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Original Research Article

Immunomodulatory Effect of Dawa-Ul-Kurkum a Unani Preparation and its Possible Mechanisms in Experimental Model of Ethanol Induced Liver Damage in Rats

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Abstract

Objective: The effects of Dawa-ul-Kurkum a polyherbal Unani formulation was evaluated on ethanol induced hepatotoxicity in rats and its possible causes were investigated.

Methods: Immunoglobulin and cytokine levels were assessed by ELISA kit manual method. Delayed type hypersensitivity reaction by Institoris *et al* method.

Results: In the vehicle treated experimental group, ethanol induced significant hydropic degeneration in the hepatocytes as evidenced by increased level of cytokine as well as immunoglobulin, alterations in liver weight and changes in body weight from controls. Dawa-ul-Kurkum (DK), at two different doses, demonstrated strong immunomodulatory and protective effects against ethanol-induced liver damage. DK as well as hydro-alcoholic extarct has immunomodulatory effects comparable to those brought on by silymarin treatment.

Conclusion: It concludes that ethanol-induced hepatotoxicity can be treated with Dawa-ul-Kurkum for immunomodulatory benefits, and it suggests that the polyherbal's attenuation may be the mechanism of action for such effects.

Keywords: Hepatotoxicity, ethanol, Dawa-ul-Kurkum, Immunomodulatory

Introduction

The liver regulates physiological processes and carries out numerous essential tasks like metabolism, secretion, and storage. Additionally, the liver is in charge of detoxifying medicines and xenobiotics. Consequently, the emergence of liver illnesses might have a negative impact on one's health. These conditions are categorised as non-inflammatory illnesses, cirrhosis, acute or chronic liver inflammation, or degenerative disorders (hepatosis) [1]. Infections, autoimmune diseases, excessive alcohol intake, or toxic compounds including peroxidized oil,

medications, antibiotics, chlorinated hydrocarbons, and carbon tetrachloride can all cause liver issues. Alcoholism and alcohol abuse are widespread global issues that cross all cultural boundaries. Alcoholic liver disease (ALD) is a leading global cause of chronic liver damage brought on by excessive alcohol intake [2].

Following an alcohol overdose, a hangover causes momentary physical and psychological issues as headache, perspiration, gastrointestinal difficulties, and exhaustion. These negative consequences are caused by a concoction of the alcohol and acetaldehyde primary metabolic products [3,4]. Alcohol causes liver damage due to increased reactive oxygen species (ROS), which are formed as a result of alcohol metabolism because the liver's antioxidant function is compromised [5,6]

Recent research in animal models imply that oxidative stress, which causes fibrosis, reduced liver functioning, and increased apoptosis, is the cause of liver injury in chronic alcoholics [7]. Since there are now no effective treatments to delay or stop the progression of alcoholic liver disease, new treatments are required to halt the sickness.

Researchers are interested in medicinal plants and their active phytochemicals as prospective treatments for alcoholic liver damage because of their antioxidant capacity and mild adverse effects. There is currently not enough scientific evidence to support the use of medicinal plants as a therapy or a preventative measure for many illnesses, despite the great rise in popularity of their use in recent decades [8].

The use of medicinal plants in complementary and alternative medicine practises for illness prevention and treatment has grown in popularity over the past ten years. The integration of contemporary medical technology with traditional medicine has also demonstrated the efficacy of these polyherbal drugs in a range of complex pathophysiological situations. The effects of the numerous therapeutic herbs that were once employed for hepatoprotection and immunomodulation must now be confirmed by means of cutting-edge scientific techniques. A polyherbal Unani remedy called Dawa-ul-Kurkum is effective for treating abdominal pain, anorexia, ascites, and liver malfunction. Sunbul-ut-Teeb, Mur Makki, Saleekha, Qust, Shagufa-e-Izkhir, Darcheeni, Zafran, along with Sharab-e Musallas and Qand Safaid make up this polyherbal [9]. This study aims to investigate the potential immunomodulatory effect of Dawa-ul-Kurkum and the underlying mechanisms in rats with ethanol-induced liver injury.

Materials and methods

Drugs and Chemicals

The medication and chemicals are obtained from many providers, including SRL, New Delhi, Silymarin, and KLH from Sigma, and Dawa-ul-Kurkum from the Central Research Institute of Unani Medicine in Hyderabad.

Animals

A male or female Wistar strain was used in the investigation. Animals were taken from the Hamdard University's Central Animal House Facility and held under strict surveillance. They were given unlimited amounts of food and beverages. The Institutional Animal Ethics Committee approved the CPCSEA guidelines for animal utilisation in the care of the animal's protocol number 1768 (173/GO/ReBi/S/2000/CPCSEA).

The Research Drug

The research drug Dawa-ul-Kurkum was provided by Central Research Institute of Unani Medicine (CRIUM), Ministry of AYUSH, Government of India, under batch number 3-1/2018-19/CRIUM. It is made up of Sunbul-ut-Teeb, Mur Makki, Saleekha, Qust, Shagufa-eIzkhir, Darcheeni, Zafran with Sharab-e Musallas, and Qand Safaid

Q.S. make up this preparation. The recipe is well-documented in traditional Unani literature [10] and CRIUM has verified that it was developed in accordance with the classical Unani text. Ingredients used to make the semi-solid preparation known as "Dawa-ul-Kurkum" include those listed below (Table 1)

Experimental procedure

In this model, the animals were fed on standard rat chow, provided water ad libitum, and randomly divided into 7 groups of 5 rats each. Group I served as control and each rat was given an additional 2 ml/100 gm/day distilled water. The experimental Group II was given 2 ml (0.5 g)/100 gm body weight per day of 30% v/v of aqueous solution of ethanol for 6 weeks.

Group-III positive control given silymarin at a dose of 50 mg/kg p.o., Group-IV and V given the Dawa-ul-Kurkum as per Unani specifications (250 and 500mg/kg, p.o.); Group-VI and VII given the HA extract of Dawa-ul-Kurkum as per Unani specifications (500 and 1000mg/kg, p.o.). After 6th week, blood samples were collected from all animals by cardiac puncturing under mild anesthesia. The blood samples analyzed for immune and cytokine markers of hepatic injury [11].

Cytokine and Immunoglobulin levels

Serum Interleukin (IL-4), Tumor Necrosis Factor (TNFα) and Interferon (IFNγ) were estimated as per the instruction of the Kit (Diaclone), serum Immunoglobulin E(IgE), Immunoglobulin G(IgG), Immunoglobulin M(IgM), Immunoglobulin A(IgA) and Interleukin (IL-13) were estimated as per the instruction of the Kit (QAYEE-BIO).

Delayed type hypersensitivity (DTH) reaction

DTH assay was carried out to assess the cell mediated immune response of CPS or CUS exposed rats. DTH reaction was estimated by the method as described by Institoris *et al*. with some minor challenge dose modification. Animals were immunized subcutaneously at the base of the tail by 1 mg KLH in 0.4 ml of antigen preparation (KLH was dissolved in equal volume of PBS and FCA to form antigen suspension for immunization) on day 0. After various treatments from day 0 to day 14, animals were challenged by injecting 100µg of KLH in 0.08 ml sterile PBS into the left hind paw and equal volume of PBS was administered in the right hind paw which served as control. Paw volume was measured at time 0 just before challenging and about 24 h after, using UGO basile plethysmometer (model no. 7140) and specific paw swelling (D%) was calculated as described by Siroki *et al*. [12].

$$D\% = \frac{(d24L - d0L) - (d24R - d0R)}{D0L} \times 100$$

D% = Specific paw swelling.

d24 = Paw volume 24 h after challenge (Time = 24 h).

d0 = Paw volume before challenge (Time = 0 h).

L and R = Left and right hind paw.

Statistical Analysis

Analysis was done by using Graph pad prism (5.0) software. The values were expressed as mean ± standard error 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.

Result

Effects of Dawa-ul-Kurkum and its hydro-alcoholic extract on whole body and liver weight in ethanol induced liver damage in rats:

The body weight was measured in all groups at the start of experiment and at the end of 6th week and liver weight was also measured on sacrifice day after various drug

treatments. The results concluded that ethanol daily dose caused less change in the body weight and also in the liver weight when compared to that control group. Following treatment with Dawa-ul-Kurkum, 50% hydro-alcoholic extract, and silymarin, the effects of ethanol were stopped, and the body weight increased without noticeably changing the weight of the liver. The improvement in body weight due to improvement in appetite which may have due to hepatoprotective or immunomodulatory effect of Dawa-ul-Kurkum. The results are shown in (Table 2)

Effects of Dawa-ul-Kurkum and its hydro-alcoholic extract on Immunoglobulin and cytokine levels:

In experimental control group, 6weeks daily dose of ethanol resulted in significant increase in the levels of IgM, IgG, IgE and IgA a marker for Immunoglobulin ($p<0.05$) in serum, similarly significant increase in cytokine levels of IL-13, Interferon- **γ**, TNF- α and IL-4 (##$p<0.01$) in comparison to control rats. This validated our model of ethanol induced liver damage in rats.

In treatment Group 4 and 5, Dawa-ul-Kurkum at two different doses for 6weeks significantly attenuated the effects of ethanol and significantly reduced level of IgM ($p<0.05$ for 500mg/kg doses), IgG, IgA ($p< 0.05$ for 250mg/kg dose) as well as in IgE ($p < 0.05$ for 250mg/kg dose) and significant decrease in IL-13($p < 0.01$ 500mg/kg and $p< 0.05$ for 250mg/kg dose), TNF- α($p < 0.05$ for both doses), IL-4 ($p<0.01$ 250 mg/kg and $p< 0.05$ 500mg/kg dose) and Interferon- **γ** ($p < 0.05$ for both doses) as comparison to experimental control group.

Concurrently treatment, in Group 6 and 7 with two different doses of hydro-alcoholic extract attenuated the effects of ethanol and significantly reduced the levels of IgM ($p < 0.05$ for 1000mg/kg dose), IgG ($p<0.05$ for 500mg/kg and $p<0.01$ 1000mg/kg dose), IgA($p<0.05$ for 1000mg/kg dose) and IgE ($p<0.05$ 500mg/kg and $p<0.01$ for 1000mg/kg dose), similarly cytokine levels also reduced significantly IL-13 ($p<0.05$ for both doses), Interferon- **γ** (**p**<0.01 500mg/kg and $p<0.05$ for 1000mg/kg dose), TNF- α ($p < 0.05$ for both doses) and IL-4 ($p < 0.01$ for both doses) as compared to that in experimental control group.

Pre-treatment with silymarin also significantly reduced the levels of IgE ($p<0.01$) and non-significant IgM, IgA, IgG, similarly in Interferon- **γ** ($p<0.05$)**,** TNF-α($p<0.05$) and IL-4 ($p<0.05$) & IL-13($p<0.01$) as compared to that in experimental controls which is suggestive of the immunomodulatory effects of the drug in this model.

The treatment of Dawa-ul-Kurkum and its hydro-alcoholic extract was found to be comparable to that of silymarin, according to the findings. These results are summarized in (Table 3, 4 & Figure 1)

Effects of Dawa-ul-Kurkum and its hydro-alcoholic extract on Delayed type hypesensitivity response in ethanol induced liver damage in rats:

In experimental control group, daily administration of ethanol for 6 weeks resulted in decrease in delayed type hypersensitivity response as comparison to control group. In treatment Group IV and V, Dawa-ul-Kurkum considerably lessened the effects of ethanol when taken for six weeks in two different doses and increased the delayed type hypersensitivity response as compared to that in experimental control group.

When compared to the experimental control group, treatment Groups VI and VII received two different doses of its hydro-alcoholic extract, which mitigated the effects of ethanol and raised the delayed type hypersensitivity reaction. Pretreatment with silymarin also increase the delayed type

hypersensitivity response as compared to that in experimental controls which is suggestive of the hepatoprotective effects of the drug in this model.

These results are summarized in (Table 5).

Table 1: Formulation Composition

S. No.	Name	Botanical/sci. name	Qty	Part
1.	Sumbul-ut-tib	*Nordostachys jatamansi* DC. Syn. *Valeriana jatamansi*	1 Part	Dried Rhizomes
2.	Murmakki	*Commiphora myrrha* (Nees) Engl.	1 Part	Gum resin
3.	Saleekha	*Cinnamomum cassia* Blume	1 Part	Bark
4.	Qust	*Saussurea lappa* C.B. Clarke	1 Part	Dried roots
5.	Shagofa Izkher	*Cymbopogon jwarancusa* Schult Syn. *Andropogon Jwarancusa* Jones	1 Part	Flower
6.	Darchini	*Cinnamomum zeylanicum* Blume	1 Part	Bark
7.	Zafran	*Crocus sativus* Linn.	1 Part	Style and stigma
8.	Sharab Musallas	-	Q.S	-
9.	Asal OR Qand Safaid	-	Q.S	-

Table 2: Effect of Dawa-ul-Kurkum and its hydro-alcoholic extract on body and liver weight

Treatment	Initial body weight (g)	Final body weight (g)	% change in body weight	Liver weight (g)	Liver index (%)
Control	252.6 ± 15.89	279.6 ± 12.61	9.65	7.090 ± 1.583	2.53
Experimental control	230.8 ± 23.24	236.8 ± 18.73	2.53	10.08 ± 0.756	4.25
Silymarin	277.2 ± 10.82	285.4 ± 13.75	2.87	8.962 ± 0.326	3.14
DK250	282.6 ± 12.42	299.0 ± 27.23	5.48	9.390 ± 0.933	3.14
DK500	275.0 ± 17.47	289.4 ± 26.05	4.97	9.334 ± 0.962	3.22
HA500	269.4 ± 15.90	272.6 ± 18.09	1.17	7.920 ± 0.620	2.90
HA1000	263.6 ± 30.90	277.4 ± 23.00	4.97	8.806 ± 0.451	3.17

Liver index was calculated as (liver weight/body weight×100%). The values were expressed as mean ± SEM

Table 3: Effects of Dawa-ul-Kurkum and its hydro-alcoholic extract on Immunoglobulin level

Treatment	IgM (µg/ml)	IgG (µg/ml)	IgA (µg/ml)	IgE (µg/ml)
Control	0.1074± 0.0008	0.0678± 0.0008	0.0935± 0.0004	0.1452± 0.0004
Experimental control	0.1102± 0.0010#	0.0720±0.0008#	0.0978± 0.0007#	0.1484± 0.0006#
Silymarin	0.1086± 0.0002	0.0684± 0.0015	0.0945± 0.0011	0.1450± 0.0003**
DK 250	0.1081± 0.0005	0.0682± 0.0005	0.0932± 0.0005*	0.1455± 0.0005*
DK500	0.1069± 0.0003*	0.0683± 0.0011	0.0938± 0.0007	0.1453± 0.0011*
HA500	0.1083± 0.0002	0.0674± 0.0004*	0.0945± 0.0013	0.1454± 0.0003*
HA1000	0.1071± 0.0005*	0.0663± 0.0004**	0.0928± 0.0010*	0.1445± 0.0003**

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

Table 4: Effects of Dawa-ul-Kurkum and its hydro-alcoholic extract on Cytokine levels

Treatment	IL-13 (pg/ml)	Interferon-`γ (pg/ml)	TNF α (pg/ml)	IL-4 (pg/ml)
Control	0.1044± 0.0002	0.1259± 0.0002	0.0600 ± 0.0003	0.0168±0.0005
Experimental control	0.1070± 0.0005##	0.1283± 0.0004##	0.0642± 0.0016##	0.0189± 0.0001##
Silymarin	0.1047± 0.0002**	0.1267± 0.0002*	0.0607± 0.0002*	0.0175±0.0004 *
DK 250	0.1049± 0.0003*	0.1263± 0.0001*	0.0609± 0.0002*	0.0168± 0.0002**
DK500	0.1046± 0.0002**	0.1265± 0.0001*	0.0607± 0.0001*	0.0170± 0.0001*
HA500	0.1050± 0.0001*	0.1261± 0.0001**	0.0611± 0.0002*	0.0163± 0.0001**
HA1000	0.1051± 0.0002*	0.1265± 0.0005*	0.0608± 0.0001*	0.0165± 0.0002**

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

Table 5: Effects of Dawa-ul-Kurkum and its hydro-alcoholic extract on Delayed type hypersensitivity (DTH) response

Treatment	DTH% (Siroki et al)
Control	46.80 ± 27.47
Experimental control	15.60 ± 37.94#
Silymarin	53.67 ± 76.42*
DK250	41.86 ± 33.36*
DK500	69.80 ± 73.03
HA500	58.00 ± 210.1*
HA1000	74.00 ± 234.5

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

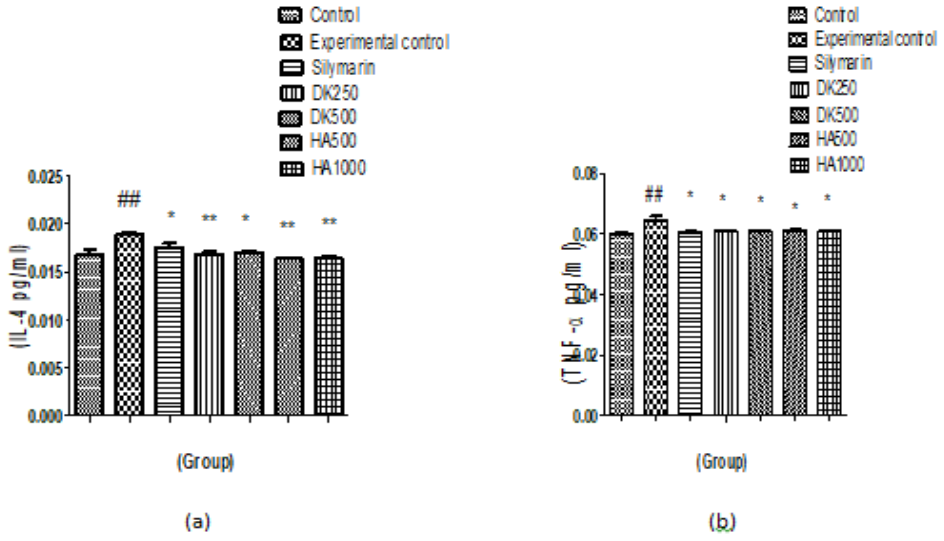

Figure 1: (a) IL_4 ; (b) TNF-α

Discussion

Chronic ethanol injection altered the rate of body weight increase in rats. Chronic oral administration of ethanol caused a significant decrease in mean body weight growth as compared to the control groups. Previously, Macdonald, Olusola, and Osaigbovo noted that rats' mean body weight gain was lessened after continuous ethanol administration [13]. The reduced rise in bodyweight could be explained by energy loss associated with ethanol metabolism via the microsomal ethanol oxidising process (MEOS). The main mechanisms for metabolising alcohol are MEOS and alcohol dehydrogenase. In the presence of ongoing alcohol use, the MEOS pathway accounts for the majority of ethanol metabolism. Due to the induction of MEOS, which oxidises ethanol without generating chemical energy, there is a change in energy consumption and weight gain [14]. Serum immunoglobulin levels are frequently modified in hepatotoxicity and are utilized as markers of hepatic damage. Previous studies have demonstrated a correlation between cirrhosis, primary biliary cirrhosis, and chronic active hepatitis and a rise in blood IgA, IgM, and IgG levels. The severity of injury has been evaluated using immunoglobulin. For the diagnosis and prevention of liver disease, immunoglobulin levels are still recommended. A considerable rise in blood IgE levels was discovered in a different research of several patients with hepatic disorders. Although it has been researched, the primary mechanism causing elevated serum immunoglobulins in hepatic disease is still unknown.

Cell stress, mitochondrial damage, and an inflammatory reaction are all results of DILI. The liver will be heavily taxed because it is the organ responsible for cell detoxification. Stress causes the liver's hepatocytes to create more inflammatory cytokines. As a result, liver cells are more vulnerable to apoptosis caused by TNF-α and IFN-γ. These effects can be countered by Bcl-2 inhibitors or inhibitors of apoptosis proteins (IAPs). IL-4 is one cytokine that has been linked to the emergence of immune-mediated DILI and the accompanying antibodies. In a prior investigation, variant IL-4 alleles were connected to the emergence of immune-mediated DILI brought on by diclofenac [15].

The current research showed that the rise in serum immunoglobulin and cytokine levels was greatly inhibited when treatment of Dawa-ul-Kurkum and its Hydro-alcoholic extract were coupled with ethanol. On the immunoglobulin and cytokine, the DK exhibited the same effects as the HA extract. Dawa-ul-Kurkum and HA extract, on the other hand, both revealed an immunomodulatory effect.

These results demonstrated the efficacy of Dawa-ul-Kurkum and it’s HA formulation as immunomodulatory drugs that help prevent liver necrosis. With the treatment of Dawa-ul-Kurkum and its extract, the level of delayed type hypersensitivity was also elevated.

Conclusion

A potential hepatotoxic effect of ethanol on Wistar rats was discovered, as shown by modifications in immunomodulatory and cytokine markers. In rats with ethanol-induced liver damage, the treatment of Dawa-ul-Kurkum and its hydro-alcoholic extract was found to considerably reduce immunomodulatory effect. Such translational research utilizing the reverse pharmacology approach may assist in integrating conventional and contemporary medical principles in the larger goal of drug development and appropriate use.

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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 idea, planning, and design. She also contributed to the data analysis and paper review. Saman Anees, Maaz Naqvi and Nafaa Hasan also help during experimental work. 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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