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Protection of hepatic cells from CCl_4 induced cytotoxicity by *Ficus carica* in liver slices culture *in vitro*

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ABSTRACT

The present study was aimed to evaluate the hepatoprotective activity of methanolic extract of leaves of *Ficus carica* Linn. Liver slice culture model have been used to demonstrate hepatoprotective activity of *Ficus carica* leaves extract *in vitro*. CCl_4 (20mM) has been used as a hepatotoxin and the cytotoxicity of CCl_4 is estimated by quantization the release of 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 Plant extract (10ìg/ml). The results clearly point out that *Ficus carica* leaves extract mitigates the CCl_4 induced liver damage by decreasing LDH level.

Key words: Hepatoprotective, Ficus carica, Cytotoxicity, Liver slices culture

INTRODUCTION

Liver plays a fundamental role in metabolizing a large number of organic, inorganic chemicals and drugs[1]. 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 by CCl_4 [2].

Herbals play an essential role in traditional and modern system of medicine. *Ficus carica* Linn plant is one of the great herbals used in folk and tribal medicines. It belongs to the family *Moraceae,* commonly known as *Anjir*. The plant is considered to be native of carica in Asia Minor and grown in nearly all topical and sub-tropical countries. Chemically *Ficus carica* containing proteins, carotene, nicotinic acid, riboflavin, citric acid, acetic acid, resin, gum, mucilage, pentogen, sugar etc. Biologically *Ficus carica* have broad spectrum activities like antispasmodic, antiplatelet[5], Antimutagenic [6], antidiabetic [7] etc. It is also used to treat wounds, inflammation, constipation, piles, cough, asthma, and chest pain etc [8, 9].

MATERIAL AND METHODS

Plant material

Ficus carica Linn leaves was collected from the Coimbatore district Tamilnadu. The herbarium of this plant was identified and authenticated by the taxonomist, Botanical Survey of India, Tamilnadu Agricultural University (TNAU), Coimbatore.

Preparation of plant material

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 70% alcohol. Then solvent was evaporated at low temperature under reduced pressure.

Drugs and chemicals

Anesthetic Ether was obtained from Hi-Pure Fine Chemical Industries, Chennai. HEPES Buffer was obtained from Sisco Research Laboratory, Mumbai. Nicotinamide Adenine Dinucleotides were 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.

Liver slice culture *in vitro*[3, 4]

Liver slice culture was maintained following the protocol developed by Wormser *et al*. (1990). The rat was dissected open after cervical dislocation, and liver lobes were removed and transferred to pre-warmed KRH medium. Liver was then cut into thin slices using surgical blades. The slices were weighed and each slice weighing between 4 and 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°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 appropriate volume of 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 rpm 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.

- Plant extract (10ìg/ml) was present for 0.5 hr. only during preincubation.
- Plant extract was present for 0.5 hr. during preincubation and also for next 2 hr. with CCl_4 (20mM).
- Plant extract was present for 2 hr. along with CCl_4.
- Control group.
- CCl_4 (20mM) alone.

Estimation of lactate dehydrogenase [3]

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.

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 m moles of pyruvate liberated/minute.

RESULTS

The protection of liver cells from carbon tetrachloride cytotoxicity by *Ficus carica* leaves extract (FCLE) in liver slice culture *in vitro*.

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 three times more LDH was released by 2 hours compared to untreated liver slices.

Assessment of hepatoprotection of FCLE against CCl_4 cytotoxicity.

Ficus carica was found to be non-toxic to the liver cells at a concentration of 10 mg/ml. Release of LDH in FCLE treated slice was found to be similar to that in case of control untreated slice. Liver slices released three times 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 FCLE for 0.5 hours protect liver tissue against CCl_4 cytotoxicity, but prolonged treatment with FCLE 2 hrs offers better protection (Table 1).

Table 1: Concentration of LDH released in different groups

Treatment	Concentration release of LDH
Control	0.025 ± 0.02
Carbon Tetrachloride (CCl_4)	0.068 ± 0.05
Ficus carica leaves extract	0.034 ± 0.03
Ficus carica leaves extract + CCl_4 (0.5h)	0.046 ± 0.02
Ficus carica leaves extract + CCl_{-4} (2h)	0.021 ± 0.04

Results are mean ±SD of three parallel measurements

DISCUSSION

CCl_4 is one of the most commonly used hepatotoxin in the experimental study of liver diseases [10]. The lipid peroxidative degeneration of bio membranes is one of the principal causes of hepatotoxicity of CCl_4. Liver slice culture is a suitable model for the experimental analysis of hepatotoxic and hepatoprotective agents [11]. Employing this model, the CCl_4 toxicity was conformed 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 three times more LDH into the medium than untreated cells over a period of 2 h. FCLE added to liver slices either before or along with CCl_4 lowered the enzyme release. Thus it can be inferred that *Ficus carica* leaves may be a promising hepatoprotective agent and this activity may be due to its antioxidant activity.

CONCLUSION

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. *Ficus carica* leaves offer vast possibilities in the treatment of various liver disorders. This may be attributed to the high level of antioxidant activity.

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