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HEPATOPROTECTIVE ACTIVITY OF SHARBAT CHYLOSIN A POLYHERBAL FORMULATION AGAINST CARBON TETRACHLORIDE-INDUCED HEPATOTOXICITY IN RATS

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ABSTRACT

Carbon tetrachloride (CCl_4) is the most commonly used hepatotoxin agents in the experimental studies for evaluate hepatoprotective activity. The present study was designed to evaluate the hepatoprotective activity of *Sharbat Chylosin* on 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 a suspension of CCl_4 in liquid paraffin in a ratio of 2:1 (v/v) in an uniform dose of 1 ml/kg body weight intraperitoneally for consecutive 14 days. Group III and IV received CCl_4 1ml/kg body weight intraperitoneally plus *Silymarin*, in dose 50 mg/kg orally and *Sharbat Chylosin* 1ml/kg body weight of rat respectively for the same 14 consecutive days. End of study hepatoprotective activity was determination by the levels of total bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP). Histopathological study also carried out on rat liver. The result showed that polyherbal formulation exhibited a significant hepatoprotective effect by total bilirubin, ALT, AST and ALP when compared with standard *Silymarin*. Good hepatoprotection also showed in histopathological study. Therefore this study suggests that *Sharbat Chylosin* might be beneficial in CCl_4 induced hepatotoxicity and may be attributed to the antioxidant properties of selected polyherbal formulation.

KEYWORDS: Polyherbal Formulation, *Sharbat Chylosin*, Carbon tetrachloride, *Silymarin*, Hepatoprotective.

1. INTRODUCTION

Liver is a glandular organ and plays a pivotal role in regulating various physiological processes in the body, such as metabolism, secretion and storage.[1] More than 30,000 deaths annually are counted due to liver diseases and over 2, 50,000 new cases of hepatocellular carcinoma usually found in each year.[2,3] In Bangladesh about 35,000000 people are sufferings from liver diseases and is increasing day after day in alarming rate. Among them about 15,000000 are suffering from the chronic hepatitis-B virus, 8,00000 from hepatitis-C virus and nearly 20,000000 people are suffering from other liver- related diseases like liver cirrhosis, cancer and fatty liver disease. Due to these diseases the hospital admission is nearly 43 percent with acute hepatitis and jaundice cases by hepatitis E virus, 22 percent cases by hepatitis B, 8 percent hepatitis A and 3 percent was hepatitis C.[4] So it a global and nationwide burden and needs reliable scientific based management. The synthetic drugs used in the treatment of liver disease are inadequate and can have serious adverse effects.[5] Numerous medicinal plants are being used for the treatment of liver disorders.[6] *Sharbat Chylosin* is a polyherbal formulation available in local market of Bangladesh and asserts to have hepatoprotective function but there is no scientific data regarding pharmacological evaluation of this selected formulation. So the present study was aimed to screen *Sharbat Chylosin* for its hepatoprotective activity in experimental rats.

2. MATERIALS & METHODS

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

Tested Formulation: The tested formulation *Sharbat Chylosin* is a unique combination of most effective natural herbs and contains the aqueous extracts of ten indigenous medicinal plants (Table 1). It was collected from local market, Bangladesh.

Table 1: Composition of 5 ml of *Sharbat Chylosin* Using Extracts from Medicinal Plants.

Plant	Amount (mg)
Foeniculum vulgare Mill.	100
Carum roxburghinum Benth. (Seed)	150
Carum roxburghinum Benth. (Root)	200
Cichorium intybus Linn.	150
Cassia fistula Linn.	150
Hygrophila auriculata (Schum.) Heyne.	100
Boerhaavia diffusa Linn.	100
Andrographis paniculata (Burm. f.) Wall.	100
Terminalia chebula (Gaertn.) Retz.	100
Artemisia absinthium Linn.	100
Leonurus cardiaca Linn.	100

Note: Other ingredients (water, sugar, sodium benzoate) are added in sufficient quantity to prepare the concoction.[7]

Experimental animals: Male Long-Evans albino rats were collected from International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDR, B). Rats were reared for three weeks when weighing about 150–200 gm were examined for inclusion in the study. The animals were housed in clean metabolic cages and maintained in temperature (27±2ºC) and light dark cycle (12 h light and 12 h dark). They were fed commercial pellet diet and water.

Grouping and manipulation: Through the process of randomization, the experimental rats were divided into four groups namely group I, II, III and IV. Each group contained 6 numbers of rats. Neither formulation nor carbon tetrachloride received in Group I. Group II received a suspension of CCl_4 in liquid paraffin in a ratio of 2:1 (v/v) in an uniform dose of 1 ml/kg body weight intraperitoneally form day ‘zero’ of the experiment for consecutive 14 days. Group III and IV received CCl_4 1 ml/kg body weight intraperitoneally along with *Silymarin*, in dose 50 mg/kg orally and *Sharbat Chylosin* 1ml/kg body weight of rat respectively for the same 14 consecutive days.

Collection of serum and tissue sample: For sample collection, rats were anesthetized with chloroform followed by cervical decapitation then blood was withdrawn directly from the heart after dissecting the thorax and was allowed to clot for 15−20 minutes. The serum was separated at 3000 rpm (micro centrifuge) for 10 minutes and subjected to biochemical investigations. Then 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.

Assessment of Hepatoprotective activity: The serum was used for assay of biochemical parameters like total bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatise (ALP). Histopathological parameters in the liver architecture like focal necrosis, fatty changes, and inflammatory cell in filtration were evaluated.

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.

3. RESULT AND DISCUSSION

End of study the level of serum total bilirubin, ALT, AST and ALP was showed significant increase in only CCl_4 treated group II when compared with the normal control group that seen in table 2. Histopathological study also showed that high degree of damage characterized by congestion of central vein and portal triads, and cloudy degeneration in group II (fig.1b).

Table 2: Effects of *Sharbat Chylosin* on different biochemical parameters in the serum of rats.

Group	Treatment	Total Bilirubin Mean ±SD	ALT Mean ±SD	AST Mean ±SD	ALP Mean ±SD
Group I	Control	0.20±0.09	61.00±3.46	73.333±7.15	181.00±9.25
Group II	CCl_4 only	2.08± 0.36*	354.50±35.87*	396.83±25.14*	515.00±18.10*
Group III	CCl_4 + Silymarin	0.17 ±0.08*	102.33±13.76*	136.67±11.20*	224.67±12.85*
Group IV	CCl_4+*Sharbat Chylosin*	0.20±0.09*	131.50±14.11*	154.50±11.97*	208.50±14.12*

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 significant measured when p value is *=$p<0.05$, ns= not significant.

Because CCl_4 mediates the changes of liver functions ultimately leads to destruction of hepatocellular membrane. It is biotransformed by Cytochrome P-450 to its active metabolite, the trichloromethyl (CCl_3) radical, which readily reacts with oxygen to form a trichloromethylperoxyl radical (CCl_3O_2). These free radicals generate to cell damage through two mechanisms viz., covalent bonding to cellular macromolecules and peroxidative degradation of membrane lipids and endoplasmic reticulum rich in polyunsaturated fatty acids. This leads to the formation of lipid peroxides, which in turn yield products like malondialdehyde (MDA), that cause loss of integrity of cell membranes and damage to

hepatic tissue. Extent of hepatic damage is assessed by elevated levels of marker enzymes mainly ALT, AST, ALP and bilirubin.[8] On the other hand in group III and group IV the level of serum total bilirubin, ALT, AST and ALP was showed significant decrease when compared with group II. Histological study also showed the progressive recovery against CCl_4 induced damage in group III and IV as compared to normal control. (fig. 1c, 1d).

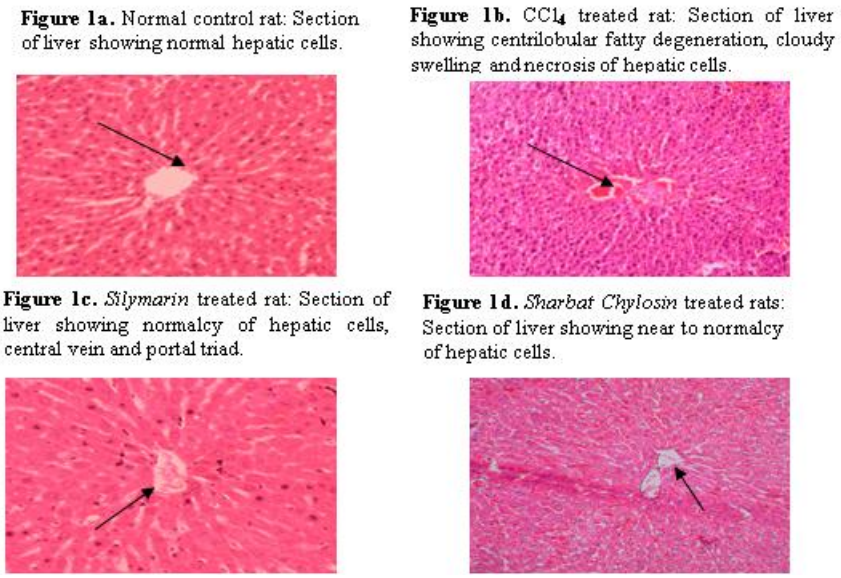

Figure 1: Effect of *Sharbat Chylosin* treatment on CCl_4 induced histopathological changes in rat liver.

Group III was received CCl_4 along with *Silymarin* and it has multiple actions on hepatoprotective action due to its antioxidant property and cell-regenerating functions as a result of increased protein synthesis.[9] The polyherbal formulation *Sharbat Chylosin* was administered in group IV which restored the liver enzyme parameters showing a dose dependent effect. Polyherbal formulation *Sharbat Chylosin* contains the extract of several medicinal plants such as *Foeniculum vulgare,*[10] *Carum roxburghinum, Cichorium intybus,*[11] *Cassia fistula,*[12] *Hygrophila auriculata,*[13] *Boerhaavia diffusa,*[14] *Andrographis paniculata,*[15] *Terminalia chebula,*[16] *Artemisia absinthium and Leonurus cardiaca* that contains specific therapeutically active principles and individually reported for hepatoprotective activity. This combined synergistic action of all ingredients of tested herbal formulation may help to normalize the liver function.

4. CONCLUSION

In conclusion, the results of this study demonstrate that *Sharbat Chylosin* a polyherbal formulation has a potent hepatoprotective effect on CCl_4 induced hepatic damage in rats at

normal dose. In order to confirm their antioxidant potential and to identify various enzymes involved in generating oxygen free radicals further studies are essential.

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