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Research Article

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

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Abstract

Dawa-Ul-Kurkum, a traditional Unani formulation was evaluated for the hepatoprotective effects in a model of liver damage induced by paracetamol in rats and the possible mechanisms were investigated. The polyherbal preparation has been formulated by CRIUM, Hyderabad. The Liver functions were assessed by measuring Serum glutamic-oxaloacetic transaminase (SGOT), serum glutamic-pyruvic transaminase (SGPT) and serum alkaline phosphatase (ALP). They were estimated by Kinetic method and serum bilirubin and total protein were assessed by End Point assay as per the instruction of the Kit manual. Malondialdehyde MDA, a marker of the lipid peroxidation was measured spectrophotometrically. Reduced glutathione (GSH), an antioxidant was estimated by the method of Ellman. Nitrates and Nitrites (NOx concentrations were estimated by using the Griess reaction as described by Tracey., *et al*. Paracetamol was administered in high dose of 2 g/kg, orally for 14 days to induce liver damage in Wistar rats and the effects of various drug treatments were assessed on morphological, biochemical and histological markers of liver toxicity. In experimental group, administration of paracetamol induced significant derangements in liver function as indicated by increased levels of SGOT, SGPT ALP and bilirubin; and reductions in body weight and increased liver weights vs control rats. Histopathological examination showed Periportal necrosis with haemorrhages in experimental control. Oral administration of Dawa-Ul-Kurkum (paste dissolved in distilled water) for 14 days showed reversal of the biochemical and histopathological derangements of liver function following paracetamol administration. Such effects were also seen after the hydroalcoholic extract (HA) of Dawa-Ul-Kurkum which showed marked protective effects on biochemical and histopathological parameters. The hepatoprotective effects of Dawa-Ul-Kurkum and HA were similar to that observed after silymarin therapy. Liver damage induced by paracetamol was accompanied with elevated levels of MDA and NOx and reduced GSH levels as compared to controls. Treatments with Dawa-Ul-Kurkum and HA induced differential degrees of attenuations in these oxidative stress markers. Both Dawa-Ul-Kurkum and HA were found to be effective against paracetamol induced liver damage in rats.

Keywords: *Hepatotoxicity; Paracetamol; Dawa-Ul-Kurkum; Histopathology*

Introduction

Liver is the most important organ regulating various physiological metabolic processes in the whole body. It is involved in various essential functions such as metabolism, secretion and storage. It has prominent capacity to detoxicate toxic substances and synthesize functional ones [1]. Paracetamol (acetaminophen) is most commonly medicine used as antipyretic and analgesic, when it is taken in overdoses leads to acute liver damage. Mostly paracetamol is metabolized in liver to excretable glucuronide and sulphate conjugates [2,3]. The liver toxicity that is due to paracetamol has been allocated to the formation of toxic metabolites when a part of paracetamol is activated by hepatic cytochrome P-450 to a highly reactive metabolite N-acetyl-P-benzoquinone imine (NAPQI) [4]. At low concentration of NAPQI, reduced glutathione (GSH) conjugates with it to form mercapturic acid and leads to its detoxification [5]. But, when the rate of NAPQI development is more than the rate of detoxification by GSH, it oxidizes tissue macromolecules like lipid protein and changes the homeostasis of calcium.

Paracetamol in overdose s known to be connected with inflammation, increase in inflammatory cytokines as well as the upregulation of nitic oxide (NO) in serum, macrophages and hepatocytes [6]. The disturbance of prooxidant-antioxidant balance in tissues has been reported to be the mechanism which results in increased levels of reactive oxygen species (ROS) and oxidative damage of macromolecules [7]. It can cause various pathological conditions in humans and animals for example, hepatic and renal dysfunction, testicular damage, respiratory disorders, and cancer [8]. In other words, there are lot of reports suggesting that paracetamol-mediated oxidative stress or liver toxicity is ameliorated by use of naturally occurring antioxidants or free radical scavengers, vitamins, medicinal plants or natural products [9,10].

Herbal or traditional medicines are being encouraged as strong alternatives to modern medical treatment. The comparative vary few adverse effects of traditional medicines combined with the regulatory issues arising out of the TRIPS agreement have resulted in a renewed interest in the traditional remedies. In recent years, a lot of research is being done on complementary and alternative medicinal using medicinal plants for prevention and treatment of diseases and thus gaining popularity [10]. Various medicinal plants are used traditionally for immunomodulation and hepatoprotection in Unani system of medicine. These effects need to be validated following modern scientific methodology, so they can be a part of the main stream of health care system for complex pathophysiological states. The polyherbal formulation of Dawa-Ul-Kurkum is used in cases of liver dysfunction, anorexia, ascites and abdominal pain by Unani physicians. Therefore, this study has been designed to evaluate the hepatoprotective and immunomodulatory effects of Dawa-Ul-Kurkum using the modern methodology and to delineate the possible cellular mechanism. The preparation Dawa-Ul-Kurkum is composed of following 9 herbs - 1) Sunbul-ut-Teeb (*Nardostachys jatamansi* DC), 2) Mur Makki (*Commiphora myrrha* Nees), 3) Saleekha (*Cinnamomum tamala*), 4) Qust (*Saussurea lappa*), 5) Shagufa-eIzkhir (*Cymbopogon schoenanthus*), 6) Darcheeni (*Cinnamomum zylenicum* bark), 7) Zafran (*Crocus sativus*), 8) Sharab-e-musallas (*Saussurea costus*) and 9) QandSafaid (*Saccharum officinarum*) Q.S. [11,12].

Materials and Methods

Drugs and chemicals

The polyherbal unani drug Dawa-Ul- Kurkum has been formulated by CRIUM, Hyderabad. Silymarin and paracetamol were purchased from Sigma-Aldrich and Cipla LTD respectively. Other routine chemicals were procured from SRL, New Delhi. Biochemical kits were purchased from ERBA Diagnostics Mannheim Gmbh.

Animals

Wistar rats of either gender (180 - 250g) were used for the study. The animals were maintained in the animal House of Vallabhbhai Patel Chest Institute, University of Delhi, at a constant temperature (25 ± 2°C) under standard laboratory conditions. The animals had free

access to food and water and 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).

The investigational drug

The standardized drug, Dawa-ul-Kurkum (paste), was prepared and provided by Central Research Institute of Unani Medicine (CRIUM), Hyderabad, Ministry of AYUSH, Govt. of India with a batch no. 3-1/2018-19/CRIUM. This polyherbal formulation is composed of 9 herbs as mentioned above. 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.

The 50% hydroalcoholic extract was prepared by mixing 100g of Dawa-Ul-kurkum paste with 100 ml ethanol (99% alcohol) + 100 ml distilled water. The mixture was boiled for 9h, filtered and the filterate was heated till the volume was reduced to half [1]. The extract was used for further comparative studies with the Dawa-Ul-Kurkum.

Experimental procedure

Paracetamol induced liver damage in rats

The experimental model of liver damage was induced in wistar rats by administration of paracetamol (2 g/kg, orally) daily for 14 days [13]. Animals were divided into seven groups and each group contained 5 rats.

Group 1 received only water and served as control; Group 2 was administered paracetamol which served as experimental control; Group 3 received Silymarin (50 mg/kg, orally) [14] + paracetamol and served as positive control and; Group 4 and 5 animals were administered Dawa-Ul-kurkum at dose (250 or 500 mg/kg paste dissolved in distilled water, orally) respectively + paracetamol; Group 6 and 7 animals were administered with 50% hydroalcoholic extract of Dawa-Ul-Kurkum (HA) at dose (500 or 1000 mg/kg, orally) + paracetamol. The dose of Dawa-Ul-Kurkum was calculated on the basis of the human dose being prescribed by the Unani physicians. All treatments were given for 14 days. Paracetamol was administered in the dose of 2 g/kg, orally daily for 14 days in all groups except group 1. On 15th day, animals were anesthetized and blood was collected by cardiac puncture, centrifuged and stored at -80ºC. After blood collection, rats were sacrificed and liver was removed and stored at -80^{0}C for estimation of biochemical and oxidative stress parameters and histopathological studies.

Biochemical estimations

The markers of liver function, i.e. Serum alanine aminotransferase, serum aspartate aminotransferase and serum alkaline phosphatase were estimated by using Kinetic method of International Federation of Clinical Chemistry. The 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 oxidative stress marker of lipid peroxidation in biomedical research was measured spectrophotometrically as 2-thiobarbituric acid-reactive substance (TBARS) in supernatant of liver homogenate [8]. 0.1 ml of homogenate supernatant was added to 0.2 ml of sodium dodecyl sulfate (8.1%), 1.5 ml of acetic acid (20%) and 1.5 ml of 2-thiobarbituric acid (0.8%). The total mixture was finally made up to 4.0 ml with distilled water and vortexed. The samples were incubated for 1h at 95°C and cooled with tap water. 1.0 ml of distilled water and 5.0 ml of mixture of butanol-pyridine 15:1 (v/v) were added to the sample and shaken for 10 min. and centrifuged for 10 min at 4000 rpm. Butanol-pyridine layer is measured spectrophotometrically at 532 nm. 1, 1, 3, 3-tetramethoxypropane (TMP) was used as the standard for comparative purpose [15].

Assay of reduced glutathione (GSH) levels

Glutathione (GSH) levels were measured by method described by Ellman [16]. This assay is based on the enzymatic recycling in which glutathione was sequentially oxidized by the DTNB and reduced by NADPH in the presence of glutathione reductase. An equal amount of sample was mixed with 10% trichloroacetic acid and centrifuged to separate the proteins. To 0.1 ml of this homogenate 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 min. The measured reduced glutathione is expressed as µmol/mg protein.

Nitrates and nitrites (NOx) assay

NOx concentrations were measured by using the Griess reaction as described by Tracey., *et al* [17]. 6 µl of homogenate 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% 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. The method of Lowry., *et a*l. [18] was used to estimate Total protein and concentration of total nitrate and nitrite (NOx) in liver homogenates was expressed as nM/mg protein.

Histopathological examination

The liver collected from all the rats after completion of respective drug treatments were subjected to histopathological examination. The microscopic examination was done by a pathologist using hematoxylin and eosin staining in a blinded fashion.

Statistical analysis

The results are expressed as mean ± standard error of the mean. One-way analysis of variance (ANOVA) followed by Tukey test was 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) during paracetamol induced liver damage in rats

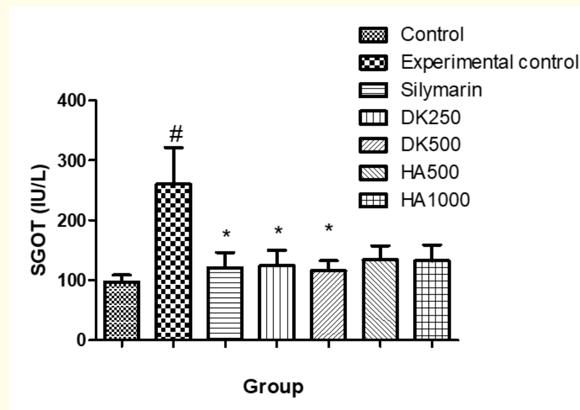
In group 2, paracetamol (2 g/kg, orally) was given daily for 14th days that resulted in significant increase in serum levels of Serum glutamic-oxaloacetic transaminase (SGOT), serum glutamic-pyruvic transaminase (SGPT), serum alkaline phosphatase (ALP), total bilirubin, direct bilirubin ($p < 0.05$ for each parameter) and reduction in total protein as compared to that in control rats. This confirms and validates our model of hepatotoxicity induced by high dose of paracetamol in rats. In Group 4 and 5 (Dawa-Ul-Kurkum at doses 250 and 500 mg/kg respectively) for 14 days significantly attenuated the damaging effects of paracetamol on liver function parameters and reduced level of serum SGOT ($p < 0.05$ at each dose), SGPT ($p < 0.05$ at 500mg/kg dose), ALP, total bilirubin and direct bilirubin ($p < 0.05$ at each dose) and increased level of serum total protein ($p < 0.05$ at 250 mg/kg dose) as compared to that in Experimental group treated with paracetamol alone. Also, in Group 6 and 7, administration of hydro-alcoholic extract in doses of 500 or 1000mg/kg produced hepatoprotective effect as it reduced the levels of serum SGOT, SGPT and total bilirubin vs Experimental control. However, significant change was observed in the levels of ALP ($p < 0.05$ at 1000 mg/kg), direct bilirubin ($p < 0.05$) and total protein ($p < 0.05$ at each dose). In group 3 also significant reduction of the hepatotoxic effects of paracetamol was observed by silymarin as evident from the reduced levels of serum SGOT ($p < 0.05$), SGPT ($p < 0.005$), ALP ($p < 0.05$), Total bilirubin and Direct Bilirubin ($p < 0.05$) as compared to that in Experimental control. The results of both 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.

Treatment	SGOT(IU/L)	SGPT (IU/L)	ALP(IU/L)
Control	97.31 ± 11.19	30.10 ± 15.19	86.79 ± 13.55
Experimental control	260.1 ± 61.28#	73.64 ± 4.53#	197.8 ± 23.65#
Silymarin	120.6 ± 25.64*	35.70 ± 7.02*	99.65 ± 13.44*
DK250	124.0 ± 25.80*	42.11 ± 5.74	110.2 ± 17.67
DK500	116.0 ± 16.62*	33.92 ± 6.13*	96.24 ± 11.15*
HA500	134.3 ± 23.32	39.41 ± 4.44	118.4 ± 12.66
HA1000	132.4 ± 26.54	46.50 ± 7.55	104.6 ± 28.43*

Table 1: *Effects of Dawa-Ul-Kurkum and its hydro-alcoholic extract on markers of liver function (SGOT, SGPT and ALP) during hepatotoxicity induced by paracetamol in rats.*

The values are expressed as mean ± SEM; DK-Dawa-Ul-kurkum; HA-Hydroalcoholic extract of DK. All groups except control group were treated with paracetamol 2 g/kg.

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



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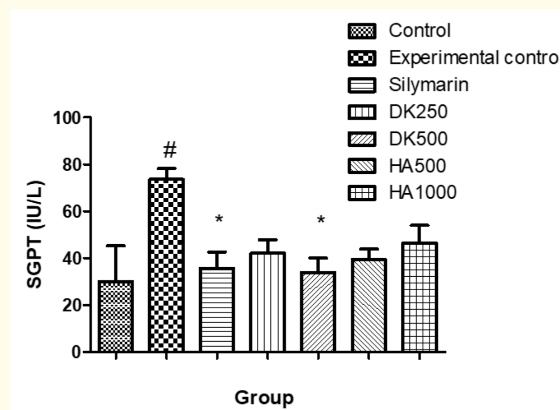

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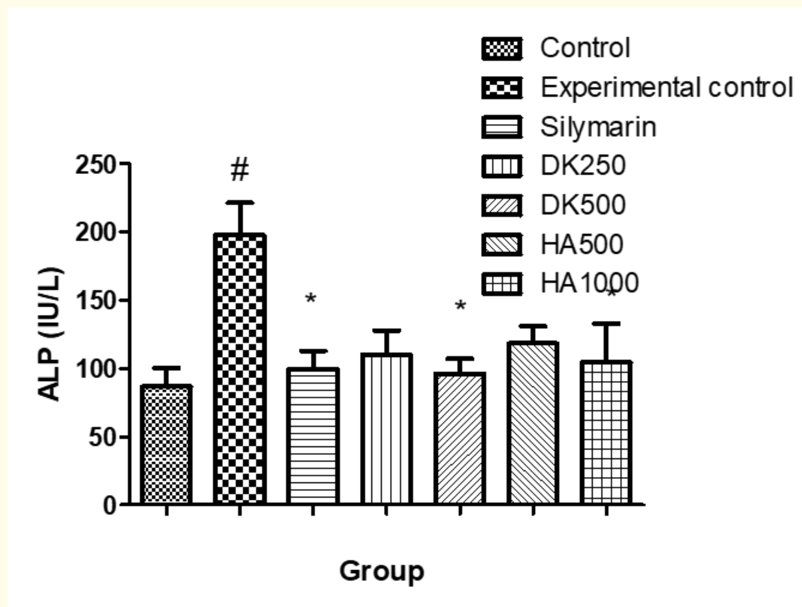

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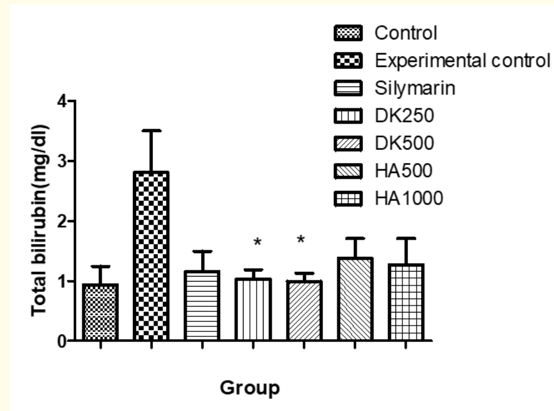
Figure 1a-1c: *Effects of Dawa-Ul-Kurkum and its hydro-alcoholic extract on (a) SGOT (b) SGPT and (c) ALP in experimental model of paracetamol induced hepatotoxicity in rats. DK-Dawa-ul-kurkum; HA-Hydroalcoholic extract of DK.*

Treatment	Total bilirubin (mg/dl)	Direct bilirubin (mg/dl)	Total protein (g/dl)
Control	0.936 ± 0.307	0.646 ± 0.224	6.217 ± 0.372
Experimental control	2.810 ± 0.691	1.887 ± 0.503#	2.697 ± 0.763
Silymarin	1.160 ± 0.333	0.744 ± 0.172*	6.246 ± 0.604
DK250	1.034 ± 0.154*	0.784 ± 0.156*	6.864 ± 0.791*
DK500	0.996 ± 0.134*	0.668 ± 0.190*	6.444 ± 0.670
HA500	1.380 ± 0.328	0.882 ± 0.189	6.656 ± 0.783*
HA1000	1.272 ± 0.436	0.804 ± 0.181*	6.914 ± 0.918*

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

The values are expressed as mean ± SEM; DK-Dawa-Ul-kurkum; HA-Hydroalcoholic extract of DK. All groups except control group were treated with paracetamol 2 g/kg.

*#: ($p < 0.05$) vs control group; *: ($p < 0.05$) vs Experimental control. The data were analyzed using one way ANOVA followed by Tukey test.*



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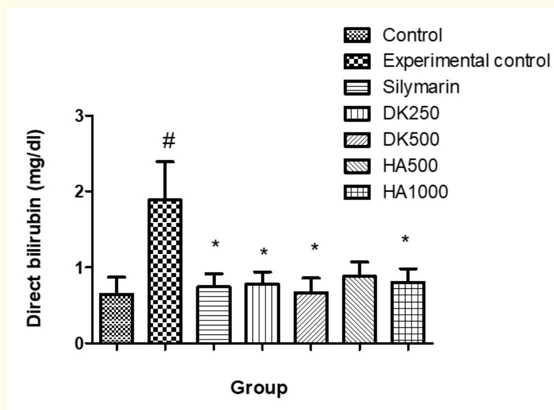

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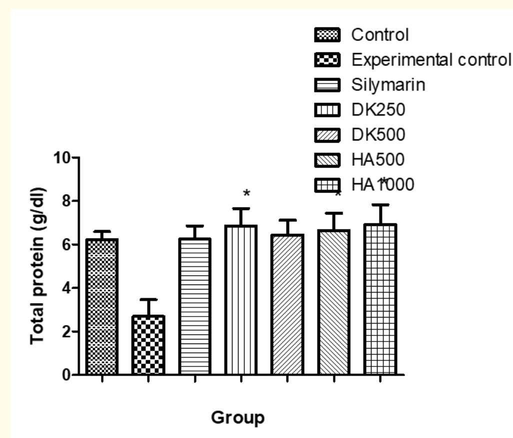

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Figure 2a-2c: *Effects of Dawa-Ul-Kurkum and its hydro-alcoholic extract on (a) Total bilirubin (b) Direct bilirubin (c) Total protein in experimental model of paracetamol induced hepatotoxicity in rats. DK-Dawa-ul-kurkum; HA-Hydroalcoholic extract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

Effects of Dawa-Ul-Kurkum and its hydro-alcoholic extract on body and liver weight in paracetamol induced liver damage in rats

The body weight of the rats of all groups was noted on 0 and 15th day. After completion of various drug treatments the rats were sacrificed on 15th day and liver were removed and weighed. The results showed that treatment with paracetamol (2 g/kg) for 14 day caused significant reduction in the body weight ($p < 0.01$) with no significant change in the liver weight when compared to corresponding control rats. Treatment with Dawa-Ul-Kurkum (250 and 500 mg/kg), hydro-alcoholic extract (500 and 1000 mg/kg) and silymarin blocked the effects of paracetamol and resulted in significant increase in the body weight. The increase in body weight can be due to improvement in appetite which may have resulted from 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	136.7 ± 1.20	148.7 ± 7.53	8.06	5.53 ± 0.81	3.71
Experimental control	155.0 ± 18.18	152.0 ± 15.04	-1.97##	6.00 ± 0.57	3.94
Silymarin	189.0 ± 23.21	190.2 ± 27.07	0.63*	5.90 ± 0.47	3.10
DK 250	163.8 ± 11.19	165.2 ± 8.07	0.84*	5.68 ± 0.20	3.43
DK500	174.0 ± 17.58	176.2 ± 14.19	1.24*	6.74 ± 0.29	3.82
HA500	151.6 ± 4.46	152.2 ± 7.31	0.39*	5.92 ± 0.29	3.88
HA1000	180.2 ± 13.99	182.8 ± 16.05	1.42*	6.44 ± 0.60	3.52

Table 3: *Effects of Dawa-Ul-Kurkum and its hydro-alcoholic extract on body and liver weight in paracetamol induced liver damage 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 paracetamol 2 g/kg. Liver index was calculated as (liver weight/body weight×100%); ##: ($p < 0.01$), when compared with control group; *: ($P < 0.05$), when compared with Experimental control group.*

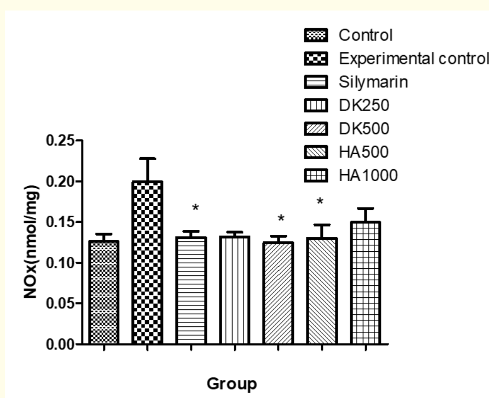
Effects of Dawa-Ul-Kurkum and its hydro-alcoholic extract on oxidative stress parameters in paracetamol induced liver damage in rats

Administration of paracetamol (2 g/kg, orally) daily for 14 days in group 2 resulted in increase in levels of NOx and MDA ($P < 0.05$) in supernatant of liver homogenates and significant reduction in GSH ($P < 0.05$) as compared to group 1. This confirms liver toxicity and tissue injury in the rat liver and corroborated to validate this model of hepatotoxicity. In group 4 and 5 (Dawa-Ul-Kurkum at doses 250 and 500 mg/kg) respectively for 14 days significantly attenuated the effects of paracetamol and reduced level of NOx ($p < 0.05$ at 500 mg/kg doses), MDA ($p < 0.05$ at 500 mg/kg doses) and significantly increased GSH ($p < 0.05$ at each dose) in liver homogenate as compared to that in group 2. Also, in group 6 and 7, treatment with hydro-alcoholic extract (500 and 1000 mg/kg) produced hepatoprotective effect as it significantly reduced the levels of NOx in liver homogenate supernatant ($p < 0.05$ at dose 500 mg/kg), MDA ($p < 0.05$ at dose 500 mg/kg) as compared to that in group 2. In group 3 silymarin also significantly reduced the hepatotoxic effects of paracetamol and reduced the levels of NOx ($p < 0.05$), MDA ($p > 0.05$) and increased GSH ($p < 0.05$) as compared to that in group 2. The results of Dawa-Ul-Kurkum and its hydro-alcoholic extract are comparable to that of Silymarin [19]. The results are shown in table 4 and figure 3.

Treatment	NOx (nmol/mg) protein	MDA (nmol/mg) protein	GSH (μmol/mg) protein
Control	0.126 ± 0.009	0.282 ± 0.041	3.141 ± 0.399
Experimental control	0.199 ± 0.028	0.489 ± 0.005#	1.566 ± 0.294#
Silymarin	0.131 ± 0.007*	0.301 ± 0.035*	3.053 ± 0.204*
DK250	0.131 ± 0.006	0.322 ± 0.055	2.980 ± 0.352*
DK500	0.124 ± 0.008*	0.306 ± 0.029*	3.159 ± 0.299*
HA 500	0.130 ± 0.016*	0.294 ± 0.013*	2.810 ± 0.169
HA 1000	0.149 ± 0.017	0.319 ± 0.026	2.728 ± 0.252

Table 4: *Effects of Dawa-Ul-Kurkum and its hydro-alcoholic extract on oxidative stress parameters in paracetamol 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; *: (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 paracetamol 2 g/kg.*



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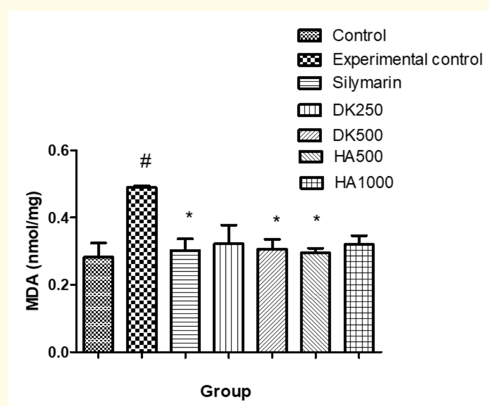

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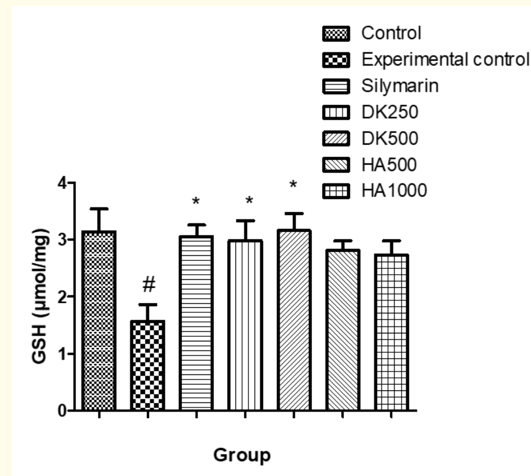

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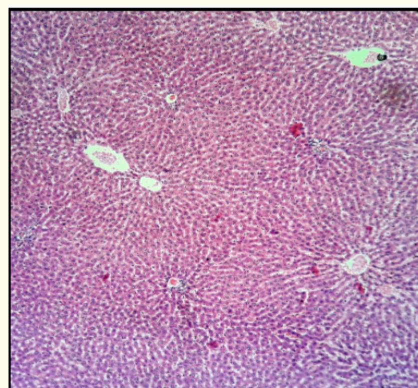
Figure 3a-3c: *Effects of Dawa-Ul-Kurkum and its hydro-alcoholic extract on (a) stable metabolites of nitric oxide (NOx), (b) MDA and (c) GSH in paracetamol induced liver damage. DK-Dawa-ul-kurkum; HA-Hydroalcoholic extract of DK.*

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

Histopathological examination of the liver sections was conducted to evaluate the effects of Dawa-ul-kurkum and HA extract. The liver section of control group rats showed well preserved lobular architecture with no cellular degeneration, inflammatory cell infiltrate or haemorrhage. In experimental control group, administration of paracetamol (2 g/kg, orally) given daily for 14 days showed Periportal necrosis with haemorrhages. This confirms liver toxicity and tissue injury in the rat liver and validated our model of paracetamol induced hepatotoxicity. Liver sections of rats of group 3 showed no degenerative changes of hepatocytes. In group 4 and 5 administration of Dawa-Ul-Kurkum at doses 250 and 500 mg/kg for 14 days showed no inflammatory and degenerative changes in hepatocytes. In group 6 and 7 (hydro-alcoholic extract 500 and 1000 mg/kg) also showed minimal inflammation and degenerative changes in hepatocytes and hydropic to fatty degenerative changes of hepatocytes. The results are shown in figure 4.



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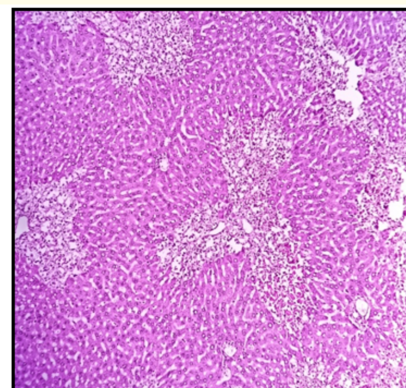

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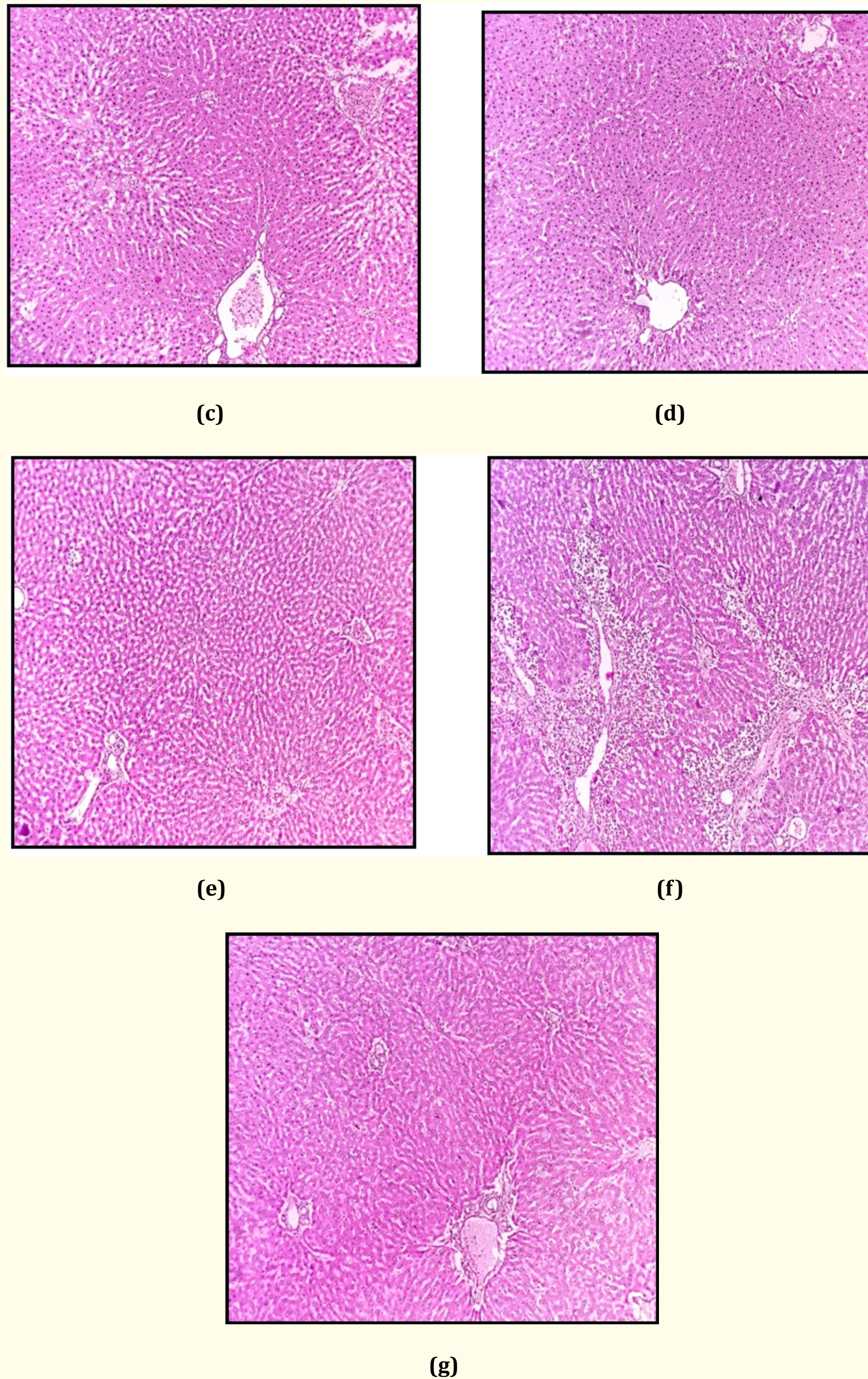

Figure 4a-4g *Histopathological picture of liver sections after various drug treatment in rats. a) Control; b) Experimental control; c) Silymarin; d) DK250; e) DK500; f) HA500; and g) HA1000. All groups except control group were treated with paracetamol (2g/kg, orally). DK-Dawa-Ul-kurkum; HA-Hydroalcoholic extract of DK.*

Discussion

Paracetamol is a well known freely available antipyretic drug. It is safe when used in low to moderate dose but leads to hepatic damage when used in overdoses [20-22]. The main dynamics of paracetamol toxicity on the liver is due to covalent binding of its toxic metabolite, n-acetyl-p-benzoquinone-amine to the sulfhydryl group of protein which causes cell necrosis and lipid peroxidation [23]. The results of the present study showed that paracetamol induced increase in liver weight, change in animal body growth and liver function, which may be due to obstruction of secretion of hepatic triglyceride into plasma [24]. After treatment with polyherbal preparation, there was improvement in the liver weight and the percentage of liver index as observed to be increased in paracetamol group, were restored.

During liver damage that is produced by overdose of paracetamol, the functions of hepatocytes are interrupted and result in the leakage of the plasma membrane [25], thus causing increase in levels of hepatic enzymes. Liver enzymes such as SGOT, SGPT, ALT, direct bilirubin, total bilirubin and total protein have still remained the gold standards for the assessment of liver injury [26]. Hepatic damage is always associated with cell necrosis which results in increase in tissue MDA and depletion in level of antioxidant, reduced glutathione (GSH). The results showed that oxidant and antioxidant ratio is disrupted due to overdose of paracetamol which leads to excess free radical generation and hepatic injury. The observation is supported by the earlier reports that paracetamol causes oxidative stress and alteration in endogenous antioxidant enzyme activities in rat [27]. Reduced glutathione (GSH) is an endogenous antioxidant which scavenges free radicals and thus mitigates oxidative damage induced by them. Reduced cellular GSH levels and capacity for GSH synthesis sensitize cells to radiation and to certain drugs [28].

The present research showed that oral administration of both Dawa-Ul-Kurkum and HA (syrup significantly attenuated the rise in the level of serum SGOT, SGPT, ALP, total bilirubin, direct bilirubin and decrease in total protein in response to administration of overdose of paracetamol. Further, oxidative stress parameters measured in the liver homogenates showed preventive effects of Unani preparation Dawa-Ul-Kurkum against raised levels of MDA and NOx (reactive oxygen and nitrogen species) in response to paracetamol and elevated the levels of GSH. The protective effect with the DK was greater in magnitude as compared to the HA extract on oxidative stress parameters. Histopathological examination of liver showed that Dawa-Ul-Kurkum reduced the inflammatory and degenerative changes in hepatocytes as seen in paracetamol treated rats. Thus, histopathological studies reemphasized the protective effect of this formulation against paracetamol induced liver damage as was also evident from biochemical estimations of liver functions. Also, as seen in the biochemical studies, the HA administration showed preventive effects on histopathological changes induced by paracetamol with minimal inflammation and degenerative changes in hepatocytes and hydropic to fatty degenerative changes of hepatocytes. These results showed that both Dawa-Ul-Kurkum and its HA preparation are effective hepatoprotective agents and prevented the paracetamol induced liver toxicity. The effect may be mediated through maintenance of the oxidant-antioxidant homeostatic balance.

Conclusion

Taken together, it can be concluded that both Dawa-Ul-Kurkum and its hydro-alcoholic extract are potentially hepatoprotective when given for 14 days, as proven by changes in markers of liver functions, oxidative stress and histopathological studies against liver damage induced by paracetamol in Wistar rats.

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