

Original Article

Hepatoprotective activity of ethanolic extract of stem bark of *Berberis aristata* against carbon tetrachloride (CCl₄) induced hepatotoxicity on albino wistar rats

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ABSTRACT

Berberis aristata DC (Berberidaceae) is used in Indian traditional medicine. The hepatoprotective activity of *Berberis aristata* stem bark suspension was studied using CCl₄ overdose induced liver damage in rats. The activity was assessed by monitoring various liver functions testing enzymes viz, Serum Glutamate Pyruvate transaminase (SGPT), Serum Glutamate Oxaloacetate Transaminase (SGOT), Serum Alkaline Phosphates (SALP) and total and direct bilirubin in blood serum. When the vehicle treated group 1 was compared with control group 2 (treated with CCl₄), there was a significant rise in the level of serum marker enzyme ALP, but no significant difference observed between the vehicle treated group and standard drug treated group. Increased levels of serum SGOT, SGPT, ALP and Bilirubin were observed in CCl₄ treated control group. But in different dose levels of EEBA (100 & 300 mg/kg of body weight) treated groups, a significant ($p < 0.001$) reduction of serum SGOT (94.5 ± 3.09 , 90.20 ± 0.04), SGPT (45.67 ± 1.58 , 43.65 ± 0.08), ALP (61.23 ± 1.59 , 58.8 ± 3.25) and bilirubin (total & direct 1.30 ± 0.02 , 1.10 ± 0.01) & (0.81 ± 0.02 , 0.53 ± 0.03) were observed.

1. INTRODUCTION

The liver is the key organ of metabolism, secretion and excretion which is continuously and widely exposed to xenobiotics, environmental pollutants and chemo therapeutic agents because of its strategic location in the body [1]. Liver diseases remain one of the major threats to public health and are a worldwide problem [2]. The hepatotoxicity mainly caused by toxic chemicals such as carbon tetra chloride (CCl₄), alcohol, drugs such as paracetamol, antidiabetic drugs. Most of the chemicals damage the liver cell mainly by inducing lipid peroxidation and other oxidative damages [3].

Conventional drugs used in the treatment of liver diseases are sometimes inadequate and can have serious adverse effects. It is, therefore, necessary to search for alternative drugs for the treatment of liver disease to replace currently used drugs of doubtful efficacy and safety [1]. The use of plant extracts for the treatment for liver diseases are now on the increase. Plants that were once considered of no value are now being

investigated, evaluated and developed in to drugs with no side effects [3]. One of such plant is *Berberis aristata* which is a spinous shrub native to northern Himalayan region. It is widely distributed fro Himalaya to Srilanka, Bhutan and hilly areas of Nepal in Himalayan region. It grows at a height of about 2000-3000 m especially in Kumaon and Chammba region of Himachal Pradesh. It is also found in Nilgiris hills in south India. It is used in Ayurvedic medicines from very long time. The plant is used traditionally in inflammation, wound healing, Skin disease, menorrhagia, diarrhea, joundice and affection of eyes. Its properties are said to be analogous to those of turmeric. A very valuable ayurvedic preparation “Rashut” is prepared by this plant [4].

2. EXPERIMENTAL

Collection and Authentication of the Plants

Samples of the whole plant of *Berberis aristata* was obtained from Bhimtal (Uttarakhand) in the month of April-May 2011

and identified by Dr. Tirath Kumar, Asst. Professor, Dept. of Pharmaceutical Science, Kumaon University, Bhimtal (Uttarakhand). The plant material was dried, powdered and store in air tight container for further studies.

Preparation of the Extracts

The barks of *Berberis aristata* were collected and dried at room temperature and coarsely powdered. The dried powders were defatted using petroleum ether (60-80°C) and subjected to extraction by ethanol in a Soxhlet apparatus. The extracts were distilled and concentrated under reduced pressure until all solvent has been removed to give an extract sample and dried completely. Extracts were stored in vacuum desiccators.

Experimental Animals

Male albino wistar rats (150-250 g) were obtained from the approved animal house of School of Pharmacy, Bharat Institute of Technology, Meerut, (India) after obtaining approval from Institute's Animal Ethical committee. They were housed in standard environmental condition (at room temperature 25±2°C and 50±5% relative humidity) in standard polypropylene cage and maintained on standard pellets, germinated grams and water *ad libitum*. Prior to experimentation the animals were fasted for 12 h but free access to drinking water.

Acute Toxicity Studies

Acute oral toxicity study was conducted as per the OECD guideline 425 [5]. Male albino wistar rats (150-200 gm) maintained under standard laboratory condition was used. A total of five animals were used for this study which received a single oral dose (1000 mg/kg, Body weight) of the extract. Animals were kept overnight fasting prior to drug administration. After the administration of the extract, food was withheld for further 3-4 hrs. animals were observed individually once during the first 30 minutes after dosing, periodically during of 14 days. Once daily cage side observations included changes in eyes and mucus membrane, skin and fur, respiratory rate, circulatory, autonomic, CNS changes and gross pathological examinations were carried out.

Dose Selection

LD₅₀ was determined as per the OECD guideline 425 for fixing the dose for pharmacological evaluation. There were no signs of toxicity at the dose of 1000 mg/kg. Hence, the pharmacological evaluation of the extract was carried out at two dose levels of 100 mg/kg and 300 mg/kg.

Experimental Design of Hepatoprotective Activity

Albino wistar rats (150-250 g) were randomly divided into five groups with five animals in each group where Group I served as vehicle treated rats received 0.5% SMC (2 ml/kg of body

weight) once daily orally. Group II served as carbon tetrachloride treated rats received (0.5 ml/kg of body weight) once daily I.P. Group III served as Standard treated rats received (100mg/kg body weight) once daily orally. Group IV & V served as test drug treated group which received 100 mg/kg and 300 mg/kg dose of ethanolic extract of *Berberis aristata* Stem bark (EEBA) once daily orally.

Blood Collection and Serum Separation

Blood from the retro-orbital plexus was collected and centrifuged at 3000 rpm for 10 minutes.

Estimation of Biochemical Parameter

The biochemical parameters like serum enzyme were estimated. They include Serum glutamic oxaloacetic transaminase (SGOT, AST) and Glutamic pyruvic transaminase (SGPT, ALT), serum alkaline phosphatase (SALP) and Bilirubin by using Auto-analyzer (RMS, model no.BCA-201) [6].

3. RESULTS AND DISCUSSION

On comparison of standard drug Silymarin 100 mg/kg and the ethanolic extract of *Berberis aristata* (stem bark) of doses 100 and 300 mg/kg treated group IV & V, it was found that ethanolic extract showed a significance result against CCl₄ induced alteration in the serum enzyme levels (Table 1).

Table 1. Effect of ethanolic Extract of *Berberis Aristata* (Stem bark) on serum biochemical parameters

Treat-ment	SGOT (U/ml)	SGPT (U/ml)	ALP KA unit	Total bilirubin mg/dl	Direct bilirubin mg/dl
Control	89.3 2± 4.19	41.3 ± 3.05	55.5 ± 1.35	0.35 ± 0.05	0.48 ± 0.01
Silymarin 100 mg/kg	87.90 ± 2.39*	30.8 ± 1.20*	45.39 ± 1.28*	0.28 ± 0.03*	0.30 ± 0.03*
CCl ₄ control	138.52 ± 11.03*	152.2 ± 13.01*	90.38 ± 9.09*	4.90 ± 0.09*	0.99 ± 0.01*
EEBA 100 mg/kg	94.51 ± 3.09*	45.67 ± 1.58*	61.23 ± 1.59*	1.30 ± 0.02*	0.81 ± 0.02*
EEBA 300 mg/kg	90.20 ± 0.04*	43.65 ± 0.08*	58.8 ± 3.25*	1.10 ± 0.01*	0.53 ± 0.03*

N = 6, Values are Mean ± SEM **P<0.01(significant), values are compared with control group.

Carbon tetrachloride (CCl₄) is a potent hepatotoxin, and on a single exposure to liver it can rapidly lead to an increase in the level of several enzymes, severe centrilobular necrosis and steatosis [7]. The hepatotoxicity induced by CCl₄ is due to its metabolite CCl₃[·], a free radical which binds to lipoprotein and leads to peroxidation of lipids of the endoplasmic reticulum [8]. The ability of a hepatoprotective drug to reduce the

injurious effects or to preserve the normal hepatic physiological mechanisms, which have been disturbed by a hepatotoxin, is the index of its protective effects. The lowering of enzyme level is a definite indication of the hepatoprotective action of the drug. Carbon tetrachloride induced hepatic damages was observed by recording SGOT, SGPT, ALP and bilirubin levels in different groups [9]. The transport function of the hepatocytes is disturbed in hepatic injury, causing the leakage of enzymes due to altered membrane permeability [10].

4. CONCLUSION

In case of the hepatoprotective activity, the EEBA was tested against hepatotoxicity in rats induced by carbon tetra chloride (CCl_4). Liver functions were assessed by the determination of SGOT, SGPT, ALP and bilirubin. The serum biochemical analysis results suggest that the use of ethanolic extract of *Berberis aristata* exhibit significant protective effect from hepatic damage in CCl_4 induced hepatotoxicity model.

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