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Hepatoprotective Activity of Sharbat Deenar, against Carbon Tetrachloride-Induced Hepatotoxicity in Rats

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***Abstract*—**Polyherbal formulation *Sharbat Deenar* is a very popular unani medicine in Bangladesh. It is usually used for different kinds of liver disorders. In absence of reliable and inadequate hepatoprotective agents in conventional medicine, the herbal preparations are preferred for liver diseases. The present study was designed to evaluate the hepatoprotective activity of *Sharbat Deenar* on carbon tetrachloride (CCl_4) induced hepatotoxicity in male Long-Evans albino rats. Group I served as normal control and received neither formulation nor carbon tetrachloride. Group II received only CCl_4 1mL/kg body weight of rat intraperitoneally for consecutive 14 days. Group III received CCl_4 1mL/kg body weight of rat intraperitoneally and *Silymarin*, in dose 50mg/kg body weight of rat orally. Group IV received CCl_4 1mL/kg body weight of rat intraperitoneally and *Sharbat Deenar* 1mL/kg body weight of rat for the same 14 consecutive days. At the end of the study, hepatoprotective activity was evaluated by the levels of total bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP). Histopathological study of rat liver was also carried out. The results showed that polyherbal formulation *Sharbat Deenar* exhibited a significant hepatoprotective effect. Such an outcome seems to be the synergistic effect of all ingredients of tested herbal formulation. Although this study suggests that *Sharbat Deenar* may be used to cure or minimize various liver diseases, it needs further study to attain the clarity of mechanism and safety.

***Keywords*—**Carbon tetrachloride, Hepatoprotective, *Sharbat Deenar, Silymarin*.

I. Introduction

LIVER disease is a major problem that causes significant morbidity and mortality worldwide [1]. Globally approximately 30,000 people die due to liver diseases and over 250,000 new cases of hepatocellular carcinoma are detected each year [2]. In 2013, 29 million people in the European region and more than 30 million Americans suffered from a chronic liver disease [3]. In the 2010, more than one million deaths were due to liver cirrhosis [4]. The hepatitis B virus is the major cause for liver cirrhosis and hepatocellular carcinoma. Hepatocellular carcinoma the sixth most common cancer globally and the second leading cause of cancer-related death worldwide. Hepatitis B virus is a major threat for all age groups of Bangladesh and other South Asian countries. It is estimated that 240 million people are chronically infected with hepatitis B. Hepatitis B causes both acute and chronic liver disease and, estimated to cause about 800,000 deaths per year mostly from liver cancer and cirrhosis [5].

In Bangladesh the most common liver disease is viral hepatitis. According to one estimate, more than 10 million people are suffering from hepatitis B in Bangladesh [6]. Nonalcoholic fatty liver disease (NAFLD) is another most common cause of liver disease with worldwide prevalence of 25.24%. The prevalence of NAFLD has increased remarkably over the years affecting up to 30% of the general population in the Asia-Pacific region. Important independent risk factor for NAFLD is more prevalent for Bangladeshi ethnicity which is up to 49.8% in diabetic patients [5].

So, it is a serious threat to health and may cause a disastrous health problem in Bangladesh if not controlled in time. Hence, to face such challenges, a reliable scientific treatment and relevant study is needed. Conventional or synthetic drugs used in the treatment of liver diseases are inadequate and can have serious adverse effects [7]. In this regard, natural medicines from medicinal plants have shown effective and safe alternative treatment for liver disorders [8].

Sarbat Deenar is a polyherbal unani formulation available in local market of Bangladesh which is very commonly used for different kinds of liver diseases. However, no scientific data are available to justify the hepatoprotective activity of this selected formulation. So, the present study was undertaken to investigate the hepatoprotective effects of *Sharbat Deenar* in experimental rats.

II. Materials and Methods

A. Drugs and Chemicals

Silymarin, a standard drug was collected from a pharmaceutical industry in Dhaka through personal contact. Carbon tetrachloride was collected from the Department of Pharmacy, Rajshahi University, and preserved in normal temperature in a strong air tight amber glass bottle. All biochemical kits were purchased from Randox company and preserved in a freezer. All other chemicals and reagents were analytical grade purchased from the local market. The study was done at the University of Rajshahi with the collaboration of Bangladesh Agricultural University.

B. Tested Formulation

Sharbat Deenar is a unique and well-known formulation in Bangladesh for hepatoprotection [9]. It is useful for the diseases such as hepatitis, obstructive jaundice, ascites,

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constipation, and pleurisy. *Sharbat Deenar* was collected from the local market, Mymensingh, Bangladesh. It contains the aqueous extract of the following medicinal plants.

TABLE I
COMPOSITION OF 5 ML OF *SHARBAT DEENAR* USING EXTRACTS FROM MEDICINAL PLANTS

Plants	Local Name	Amount (mg)
Cichorium intybus Linn. (root)	Kasni	400
Cichorium intybus Linn. (seed)	Kasni	200
Rosa damascena Mill.	Golap	200
Nymphaea alba Linn.	Shapla	100
Borago officinalis Linn.	Gaojoban	100
Cuscuta reflexa Roxb.	Swarnalata	150
Rheum emodi Wall. ex Meissn.	Rheuchini	300

Note: Other ingredients (water, sugar, sodium benzoate) are added in required quantity to prepare the concoction.

C. Experimental Animals

Long-Evans albino male rats were collected from International Centre for Diarrhoeal Diseases Research, Bangladesh (ICDDR,B). After three weeks of rearing, these rats weighing about 150–200 gm were examined for inclusion in the study. The animals were housed in clean metallic cages and maintained in controlled temperature (27±2ºC) and light-dark cycle (12 h light and 12 h dark). They were fed with commercial pellet diet and water.

D. Grouping and Manipulation

The experimental rats were divided into four groups namely Group I, II, III and IV through the process of randomization. Each group contained 6 numbers of rats. Group I served as normal control and received only normal diet and water. Group II received a suspension of CCl_4 in liquid paraffin in a ratio of 2:1 (v/v) in a uniform dose of 1mL/kg body weight of rat intraperitoneally form day 'zero' of the experiment for consecutive 14 days. Group III received CCl_4 1mL/kg body weight of rat intraperitoneally and *silymarin*, in dose 50 mg/kg body weight of rat orally for 14 consecutive days. Group IV received CCl_4 1mL/kg body weight of rat intraperitoneally and *Sharbat Deenar* 1mL/kg body weight of rat for the same 14 consecutive days.

E. Collection of Serum and Tissue Sample

At the end of the treatment period, the rats were euthanized after being lightly anesthetized with chloroform 30 ppm followed by cervical decapitation. Then blood was withdrawn directly from the heart after dissecting the thorax. Blood was allowed to clot and centrifuged for 15−20 minutes at 3,000 rpm to separate out the serum. Then the animals were sacrificed and biopsy samples from liver was rapidly excised and serially sectioned. The tissue was fixed in 10% formalin and consecutive sections were stained by haematoxylin and eosin for histological examination.

F. Assessment of Hepatoprotective Activity

The biochemical parameters like total bilirubin, ALT, AST and ALP and histopathological study of liver architecture like focal necrosis, fatty changes, inflammatory cell infiltration were assessed at the end of the study.

G. Statistical Analysis

The obtained data were analyzed using student's *t* test. The value was expressed as mean ± SD (standard deviation). Probability level of less than 5% ($p<0.05$) was considered for significant values.

III. RESULT AND DISCUSSION

The result showed that the level of serum total bilirubin, ALT, AST and ALP significantly increased only in the CCl_4 treated Group II when compared with the normal control. Histopathological study also showed that high degree of damage characterized by congestion of central vein and portal triads, and cloudy degeneration in Group II occurred. Because Group II received only CCl_4 that mediate the changes of liver functions and ultimately leading to destruction of hepatocellular membrane. Carbon tetrachloride CCl_4 is widely used for experimental induction of liver damage [10].

Carbon tetrachloride metabolically activated by the cytochrome P-450 and reacts with oxygen in the endoplasmic reticulum to form trichloromethyl free radical (CCl_3) that combined with cellular lipids and proteins in the presence of oxygen and induces lipid per-oxidation. As a result, changes of the structures of the endoplasmic reticulum and other membrane, loss of metabolic enzyme activation, reduction of protein synthesis and loss of glucose-6-phosphatase activation finally led to liver injury [11].

On the other hand, treatment with the reference drug *silymarin* as well as the test drug *Sharbat Deenar* significantly reduced the elevation of the level of serum total bilirubin, ALT, AST and ALP in Group III and Group IV respectively, when compared with Group II. Histopathological study also showed the progressive recovery of liver against CCl_4 induced damage in Groups III and IV as compared to normal control in Group I. In Group III, the standard drug *silymarin* was administered along with CCl_4. Silymarin is a worldwide complementary alternative medicine for hepatic diseases that derived from *Silybum marianum.* This compound from Asteraceae family and native to southern Europe, northern Africa, and the Middle East region which is characterized by thorny branches and a milky sap, with its oval leaves reaching up to 30 cm.

The flowers are bright pink and can measure up to 8 cm in diameter. The plant contains at least seven flavolignans. The most important flavolignans are silybin, silydianin, and silychristine [12]. Silymarin offers good protection in various toxic model of experimental liver diseases in laboratory animal. It possesses antioxidative, anti-inflammatory, antifibrotic, antilipid peroxidative, membrane stabilizing and liver regenerating activities. Silymarin and its main components silybinin are used almost exclusively for hepatoprotection in humans [13]. Therefore, the results showed that *silymarin* had the expected hepatoprotective action as reported earlier [8].

The polyherbal formulation *Sharbat Deenar* was administered to Group IV which also showed hepatoprotective function against CCl_4 induced hepatotoxicity. As a result, the levels of serum total bilirubin, ALT, AST and ALP were not elevated more like in Group II. It might be attributed due to its herbal ingredients of *Cichorium intybus, Rosa damascena*, *Nymphaea alba*, *Borago officinalis, Cuscuta reflexa* and *Rheum emodi* which have very potent active constituents and antioxidant properties for hepatoprotection.

Cichorium intybus, is a perennial herb from Asteraceae family, native of Europe and Asia usually rough and more or less glandular herb stems 0.3-0.9m, angled or grooved, branches tough, rigid spreading radical and lower leaves 7.5-15 cm, pinatified, lobes toothed, pointing downwards, upper leaves alternate [14]. Its various plant parts have beneficial role in treating liver diseases, enlargement of spleen, as bitter tonic effective in jaundice, liver enlargement etc because the roots of *Cichorium intybus* contain sesquiterpene lactones, inulin (up to 60%), phlobaphenes caffeic acid, cichoric acid, sugar, pactin, fixed oil, choline and reducing sugars. Past studies showed that the methanolic fraction and a phenolic compound of seed of *Cichorium intybus* were found to possess a potent antihepatotoxic activity comparable to the standard drug silymarin. Another study showed that paracetamol induced rise in serum enzymes was protected by pre-treatment of rats with Esculetin (6 mg/kg), a phenolic compound found in *Cichorium intybus* and *Bougainvllra spectabillis*. It has also been reported to prevent the rise of serum enzymes against CCl_4 induced hepatotoxicity [15].

Rosa damascene is a widely cultivated ornamental plant in Asia which is from Rosaceae family. Its chemical constituents are volatile essential oils, fats, resins, malic, tartaric and tannic acids. The petals contain an aromatic volatile oil, a glucoside quercetin, gallic acid and quercetinic acid. Petals of the flowers are employed for the production of rose water and attar (otto or oil) of roses. It possesses good antioxidant activity. Experimental study showed that the hepatoprotective activity from free radical mediated disease processes [16].

Cuscuta reflexa is another plant is available in Bangladesh and other part of South- Asian region. Past study showed that the chloroform and ethanol extract of *Cuscuta reflexa* exhibits significant hepatoprotective properties [17].

Nymphaea alba is from Nymphaeaceae family generally found in tanks and ponds distributed in Europe, North Africa, Southwest Asia, India, China and Russia. It is rich in tannic acid, gallic acid, alkaloids, sterols, flavonoids, glycosides, hydrolyzable tannins and high–molecular-weight polyphenolic compounds. The past study showed that the ethanolic extract of flower of *Nymphaea alba* possess strong and potential Hepatoprotective activity in rats [18].

Rheum emodi from Polygonaceae is a leafy perennial herb distributed in Himalayas from Kashmir to Sikkim in India. The herb has been traditionally used to treat pathological ailments like fevers, ulcers, bacterial infections, fungal infections, jaundice and liver disorders. The methanolic extracts of *Rheum emodi* showed the prevention of cellular proliferation, fibrosis, cirrhosis, and cancer of the liver due to its various phyto constituents like alkaloids, terpinoids, glycosides, steroids, triterpenoids, flavonoids, carbohydrates, saponins and tannins [19].

Borago officinalis is from Boraginaceous family usually used as an ornamental plant. It possesses a high antioxidative activity due to its high content of phenolic compounds officinalioside and kaempferol 3-O-β D-galactopyranoside. Past studies showed that the *Borago officinalis* potential hepatoprotective effects against chronic liver injury, which we propose to be due to the antioxidant and anti-inflammatory effects in rats [20].

Some earlier studies showed that the ingredients of *Sharbat Deenar* like *Cichorium intybus*, [21] *Borago officinalis* [22], *Rosa damascena* [23], *Nymphaea alba* [24], *Cuscuta reflexa* [25] and *Rheum emodi* [26] individually reported for hepatoprotective activity. So, this combined synergistic action of all ingredients of tested herbal formulation may help to normalize the liver function.

TABLE II
EFFECTS OF *SHARBAT DEENAR* ON DIFFERENT BIOCHEMICAL PARAMETERS IN THE SERUM OF RATS

Group	Treatment	Total Bilirubin Mean ±SD	ALT Mean ±SD	AST Mean ±SD	ALP Mean ±SD
I	Control	0.20±0.09	61.00±3.46	73.333±7.15	181.00±9.25
II	CCl_4 only	2.08± 0.36*	354.50±35.87*	396.83±25.14*	515.00±18.10*
III	CCl_4 + *Silymarin*	0.17 ±0.08*	102.33±13.76*	136.67±11.20*	224.67 ±12.85*
IV	CCl_4+ *Sharbat Deenar*	0.20±0.06*	138.50±16.00*	145.67 ±14.55*	218.50 ±17.18*

Note: Values are expressed as mean ± SD of 6 animals in each group, Group II compared with Group I and Group III, IV compared with Group II.
Student's *t* test was followed, and the value was considered significant (*) when the p value was ≤0.05.

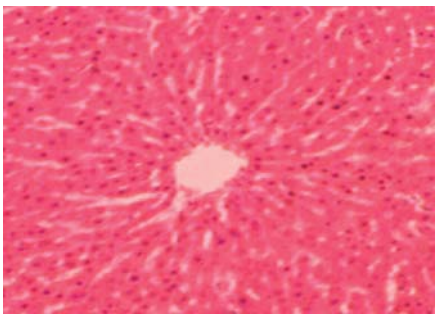

Fig. 1 Normal Control Rat: Section of liver showing normal hepatic cells

IV. CONCLUSION

In conclusion, the polyherbal formulation *Sharbat Deenar* has demonstrated a very good hepatoprotective effect against CCl_4 induced hepatic damage in rats. However, further studies need to be carried out to determine the detailed phytochemical analysis and mechanisms of action and clinical study that might be related to its hepatoprotective action.

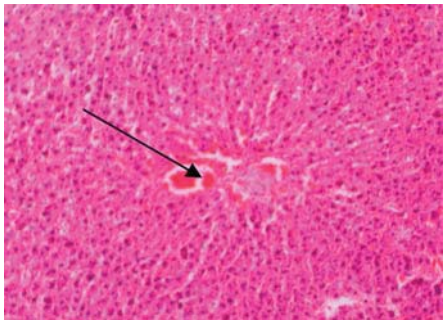

Fig. 2 CCl_4 treated rat: Section of liver showing centrilobular fatty degeneration, cloudy swelling, and necrosis of hepatic cells

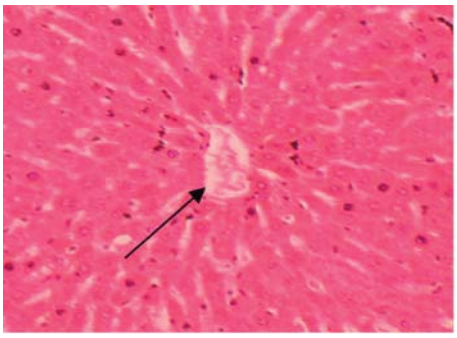

Fig. 3 *Silymarin* treated rat: Section of liver showing near normalcy of hepatic cells, central vein, and portal triad

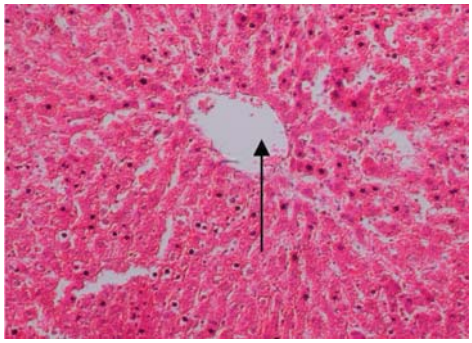

Fig. 4 *Sharbat Deenar* treated rats: Section of liver showing near to normalcy of hepatic cells

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