

Hepatoprotective and Anti-Hepatitis effect of non pharmacopoeial compound formulation on CCl₄-induced hepatotoxicity in albino rats

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To evaluate the anti-hepatitis activity of non-pharmacopoeial compound formulation on CCl₄ induces hepatotoxicity in albino rats. CCl₄ is used to produce hepatic damage in albino rats in the dose of 2 mL/kg BW, i.p. for 6 days. The anti-hepatitis effect of low dose (70 mg/100 g), high dose (140 mg/100 g) and 50% ethanolic extract (15 mg/100 g) of non-pharmacopoeial compound formulation (*Majoon*) was assessed in CCl₄ (2 mL/kg BW i.p.) induce hepatic damage in albino rats. The biochemical parameters such as SGOT (AST), SGPT (ALT), ALP, total bilirubin and total protein were estimated and the antioxidant activity was also estimated. These biochemical observations were supplemented by histopathological study of liver sections. Silymarin (10 mg/100 g B.W.p.o.) was used as a standard hepatoprotective drug for positive control. Data was analysed by one way ANOVA test. Administration of non-pharmacopoeial compound formulation and its extract significantly prevented CCl₄ induced elevation of serum ALT, AST, ALP, total bilirubin and total protein level. The histopathological study revealed hepatocytes regeneration. The test drug also decreases lipid peroxidation. So, the test drug was found effective as anti-hepatitis and antioxidant effect. The result was comparable to that of Silymarin. The results of present study show that the compound formulation has significant anti-hepatitis activity.

Keywords: Anti-hepatitis, Antioxidants, CCl₄, Hepatoprotective, Non-pharmacopoeial compound formulation

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Liver is the most important organ of metabolism and excretion¹. Liver is a key organ in coordinating the homeostasis of the body². Alteration in normal physiological roles may lead to impaired liver function³. According to the WHO data published in May 2014, liver disease deaths in India reached 2,16,865 or 2.44% of total deaths. Hepatocellular carcinoma is one of the ten most common tumors in the world with over 2,50,000 new cases per year¹. Hepatitis is the commonest liver disorder which prevails throughout the world. Modern medicine offers little by way of cure, but, so far, there is no effective treatment available for hepatitis and to stop the progression of Cirrhosis in patients with liver disease⁴. Herbs are known to play a major role in the management of various liver diseases. Liver protective drugs contain a variety of chemicals constituents in which flavonoids and volatile oils are the most commonly encountered liver protective active constituents¹. In *Ayurveda* about 77 and in *Tibb-e-Unani* about 42 herbal drugs are used as hepatoprotective agents. Nearly 160 phyto-

constituents obtained from 101 plants belonging to 52 families have been reported to have antihepatotoxic activity¹. In India more than 87 medicinal plants are used in different combinations for this purpose⁵. Researchers explore the potential in the traditional medicine (*Unani* medicine) as it possesses number of safe curative agents for liver disorders. The present study was conducted on a non-Pharmacopoeial compound formulation (NPCF). This is a new formulation based on ten ingredients viz. Rose petals (*Rosa damascene* Mill.), Rubarb root (*Rheum emodii* Wall.), Cassia bark (*Cinnamomum cassia* Blume.), *Jatamansi* root (*Nardostachys jatamansi* DC.), *Kasni* seeds (*Cichorium intybus* L.), *Punarnawa* root (*Boerhavia diffusa* L.), *Asaroon* root (*Asarum europoeum* L.), *Orris* root (*Iris ensata* Thunb.), Saffron (*Crocus sativus* L.) and Lac resin (*Laccifer lacca* Kerr.) (Table 1). They are hepatoprotective, tonic, anti-inflammatory, diuretic, deobsturent and antioxidant in property⁶⁻¹⁰. This formulation was a semi-solid paste called as '*Majoon*' in *Unani* Medicine. The *Majoon* and its hydroalcoholic extract from Soxhlet apparatus were used for the study in albino rats.

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Table 1 — List of ingredients of *Majoon*

S No	Unani name	Amount	Botanical name	Family	Parts used
1.	Gul-e-surkh	30g	Rosa damascene Mill.	Rosaceae	Flower
2.	Rewandchini	10g	Rheum emodi Wall.	Polygonaceae	Rhizome
3.	TajQalmi	10g	Cinnamomum cassia Blume.	Lauraceae	Bark
4.	Balchad	10g	NardostachysjatamansiDC.	Valerianaceae	Root
5.	Kasni	10g	Cichoriumintybus L.	Asteraceae	Seed
6.	Punarnawa	10g	Boerhaviadiffusa L.	Nyctaginaceae	Root
7.	Asaroon	10g	Asarumeuropoeum L.	Aristolochiaceae	Root
8.	Irsa	10g	Iris ensataThunb.	Iridaceae	Root
9.	Zafran	3g	Crocus sativus L.	Iridaceae	Stigma,Style
10.	Luk-e-Maghsool	10g	From an insect, Lacciferlacca Kerr.	Lacciferidae	Resinous secretion

Materials and Methods

Chemicals

Silymarin was purchased from Sigma-Aldrich, Germany. Carbon tetrachloride (CCl₄) was purchased from Ranbaxy lab. Ltd. India. ALT, AST, ALP, Serum bilirubin, Total protein kits were purchased from Siemens pvt. Ltd.

Collection of material

Majoon's ingredients were procured from Dwakhana Tibbiya College, AMU, Aligarh and were identified and authenticated in the Pharmacognosy section of Department of Ilmu Advia, Faculty of Unani Medicine, AMU Aligarh, followed by preparation of *Majoon*.

Preparation and Administration

The *Majoon* (NPCF) was prepared according to the standard method described in *Unani Formulary*¹¹ and *Pharmacopoeia*. Before preparation of *majoon* the single plant drugs of formulation were dried in oven at different temperatures such as roots and barks at 50 – 60°C, flowers and seeds at 20–30°C¹². After that the drugs were cleaned and crushed to make fine powder in electric grinder at 80 meshes sieves except *Luk* and *Zafran*.

The whole *majoon* in low and high dose was administered per orally as per route of choice of *Unani* Medicine. The 50% ethanolic extract was prepared by Soxhlet's apparatus¹³. All forms of test drug were dissolved in distilled water and administered orally by a feeding canula after shaking the solution well. The doses of the test drug for the study were calculated by multiplying the clinical doses with appropriate conversion factor of 7 for rats¹⁴.

The clinical dose of *Majoon*: 5-10 g.

Animals

Acute study was conducted for NPCF (Test drug) on 36 Albino rats of Wistar strain of either sex,

weighing 150-200 g which were divided into 6 groups of 6 animals each. The animals were kept under standard laboratory conditions of 12 h light and dark period. Standard rat diet and water were given *ad libitum*. The study protocol was approved by the institutional animal ethics committee.

Experimental design

The animals were deprived of food for 12 h before the treatment. Test drugs were administered to the animals by oral feeding canula for 6 days. Carbon tetrachloride (CCl₄), a hepatotoxic agent, was injected in the form of suspension to the animals intraperitoneally in a dose of 2 mL/kg B.W. on the 6th day except group I (Plain Control). On the seventh day all the animals were sacrificed by ether anaesthesia.

The animals groups were treated as follow

- Group I: Served as normal control and administered distilled water orally for 7 days.
- Group II: Served as Negative control, CCl₄ was given as 1:1 in Olive oil in the dose of 0.2 mL/100g B.W. i.p. on 6th day.
- Group III: Served as Standard control, Silymarin was given in the dose of 10mg/100 g B.W. orally + CCl₄ (0.2 mL/100g) on 6th day.
- Group IV: Low dose of NPCF (70mg/100g B.W.) orally + CCl₄ on 6th day.
- Group V: High dose of NPCF (140mg/100g B.W.) orally + CCl₄ on 6th day.
- Group VI: 50% Alcoholic extract of NPCF (15mg/100g B.W.) orally+ CCl₄ on 6th day.

Collection of biological samples

After sacrificing the animals, the blood was collected in serum separating tubes (gel and clot

activator) and centrifuged at 7000 rpm for 15 min and stored at 2-4°C. The liver was also taken immediately and fixed in 10% formalin. Biochemical markers and protein were estimated in serum.

Estimation of biological parameters

The protective effect of the test and standard drugs was studied by estimating the concentration of biochemical markers of liver function, viz. serum bilirubin, serum alanine transaminase (ALT/SGPT), serum aspartate transaminase (AST/SGOT), serum alkaline phosphatase (SALP) and serum total protein (STP).

Histopathological study

For histological study, the sections of liver were removed and fixed in 10% formaline. The fixation was done immediately to check autolysis, to preserve as nearly as possible the natural state of the tissue cells. Care was taken to maintain the correct volume of the fixative¹⁵. The tissue was processed and sections were cut. The slides were prepared and stained with haematoxyline and eosin stain and studied for histopathological changes by light microscopy under various magnifications.

Statistical analysis

The concentrations of each parameter in various animal groups were statistically compared for determining significance of difference by one-way ANOVA Test followed by Tukey: Compare all pairs of column. The analysis was carried out by using In Stat Graph Pad software. Values were expressed as Mean±SEM. A probability (P) values of less than 0.05 were considered as statistically significant.

Results

The effect of the test drug on serum marker enzymes is presented in Table 2. The level of serum bilirubin, ALT, AST, ALP were significantly increased while total protein was found to be significantly decreased (p<0.001) in CCl₄ treated rats as compare to plain control group, indicating liver damage Fig. 1. Silymarin was found to significantly decrease (p<0.001) the biochemical parameters of liver function as compared to the group II, and serum total protein was increased significantly (p<0.01). It confirms that the Silymarin possesses potent hepatoprotective activity. In comparison of low dose, high dose and hydroalcoholic extract of the test drug, it was observed that the decrement produce by the high dose (140 mg/100g) and

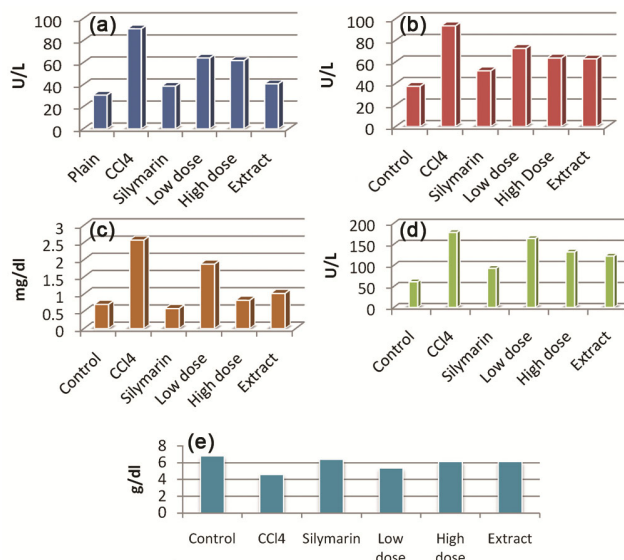


Fig. 1 — Graphs showing effect of CCl₄, Silymarin and Test drugs on (a) ALT, (b) AST, (c) Bilirubin, (d) ALP, (e) Total Protein

Table 2 — Effect of test drug (NPCF) and standard drug on biochemical parameters of liver function in CCl₄ induced hepatotoxicity

Groups	S. ALT / SGPT (Unit/L) Mean±SE	S. AST / SGOT (Unit/L) Mean±SE	Serum Bilirubin (mg/dl) Mean±SE	S. Alkaline Phosphatase (U/L) Mean±SE	Total Protein (g/dl) Mean±SE
Plain control (Distilled water)	30.618±2.44	37.39±1.49	0.694±0.219	61.064±3.33	6.756±0.279
Hepatotoxic CCl ₄ 0.2mL/100g	90.762±3.594 x ³	93.5±2.00 x ³	2.568±0.155 x ³	178.34±2.832 x ³	4.57±0.252 x ³
Standard (Silymarin) 10mg/100g	38.74±2.52 y ³	51.73±2.64 x ³ y ³	0.584±0.114 y ³	93.074±2.11 x ³ y ³	6.385±0.329 y ²
Low dose (70mg/100g)	64.31±3.179 x ³ y ³ z ³	72.61±2.74 x ³ y ³ z ³	1.866±0.105 x ³ y ¹ z ³	163.818±2.062 x ³ y ¹ z ³	5.318±0.129 x ² z ¹
High dose (140mg/100g)	62.019±0.900 x ³ y ³ z ³	63.63±0.98 x ³ y ³ z ² a ¹	0.822±0.157 y ³ a ³	131.856±3.804 x ³ y ³ z ³ a ³	6.131±0.249 y ²
50%Alcoholic extract (15mg/100g)	40.86±2.901 y ³ a ³ b ³	62.78±1.51 x ³ y ³ z ² a ¹	1.022±0.174 y ³ a ¹	122.03±3.82 x ³ y ³ z ³ a ³	6.108±0.144 y ²

N=6, x = Against Plain control, y = Against CCl₄, z = Against Standard, a = Against Low dose, b = Against High dose, c = 50%Aloholic extract, 1 = p< 0.05, 2 = p< 0.01, 3 = p< 0.001

the hydroalcoholic extract (15 mg/100g) in biochemical and metabolic parameters of liver function was greater than the low dose (70 mg/10g). It showed that the effect of high dose and hydroalcoholic extract of the formulation are on the whole equivalent to the effect of Silymarin, because Silymarin, high dose and extract significantly reduced ($p < 0.001$) the serum ALT [Fig. 1(a)], AST [Fig. 1(b)], bilirubin [Fig. 1(c)] and ALP [Fig. 1(d)]. The high dose produced slightly greater reduction ($p < 0.001$) in serum bilirubin [Fig. 1(c)] and ALP [Fig. 1(d)] than low dose. The hydroalcoholic extract of the test drug produced greater reduction in serum ALT [Fig. 1(a)] and ALP [Fig. 1(d)] ($p < 0.001$) than low dose. Administration of test drug as a whole in single and double dose or its hydroalcoholic extract remarkably prevented CCl_4 induced hepatotoxicity in rats by significantly decreasing serum bilirubin, ALT, AST, ALP and by increasing total protein [Fig. 1(e)] and (Table 2).

Histopathological studies of the liver sections of control and experimental animals shown in Fig. 2 were carried out to test the hepatoprotective and anti-hepatitis effect of the test drug.

The Fig. 2(a) shows the liver section of Group I (Normal control) animals, which has normal architecture, where the central vein, portal tracts, hepatocytes and sinusoids appear normal. Fig. 2(b)

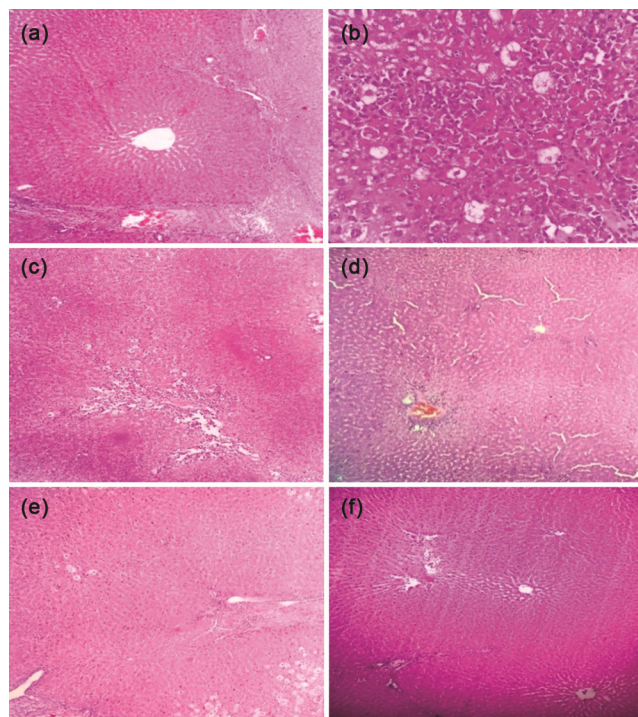


Fig. 2 — Liver histopathology of **A**-normal control group, **B**- CCl_4 treated group, **C**-Silymarintreated group, **D**-Low dosetreated group, **E**-High dosetreated group, **F**-Hydroalcoholic treated group

(Group II) shows hepatocellular damage, there was hepatocyte necrosis, degeneration, presence of fatty vacuoles, infiltration of inflammatory cells. Fig. 2C (Group III) shows regeneration of hepatocytes, no fatty degeneration, absence of fatty vacuoles. Fig. 2D & 2E (Group IV & V) shows significant liver protection against toxicant as evident by the presence of normal hepatocytes, absence of necrosis and less fatty infiltration. Fig. 2(f) (Group VI) shows regeneration of hepatocytes and their arrangement around central vein with mild fatty changes. Hepatic architecture was similar to that of control. The histopathological observation showed that the test drug has an ability to initiate regeneration of hepatocytes. All the results showed that the drug improves the functional and structural status of liver following the exposure to known liver toxicant, CCl_4 .

Discussion

This study was under taken to demonstrate the hepatoprotective ability and anti-hepatitis activity of non pharmacopoeial compound formulation (*Majoon*) on liver damage induced by CCl_4 . Biochemical markers of liver function and histopathological features were taken as the indicators of liver toxicity or hepatoprotection in light of their deviation from normal values or their proximity with the normal. Estimating the activities of serum marker enzymes like ALT and AST can make assessment of liver function. When hepatocytes plasma membrane is damaged, various enzymes normally present in the cytosol are released into the blood stream. Their estimations in the serum are a useful quantitative marker of the extent and type of hepatocellular damage¹⁶. Histopathological studies give the supportive evidence for the biochemical analysis¹⁷. In this study, the use of low (single) dose, high (double) dose and hydroalcoholic extract of the test drug protects liver from damage by CCl_4 as evident by improved histological picture and biochemical markers of hepatic damage. This formulation exerted its hepatoprotective and anti-hepatitis effect might be by inhibiting lipid peroxidation mediated by CCl_4 due to its anti-oxidant activity. The presence of flavonoids, tannins, phenols, terpens and sterols in this formulation explain its role in hepatoprotection by inhibiting the free radicals mediated damage¹⁸, claimed that flavonoids, terpens and tannins were antioxidant agents and may interfere with free radical formation¹⁹.

Conclusion

In the view of above discussion, it was concluded that the test drug, viz. Non-pharmacopoeial compound

formulation and its hydroalcoholic extract possess significant hepatoprotective and anti-hepatitis effect against acute hepatic damage induced by CCl₄. The effect of high dose and hydroalcoholic extract were more marked, which was almost equivalent to the effect of Silymarin. The test drug (NPCF) produced dose dependent response as low and high doses possessing significant hepatoprotective effect, but high dose is slightly better. The test drug also showed hepatocytes regeneration. So, it may be effective in hepatitis and chronic liver disease.

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