

Evaluation of Hepatoprotective Effect of a Polyherbal Formulation against Carbon Tetrachloride-Induced Hepatotoxicity in Rats

Muhammad Nazmul Huda^{1*} and Ashik Mosaddik²

¹Department of Ayurvedic Medicine, Government Unani and Ayurvedic Medical College and Hospital, Dhaka 1221, Bangladesh

²Department of Pharmacy, University of Rajshahi, Rajshahi, Bangladesh

*Corresponding author: Muhammad Nazmul Huda, Department of Ayurvedic Medicine, Government Unani and Ayurvedic Medical College and Hospital, Section 13, Mirpur, Dhaka 1221, Bangladesh, Tel: 8801712720025, E-mail: mdnazmulhudabd@yahoo.com

Received date: 07 April 2018, Accepted date: 09 April 2018, Published date: 16 April 2018

Copyright: © 2017 Md Nazmul H, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original author and source are credited.

Abstract

Background: Millions of people suffer and die from liver diseases every year in worldwide. Viral hepatitis is a very common problem in Bangladesh however management is problematic and expensive in conventional medicine. So Ayurvedic medicine is way to solve the problem.

Methods: This study was carried out to investigate the hepatoprotective effect of Rohitakarista, a polyherbal formulation against carbon tetrachloride induced hepatotoxicity in rats. Male albino rats (Wistar strain) were divided into 4 groups namely groups I, II, III and IV. Group I served as normal control and received neither formulation nor carbon tetrachloride. Group II received CCl₄ in dose of 1ml per kg body weight intraperitoneally. Group III and IV received CCl₄ 1ml per kg body weight intraperitoneally plus Silymarin, in dose 50 mg/kg orally and Rohitakarista 1ml per kg body weight of rat respectively. After 14 days, blood was obtained for the determination of serum bilirubin, alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase. The histological study was also carried out.

Result: The result showed that polyherbal formulation exhibited a significant hepatoprotective effect when compared with standard Silymarin.

Conclusion: Therefore this study suggests that polyherbal formulation Rohitakarista may have hepatoprotective activity.

Keywords Polyherbal formulation; Rohitakarista; Carbon tetrachloride; Silymarin; Hepatoprotective

Introduction

Liver disease remains one of the serious health problems [1]. Millions of people suffer and die from liver diseases every year. The incidence of different kinds of liver disease like hepatitis, liver cirrhosis, liver cancer and other related diseases are very common in Bangladesh. Most common liver diseases in Bangladesh are different types of viral hepatitis. Virus related liver diseases are important causes of morbidity in Bangladesh [2]. It has been reported that about 7 percent to 10 percent (9.1 million to 13 million) of the population have hepatitis B and at least 2 percent to 3 percent (2.6 million to 3.9 million) have hepatitis C infection. Some have multiple viral infections. Another study showed that about 3.5 percent of pregnant mothers have hepatitis B infection.

About 90 percent of mothers infected with Hepatitis B and are 'e' antigen (HBeAg) positive may transmit this virus to their children [3]. The HBsAg is positive in 7.5 percent of healthy adult jobseekers [2]. So, it is easily understood that only hepatitis B may produce a disastrous health situation in Bangladesh and throughout the world if is not timely controlled. This is a problem of grave concern and needs reliable scientific based management.

Management options for common liver diseases such as cirrhosis, fatty liver and chronic hepatitis are problematic. The effectiveness of the treatment with interferon, colchicines, penicillamine and corticosteroids are inconsistent and found to have profound side effects [4]. But medicinal herbs play a vital role in the management of various liver disorders and there are number of medicinal preparations in Ayurveda recommended for the treatment of liver disorders [5].

Medicinal plants are widely used all over the world including Bangladesh for production of both traditional and modern drugs, and development of new drugs. There are more than 300 traditional medicine manufacturers, including Unani, Ayurvedic and Homeopathy in Bangladesh. Maximum companies produce their patent drugs according to National Formulary. They claim that some of their drugs are effective in various hepatic disorders. Most of these claims are based on old literature, folk sayings, occasional experiences, and traditional uses, but not on any significant clinical or pharmacological studies and statistical data.

So, the present study was undertaken to investigate the effects of one polyherbal formulation named Rohitakarista on the status of hepatoprotection and release of marker enzymes in the serum after inducing hepatotoxicity by administering carbon tetrachloride (CCl₄) in experimental rats.

Materials and 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, Rajshahi University, 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 work was done at the University of Rajshahi, Bangladesh

Tested formulation

Rohitakarista is a polyherbal hepatoprotective Ayurvedic medicine. It contains the aqueous extract of ten indigenous medicinal plants (Table 1). It was collected from local market, Bangladesh [6].

Plant	Amount (gm)
<i>Aphanamixis polystachya</i> (Wall.) Parker	1.52
<i>Woodfordia fruticosa</i> (Linn.) Kurz.	0.24
<i>Piper longum</i> Linn. (Root)	0.15
<i>Piper longum</i> Linn. (Seed)	0.15
<i>Zingiber officinale</i> Rosc.	0.15
<i>Cinnamomum tamala</i> Ness.	0.15
<i>Cinnamomum zeylanicum</i> Bl.	0.15
<i>Elettaria cardamomum</i> (Linn.) Maton.	0.15
<i>Phyllanthus emblica</i> Linn.	0.15
<i>Terminalia chebula</i> (Gaertn.) Retz.	0.15
<i>Terminalia bellerica</i> Roxb.	0.15

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

Table 1: Composition of 5 ml of Rohitakarista Using Extracts from Medicinal Plants.

Experimental animals

Long-Evans albino male of age about 9 weeks were collected from International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDR, B). These were reared for three weeks. At the end of three weeks of rearing, these rats when rats weighing about 150 gm to 200 gm were examined for inclusion in the study. The animals were housed in clean metabolic cages and maintained in controlled temperature ($27 \pm 2^\circ\text{C}$) and light-dark cycle (12 h light and 12 h dark). They were fed with 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 rats. Group I served as normal control and received neither formulation nor carbon tetrachloride 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 an uniform dose of 1 ml per kg body weight intraperitoneally from day 'zero' of the experiment for consecutive 14 days.

Group III and IV received CCl_4 1 ml/kg body weight intraperitoneally plus Silymarin, in dose 50 mg/kg orally and Rohitakarista 1 ml/kg body weight of rat respectively for the same 14 consecutive days.

Collection of serum and tissue sample

End of treatment rats were euthanized after being lightly anesthetized with chloroform 30 ppm followed by cervical decapitation. After anesthetized blood was withdrawn directly from the heart after dissecting the thorax. Blood was allowed to clot and centrifuged for 15 minutes to 20 minutes at 3,000 rpm to separate out serum. 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 phosphatase. Histopathological study of liver architecture like focal necrosis, fatty changes and inflammatory cell in filtration was evaluated.

Statistical analysis

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

Results

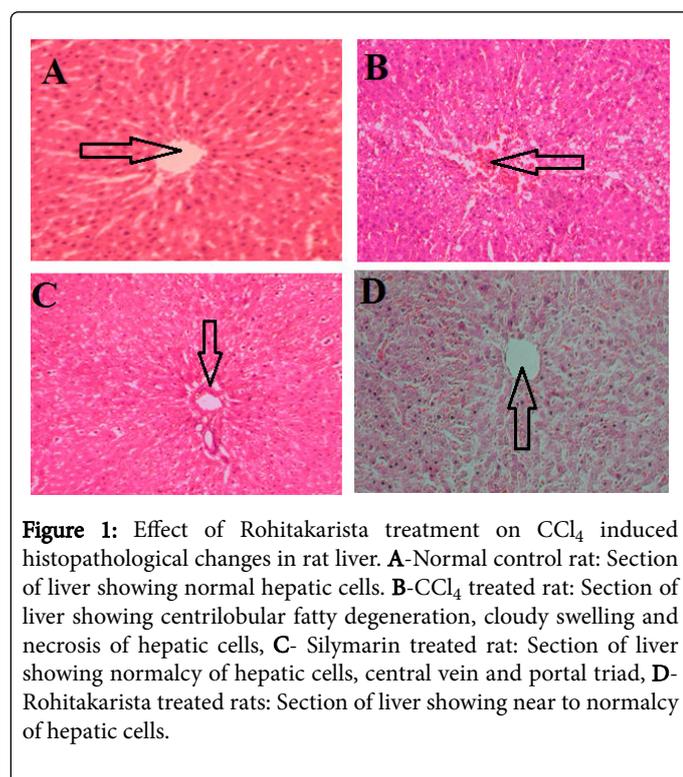
From Table 2 showed that only CCl₄ treated rats developed significant hepatocellular damage as evidenced from increase in serum levels of total bilirubin, ALT, AST and ALP in group II when compared with the normal control group. Treatment with Silymarin 50 mg/kg body weight in group III and compound formulation Rohitakarista 1 ml per kg body weight in group IV caused reduction in increased serum levels of total bilirubin ALT, AST, ALP when compared to group

II. Histological section of normal control group I showed normal hepatic cells with well-preserved cytoplasm, prominent nucleus and conspicuous central vein (Figure 1A). Only CCl₄ treated group II showed that high degree of damage characterized by congestion of central vein and portal triads, and cloudy degeneration (Figure 1B). Histological section of group III and IV showed the progressive recovery against CCl₄ induced damage as compared to normal control. (Figures 1C and 1D).

Group	Treatment	Total Bilirubin	ALT	AST	ALP
		Mean ± SD	Mean ± SD	Mean ± SD	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 ± 13.77*	136.67 ± 11.20*	224.67 ± 12.82*
Group IV	CCl ₄ + Rohitakarista	0.15 ± 0.06*	94.67 ± 18.96*	128.17 ± 9.33*	212.17 ± 19.02*

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 followed by Student's t test when p value is *p<0.05, ns= not significant

Table 2: Effects of Rohitakarista on different biochemical parameters in the serum of rats.



Discussion

In the present study liver enzymes level and liver histopathology of the rats were evaluated in order to explore the polyherbal formulation Rohitakarista against CCl₄ induced hepatotoxicity in rats. CCl₄ is well known hepatotoxin which is widely used to induce toxic liver injury in laboratory animals. Carbon tetrachloride is metabolically activated by the cytochrome P-450 dependent mixed oxidase in the endoplasmic

reticulum to form trichloromethyl free radical (CCl₃) which combined with cellular lipids and proteins in the presence of oxygen to induce lipid peroxidation. These result in changes of structures of the endoplasmic reticulum and other membrane, loss of metabolic enzyme activation, reduction of protein synthesis and loss of glucose -6-phosphatase activation, leading to liver injury [7].

This is evidenced by an elevation in the serum marker enzymes namely ALT, AST and ALP by CCl₄ [8]. The elevation of liver enzyme especially ALT has more importance as a specific marker of liver injury due to toxic drugs, alcohol and virus [9]. In this study it showed that treatment with CCl₄ only significantly increased the serum enzyme levels, namely ALT, AST and ALP that indicates chemical induced hepatocellular toxicity in group II. But post treatment with Silymarin and polyherbal formulation Rohitakarista in group III and group IV respectively restored the liver enzyme parameters showing a dose dependent effect. The protective effect may be the result of stabilization of plasma membrane thereby preserving the structural integrity of cell as well as the repair of hepatic tissue damage caused by CCl₄.

As observed in present study CCl₄ treatment produced various histological changes in the hepatocytes including centrilobular necrosis with congestion of sinusoids, ballooning degeneration, cell inflammation, and infiltration of inflammatory cells. Treatment with Rohitakarista prevented CCl₄ induced change in the hepatic architecture and protected the liver tissue from necrotic, fatty and degenerative change. This might be the hepatoprotective effect of Rohitakarista attributed to its herbal ingredients which possess very potent antioxidant and hepatoprotective phytoconstituents and their combined synergistic action of all the ingredients helps to normalize the liver function and thus cure complex liver disorders.

In conclusion it justified that Rohitakarista is a potent hepatoprotective formulation against CCl₄ induced hepatotoxicity in rats. So it can be used in drug induced hepatotoxicity but phytochemical study is recommended for further studies.

References

1. Selvakumar D, Natarajan P (2008) Hepato-protective activity of *Moringa oleifera* Lam leaves in carbon tetrachloride induced hepato-toxicity in albino rats. *Phcog Mag* 4: 97–98.
2. Khan M, Ahmad N (1996) Seroepidemiology of HBV and HCV in Bangladesh. *Intern Hepatol Commun* 5:27–29.
3. Liver Foundation of Bangladesh (2005) Liver disease: Bangladesh perspective.
4. Luper S (1998) A review of plants used in treatment of liver disease: Part 1. *Altern Med Rev* 3 (1998): 410–421.
5. Samudram P, Hari R, Vasuki R, Geetha A, Sathiyamoorthi P (2008) Hepatoprotective activity of bi-herbal ethanolic extract on CCl₄ induced hepatic damage in rats. *Asian J Biochem Res* 2: 61-65.
6. Rohitakarista in Bangladesh National Formulary of Ayurvedic medicine (1992) Dhaka (Bangladesh): Board of Unani and Ayurvedic System of medicine.
7. Ahsan MR, Islam KM, Bulbul IJ, Musaddik MA, Haque E (2009) Hepatoprotective activity of methanol extract of some medicinal plants against carbon tetrachloride-induced hepatotoxicity in rats. *Euro J Sci Res* 37: 302-310.
8. Bhattacharyya D, Pandit S, Mukherjee R, Das N, Sur TK (2003) Hepatoprotective effect of Himoliv®, A Polyherbal formulation in Rats. *Indian J Physiol Pharmacol* 47: 435–440.
9. Girish C, Koner BC, Jayanthi S, Rao KR, Rajesh B, et al. (2009) Hepatoprotective activity of six polyherbal formulations in paracetamol induced liver toxicity in mice. *Indian J Med Res* 129: 569-578.