ISSN: 2230-9926

Available online at http://www.journalijdr.com

IJDR

International Journal of Development Research
Vol. 5, Issue, 04, pp. 4097-4102, April, 2015

International Journal of
DEVELOPMENT RESEARCH

Full Length Research Article

STUDY OF PROTECTIVE EFFECT OF *DAWA-UL-QUST* (A UNANI COMPOUND FORMULATION) AGAINST CCL_4 INDUCED ACUTE HEPATIC INJURY IN RATS

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ARTICLE INFO

Article History:
Received 24th January, 2015
Received in revised form
28th February, 2015
Accepted 30th March, 2015
Published online 29th April, 2015

Key words:
Hydro-alcoholic Extract,
CCl_4,
Alanine amino transaminase (ALT),
Aspartateaminotransaminase (AST),
TBARS

ABSTRACT

A Unani compound formulation known as *Dawa-ul-Qust*was evaluated for its hepatoprotective effects by crude as well as 50% hydro-alcoholic extract against Carbon tetra chloride (CCl_4) induced liver injury in rats. The animals were divided into five groups of 6 animals each – I (Plain control), II Negative control (CCl_4 treated group), III (Silymarin treated), IV *DQ* (Crude treated)and V *DQ* (Extract treated). Hepatotoxicity was induced by single administration of CCl_4 (2ml/ kg I.P. 1:1 in olive oil) 24 hours before the sacrifice.The standard group was treated with Silymarin orally in the dose of 100 mg/kg body wt, once daily for 7 days.The test drug (*Dawa-ul-Qust*) was administered at the doses of 500 mg/kg and 74.9 mg/kg of body weight respectively in crude as well as in extract formsonce daily, per oral for 7days. On the 8thday all the animals were sacrificed by ether anaesthesia and the blood was collected for biochemical estimations and liver was dissected out for histological studies.The elevation of enzyme markers and structural changes in histological reports of liver sections were taken as the indicators of hepatic injury. The serum of each animal of all groups were estimated for, ALT, AST, and TBARS. While the liver was dissected out for histological studies to support the above parameters. The serum of each animal of all groups estimated for, the mean serum ALT, AST, and TBARS were decreased significantly as compared to CCl_4 treated group. The histopathological study showed signs of recovery and regeneration in damaged liver cells as compared to CCl_4 group. The study demonstrated significant hepatoprotective activity of *Dawa-ul-Qust* probably due to combined action of all ingredients.

INTRODUCTION

A large number ofsingle and compoundUnani drugs that are highly effective and safe, possesses hepatoprotective effect have been used in liver pathologies since centuries. So investigating these drugs for their hepatoprotective activity to make an effective medicine in the treatment of liver toxicity or dysfunction is promising.Since liver diseases entail a lot of complexities and diverse clinical manifestations therefore, the use of a single drug is unlikely to be effective in all types of severe liver disorders (Arvind *et al*., 2010). That's why plantmedicines are more often used in combination rather than in a single in order to get maximum benefit from their combined strengthand the use of compound formulation appears to be more appropriate therefore, an effective formulation has to be developed using medicinal plants, with proper pharmacological experiments (Dandagi *et al*., 2008). There are some compound formulations such as Jigrine (Abul *et al.*, 2004), Icterene (Nasreen, 1999), Majoon-Dabeedul-ward (Naeem, 1995), Hepatogard, Biliarin, Livol (Tareque, 2001), Livergen (Naseem, 2003) etc, which have been proven scientifically for their activities against the liver injury. Compound drugs (Murakkabat) with therapeutic effectiveness are given to achieve maximum and quick results to complex therapeutic objectives, but modern scientific research and even the study of *Tibb-e-Unani* have been devoted mainly to single drugs and compound drugs are generally ignored. However, only a small portion of the hepatoprotective plants as well as formulation used in traditional medicine are pharmacologically evaluated for their efficacy and a number of drugs particularly compound drugs have still not been scientifically evaluated for their described effects (Handa *et al*., 1989). *Dawa-ul-Qust* is one such compound preparation described to be effective in liver diseases (Khan, 1921), and

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prescribed commonly by the Unani physicians has not been investigated so far, for its effect in hepatic diseases.Qust (*Sausurialappa*) is its chief ingredient combined with other ingredients viz. Dar cheeni (*Cinnamomumzelaynicum*), Saleekha (*Cinnamomumtamala*), Zafraan (*Crocus sativus*), Badyan (*Foeniculumvulgare*), Karafs (*Apiumgraveolens*) Tagar (*Aquillariaagallocha*), Rewandchini (*Rheum emodi*) Shagoofa-e-Izkhar (*Andropoganschaerranthus*), and Murmakki (*Dendron myrrh*) (Khan, 1921). All the ingredients have been mentioned to possess properties that are effective directly or indirectly in liver disease (IbneSina, 1906, Ghani, 1921; Azam, 1313 H; Antaki, 1317 H; Sharif, 1280 H; Momin, 1272 H) and the combination has even more strong hepatoprotective effect. In view of these points, the present study has been designed to investigate its protective effect in chemically induced hepatic damage in rats. The damage produced by Carbon tetra chloride (CCl_4) is described to be similar to the pathological changes seen in infective and viral hepatitis and in many other liver diseases (Berger *et al.*, 1986) therefore, it was used to produce liver damage in rats for evaluation of the effect of the test drug.

MATERIALS AND METHODS

Plant materials

Ingredients of *Dawa-ul-Qust* (Khan, 1921)

1.	Dar cheeni	(*Cinnamomumzelaynicum*)	85 gm
2.	Saleekha	(*Cinnamomumtamala*)	85 gm
3.	Qust	(*Saussurialappa*)	85 gm
4.	Zafraan	(*Crocus sativous*)	24 gm
5.	Badiyan-e- Roomi	(*Foeniculumvulgare*)	30 gm
6.	Tukhm-e- Karafs	(*Apiumgraveolans*)	30 gm
7.	Tagar	(*Aquillariaagallocha*)	4.5gm
8.	Rewande Cheeni	(*Rheum emodi*)	30 gm
9.	ShagoofaeIzkhar	(*Andropoganschaerranthus*)	70 gm
10.	Murmakki	(*Dendron myrrh*)	70 gm

Preparation of Powder and Extract

All the ingredients of *Dawa-ul-Qust* (*DQ*) were procured from the herbal market in Aligarh and New Delhi and after identification and authentication of the crude ingredients of the test drug, was crushed to make a powder and homogenized in water for crude administration in aqueous medium. A 50% ethanol extraction was made through Soxhlets Apparatus (Anonymous, 1968; Anonymous, 1987) and dissolved / suspended in water for oral administration to the animal. Both the forms of *Dawa-ul-Qust* (DQ) collectively were used for screening the protective effects against CCl_4 induced liver damage. The doses for animals were determined by extrapolating the Unani human dose range by multiplying it by conversion factor of 7 (Dhawan, 1982). The doses of *Dawa-ul-Qust*(DQ) thus calculated for albino rats, was found to be 500 mg/kg and 74.9 mg/kg of body weight respectively in crude as well as in extract forms.

Animals

Albino rats weighing 175-200 gm of either sex were obtained from the Meerut animal house and they were housed in clean polypropylene cages. The rats had free access to standard diet and water *ad libitum* throughout the experiment with the exception in which the animals were deprived of food, but not water, 12 h before the experiments. The room temperature was maintained at 25 ± 1^0 C with 12 hour light and dark cycle. The rats were randomly selected and were divided into five groups with six animals in each group and were left for one week for acclimatization to experimentation room. The experimental protocol was approved by the Institutional Ethics Committee.

Experimental design

The animals were divided into five groups containing six animals in each group. The normal control group received normal saline orally in equal volume of test drug. The standard group received Silymarin 100 mg/kg orally for 7 days. The animals kept in group IV & V received treatment of *Dawa-ul-Qust* (test drug) suspended in water at doses of 500 mg/kg and 74.9 mg/kg of body weight respectively in crude as well as in extract forms orally. On 7^{th} day carbon tetrachloride (CCl_4) was injected (2 ml/kg of body wt) in 50% v/v in olive oil intraperitoneally to all groups except normal control (CCl_4 treated, standard, *DQ* crude and extract treated) to induce hepatotoxicity along with their routine treatment. On the 8^{th} day all the animals were sacrificed under the ether anesthesia and blood was collected from each animal for serum analysis and liver were removed and fixed in 10% formalin for histopathological studies of the liver to determine the degree of hepatic damage (Devaraj *et al*., 2011).

Drugs and Chemicals

CCl_4, n-butanol, Acetic acid (Thomas Baker Pvt. Limtd. Mumbai), sodium dodecylesulphate, thiobarbituric acid (Otto Kemi Mumbai), 1, 1, 3, 3-tetraethoxypropane (Sigma USA), Silymarin (Sigma-Aldrich, Germany), Folin's reagent (CDH, Mumbai), AST, ALT, estimation kits (Span Diagnostic Ltd, Surat).

Preparations of Samples for Biochemical studies

The blood and liver were collected after sacrificing the animals. The blood was kept for 30 minutes without disturbing and was centrifuged for 15-20 minutes at 5000 rpm to separate the sera and stored at 4^0C. The serum of each animal of all groups were estimated for, Serum transaminases [aspartate aminotransferase (AST) and alanine aminotransferase (ALT (Reitman and Frankel 1957), and)] and Malondialdehyde (Okhawa *et al.,*1979) which is an index of lipid peroxides (Lowry *et al.,* 1951)

Histopathological Observation

The small pieces of liver of all groups were removed immediately and fixed in 10% formalin. Care was taken to keep the volume of the fixative (Mukherjee, 1988). The tissue was processed and sections were cut. The slides were prepared and stained with hematoxyline and eosin stain and observed the histopathological changes by a photomicroscope under various magnifications.

Statistical Analysis

Data was presented as mean ± standard error and analyzed using one way ANNOVA test, followed by pair-wise comparison of various groups by LSD. The analysis was carried out by using the software of the website, www. Analyse it.com. P<0.05 or less was considered significant.

RESULTS

Biochemical Parameters

The results of hepatoprotective activities of *Dawa-ul-Qust*at doses of 500 mg/kg and 74.9 mg/kg b.wt., respectively crude as well as extract form son rats intoxicated with carbon tetrachloride are illustrated in the Table 1. The table shows the comparison of effects among the untreated (normal control) and carbon tetrachloride treated (induction control) group with the drug treated group of rats. The values of biochemical parameters and MDA level were found significantly higher in the Group II (Carbon tetrachloride group) as compared to group I and other pre- treated groups (p< 0.001). The values of ALT, AST and MDA were found lower in the pre-treated Silymarin and crude as well as extract treated Groups than the CCl_4 control group II (p<0.001). While comparing group I with group III, IV and V, it was observed that values of all the biochemical parameters had significantly increased (p<0.001). However, while comparing group III, IV and V no statistically significant difference was observed (p>0.05).

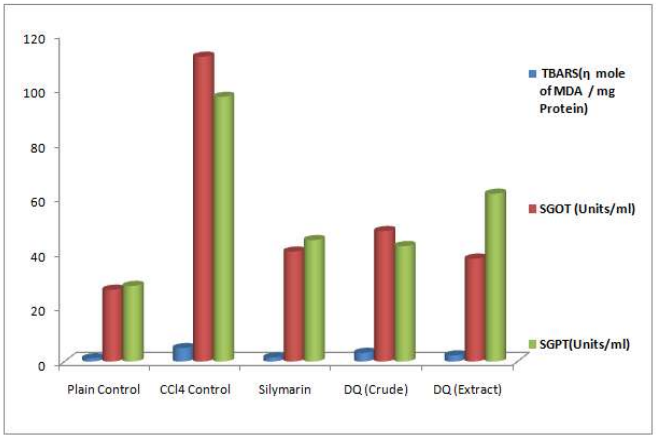
Table 1. Protective effect of *Dawa-ul-Qust* in CCl_4 mediated hepatic damage

Groups	TBARS (η mole of MDA / mg Protein)	SGOT (Units/ml)	SGPT (Units/ml)
Plain Control	1.18 ± 0.095	26.3 ± 2.94	27.7 ± 3.40
CCl_4 (2 ml/kgm)	4.92 ± 0.45	111.7 ± 3.60	97 ± 6.61
Silymarin (100 mg/kgm)	1.48 ± 0.05	40.3 ± 3.40	44.5 ± 2.06
DQ(Crude) (500 mg/kgm)	3.18 ± 0.21 a^3	47.8 ± 3.84 a^3	42.3 ± 1.94 a^3
DQ (Extract) (74.9 mg/kgm)	2.25 ± 0.11 a^3	37.7 ± 4.24 a^3	61.5 ± 6.71 a^3

(n=6)
1 = P < 0.05, 2 = P<0.01, 3 = P<0.001
a= against CCl_4, b=against plain control, c=against Silymarin

Histological study

Results of histopathological studies provided supportive evidence for biochemical analysis. Histology of liver section of normal control animal (group 1) exhibited normal hepatic cells each with central blood vessels and radiating cords of hepatocytes as well as the vascular sinusoids with no evidence of fatty changes, necrosis or inflammation (Figure A), whereas that of CCl_4 intoxicated group animal showed centrilobular necrosis and vascular congestion (Figure B). The animals treated with Silymarin (Group III) showed mild vascular congestion and peri-vascular infiltrate of mono nuclear cells and fibroblast, no fatty changes were seen (Figure-C). Animals administered with crude form of test drug (Group IV) exhibited mild vascular congestion and regenerating hepatocytes (Figure. D). The animals received extract form of test drug (Group V) showed mild vascular congestion and peri-vascular infiltrate of mono nuclear cells and fibroblast (Figure. E). Maximum protection against hepatic damage was achieved by the both forms of test drug. However, the Silymarin and test drug especially extract form protect the liver structure and showed excellent protection of liver architecture.



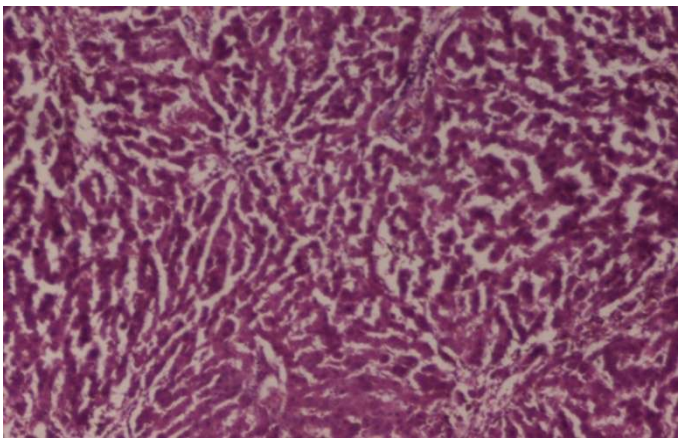


Fig.A. Plain control (Water only)

Central blood vessels and radiating cords of hepatocytes as well as the vascular sinusoids with no evidence of fatty changes, necrosis or inflammation.

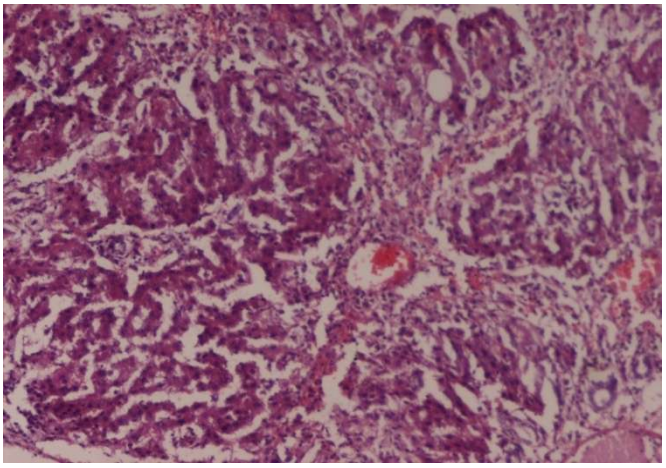


Fig. B. Negative Control (CCl_4 only)

Centrilobular (Acidophilic) necrosis and vascular congestion

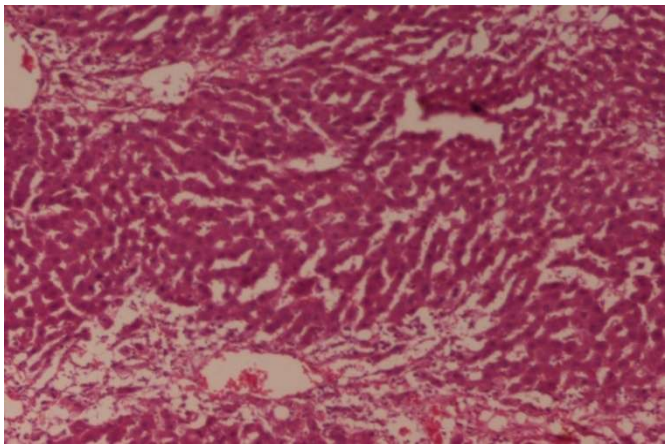


Fig. C. Standard (Silymarin) + CCl4

Mild vascular congestion and peri-vascular infiltrate of mono nuclear cells and fibroblast. No fatty changes

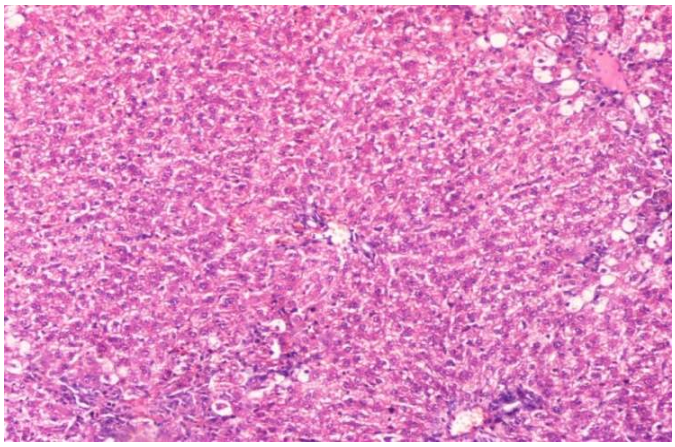


Fig. D. *Dawa-ul-Qust* (Crude) +CCl4

Mild vascular congestion and regenerating hepatocytes

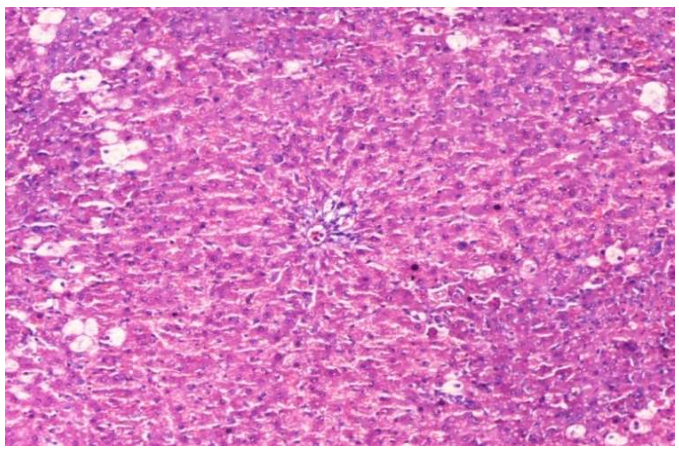


Fig. E. *Dawa-ul-Qust* (Extract) +CCl4

Mild vascular congestion and peri-vascular infiltrate of Mono nuclear cells and fibroblast

DISCUSSION

Hepatoprotective study was conducted to investigate the efficacy of crude as well as extract forms of *Dawa-ul-Qust* in protecting the liver damage caused by a single dose of CCl_4. In this study, liver damage was induced after the application of standard drug and test drug. Hepatotoxicity of the CCl_4 in the rats was determined by changes in serum parameters by estimating the levels of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) which are enzymes originally present at higher concentration in the cytoplasm (Mohamed *et al*., 2009). When there is hepatopathy, these enzymes leak into the blood stream in conformity with the extent of liver damage (Nkosi *et al*., 2005). The CCl_4 damaged liver toxicity was also associated with marked increase in liver Malondialdehyde (MDA) level and the elevation of MDA has been well accepted reliable marker of lipid peroxidation. Hence, in the present study MDA level was also estimated to evaluate the protective properties of test drug. A significant difference in liver marker enzymes and MDA level was observed (Table-1). Histological studies of the liver also showed severe damage to the hepatocytes. Necrosis of the hepatocytes is quite prominent in rats in CCl_4 control group (Figures-B) as compared to other pretreated groups (Figure-C, D and E).

Crude as well as extract of *Dawa-ul-Qust* showed significant hepatoprotective effect by lowering the liver marker enzymes alanine aminotransferase (ALT), aspartate aminotransferase (AST) and a significant decrease in Malondialdehyde (MDA) level was also observed in both the forms of test drug treated groups as compared to the CCl_4 control group. In histological examination of the liver sections of rats treated with CCl_4 with the silymarin and test drug (crude and extract forms of *DQ*) there were observed that architectural liver pattern was restored. CCl_4 can cause damage to many tissues in the body, however, the most important primary target organ for CCl_4 induced toxicity in many species is the liver. CCl_4 when metabolized in the body is changed into very reactive free radicals (halogenated free radical) by cytochrome P_{450} mixed function oxidase system. These reactive species then induce hepatic damage. Many latest evidences show that oxidative stress caused by free radicals may induce peroxidation and damage to biomolecules (lipid protein and nucleic acids). This may further leads to aging, cancer, severe fatty changes in liver and many other diseases in human (Afaf *et al.,* 2008).

Hepatoprotective properties of plants or plants extracts are generally attributed to the presence of chemicals which act as antioxidants or inhibitor of the microsomal drug metabolizing enzymes (Gopinathan *et al*., 2004). As it is widely accepted that CCl_4 is metabolically activated by hepatic microsomal cytochrome P_{450} mediated reactions to the trichloromethyl radical (Slater, 1984). Therefore, the inhibitors of cytochrome P_{450} can impair the bioactivation of CCl_4 into its toxic species and thus provide protection against hepatocellular damage (Nelson *et al*., 1980). Hepatoprotective activity in *DQ* may be due to the presence of certain antioxidants which act as scavengers and remove the free radicals formed. These antioxidants also have the ability to prevent the process of peroxidation and improve the health of hepatocytes. The result of serum biochemical parameters, level of MDA and histopathological studies in the pre-treatment groups support the highly potenthepatoprotective activity of the crude as well as extract of *Dawa-ul-Qust* against the CCl_4 induced liver injury.

Conclusion

It can be concluded that both the doses forms of test drug (*Dawa-ul-Qust*) possess significant hepatoprotective activity against acute hepatic damage induced by CCl_4. However, the effect produced by the extract was more marked by decreasing the lipid peroxidation and SGOT which was almost equal to that of Silymarin. Further it should be evaluated in the human studies in order to have the proper treatment for the liver diseases.

Acknowledgement

We are very thankful to Central Council for Research in Unani Medicine (CCRUM) Ministry of AYUSH, Govt. of India, New Delhi for providing financial assistance for the research work. We are also thankful to Professor Nafees Ahmad Farooqi Department of Anatomy and Prof. ShaistaVasenwala Department of Pathology JawaharLal Nehru Medical College, Aligarh Muslim University, Aligarh for histological studies and special thanks to Dr. Ghufran Ahmad, Department of IlmulAdvia, Ajmal Khan Tibbiya College, Aligarh Muslim University, Aligarh for his critical suggestions, encouragement and valuable guidance.

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