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Cajanus cajan Linn. (Leguminosae) prevents alcohol-induced rat liver damage and augments cytoprotective function

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ABSTRACT

Aim of the study: Cajanus cajan Linn. (Leguminosae) is a nontoxic edible herb, widely used in Indian folk medicine for the prevention of various liver disorders. In the present study we have demonstrated that methanol-aqueous fraction (MAF2) of *Cajanus cajan* leaf extract could prevent the chronically treated alcohol induced rat liver damage.
Materials and Methods: Chronic doses of alcohol (3.7g/ kg) orally administered to rats for 28 days and liver function marker enzymes such as GPT, GOT, ALP and anti-oxidant enzyme activities were determined. Effect of MAF2 at a dose of 50mg/kg body weight on alcohol treated rats was noted.
Results: Alcohol effected significant increase in liver marker enzyme activities and reduced the activities of anti-oxidant enzymes. Co-administration of MAF2 reversed the liver damage due to alcohol; it decreased the activities of liver marker enzymes and augmented antioxidant enzyme activities. We also demonstrate significant decrease of the phase II detoxifying enzyme, UDP-glucuronosyl transferase (UGT) activity along with a three- and two-fold decrease of UGT2B gene and protein expression respectively. MAF2 co-administration normalized UGT activity and revived the expression of UGT2B with a concomitant expression and nuclear translocation of Nrf2, a transcription factor that regulates the expression of many cytoprotective genes.
Conclusion: Cajanus cajan extract therefore shows a promise in therapeutic use in alcohol induced liver dysfunction.

1. Introduction

Alcoholism is the most serious addictive disease prevalent in our society and accepted to be a worldwide drinking related health problem. Despite the fact that the population in general is well aware of the adverse and often fatal consequences of alcohol consumption, it is estimated that only in USA; more than 7% of adult population has alcohol related health problems (Mc Ginnis and Foege, 1993). The liver is one of the major alcohol target organs known to be severely damaged due to chronic alcohol intake (Lieber, 1994). Alcohol-induced liver pathogenesis is due to the accumulation of toxic substances generated during alcohol metabolism, which in turn generates reactive oxygen species (ROS) and other free radicals (Moncada et al., 1994; Albano et al., 1999). This results into covalent modification of cellular macromolecules, morphological changes leading to tissue damage and aberrant biochemistry of liver (Lieber, 1991; McCuskey, 1991).

Recently, there has been a growing interest in establishing the therapeutic potentials of plants and plant derived molecules for the purpose of drug development. *Cajanus cajan* Linn. (Leguminosae), a nontoxic edible herb (Duke, 1981; Nene et al., 1990) locally available in India is one of those plants which has many folk medicinal uses. Leaves of this plant have been traditionally used by village people for liver injury and a number of other clinical disorders (Chopra et al., 1986). A 43 kDa protein isolated from *Cajanus indicus* has recently been demonstrated to have hepatoprotective effect (Sarkar et al., 2005; Ghosh et al., 2006; Manna et al.,2 2007).

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It is known that both chronic *in vivo* and acute *in vitro* doses of alcohol treatment induces liver tissue damage and subsequent generation of oxidative stress in rats as marked by the increased tissue lipid peroxidation (LPO) and protein carbonyl production (French et al., 1993), with a subsequent reduction of reduced glutathione (GSH) level (Guerri and Grisolia, 1980; Meister and Anderson, 1983). It has also been reported that the activity of antioxidant enzymes like superoxide dismutase (SOD), catalase, glutathione reductase (GR) and glutathione-*S*-transferase (GST) tend to get reduced in response to such stressful condition (Almeida et al., 2006). Recent studies have pointed out the importance of phase II detoxification enzymes such as UDP-glucuronosyl transferases (UGTs) in the elimination of toxic metabolites and thereby reducing the toxic burden of liver undergoing stressful situation (Tukey and Strassburg, 2000). Hence, a decrease in the activity of UGTs would lead to accumulation of toxic substances resulting oxidative damage. It has also been reported that the expression of UGTs are regulated by a transcription factor Nrf2 (Motohashi et al., 2002). Therefore, the UGT-Nrf2 pathway may play a central role in the protection of liver from toxic damage (Burchell and Coughtrie, 1989; Jansen et al., 1992). However, whether UGT activity or its expression is related to the alcohol-induced stress is not known.

The major objective of this investigation is to evaluate the potentiality of *Cajanus cajan* leaf extract (methanol-aqueous fraction2, i.e., MAF2) in protecting alcohol-induced damage of rat liver.

2. Materials and methods

2.1. Materials

Kits for the measurement of serum GPT (Glutamate pyruvate transaminase), GOT (glutamate oxaloacetate transaminase) and ALP (Alkaline phosphatase) were purchased from Span Diagnostics Ltd., India. Uridine 5′-diphosphoglucuronic acid triammonium salt, glutathione reductase, PCR primers for UGT 2B were purchased from Sigma Chemical Co. (St Louis, USA). Polyclonal anti-UGT 2B (anti-goat) and polyclonal anti-Nrf2 (anti-rabbit) antibodies was purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). All other reagents used were of analytical grade.

2.2. Fractionation of Cajanus cajan leaf extract

The fresh mature leaves of *Cajanus cajan* were collected from Birbhum district of West Bengal, India and identified by Professor Samit Roy and Dr. Subrata Mondal, Department of Botany, Visva-Bharati University where a botanical voucher was deposited at Herbarium for reference (VBRS 001). The leaves (3 kg) were pulverized in a motor driven grinder with methanol (MeOH) and water (1:1) to prepare the extract. The extracted solution, after evaporation *in vacuo*, gave a residue (75 g) and was evaluated for bioactivity. The methanol extract was then partitioned into ethyl acetate (EtOAc), *n*-butanol (*n*-BuOH) and water (H_2O) and evaporated *in vacuo*. Each of the residues was then checked again for bioactivity and the aqueous part was found to be most active while the *n*-butanol part had moderate activity. The aqueous part (22 g) was subjected to Diaion HP-20 chromatography with water (100%), water:methanol (50:50), water:methanol (25:75) and methanol (100%) as eluent. Four fractions were obtained, evaporated *in vacuo* and fraction 2 (water:methanol, 50:50), hereafter referred as MAF2 (6.5 g), was found to be most active.

2.3. In vitro study

In vitro study was done using liver slice culture system following the protocol developed by Wormser and Ben Zakine (1990), with slight modification. Rat liver was perfused heavily by using Modified Hank's Medium to remove all the blood clots. The liver was then cut into small square slices, weighing 5–6 mg, and was cultured in 24 well plates (20–22 slices weighing about 100–120 mg/well) using MEM 199 medium supplemented with 0.1-g/l penicillin, 0.07-g/l streptomycin and 0.2% BSA. Liver slices were incubated in this condition for 4 h at 37 °C in 95%O_2/5% CO_2. The medium was changed at 2 h, this time gap was given to recover the shock from surgery. The control slices were kept in culture medium only, ethanol (50 mg/ml) or ethanol plus MAF2 (2.5, 5.0 and 10.0 mg/ml) or ethanol plus Silymarin (1.0, 2.0 and 3.0 mg/ml) were added to the incubation medium where mentioned. On termination of incubation at 4 h, the medium was collected and subjected to the determination of GOT and GPT for the assessment of liver damage. The liver slices from different incubations were separately collected, washed repeatedly and then homogenized in ice-cold phosphate (50 mM, pH 7.4) buffer; the homogenate from each incubation was subjected to the estimation of lipid peroxidation.

2.4. In vivo study

Adult male albino Sprague–Dawley rats weighing 180–220 g were selected for our experiments. Animals were maintained under standard laboratory conditions in polypropylene cages (3 animals/cage of 43 cm × 28 cm × 15 cm). Food and water was given *ad libitum*, under 12 h light/dark cycles, at 25 ± 2 °C. The principles of laboratory animal care were followed throughout the experimental schedule and the experimental design had the approval of the animal ethical committee of Visva-Bharati University.

Rats were divided randomly in different groups with 6 rats per group. The first group served as control and received distilled water orally. The second group received ethanol (3.7 g/kg body weight) orally for a single dose each day up to 35 days. The third, fourth and fifth group received MAF2 at a dose of 25, 50, and 100 mg/kg body weight, respectively, once a day, 3 h before administration of ethanol for 35 days. These doses were selected by chronic toxicity studies of MAF2 on rat at the Indian Institute of Chemical Biology, Kolkata for 90 days. In this study a dose up to 200 mg/kg body weight did not show any toxic effect. Since, 100 mg/kg body weight had similar effect as 150 and 200 mg/kg body weight; we selected 100 mg/kg body weight as the highest dose in our experiments. Since, co-treatment of MAF2 and alcohol for 28 days showed an optimum response, the rest of the experiments were conducted for 28 days. To examine the efficacy of MAF2 with a standard drug for liver damage treatment, we have selected Silymarin, at a dose of 5 mg/kg body weight for a single dose each day, keeping control and MAF2 treated group in a similar manner as described above.

Rats were anesthetized to perform the perfusion of livers with normal saline (0.9% NaCl) to remove the blood and re-washed thoroughly with ice-cold phosphate buffer (50 mM, pH 7.4) with saline (PBS). 10% homogenates of liver tissue was prepared in PBS using Potter Elvehjem homogenizer and then subjected to differential centrifugation to separate cytosolic and microsomal fractions according to the method of Bock et al. (1973). Protein estimation of the samples was done by using the method of Lowry et al. (1951).