IN VITRO HEPATOPROTECTIVE ACTIVITY OF *BAUHINIA VARIEGATA*

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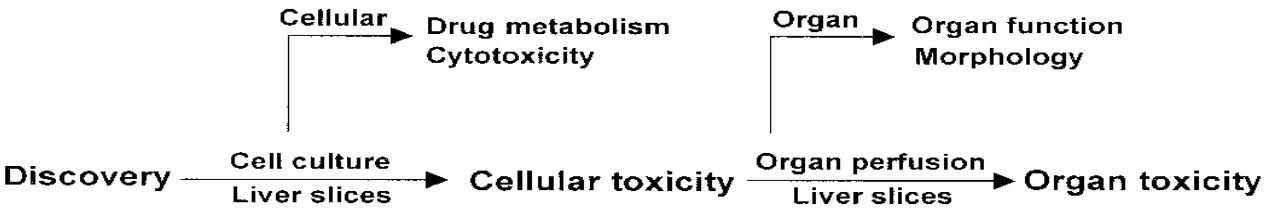
Summary

Bauhinia variegata (Family *Leguminose or Fabaceae (Pea Family)*, Genus *Bauhinia*) is an herbaceous plant, found throughout India. The plant is known as Kachnara in Sanskrit, and Kachnar in Hindi. A paste of the bark is useful in the treatment of cuts and wounds, skin diseases, scrofula and ulcers. We have used liver slice culture model to demonstrate hepatoprotective activity of BVLE *in vitro*. CCl_4 (20mM) has been used as a hepatotoxin and the cytotoxicity of CCl_4 is estimated by quantitating the release of lactate dehyrogenase LDH in the medium. CCl_4 induces twice the amount release of LDH from the liver as compared to the cells from untreated liver tissue and this was significantly reduced in presence of BVLE (10µg/ml). Our results clearly point out that BVLE mitigates the CCl_4 induced liver damage by decreasing LDH level.
Key words: *Bauhinia variegata* ,hepatotoxin, scrofula.

Introduction

Herbal drugs are significant source of hepatoprotective drugs. Mono and polyherbal preparations have been used in various liver disorders[1]. Liver plays a major role in detoxification and excreation of many endogenous and exogenous compounds. Any impairement to its function may lead to many implications on one's health. Management of liver diseases is still a challenge to the modern scientific community [2].The greater susceptibility of the liver to damage by chemical agents appears to be a consequence of its primary role in the metabolism and deposition of foreign substances. The diverse aspects include the nature of the hepatotoxic agents, the character of the injury, the mechanism of the hepatotoxic effects, circumstances of exposure and medico-social importance. CCl_4 is one of the most commonly used hepatotoxin in the experimental study of liver disease. The lipid peroxidative degeneration of bio membranes is one of the major cause hepatotoxicity of CCl_4[3]. The increase in the levels of liver slices released twice more LDH in the medium in the presence of CCl_4 was a clear indication of cellular leakage and loss of functional integrity of cell membrane [4,5]. An attempt has been made in the present work to develop a new hepatoprotective drug from the natural plant source.

Bauhinia variegata Linn (leguminosae) is known as Kanchanar in Hindi is a medium sized tree abundant in Sub-Himalayan tract extending eastwards to Assam, Eastern, Central and South India[6]. The various parts of the plants viz., leaves, flower buds, flower, stem, stem bark, seeds and roots are used in fever, as tonic, astringent, diarrhoea, dysentery, hemorrhoids, piles, edema, laxative, anthelmintic, antileprotic, in skin diseases, wound healing, antigoitrogenic, antitumor, in obesity, stomatitis, antidote for snake poisoning, dyspepsia, flatulence and as carminative[7]. The chemical constituents isolated so far from the plant are b-sitosterol, kaempferol-3-glucoside, tannins [8], carbohydrates, amides, reducing sugars, vitamin C, crude protein, fibers [9], calcium, phosphorus[10], quercetin, rutin, quercitrin, apigenin, apigenin- 7-O-glucoside[11], heptatriacontan-12, 13-diol and dotetracontan-15-en-9-ol [12].



Materials and Methods

Collection and Preparation of plant material: The plant *Bauhinia variegata* was collected from medicinal garden of Jawaharlal Nehru Cancer Hospital and Research Centre, Bhopal, India. The plant was identified and authenticated from standard resources. Fresh leaves were collected and air dried in shade at room temperature. Dried leaves were powdered mechanically through mesh sieve. 100 g of freshly powdered leaves were evenly packed in soxhlet apparatus and the extraction was done with 50% alcohol. Then solvent was evaporated at low temperature under reduced pressure.

Animal: Swiss albino mice (Mus musculus) of either sex, 6–8 weeks old with body weight of 24 ± 2 g, were used from animal house of department of research, Cancer Hospital and Research Center, Bhopal, India, as per norms laid down by CPCSEA. Mice were given standard mouse feeding pellets and water ad libitum.

Drugs and chemicals: Hepes Buffer was obtained from Sisco Research Laboratory, Mumbai. Ether anesthetic were obtained from Hi-Pure Fine Chemical Industries, Chennai. Nicotinamide Adenine dinucleotide was obtained from SD Fine Ltd, Baisar. Dinitrophenyl Hydrazine, Lithium Lactate, Sodium pyruvate were obtained from Himedia, Mumbai. All other chemicals used were obtained commercially and were of analytical grade.

Krebs Ringer HEPES (KRH) Medium: 2.5mM HEPES pH 7.4, 118 mM NaCl, 2.85 mM KCl, 2.5 mM $CaCl_2$, 1.15 mM KH_2PO_4, 1.18 mM $MgSO_4$, 4.0 mM Glucose and Double distilled water.

Test solution Dilution of test compound should be made in water or appropriate non-toxic solvent. Compounds which are poorly soluble in water first dissolved in propylene glycol. Concentration of 16.6% propylene glycol has been used without causing any tissue damage. Vehicles other than propylene glycol are rarely used because of their toxicity. *Bauhinia variegata* extract was dissolved in water at a concentration of 10µg/ml.

Liver slice culture *in vitro* assay [4,5]

The rat was dissected open after cervical dislocation, and liver lobes were removed and placed in pre-warmed Krebs Ringer HEPES medium. Liver was then cut into thin slices. The slices were weighed between 4-6 mg was used for the experiment. Each experimental system contained 20-22 slices weighing together 100-120 mg. These slices were washed with 10ml KRH medium every 10 min over a period of 1hour. These were then pre-incubated for 60 minutes in small plugged beakers containing 2 ml KRH on a shaker water bath at 37^{o}C.

At the end of pre incubation the medium was replaced by 2ml KRH medium and incubated for 2hr at 37°C. At the end of incubation, each group of slices was homogenized in chilled potassium phosphate buffer (100mM, pH 7.8) in an ice bath to give a tissue concentration of 100mg/ml. The culture medium was collected and the homogenates were centrifuged at 10,000 rev/min for 10 min and the supernatant was used for estimation of lactate dehydrogenase (LDH), which was employed as a cytotoxicity marker.

Five different experimental conditions were used for treatment with plant extract.

a) Plant extract (10µg/ml) was present for 0.5 hr. only during pre incubation.
b) Plant extract was present for 0.5 hr. during pre incubation and also for next 2 hr. with CCl_4 (20mM).

c) Plant extract was present for 2 hr. along with CCl_4.
d) Control group.
e) CCl_4 (20mM) alone.

Estimation of lactate dehydrogenase [4]

The lactate is acted upon by lactate dehydrogenase to form pyruvate in the presence of NAD. The pyruvate forms pyruvate phenyl hydrazone with 2, 4 dinitrophenyl hydrazine. The colour developed is read in a spectrophotometer at 440 nm.

Reagents

Glycine buffer → 0.1M, pH10: 7.505g of glycine and 5.85g of sodium chloride were dissolved in 1 litre of water.

Buffered substance → 125ml of glycine buffer and 75ml of 0.1 NaOH were added to 4g of lithium lactate and mixed well.

Nicotinamide adenine dinucleotide → 10mg of NAD was dissolved in 2.0 ml of water.

2, 4 dinitrophenyl hydrazine → 20mg of DNPH was dissolved in 100ml of 1N HCL.

Standard-sodium pyruvate 1μmol/ml → 8.5-mg/10 ml buffered substrate.

1.0 ml buffer substrate was placed and 0.1 ml supernatant was added into each of two test tubes with 0.2 ml water to the blank, and then to the test added 0.2 ml of NAD. Mixed and incubated at 37°C for 15 minutes. Exactly after 15 minutes, 1.0 ml of dinitrophenyl hydrazine was added to each test and control. Left for 15 minutes, then added 10 ml of 0.4N sodium hydroxide and the colour developed was read immediately at 440 nm.

LDH activity was expressed as μmoles of pyruvate liberated/minute.

Results

The protection of liver cells from CCl_4 cytotoxicity by using *Bauhinia variegata* leaf extract (BVLE) in liver slice culture *in vitro* assay.

Assessment of carbon tetrachloride (CCl_4) hepatotoxicity: In the liver slice culture system leakage of LDH was used as a marker to study the hepatotoxicity of CCl_4. It was observed that in case of slices treated with CCl_4 there was more LDH in the medium as compared to control. Almost twice LDH was released by 2 hours compared to untreated liver slices (Fig.1).

Assessment of hepatoprotection of *Bauhinia variegata* leaf extract against CCl_4 cytotoxicity: *Bauhinia variegata* was found to be non-toxic to the liver cells at a concentration of 10 μg/ml. Release of LDH in BVLE treated slice was found to be similar to that in case of control untreated slice.

Liver slices released twice more LDH in the medium in the presence of CCl_4 when compared to control. When the liver slices were pre treated with extract for 0.5 hours this CCl_4 induced release of LDH was decreased. When extract was present along with CCl_4 during incubation for 2 hours, the LDH released was further decreased. Thus, it is clear that pretreatment with PBLE for 0.5 hours protect liver tissue against CCl_4 cytotoxicity, but prolonged treatment with *Bauhinia variegata* leaf extract 2 hour's offers better protection. (Table.1)

Table-1 . Concentration of LDH released in different groups

Treatment	Concentration release of LDH
Control	0.020 ± 0.003
CCl_4	0.053 ± 0.001
PBLE	0.031 ± 0.001
PBLE + CCl_4 (0.5h)	0.036 ± 0.009
PBLE + CCl_4 (2h)	0.027 ± 0.005

Results are mean ± SD of three parallel measurements

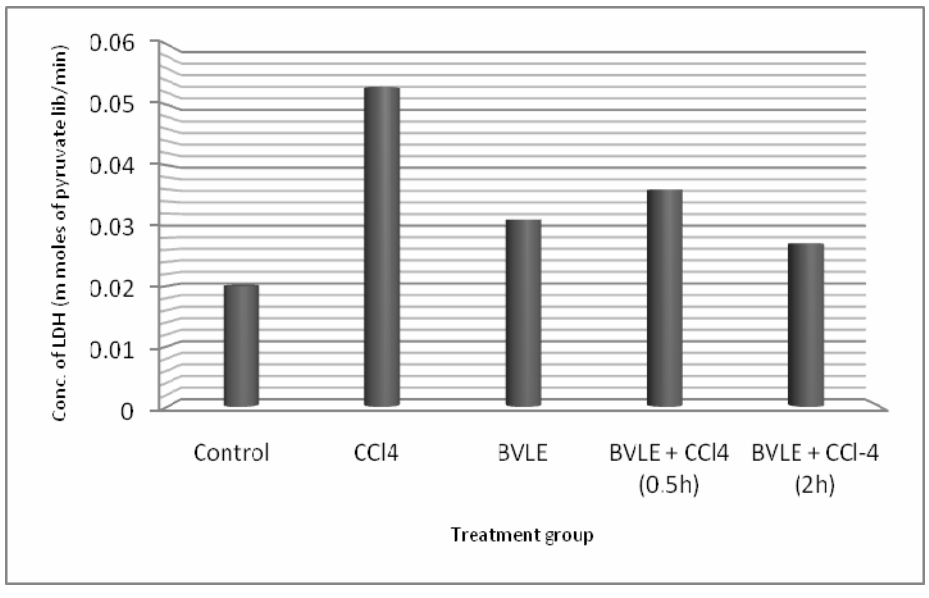


Fig-1 Release of LDH under different conditions of hepatotoxin used

Discussion

The present study is an attempt to assess the hepatoprotective and antioxidant activities of *Bauhinia variegata* leaf extract using in *vitro methods*.

CCl_4 is one of the most commonly used hepatotoxin in the experimental study of liver diseases[13]. The lipid peroxidative degeneration of bio membranes is one of the principal causes of hepatotoxicity of CCl_4 [14].

The protection of liver cells from CCl_4 cytotoxicity by *Bauhinia variegata* leaf extract *in vitro* liver slice culture was studied. Liver slice culture is a suitable model for the experimental analysis of hepatotoxic and hepatoprotective agents [15]. Employing this model, the CCl_4 toxicity was confirmed by measuring the release of LDH into the medium by liver slices. LDH is a cytosolic enzyme mainly present in periportal hepatocytes and released when the cells are lysed by hepatotoxin. The amount of enzyme released is proportional to the extent of damage caused to the cell. CCl_4 treated liver slices released twice more LDH into the medium than untreated cells over a period of 2 h. *Bauhinia variegata* leaf extract added to liver slices either before or along with CCl_4 lowered the enzyme release.

Thus it can be inferred that *Bauhinia variegata* leaves may be a promising hepatoprotective agent and this activity may be due to its antioxidant activity.

To conclude, in most of the developed and developing countries the incidence of viral hepatitis is more, so the investigation for an effective hepatoprotective agent from the natural source is an urgent necessity. *Bauhinia variegata* leaves offer vast possibilities in the treatment of various liver disorders. This may be attributed to the high level of antioxidant activity. Further studies on other models and extensive clinical trials are needed to confirm these findings.

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