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Original Article

A Study of Hepatoprotective Effect of Unani Formulation (Qurs Rewand) in Rats

Alam S [1], Khan NA [2], Nasiruddin M [3, *], Ahmad SS [4]

[1, 2] Department of Ilmul Advia, Faculty of Unani Medicine, A.M.U, Aligarh, Uttar Pradesh,, India – 202002.

[3] Department of Pharmacology, J.N.M.C.H., A.M.U, Aligarh, Uttar Pradesh, India – 202002

[4] Departments of Pathology, J.N.M.C.H., A.M.U, Aligarh, Uttar Pradesh, India – 202002

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ABSTRACT

Aim of study: To evaluate hepatoprotective effect of Unani formulation (*Qurs Rewand).* **Materials and Methods:** The study was conducted on adult Wistar albino rats of either sex weighing 150-200 g. Animals were divided into five groups of 6 animals each – I (Plain control), II (Negative control–CCl_4 treated group), III (Sylimarin treated group), IV and V (UPF treated groups). Hepatotoxicity was induced by single administration of CCl_4 (2ml/ kg I.P., 1:1 in liquid paraffin) in group II, III, IV & V on 7th day of treatment. The UPF was administered in a dose of 50 mg/kg and 100 mg/kg, once daily, orally for 7 days in group IV and V respectively. Silymarin was administered orally in the dose of 100 mg/kg body weight, once daily for 7 days in III group and served as standard control. On the 8th day all the animals were sacrificed and the blood was collected. Serum was separated for biochemical estimations. The serum was estimated for ALT, AST, bilirubin, alkaline phosphatase, total protein and TBARS. Histological study of liver was done.**Results:** The mean serum ALT, AST, bilirubin, alkaline phosphatase, and TBARS were decreased significantly as compared to CCl_4 treated group.While total protein was increased significantly as compound with CCl_4 treated group. The histological study showed signs of recovery and regeneration in damaged liver cells as compared to CCl_4 treated group. **Conclusion:** The study demonstrated significant hepatoprotective activity of *Qurs Rewand* (Unani Polyherbal formulation).

Key words*: :* Unani Polyherbal formulation, carbon tetrachloride, hepatoprotective activity and Silymarin

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Corresponding author *
Dr. Mohammad Nasiruddin*, Associate Professor, Departments of Pharmacology, J.N.M.C.H., A.M.U, ALIGARH, U.P.,INDIA–202002.
Mobile number: 9412596898; naseer_bettiah@yahoo.co.in

1. INTRODUCTION

Qurs-e-Rewand is a Unani polyherbal preparation (Table-1) [1] frequently prescribed by the physicians of Unani medicine in the management of liver diseases such as infective and other hepatitis.[2, 3, 4] *Rubia cordifolia* Linn (Rubiaceae) and *Agrimonia eupatoria* Linn. (Rosaceae) have been scientifically evaluated for

their hepatoprotective effect [5], but the compound as a whole has not been studied for its described properties. Therefore present study has been undertaken to investigate its hepatoprotective effect against CCl_4 induced hepatotoxicity in albino rats. The damage produced by CCl_4 is described to be similar to the pathological changes seen in infective hepatitis and in many other liver diseases. [6] The liver function test was used to assess the extent of liver damage and the protection induced by the test drug. Since CCl_4 is reported to damage the hepatocytes mainly by inducing lipid peroxidation, therefore thiobarbituric acid reactive substance (TBARS) test was employed to study the antioxidant property of the test drug with a view to underline its mechanism of action.[7] Silymarin (100 mg / kg) was used as the standard hepatoprotective agent to confirm the integrity of the test system and also to compare the efficacy of the test drug as, it has been used in the treatment of chronic or acute liver disease, as well as protecting the liver against toxicity.[8] The hepatoprotective properties of Silymarin have been related to the inhibition of lipid peroxides formation or scavenging of free radicals.[9] Histological study was conducted to observe the structural status of cell matrix liver.

2. MATERIALS AND METHODS

The study was conducted in the Department of Ilmul Advia, Ajmal Khan Tibbiya College, AMU in collaboration with the Departments of Pharmacology and Pathology, Jawaharlal Nehru Medical College, AMU, Aligarh, after obtaining permission from Institutional Ethics Committee.

Test drug procurement and identification:

All the ingredients of UPF were procured from Dawakhana, Tibbiya College, AMU, Aligarh and were identified by comparison for its macroscopic and microscopic characters with authentic specimens of above mentioned at Department of Ilmul Advia, Ajmal Khan Tibya College, A.M.U. Aligarh, U.P. India.(Voucher specimen No. are given below).

Botanical Name	**Voucher specimen No.**
Rheum emodi Wall	Sc-0098/09
Rubia cordifolia Linn	Sc-0097/09
Creteria lacca	Sc-0096/09
Apium graveolens Linn	Sc-0095/09
Feoniculum vulgare Mill	Sc-0094/09
Agrimonia eupatoria Linn	Sc-0093/09

Preparation of extract:

All the ingredients (Table 1) were coarsely powdered before subjection to extraction. The hydro-alcoholic extract of all the ingredients was prepared using by Soxhlet apparatus, in which they were continuously extracted for 6 hours. The extract was filtered by Whatman No. 1 filter paper and evaporated on water bath at 40 - 60^0C until it dried completely. The prepared extract was stored in the refrigerator for further use.

The dose of the test compound formulation for albino rats were calculated by multiplying its clinical doses described in Unani literature with conversion factor 7.[10]

Drugs and Chemicals

CCl_4, n-butanol, acetic acid (Thomas Baker Pvt. Limtd. Mumbai), sodium dodecyle sulphate, thiobarbituric acid (Otto Kemi Mumbai), 1, 1, 3, 3-tetraethoxypropane (Sigma USA), Silymarin (Sigma-Aldrich, Germany), Folin's reagent (CDH, Mumbai), AST, ALT, Billirubin, Alk.Phos. and Total Protein estimation kits (Span Diagnostic Ltd, Surat).

Animals

Thirty Wistar albino rats of either sex, weighing 150-200 g were divided into five groups of 6 animals each. The animals were kept under standard laboratory conditions. Commercial diet pellets and water were given *ad libitum*. The room temperature was maintained at 25 ± 1^0 C.

Treatments

Group I (Plain Control): Distilled water orally in the dose of 1ml /kg, daily for 8 days.

Group II (Negative Control): Distilled water orally in the dose of 1ml /kg, daily for 7 days.

Group III (Standard): Silymarin in dose of 100 mg / kg daily for 7 days.

Group IV & V (Test Groups): Extract of *Qurs-e-Rewand* in the dose of 50 mg/kg and 100 mg/kg respectively suspended in distilled water daily for 7 days.

On the 7th day the animals of all groups except those in group I were administered carbon tetrachloride i.p. along with their routine treatment and 24 hours later (on 8th day) all the animals including in group I were sacrificed.[11]

Collection of Samples

The blood was collected and was kept for 30 minutes without disturbing. The serum was separated by centrifugation for 15-20 minutes at 5000 rpm. The sera of each animal of all groups were estimated for, ALT & AST [12], bilirubin[13], alkaline phosphatase[14], total protein[15] and TBARS[16], which are index of lipid peroxides.[17]

Histological Examination

The liver of rats of all groups was removed immediately and fixed in 10% formalin.[18] The tissue was processed and sections were cut. The slides were prepared and stained with haematoxyline and eosin stain and the histological changes were observed by photomicroscope under high power magnification.

Statistical analysis

The results are presented as means ± S.E.M. The data were statistically compared for determining significance of difference by one-way ANOVA test, followed by pair-wise comparison of various groups by LSD. The analysis was carried out by using the software of the website, www. analyseit.com. $P< 0.05$ was considered significant.

3. RESULTS

Biochemical Parameters

A highly significant increase in levels of serum ALT, AST, bilirubin, alkaline phosphatase was observed in CCl_4 treated rats while total protein was found significantly decreased as compared to control group. There was significant reduction in all biochemical parameters and significant increase in total protein after oral administration of test drug at two different doses ($P< 0.001$). Oral administration of Silymarin also reduced the liver enzyme levels significantly as compared to CCl_4 treated group ($P< 0.001$). But, the liver enzyme levels in silymarin in dose of 100 mg / kg daily and test drug in the dose of 100mg/kg treated groups were statistically similar. It was observed that reduction produced in biochemical parameters of liver was greater in dose of 100 mg/kg than that of 50 mg/kg of test drug, and the difference was found to be significant statistically ($p< 0. 05$). Table-2

Histological Examination

Group I*:* There were central blood vessels and radiating cords of hepatocytes as well as the vascular sinusoids with no evidence of fatty changes, necrosis or inflammation. (Fig.1)

Group II: There was centri-lobular (acidophilic) necrosis and vascular congestion. (Fig.2)

Group III: There was mild vascular congestion and peri-vascular infiltrate of mono nuclear cells and fibroblast. No fatty degeneration was observed. (Fig.3)

Group IV (50 mg/kg): There was vascular congestion and peri-lobular hydropic degeneration of hepatocytes (In high magnification only vascular congestion). (Fig.4)

Group V (100mg/kg): The slides showed well preserved hepatic architecture. There was no fatty degeneration, only mild vascular congestion and peri-

vascular infiltrate of mono nuclear cells and fibroblast and regenerating hepatocytes were observed. The hepatic architecture was found similar to that observed in group III. (Fig.5)

4. DISCUSSION

The findings of the present study demonstrated that the test drug (Unani formulation) lowered the biochemical markers of liver function as well as the lipid peroxide towards normal. The histological findings also indicated protective response by bringing the derangement of liver cell matrix to very near to normalcy. Thus the remarkable reduction in CCl_4 intoxicated biochemical markers by test drug extract, supplemented by commensurate histopathological findings of rat liver sections indicated hepatoprotective effect of this herbal formulation. The likely mechanism of hepatoprotective response appears to be the anti-oxidant property.

Table 1: Ingredients of *Qurs-e- Rewand*

Botanical Name (Family) English Name Quantity (in gm)	
Rheum emodi Wall Rhubarb	(Polygonaceae) 17.5 gm
Rubia cordifolia Linn Indian Madder	(Rubiaceae) 10.5 gm
Creteria lacca Lac	(Coccoidea) 10.5 gm
Apium graveolens Linn Celery	(Apiaceae) 3.5 gm
Feoniculum vulgare Mill Fennel	(Umbelliferae) 3.5 gm
Agrimonia eupatoria Linn Agrimony	(Rosaceae) 3.5 gm

Carbonterachloride has been widely used for inducing hepatic damage due to free radical formation during its metabolism by hepatic microsomes.[19] The clinical features of carbontetrachloride induced hepatic damage resemble that of acute viral hepatitis.[20] The mechanism of producing hepatic damage by CCl_4 depends on reductive dehalogenation of CCl_4 catalyzed by cytochrome P_{450} in the liver cell endoplasmic reticulum leading to the generation of unstable complex of CCl_4 radical. This trichloromethyl radical reacts rapidly with O_2 to yield trichloromethyl peroxy radical which is reported as a highly reactive species. *Qurs-e-Rewand* appers to exert its hepatoprotective effect by inhibiting lipid peroxidation mediated by CCl_4, due to its antioxidant activity as it decreased the lipid peroxide significantly in TBARS test. The test drug further appears to exert hepatoprotective effect due to its effect against cellular leakage and loss of functional integrity of the cell membrane in hepatocytes i.e. they possess membrane stabilizing property, indicated by significant decrease in AST, ALT, Alk. phosphatase and lipid peroxidation.

Table 2: Effect of test drug (*Q.R*) and Silymarin on biochemical parameters of liver function in CCl_4 induced toxicity

Groups (n = 6)	ALT/SGPT (Units/ml)	AST/SGOT (Units/ml)	S. Bilirubin (mg/dl)	S.Alk. phosphatase (KAU/dl)	Total protein (gm/100 ml)	Lipid peroxidation (n mole of MDA/mg of protein)
Group I	25.66 ± 1.30 $y^3z^3a^3b^3$	28.16 ± 1.53 $y^3z^3a^3b^3$	0.83 ± 0.11 y^3a^2	8.57 ± 0.38 $y^3z^3a^3b^3$	6.81± 0.20 $y^3z^1a^3$	3.21± 0.35 y^3a^2
Group II	80.50 ± 3.23	78.00 ± 2.22	2.49 ± 0.16	42.87 ± 1.74	4.14± 0.18	10.67± 0.88
Group III	59.66 ± 2.08 y^3a^1	60.33 ± 2.38 y^3a^2	1.13 ± 0.16 y^3	21.30 ± 1.86 y^3	5.98 ± 0.16 y^3a^2	4.51± 0.41 y^3
Group IV	67.83 ± 2.24 y^3	69.33 ± 2.73 y^1	1.58 ± 0.16 y^3	24.40 ± 1.59 y^3	5.01± 0.32 y^1	5.93± 0.61 y^3
Group V	57.00 ± 2.76 y^3a^2	57.66 ± 2.46 y^3a^2	1.28 ± 0.23 y^3	16.54 ± 1.26 $y^3z^1a^3$	6.23 ± 0.21 y^3a^3	4.13± 0.34 y^3a^2

Values are Mean ï SE; n = 6; x = against plain control, y = against CCl_4 (2 ml/kg), z = against standard (Silymarin) (100 mg/kg); a = against *Q.R.* single dose (50 mg/kg), b = against *Q.R.* double dose (100 mg/kg); = P < 0.05, 2 = P < 0.01, 3 = P< 0.001.

The biochemical as well as histological observations demonstrated dose dependent protective action of the extract against the liver damage. The extract in dose of 100mg/kg caused greater response. The biochemical markers were found significantly lower and retention of hepatic architecture, reduction in fatty degeneration and necrosis were more marked in this group than the group treated with 50mg/kg. The overall hepatoprotective effect produced by the 100mg/kg was sientifically equal to standard drug Silymarin. Further, quite interestingly the animals treated with *Qurs-e-Rewand* showed regeneration of hepatocytes, which

provide an indication of high clinical and therapeutic value.

The protective effect demonstrated with regard to liver function (Biochemical markers), lipid peroxidation and structure (Histological study) provides conclusive proof that the test drug possesses hepatoprotective activity.

The present study provides scientific support and validation to the Unani claim regarding the hepatoprotective activity of the test drug. The study also provides the interesting information that *Qurs-e-Rewand* promotes hepatocellular regeneration, so it may be useful in diseases where liver damage is extensive such as chronic hepatitis and cirrhosis.

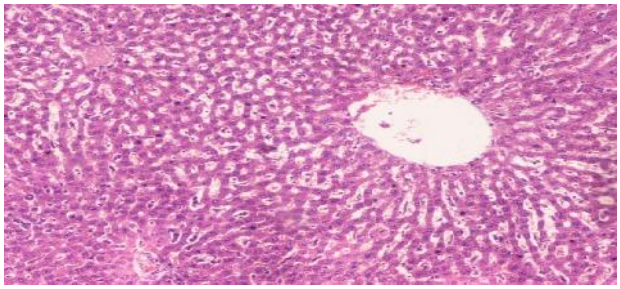

Fig 1: Section of rat liver showing central blood vessels and radiating cords of hepatocytes as well as the vascular sinusoids (H & E stain High power)

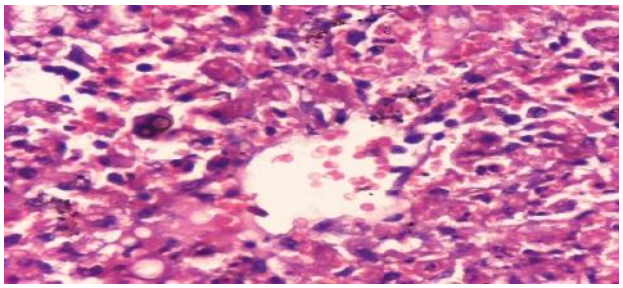

Fig. 2: Section of liver showing centrilobular (Acidophilic) necrosis, and vascular congestion and marked congestion of portal vessels (H & E stain, High power)

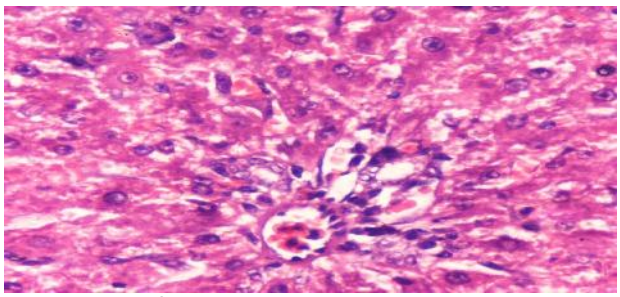

Fig. 3: Section of liver showing mild vascular congestion and peri-vascular Infiltrate of mono nuclear cells and fibroblast (H & E stain, High power)

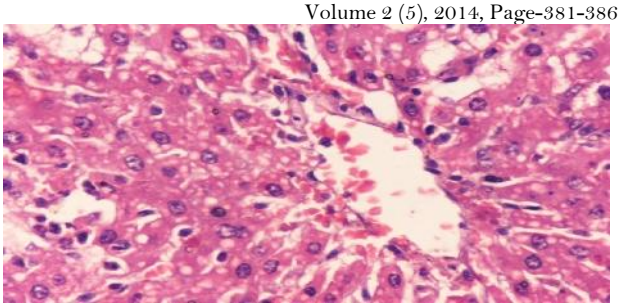

Fig 4: Section of liver showing vascular congestion (H & E stain, High power)

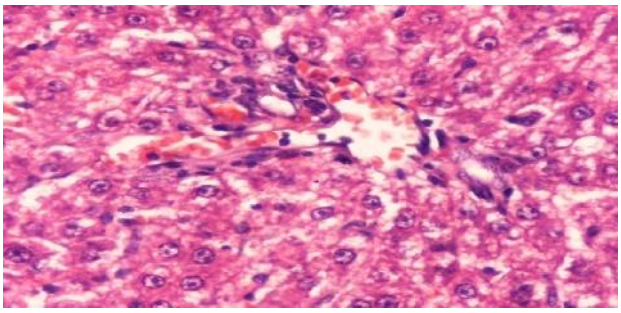

Fig 5: Section of liver showing mild vascular congestion and peri-vascular infiltrate of mono nuclear cells and fibroblast and regenerating hepatocytes (H & E stain, High power)

5. CONCLUSION

It can be concluded that both the doses of test drug (*Qurs Rewand*) possess significant hepatoprotective activity against acute hepatic damage induced by CCl_4. Further, the mechanisms and activities of compound drug require more study to understand the hepatoprotective mechanism.

6. ACKNOWLEDGEMENT

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7. REFERENCES

1. Khan S, Ilaj-ul-Amraz. Matba Munshi Naval Kishor, Lucknow. 1921; 223-224.
2. National Formulary of Unani Medicine. English Edition, Ministry of Health and Family Welfare, New Delhi, Part III, 2001; 7, 32.
3. Arzani. Qarabadeen-e-Qadri (Urdu) Matba Munshi Naval Kishor, Lucknow, 1924; 311-313.

4. Kabiruddin H. Bayaz-e-Khas Al-maroof Ilaj-ul-Amraz Aijaz Publishing House, Kucha Chelan Darya Ganj, New Delhi, 2006; 278, 466
5. Guntupalli M. Mohana Rao, Chandana V. Rao, Palpu Pushpangadan, Annie Shirwaikar., Hepatoprotective effects of rubiadin, a major constituent of Rubia cordifolia Linn. Journal of Ethnopharmacology 2006; 103(3): 484-490
6. Berger BML, Comber H, Esta BB. CCl_4 induced toxicity in isolated hepatocytes, The importance of direct solvent injury. Hepatology 1986; 6: 325-327.
7. Recknagel RO, Glende EA, Dolak JA, Walter R.L. Mechanism of Carbontetrachloride toxicity. Pharmacol Ther 1989; 43: 139-154.
8. Choski S, Patel SS, Saluja AK. Silymarin: A Promising herbal Hepatoprotective drug. Indian Drugs 2000; 37 (12): 566-69.
9. Ferenci P, Dragasics B, Dittrich H. Randomized controlled trial of Silymarin treatment in patients with cirrhosis of the liver. Journal of Hepatololgy 1989; 9: 105-113.
10. Dhawan BN. Organization of Biological Screening of Medicinal Plants with special reference to C.D.R.I programmes. Appendix-1, Lectures UNESCO-CDRI workshop on the use of Pharmacological Techniques for Evaluation of Natural Products, CDRI, Lucknow. 1982; 61-65.
11. Idris Turel et al., Hepatoprotective and anti-inflammatory activities of Plantago major L. Indian J Pharmacol 2009; 41(3): 120-124.
12. Reitman S, Frankel S. A colorimetric method for the determination of Serum Glutamic Oxaloacetic and Glutamic Pyruvic Transaminases. Am J Clin Path 1957; 28: 56-63.
13. Malloy HT, Evelyn KA. Estimation of Serum Bilirubin Level . J Biol Chem 1937; 119: 481-484.
14. Kind RRN, King EJ.. In vitro determination of Serum Alkaline Phosphatase. J Clin Path 1954; 7: 322-328.
15. Doumas BT et al., Clinical chemistry 1981; 27/10 1642-50.
16. Ohkawa H, Ohishi N, Yagi K. Assay for Lipid Peroxides in animal tissues by Thiobarbituric Acid Reaction. Analytical Biochemistry 1979; 95: 351-358.
17. Lowry OH, Rosenbrough NJ, Farr AlL, Randall RJ. Protein measurement with Folin phenol reagent. J Biol Chem 1951; 193: 265-269.
18. Mukherjee KL. Medical Laboratory Technology. Tata McGraw Hill, Publishing Company 1988; 3: 1111-1124.
19. Slater TF. Nature (London) 1966: 209: 36-40.
20. Beeson PB. Text Book of Medicine. Ist Ed, Press of Ijak shoim, Jaipur 1971; 981-988.