bilirubin (md/dl)	Direct bilirubin (mg/dl)	Total protein (g/dl)
Control	0.55 ± 0.02	0.30 ± 0.02	6.22 ± 0.48
Experimental control	1.92 ± 0.56 **	0.70 ± 0.10 **	5.27 ± 0.32
Silymarin	0.76 ± 0.11 a	0.41 ± 0.03 a	6.12 ± 0.40
DK250	1.00 ± 0.18	0.46 ± 0.07	5.83 ± 0.45
DK500	0.89 ± 0.21 a	0.41 ± 0.06 a	5.98 ± 0.43
HA500	0.86 ± 0.11 a	0.39 ± 0.03 a	5.95 ± 0.52
HA1000	0.73 ± 0.05 b	0.35 ± 0.05 a	6.07 ± 0.28

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

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 400 mg/kg.

*** (p < 0.01) vs control group; a (p < 0.05) and b (p < 0.01) vs Experimental control. The data were analyzed using one way ANOVA followed by Tukey test.*

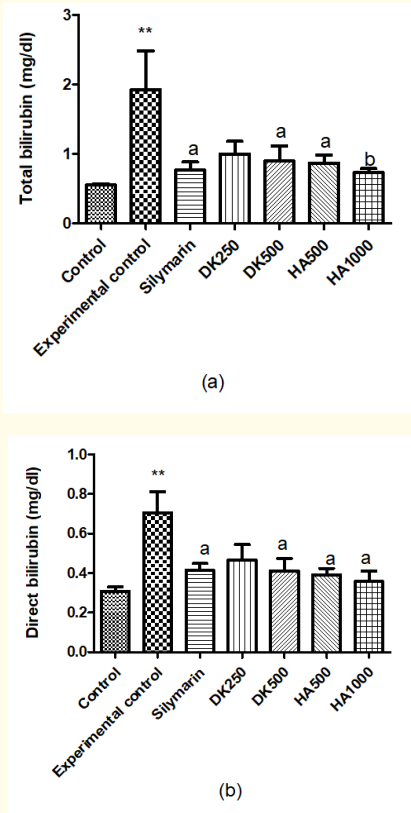


Figure 2: *Effects of Dawa-Ul-Kurkum and its hydro-alcoholic extract on (a) Total bilirubin (b) Direct bilirubin in experimental model of Galactosamine induced hepatotoxicity in rats. DK-Dawa-ul-kurkum; HA-Hydroalcoholic extract of DK.*

totoxic effects of D-Galactosamine and reduced the levels of serum SGOT ($p < 0.01$), SGPT ($p < 0.001$), ALP ($p < 0.05$), Total bilirubin ($p < 0.01$) and Direct Bilirubin ($p < 0.01$) 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, 2 and figure 1, 2.

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

The mean body weight was recorded in all groups at 0 and 15th day and liver weight was recorded on 15th day after various drug treatments. The results showed that single dose of D-Galactosamine (400 mg/kg) on 14th day caused significant reduction in the body weight ($p < 0.01$) but no significant change in the liver weight when compared to corresponding control rats. Interestingly, treatment with Dawa-Ul-Kurkum (250 and 500 mg/kg), 50% hydro-alcoholic extract of Dawa-Ul-Kurkum (500 and 1000 mg/kg) and silymarin blocked the effects of D-Galactosamine and resulted in significant 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 been 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	186.3 ± 3.844	191.3 ± 4.667	2.613	6.267 ± 0.352	3.276
Experimental control	167.7 ± 12.17	158.7 ± 11.26	-5.671*	7.933 ± 0.405	4.998
Silymarin	198.2 ± 6.328	201.8 ± 7.235	1.783 b	6.360 ± 0.538	3.151
DK 250	181.4 ± 14.60	183.0 ± 13.07	0.874 b	5.960 ± 0.278	3.256
DK500	205.4 ± 8.778	206.8 ± 6.176	0.676 b	6.760 ± 0.213	3.268
HA500	190.2 ± 5.826	193.6 ± 6.757	1.756 b	6.920 ± 0.449	3.574
HA1000	175.2 ± 10.45	178.2 ± 15.54	1.683 b	5.880 ± 0.512	3.299

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

*The values are expressed as mean ± SEM; DK-Dawa-Ul-kurkum; HA-Hydroalcoholic extract of DK. Initial and final body weight was measured on 0 and 15th day of treatment. All groups except control group were treated with D-Galactosamine 400 mg/kg. Liver index was calculated as (liver weight/body weight×100%); * ($p < 0.01$), when compared with control group; b ($P < 0.01$), when compared with Experimental control group.*

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

In experimental control group, single dose of D-Galactosamine (400 mg/kg) on 14th day resulted in increase in stable metabolites of nitric oxide (NOx) ($P < 0.05$) and MDA ($P < 0.05$) in supernatant of liver homogenates and significant reduction in GSH as compared to control rats. This is suggestive of 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 doses 250 and 500 mg/kg respectively for 14 days significantly attenuated the effects of D-Galactosamine and reduced level of homogenate supernatant NOx ($p < 0.05$ at both doses), MDA ($p < 0.05$ at both doses) and significantly increased GSH ($p < 0.05$ at dose 500 mg/kg) 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 Dawa-Ul-Kurkum (500 and 1000 mg/kg) produced hepatoprotective effect as it significantly reduced the levels of NOx in homogenate supernatant ($p < 0.05$ at dose 1000 mg/kg), MDA ($p < 0.05$ at dose 500 mg/kg) and increased GSH ($p > 0.05$) 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 [9]. The results are shown in table 4 and figure 3.

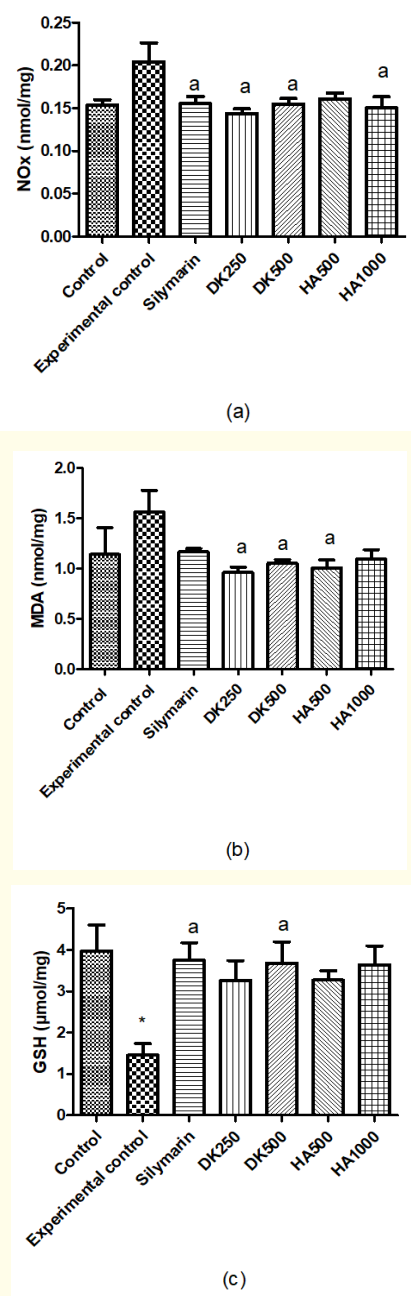


Figure 3: *Effects of Dawa-Ul-Kurkum and its hydro-alcoholic extract on a) stable metabolites of nitric oxide (NOx), b) MDA and c) GSH in D-Galactosamine induced hepatotoxicity in rats.*

Treatment	NOx (nmol/mg) protein	MDA (nmol/mg) protein	GSH (µmol/mg) protein
Control	0.153 ± 0.006	1.142 ± 0.265	3.975 ± 0.6335
Experimental control	0.204 ± 0.022	1.565 ± 0.212	1.457 ± 0.2780 *
Silymarin	0.155 ± 0.008 a	1.168 ± 0.030	3.754 ± 0.4233 a
DK250	0.143 ± 0.005 a	0.964 ± 0.051 a	3.267 ± 0.4761
DK500	0.154 ± 0.006 a	1.054 ± 0.035 a	3.683 ± 0.5152 a
HA 500	0.160 ± 0.006	1.008 ± 0.079 a	3.271 ± 0.2279
HA 1000	0.150 ± 0.012 a	1.095 ± 0.093	3.642 ± 0.4571

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

*The values are expressed as mean ± SEM; DK-Dawa-Ul-kurkum; HA-Hydroalcoholic extract of DK; * ($p < 0.05$) vs control group; a ($p < 0.05$) 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 400 mg/kg.*

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

Histopathological examination of the liver sections of vehicle treated (control) rats showed well preserved lobular architecture with normal central vein, hepatocyte arranged in radiating pattern and sinusoids. No cellular degeneration, inflammatory cell infiltrate or haemorrhages seen. In experimental control group, administration of D-Galactosamine (400 mg/kg) single dose on 14th day showed multifocal areas of inflammatory cell infiltrate in hepatic parenchyma and mild haemorrhages. Vasodilation was seen along with focal areas of hepatocytes showing hydropic changes and loss of radiating pattern of hepatocyte arrangement around some central vein. This was suggestive of notable degree of hepatotoxicity and tissue injury in the rat liver and validated our model of hepatotoxicity. Silymarin treated group showed that hepatic lobular architecture was fairly preserved were as multifocal areas of inflammatory cell infiltrate in hepatic parenchyma were present. Vasodilation and haemorrhages were not seen. In Group 4 and 5, treatment with Dawa-Ul-Kurkum at doses 250 and 500 mg/kg respectively for 14 days showed fairly preserved hepatic lobular architecture but some multifocal areas of inflammatory cell infiltrate in hepatic parenchyma. Vasodilation and haemorrhages were not seen and mild non-significant hydropic changes were seen focally. Hepatic lobular architecture was fairly preserved in most parts. In Group 6 and 7 treatment with 50% hydro-alcoholic extract of Dawa-Ul-Kurkum (500 and 1000 mg/kg) also showed that hepatic lobular architecture was fairly well preserved with some multifocal areas of inflammatory cell infiltrate in hepatic parenchyma. Although focal areas of haemorrhages were seen but the intensity is less compared to other groups. Vasodilation and haemorrhages were not seen. The results are shown in figure 4.

Discussion

Hepatic damage induced by D-Galactosamine is the standard and well accepted method to simulate xenobiotic induced hepatotoxicity and is commonly used for the screening of hepatoprotective agents. It is important for the development of safe and efficacious hepatoprotective agent of plant origin that it holds anti-inflammatory and antioxidant property to diminish tissue damages for the better management of hepatitis [15,16]. Therefore, D-Galactosamine stimulated hepatic damage was selected as the experimental model to evaluate the hepatoprotective effect of DK. Previous studies have divulged that D-Galactosamine causes shift of liver biomarker enzymes and causes notable elevation in hepatic markers [16,17]. Further D-Galactosamine is acknowledged to prevent the transcription and translation due to endotoxemia, which causes fulminant hepatitis [18]. Nitric oxide (NO) a ubiquitous free radical moiety, was first discovered in the vascular endothelium and now known to be located in many tissue/organ systems including the gastrointestinal and hepatobiliary system and increased levels are found in inflammatory conditions [19].

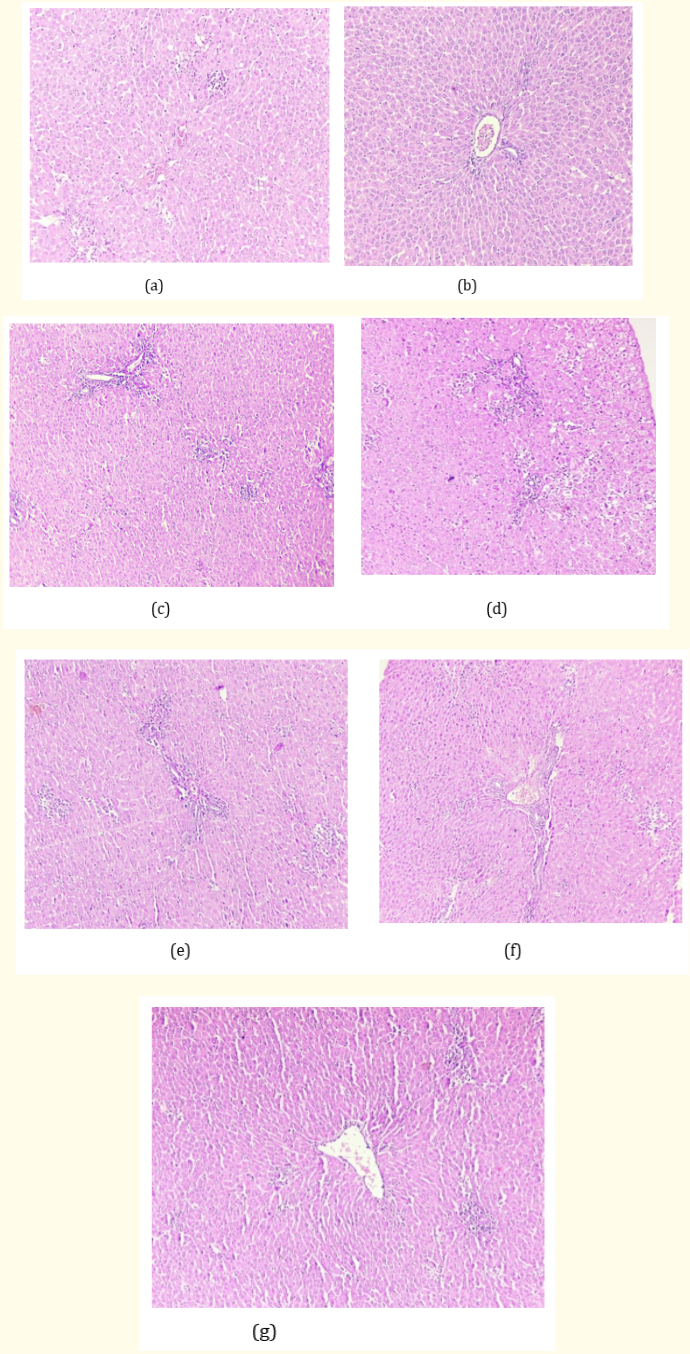


Figure 4: *Histopathological picture of liver sections after various drug treatment in rats. a) Control; b) Experimental control; c) Silymarin; d) DK250; e) DK500; f) HA500; and g) HA1000. All groups except control group were treated with D-Galactosamine (400mg/kg, i.p.). DK-Dawa-Ul-kurkum; HA-Hydroalcoholic extract of DK.*

The present result showed that in experimental control group of rats, liver damage was characterized by a rise in serum SGOT, SGPT, ALP, total bilirubin and direct bilirubin levels but decrease in serum protein level. Oxidative stress parameters like tissue MDA and NOx levels were also increased which was accompanied with lowered GSH levels. Rise in MDA, a thiobarbituric acid reactive substance (TBARS) indicated increased lipid peroxidation and tissue damage. This indicated oxidative damage as a result of failure of anti-oxidant defense system to prevent formation of excess free radicals. The decrease in level of proteins in D-Galactosamine treated group may have been due to liver dysfunction following decrease in levels of reduced glutathione in the tissues leading to inhibition of many enzymes containing SH group and inhibition of protein synthesis [20], which is an indicator of severe liver damage [21,22]. The biochemical findings were corroborated by the histopathological examination of liver tissue which showed necrosis, inflammatory, mild haemorrhages and vasodilation changes in rat hepatic tissue.

The present results showed that concurrent administration of Dawa-Ul-Kurkum and 50% Hydro-alcoholic extract of Dawa-Ul-Kurkum along with D-Galactosamine significantly prevented the rise in the level of serum SGOT, SGPT, ALP, total bilirubin and direct bilirubin. Further, measurement of oxidative stress parameters in liver homogenates showed protective effects of Unani polyherbal preparation Dawa-Ul-Kurkum against raised levels of reactive oxygen and nitrogen species in response to D-Galactosamine as seen by lowered levels of MDA and NOx and elevating the levels of GSH. The effects with the DK were more consistent as compared to the HA extract on oxidative stress parameter. Histopathological examination of liver also showed that Dawa-Ul-Kurkum with multifocal areas of inflammatory cell infiltrate, hepatic lobular architecture is fairly preserved, vasodilation and haemorrhages were not seen and thus reemphasizing the protective effect of this polyherbal formulation against D-Galactosamine induced hepatotoxicity. However, as seen in the biochemical studies, the protective effect was more prominent with the hydro-alcoholic extract of Dawa-Ul-Kurkum and its administration also showed fairly well preserved lobular architecture, and multifocal areas of inflammatory cell infiltrate in some parts. These results showed that both Dawa-Ul-Kurkum and its HA preparation is effective hepatoprotective agents and prevented the D-Galactosamine induced hepatotoxicity. The protective effects may be mediated through maintenance of the oxidant-antioxidant homeostatic balance.

Conclusion

The present study demonstrated that D-Galactosamine is potentially hepatotoxic to Wistar rats, when given in single dose as proven by changes in markers of liver functions, oxidative stress and histopathological studies. Both DK and its 50% hydro-alcoholic extract were found to be effective against Galactosamine induced hepatotoxicity as they significantly prevented the hepatotoxic damage induced in rats, with differential effects on biochemical and oxidative stress parameters. Such translational studies using the reverse pharmacology approach could help in the integration of traditional and modern medicinal concepts in the greater interest of drug development and rational therapy.

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