

## EVALUATION OF HEPATOPROTECTIVE ACTIVITY OF *BERBERIS ARISTATA* AGAINST CARBON TETRACHLORIDE INDUCED HEPATOTOXICITY IN RATS

UNKESHWAR P<sup>1</sup>, NASIRUDDIN M<sup>2</sup>, FAYAZUDDIN M<sup>2</sup>, KHAN RA<sup>2</sup>, KHAN AA<sup>3</sup>, TAJUDDIN<sup>4</sup>

<sup>1</sup>Department of Pharmacology, Rama Medical college, Ghaziabad, <sup>2</sup>Department of Pharmacology, <sup>3</sup>Department of Anatomy, J.N. Medical College, A.M.U, Aligarh and <sup>4</sup>Department of Saidla, A.K.Tibbiya Collage, A.M.U, Aligarh, 202002. Email: naseer\_bettiah@yahoo.co.in

Received: 16 July 2013, Revised and Accepted: 01 Oct 2013

### ABSTRACT

**Objective:** To evaluate the hepatoprotective activity of *Berberis aristata* against CCl<sub>4</sub>-induced hepatotoxicity in rats.

**Materials and Methods:** Twenty four Wistar albino rats of either sex, weighing 150-200g were procured from Central Animal House of J. N. Medical College, A.M.U. Aligarh. Ethanolic extract of *Berberis aristata* was prepared with the help of Soxhlet's apparatus. Rats were divided into four groups of 6 animals each. Group I was administered distilled water in the dose of 1 ml/kg, orally, daily for 8 days. Group II was also administered distilled water in the dose of 1 ml/kg, orally, daily for 7 days. Silymarin (100 mg/kg) daily was used as standard drug per oral for 7 days in group III. Ethanolic extract of *Berberis aristata* was administered in the dose of 400 mg/kg/day orally for 7 days in group IV. Carbon tetrachloride was administered in the dose of 2ml/kg as 1:1 mixture with liquid paraffin i.p. on 7<sup>th</sup> day along with their routine treatment to induce hepatotoxicity in animals of all groups except group I. On 8<sup>th</sup> day rats were sacrificed and hepatoprotective effect was assessed on the basis of biochemical parameters such as serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), alkaline phosphatase and total bilirubin along with histopathological examination of liver.

**Results:** Treatment of animals with ethanolic extract significantly reduced SGPT, SGOT, alkaline phosphatase and total bilirubin as compared with the carbon tetrachloride treated groups. Histological examination of the liver sections demonstrated the preservation of architecture of hepatic cords, reduction of necrosis and inflammatory cell infiltration in the extract treated group as compared with the carbon tetrachloride treated groups further confirming the protective effect of *Berberis aristata*.

**Conclusion:** The ethanolic extract of stem of *Berberis aristata* showed significant hepatoprotective activity against Carbon-tetrachloride induced hepatotoxicity.

**Keywords:** *Berberis aristata*, Carbon tetrachloride, Silymarin, Hepatoprotective

### INTRODUCTION

Liver is a vital organ of the body which maintains metabolic homeostasis. These functions include the synthesis of most essential serum proteins, regulation of nutrients, production of bile and metabolism of xenobiotics. As the primary organ for detoxification of endogenous as well as exogenous compounds, the liver is also a major site for their assaults resulting in distortion of metabolic functions. Liver injury may follow the inhalation, ingestion, or parenteral administration of a number of pharmacological and chemical agents. These include industrial toxins like, carbon tetrachloride, trichloroethylene, and yellow phosphorus, and pharmacological agents like paracetamol, rifampicin, isoniazid etc. used in medical therapy. Hepatotoxic drugs can injure the hepatocyte by generating free-radical or metabolic intermediate that causes peroxidation of lipid membrane resulting in liver cell injury [1].

Efforts have been made to search for effective hepatoprotective agents however no effective hepatoprotective agent is available in mainstream medicine till date, while in traditional system of medicine a number of herbal drugs are successfully being used in the treatment of liver disorders and are scientifically evaluated for their safety and efficacy [2]. Treatment options for common liver diseases such as drug induced hepatitis, fatty liver and chronic hepatitis are very few and only supportive. The effectiveness of agents available for the treatment of liver diseases are inconsistent and have greater incidence of side-effects [3]. In the absence of reliable hepatoprotective drugs in modern medicine, a number of medicinal plants and their formulations are used for the treatment of hepatic disorders in traditional systems of medicine in India. *Berberis aristata* is deciduous large shrub, belongs to the family Berberidaceae. It is commonly known as Indian barberry. It is distributed in the temperate and Sub-tropical parts of Asia, Europe and America. its occurrence is reported from middle altitude areas (1800-3000 m) of the state of Uttarakhand and Himachal Pradesh [4].

The stem or wood, root-bark and extract of Indian Barberry have been used in Indian traditional Medicine from a very remote period and its properties are said to be analogous turmeric [5]. It is reported to have antioxidant, antidiabetic and anti-inflammatory activity [6-7]. So the present study was done for scientific evaluation of hepatoprotective activity of *Berberis aristata* against carbon tetrachloride-induced toxicity in rats.

### MATERIALS AND METHODS

#### Plant Collection and Extraction

The stems of *Berberis aristata* were procured from Ramnagar (Uttarakhand). The plant material was identified and authenticated (Certificate no.1457/55 dated 16 July 2010) by a botanist, Professor H.B. Singh, Head of Raw Materials Herbarium & Museum (RHMD) of National Institute of Science Communication & Information Resources (NISCAIR), New Delhi. Stems of plant were brought in one batch. The plant material was further dried under the shade and was later finely powdered with a special herbal grinder, and was stored in air-tight bottles till use. 100g of powder was extracted in 400 ml ethanol for 72 hours with the help of Soxhlet's apparatus. The extract was dried at -40°C in a freeze dryer.

#### Chemicals

All chemicals used were of analytical grade. The kits for the estimation of serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), alkaline phosphatase (ALP) and total bilirubin (TB) were purchased from Siemens, Mumbai. Carbon tetrachloride (CCl<sub>4</sub>) was purchased from Thomas Baker Pvt. Ltd. Mumbai and Silymarin was purchased from Sigma-Aldrich, USA.

#### IAEC Approval

The study protocol was approved by the IAEC, J. N. Medical College, AMU, Aligarh, U. P. with registration number 401/CPCSEA. All animal experiments were carried out as per the

rules and regulations of IAEC & CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) of under the "Guidelines for Care and Use of Animals in Scientific Research".

### Animals

Twenty four Wistar albino rats of either sex, weighing 150-200g were procured from Central Animal House of J.N. Medical College, A.M.U. Aligarh. They were housed in polypropylene cages at ambient temperature ( $25 \pm 2^\circ\text{C}$ ), relative humidity ( $55 \pm 5\%$ ) and 12-hr light-dark cycle. Animals had free access to standard pellet diet and water ad libitum.

### Experimental design

Rats were divided into four groups of 6 animals each

Group I (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 (Positive control): Silymarin (100mg/kg) orally daily for 7 days.

Group IV (Test group): Ethanolic extract of *Berberis aristata* in dose of 400mg/kg suspended in distilled water orally daily for 7 days.

On the 7<sup>th</sup> day, animals of all groups except group I were administered  $\text{CCl}_4$  in a dose of 2 ml/kg, i.p. ( $\text{CCl}_4$  in paraffin 1 : 1 v/v) along with their routine treatment. On 8<sup>th</sup> day all the animals including group I were sacrificed under ether anesthesia..

### Collection of Samples

The blood was collected and 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 SGOT, SGPT, total bilirubin and alkaline phosphatase [8-10].

### Histological Examination

The liver of rats of all groups was removed immediately and fixed in 10% formalin. 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 [11].

### Statistical Analysis

All the values are presented as Mean  $\pm$  Standard Error of Mean (SEM). Statistical significance was calculated by one way ANOVA followed by post hoc Dunnett's test multiple comparison test  $p < 0.05$  was considered to be statistically significant.

### RESULTS

Administration of  $\text{CCl}_4$  to the rats in negative control group (II) resulted in a significant increase in total bilirubin, SGOT and SGPT, and serum ALP when compared with control group (I) ( $P < 0.01$ ). Administration of silymarin and ethanolic extract of *B. aristata* in group III and group IV respectively prevented the rise in total bilirubin, SGOT and SGPT, and serum ALP when compared with negative control group (II) ( $P < 0.01$ ). All the parameters were significantly decreased in the extract treated group as compared to negative control group following  $\text{CCl}_4$  administration and were comparable with that of standard drug silymarin treated group (Table-1).

**Table 1: Effect of *Berberis aristata* on biochemical parameters in  $\text{CCl}_4$  induced toxicity.**

Groups	SGPT (IU/L)	SGOT (IU/L)	S.ALP (IU/L)	Total Bilirubin (mg/dL)
Group-I Control	32 $\pm$ 2.5	32 $\pm$ 2.0	39 $\pm$ 4.3	0.73 $\pm$ 0.02
Group-II Negative control	137 $\pm$ 4.2*	125 $\pm$ 3*	80.17 $\pm$ 3.8*	0.83 $\pm$ 0.04*
Group-III Positive control	47 $\pm$ 2.3**	50.5 $\pm$ 3.3**	46.33 $\pm$ 2.9**	0.65 $\pm$ 0.02**
Group-IV Test group	65 $\pm$ 3.3**	58.5 $\pm$ 2.8**	52.5 $\pm$ 1.8**	0.72 $\pm$ 0.02**

Values are mean  $\pm$  SEM; N = 6;

\* $P \leq 0.01$  compared with Group I; \*\* $P \leq 0.05$  compared with Group II,

SGOT - serum glutamicoxaloacetic transaminase; SGPT- serum glutamic pyruvic transaminase, S.ALP- Serum alkaline phosphatase

### Histological Examination of liver [Figure1]

**Group I:** Normal control group showed central blood vessels and radiating cords of hepatocytes as well as the vascular sinusoids with no evidence of fatty changes, necrosis or inflammation. (A)

**Group II:**  $\text{CCl}_4$  treated animals showed centrilobular (acidophilic) necrosis and vascular congestion. (B)

**Group III:** Silymarin treated rats showed mild vascular congestion and peri-vascular infiltrate of mono nuclear cells and fibroblast. No fatty degeneration was observed. (C)

**Group IV:** Ethanolic extract treated rats showed well preserved liver architecture, no fatty degeneration, only mild vascular congestion and perivascular infiltrate of mono nuclear cells and fibroblast and regenerating hepatocytes were observed. The hepatic architecture was found similar to that observed in silymarin treated group (D).

(A) Normal Control, (B) Negative Control, (C) Positive control: Silymarin 100mg/kg (D) Test group: Ethanolic extract 400mg/kg.

### DISCUSSION

Carbon Tetrachloride induced liver injury is a commonly used model for screening of hepatoprotective drugs [12]. The toxicity caused by

$\text{CCl}_4$  is by the generation of trichloromethyl ( $\text{CCl}_3^*$ ) free radicals in the body which results in oxidative damage of cell organelles and membrane. This radical can also react with oxygen to form the trichloromethylperoxy radical  $\text{CCl}_3\text{OO}^*$ , which initiates the chain reaction of lipid peroxidation. Since the changes associated with  $\text{CCl}_4$  induced liver toxicity were similar to that of acute viral hepatitis.  $\text{CCl}_4$  mediated hepatotoxicity was taken here as the experimental model for inducing liver injury.  $\text{CCl}_4$  is a potent hepatotoxin which produces centrilobular hepatic inflammation and necrosis [13].

$\text{CCl}_4$  induced liver damage is quantified by measuring the levels of serum enzymes like SGOT, SGPT and ALP, which are released into the blood from damage hepatocytes. They are also the indicators of liver damage [14]. After administration of drugs, normalisation of the elevated markers indicates that these drugs have been able to induce regeneration of liver cells thereby reducing the leakage of the enzymes into the blood. It has been reported that the healing of liver parenchyma and hepatocytes results in return of serum transaminases to normal levels [15]. 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 [16]. The hepatoprotective properties of Silymarin have been

related to the inhibition of lipid peroxides formation or scavenging of free radicals [17]. As shown in Figure B, CCl<sub>4</sub> causes change around the central vein in the liver and other oxidative damages with the leakage of marker enzymes like SGOT, SGPT and ALP in the serum and increase in serum total bilirubin levels [18-19]. Administration of extracts of *B. aristata* stems showed significant hepatoprotective activity, which was comparable with the standard drug silymarin. The phytochemical studies on *B. aristata* have shown the presence of alkaloids, flavonoids, glycosides, saponins and sterols. Furthermore, it has been reported that the

flavonoids constituents of plant possess anti-inflammatory and antioxidant properties and was found to be useful in the treatment of liver damage [20-21]. The results indicate that the ethanolic extract of *B. aristata* has significant hepatoprotective activity. This may be probably due to the higher content of flavonoids like luteolin, rutin, and apigenin. The earlier investigators have screened the hepatoprotective activity of the flavonoid compound, rutin, which is also claimed to have free radical scavenging property and it inhibits the lipid peroxidation against CCl<sub>4</sub>-induced hepato toxicity [22].

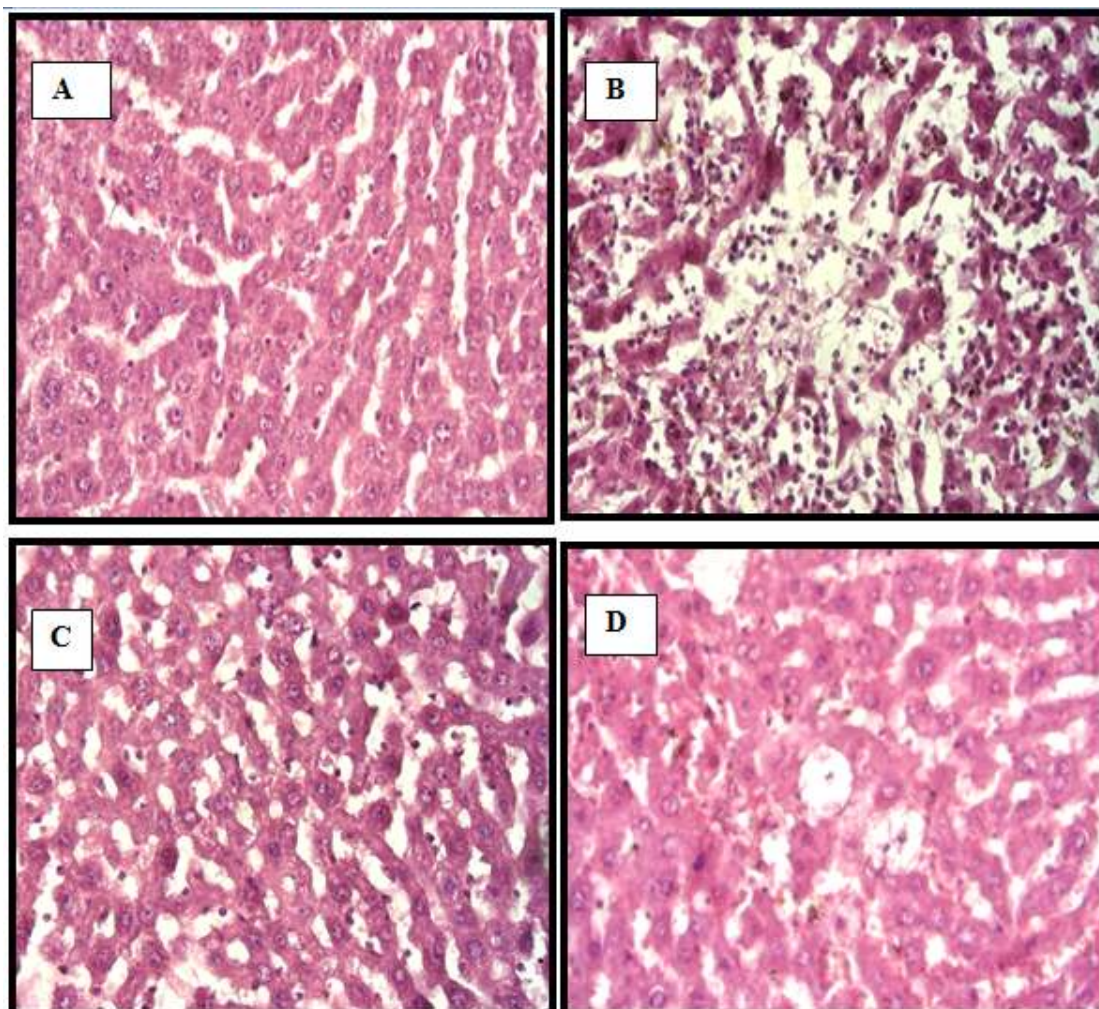


Fig. 1: Histological Examination of liver.

## CONCLUSION

In view of the findings of the study it can be concluded that ethanolic extract of stem of *B. aristata* possesses significant hepatoprotective effect against CCl<sub>4</sub>-induced hepatotoxicity.

## REFERENCES

1. Gupta NK, Lewis JH. Review article: The use of potentially hepatotoxic drugs in patients with liver disease. *Aliment Pharmacol Ther.* 2008; 23: 1021-1041.
2. Subramoniam A, Pushpangadan P. Development of phytomedicines for liver diseases. *Indian J Pharmacol.* 1999; 31: 166-75.
3. Manoj, Ahmad F, Kumar A, Yunus SM. Screening of hepatoprotective activity of Ethanolic extract of stem bark Of *Bauhinia variegata* in rats. *Int J Pharm Pharm Sci.* 2013;5: 624-628.
4. Chopra. RN, Chopra IC, Handa, KL, Kapur, LD. *Chopra's Indigenous Drugs of India.* N. N. Dhar and Sons Pvt. Ltd, Calcutta-12; 1958: p. 289.
5. Kirtikar KR, Basu BD. *Indian Medicinal Plants.* International Book Publications, Dehradun, India; 1995: p. 102-103.
6. Gupta SK, Agarwal R, Srivastava S, Agarwal P, Agrawal SS, Saxena R, Galpalli N. The anti-inflammatory effects of *Curcuma longa* and *Berberis aristata* in endotoxin-induced uveitis in rabbits. *Invest Ophthalmol Vis Sci.* 2008 Sep; 49(9):4036-40.
7. Singh J, Kakkar P. Antihyperglycemic and antioxidant effect of *Berberis aristata* root extract and its role in regulating carbohydrate metabolism in diabetic rats. *J Ethnopharmacology.* 2009 May 4; 123(1): 2-6.
8. Reitman S, S. Franke. A colorimetric methods for the determination of serum levels of glutamic oxaloacetic acid and pyruvic acid transaminases. *Am.J.Clin. Pathol.* 1957; 10: 394-399.

9. Jendrassik-Gróf method on photometric systems for in vitro determination of direct and total bilirubin. *Biochem Zeitschrift*. 1938; 297: 82-9.
10. Walton H, Marsh, Benjamin Fingerhut, Elaine Kirsch. Adaption of alkaline phosphatase method for automatic colorimetric analysis. *Clinical Chemistry*. 1959; 5: 119-126.
11. Mukherjee KL. *Medical Laboratory Technology*. Tata McGraw Hill Publishing Company. 1988; p.1111-1124.
12. Slater TF. Biochemical studies on liver injury. In *Biochemical mechanism of liver injury*. London, Academic press. 1965; p. 1-44.
13. Dhuley JN, Naik SR. Protective effect of Rhinax, a herbal formulation, against CCl<sub>4</sub> induced liver injury and survival in rats. *J Ethnopharmacol*. 1997; 56: 159- 64.
14. Pratt SD, Kaplan MM. Evaluation of liver function. *Harrison's Principles of Internal Medicine*. 16<sup>th</sup> ed. McGraw-Hill. 2005; p.1813-15.
15. Molander DW, Sheppard E, Payne MA. Serum transaminase in liver disease. *J Am Med Assoc*. 1957;163(16):1461-1465.
16. Choski S, Patel SS, Saluja AK. Silymarin: A promising herbal hepatoprotective drug. *Indian Drugs*. 2000; 37 (12): 566-569.
17. Ferenci P, Dragasics B, Dittrich H. Randomized controlled trial of Silymarin treatment in patients with cirrhosis of the liver. *Journal of Hepatology*. 1989; 9: 105-113.
18. Recnagel RO, Carbon tetrachloride hepatotoxicity status and future prospects. *Pharmacol Sci*, 1983; 4:129-31.
19. Okuno H, Hazama H, Muraze T, Shiozaki Y, Sameshima Y. Drug metabolizing activity in rats with chronic liver injury induced by carbon tetrachloride: Relationship with the content of hydroxyproline in the liver. *Japanese J Pharmacology*. 1986; 41:363-71.
20. Hesham RE, Shgeru N. Chemistry of Bioflavonoids. *Indian J Pharm Educ*. 2002; 36:191-194.
21. Fayazuddin M, Ahmad F, Kumar A, Yunus SM. An experimental evaluation of anti-inflammatory activity of *Moringa oleifera* seeds. *Int J Pharm Pharm Sci*. 2013; 5(3): 717-721.
22. Khalid HJ, Sheikh As, Anwar HG. Protective effect of rutin on paracetamol and CCl<sub>4</sub> induced hepatotoxicity in rodents. *Fitoterapia*. 2002; 73:557-63.