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Anti-oxidant and Hepatoprotective Activities of Alcoholic Extract of *Terminalia arjuna*

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Abstract – Alcoholic extract of *Terminalia arjuna* [TA] was evaluated for its hepatoprotective activity against carbon tetrachloride (CCl_4)-induced hepatic damage in rats. The hepatoprotective activity of TA was evaluated by measuring levels of serum marker enzymes like serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), serum alkaline phosphatase (SALP). The serum levels of total proteins (TP), total albumins (TAL) and bilirubin (BILN) were also estimated. The histological studies were also carried out to support the above parameters. Silymarin (SM) was used as standard drug. Administration of TA (250 and 500 mg/kg/po) markedly prevented CCl_4-induced elevation of levels of SGOT, SGPT, SALP, TP, TAL and BILN. These biochemical observations were supplemented by histopathological examination of liver sections. Alcoholic extract of TA also shown significant *in-vitro* free radical scavenging activity against 1, 1-diphenyl-2-picryl hydrazyl (DPPH) and nitric oxide (NO) radicals. Thus, the present study provides a scientific rationale for the traditional use of this plant in the management of liver diseases.
Keywords – *Terminalia arjuna, In-vitro* anti-oxidant, hepatoprotective, *Liv 52*, CCl_4.

Introduction

Liver has a pivotal role in the regulation of physiological processes such as metabolism, secretion and storage. Liver diseases are now the fifth most common cause of death after heart disease, stroke, chest disease and cancer (Williams, 2006). It is estimated that about 370 million subjects are infected with hepatitis B virus (Alter *et al.*, 2006) and 170 million with hepatitis C (Chisari, 2005). In spite of the tremendous advances made in allopathic medicine, no effective hepatoprotective medicine is available (Subramonium and Pushpangadan, 1999).

Terminalia arjuna [TA] (Roxb. Ex DC) (Combretaceae) is known as *Arjuna* in Indian Medicine. In the traditional systems of Indian Medicine, it is used in the treatment of diseases such as cardiac failure, cirrhosis of liver, asthma, bronchitis, cough, tumours, inflammations, diabetes, piles, leucorrhoea (Nadkarni and Nadajarni, 1976; Tripathy *et al.*, 1996; Sharma *et al.*, 2001). We have recently reported the anti-allergic and anti-asthmatic activities of the alcoholic extract of TA and arjunolic acid (Prasad *et al.*, 2004).

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Terminalia arjuna [TA] is one of the ingredients in the popular Ayurvedic medicine *Liv 52*. The hepatoprotective action of *Liv 52* was very well documented (Sama *et al.*, 1976; Subbarao *et al.*, 1978 and Sandhir *et al.*, 1999). However, there is lack of scientific data regarding the hepatoprotective activity of TA. In this communication, we report the hepatoprotective activity and *in-vitro* anti-oxidant activity of alcoholic extract of TA.

Experimental

Plant material – The stem bark of the plant was collected from Palayamkottai, Tamil Nadu, India, during October 2003 and was authenticated by Dr S. Usman Ali, department of botany, Central Research Institute for Siddha, Arumbakkam, Chennai-600 106. (Voucher specimen no. 128/03, deposited at the herbarium of the institute.)

Extraction – Dried and powdered stem bark of the plant (2 kg) was extracted with alcohol in the cold (48 h). The extract was filtered and distilled to a syrupy mass and dried in vacuum (yield 150 g).

Animals – Male albino wistar rats (120 - 150 g) were obtained from the institute experimental animal facility

and were kept 25 ± 5°C and relative humidity 50 ± 15%, in a well ventilated animal house under 12 h light and dark cycle. The protocol of the study is approved by Institutional Animal Ethics Committee (IEAC No. XII/13/2004-05).

Hepatoprotective activity – Carbon tetrachloride (CCl_4) induced hepatotoxic model in rats was employed (Zafar *et al.*, 1988). Twenty four wistar albino rats were taken and grouped into four containing six rats in each group. Group I received only 1% sodium carboxy methyl cellulose [SCMC] suspension and served as solvent control. Group II received the standard drug silymarin (SM) (25 mg/kg/po in 1% SCMC suspension). Group II and IV received TA (250 and 500 mg/kg/po in 1% SCMC suspension respectively). The above drug treatment was given for 5 days. All the groups except group I received CCl_4 : olive oil (1 : 1) (2 mL/kg/sc) on 3rd day, 30 minutes after the administration of the drug treatment.

Collection of serum and tissue samples – On the 5th day, after 4 h of the drug administration, animals were sacrificed by decapitation. Blood was collected by excising the jugular vein. It was allowed to clot and then centrifuged at 3000 rpm for 15 minutes. The serum samples were collected for the biochemical parameters and liver tissue samples were collected for the histopathological studies.

Biochemical estimations – Biochemical parameters like serum enzymes such as serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) (Reitman and Frankel, 1957) and serum alkaline phasphatase (SALP) (Kind and King, 1954) were determined. Bilirubin (BILN) (Malloy and Evelyn, 1937), total protein (TP) (Lowry *et al.*, 1951) and total albumin (TAL) (Wooton, 1964) were also determined.

Histopathological studies – Seven micrometer thick paraffin sections of buffered formalin-fixed liver samples were stained with haematoxylin-eosin for photo microscopic observations of the liver histological architecture of the control and treated rats.

Chemicals – 1, 1-diphenyl-2-picryl hydrazyl (DPPH) was obtained from Aldrich, USA, Naphthalene diamine dichloride received from Loba Chemie, Mumbai, India. Sulphanilic acid was obtained from Himedia laboratories Ltd., Mumbai, India. Ascorbic acid was obtained from S.D fine chemicals, Biosar, India.

DPPH method – The antioxidant activity of alcoholic extract of TA was assessed on the basis of the radical scavenging activity of the stable DPPH free radical (Blois, 1958). The reaction mixture consisted of 1 ml of 0.1 mM DPPH in ethanol, 0.95 ml of Tris-HCl buffer (pH 7.4), 1 mL of ethanol and either 0.05 mL of different concentrations alcoholic extract of TA [Linearity range 4 - 720 μg/mL] or 0.05 ml of different concentrations ascorbic acid [Linearity range 0.1 - 1 μg/mL]. After incubation at 37 °C for 30 minutes, the absorbance of each solution was measured at 517 nm using the spectrophotometer and the corresponding blank readings were also taken and the IC_{50} value of the alcoholic extract was calculated (Hwang *et al.*, 2001). IC_{50} value is the concentration of the sample required to scavenge 50% DPPH free radical.

Nitric Oxide [NO] radical inhibition assay – Sodium nitroprusside in aqueous solution at the physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions and the same can be estimated by the use of Griess Illosvoy reaction (Garrat, 1964). In the present investigation Griess Illosvoy reagent is modified by using naphthyl ethylenediamine dihydrochloride (0.1% w/v) instead of 1-naphthylamine (5% w/v). Scavengers of NO compete with oxygen, leading to reduced production of nitric oxide (Marcocci *et al.*, 1994). The reaction mixture (3 mL) containing sodium nitroprusside (10 mM, 2 mL), standard phosphate buffer 0.5 mL (0.025 M, pH 4) and either 0.5 ml of various concentrations alcoholic extract of TA [Linearity range 5 - 640 μg/mL] or 0.5 mL of ascorbic acid solution [Linearity range 0.1 - 1 μg/mL] was incubated at 25 °C for 150 minutes. After incubation, 0.5 mL of the reaction mixture containing nitrite ions was pipetted and mixed with 1 mL of sulphanilic acid reagent (0.33% w/v in 20% v/v glacial acetic acid) and allowed to stand for 30 minutes. A pink coloured chromophore was formed in diffused light. The absorbance of these solutions was measured at 546 nm against the corresponding blank solutions using spectrophotometer and the IC_{50} value of the alcoholic extract was calculated (Marcocci *et al.*, 1994). IC_{50} value is the concentration of the sample required to inhibit 50% of NO radical.

Statistical Analysis – In case of *in-vitro* anti-oxidant studies, the statistical analysis was performed by students 't' test. The differences in the absorbance between the blank solutions and the test solutions are considered significant when $p < 0.05$. In case of hepatoprotective activity the statistical analysis was performed by one way ANOVA followed by Dunnet's 't' test. The results were expressed as the mean ± SEM to show variations in a group. Differences are considered significant when $p < 0.05$.

Results and Discussion

CCl_4 induced hepatotoxicity in rats represents an

Table 1. Hepatoprotective activity of alcoholic extract of *T. arjuna* [TA] and silymarin [SM] on CCl_4 induced hepatoxicity in rats

Treatment	Dose	SGOT [IU/L]	SGPT [IU/L]	SALP [IU/L]	TAL [g/dl]	TP [g/dl]	BILN [mg/dl]
1% w/v SCMC	10 ml/kg	83.66 ± 1.31	58.50 ± 1.65	125.33 ± 3.41	3.62 ± 0.13	6.43 ± 0.14	0.64 ± 0.07
CCl_4	2 ml/kg	242 ± 5.74	184 ± 3.51	347.50 ± 4.64	1.28 ± 0.08	4.67 ± 0.14	3.89 ± 0.19
Silymarin	25 mg/kg	90.16 ± 1.99*	63.16 ± 1.55*	151.16 ± 3.52*	5.24 ± 0.19*	7.57 ± 0.11*	0.91 ± 0.05*
TA	250 mg/kg	169.50 ± 1.60*	132 ± 1.94**	251.00 ± 6.54	3.71 ± 0.08	5.48 ± 0.06	2.36 ± 0.06*
TA	500 mg/kg	118.16 ± 3.35*	97 ± 1.73*	180.00 ± 4.61*	4.42 ± 0.09*	6.60 ± 0.16	1.55 ± 0.09*

Results are expressed as Mean ± SEM. The statistical difference between control and treated groups were tested by one way ANOVA followed by Dunnett's 't' test. * $P < 0.0001$, ** $P < 0.01$, *** $P < 0.05$.

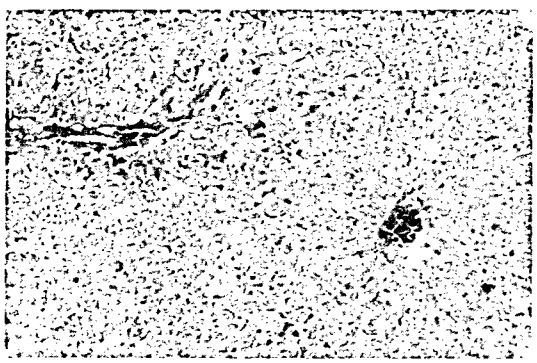
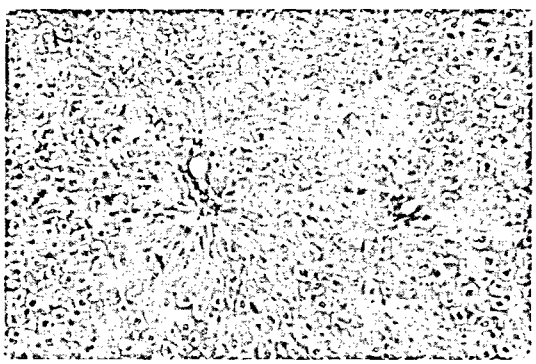


Fig. 1.



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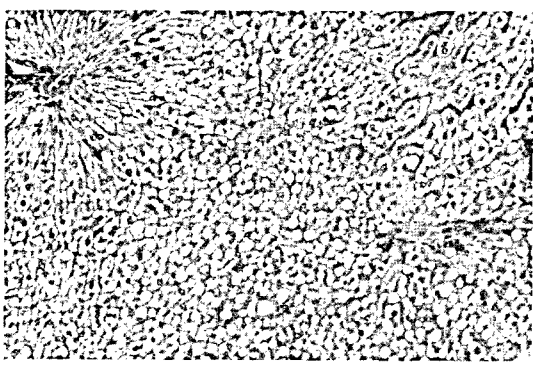

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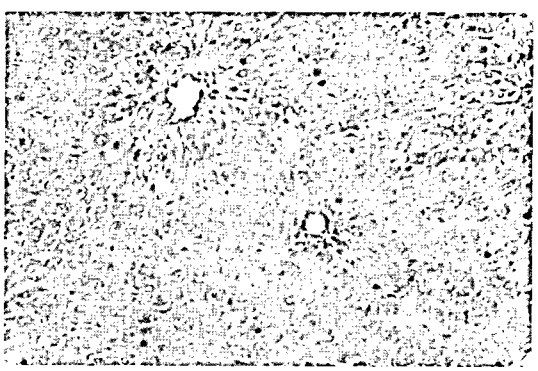

Fig. 4.

adequate experimental model of cirrhosis in man and hence it is used for the screening of hepatoprotective drugs (Perez-Tamayo, 1983). Hepatotoxic compounds such as CCl_4 are known to cause marked elevation in serum enzymes such as SGOT, SGPT, SALP and bilirubin levels (Zimmerman and Seef, 1970). In the present investigation also it was seen that administration of CCl_4 elevated the levels of serum hepatic marker enzymes SGOT, SGPT, SALP and bilirubin (Table 1). The levels of total protein and albumin were lowered. *Terminalia arjuna* (250 & 500 mg/kg) and silymarin (25 mg/kg) treated groups decreased the elevated levels of SGOT, SGPT, SALP and bilirubin significantly as compared with CCl_4 alone treated rats. The stabilization of serum SGOT, SGPT, SALP and bilirubin by alcoholic extract of TA is a clear indication of the improvement of the functional status of the liver cells.

Histopathological studies also support the hepatoprotective property of the alcoholic extract of TA. Normal lobular architect of the liver with central vein and portal tracts and cords of hepatocytes with sinusoids in between was observed in vehicle treated rats (Fig. 1). The liver of

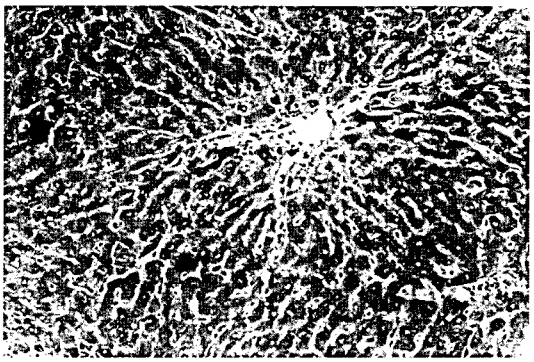

Fig .5.

the CCl_4 intoxicated rats showed fatty degeneration of hepatocytes (Fig. 2). In silymarin (25 mg/kg) treated group showed early reversal of fatty changes (Fig. 3). In TA (250 mg/kg) treated group the fatty changes are seen more in perivenular region. Larger areas of normal hepatocytes are also evident (Fig. 4). In TA (500 mg/kg) treated animals relatively normal hepatocytes with minimal fatty changes were observed (Fig. 5).

Alcoholic extract of TA was tested for free radical scavenging potential using DPPH and NO methods. The IC_{50} values of TA by DPPH and NO radical inhibition assay methods were found to be 386 and 394 μg/ml respectively, whereas the IC_{50} values of ascorbic acid by DPPH and NO radical inhibition assay methods were found to be 0.68 and 0.72 μg/mL respectively. It is well established that the hepatotoxicity of CCl_4 is due to the release of CCl_3 free radical in the liver endoplasmic reticulum by cytochrome P 450 (Recknagel, 1967). It was well documented that free radicals are involved in a variety of pathological events and aging process (Bulkley *et al.*, 1983). Therefore herbal drugs which possess anti-oxidant or free radical scavenging activity has become a central focus for research designated to prevent or ameliorate tissue injury and may have a significant role in the protection of free radical mediated diseases such as hepatic damage (Halliwell *et al.*, 1984).

Triterpenoids mainly oleanolic acid and arjunolic acid are reported from the plant (Anjaneyulu *et al.*, 1982; King *et al.*, 1954). The hepatoprotective effect of oleanolic acid isolated from the plants has been reported (Ma *et al.*, 1982). The proposed mechanism of hepatoprotection of oleanolic acid include suppression of cytochrome P-450 and inhibition of lipid peroxidation (Zhang and Li, 1992), enhancement of hepatic glutathione system (Liu *et al.*, 1995), prevention of fibrosis and stimulation of liver generation (Han *et al.*, 1981). The observed hepatoprotective mechanism of alcoholic extract of TA may be due to the presence of triterpenoids such as oleanolic acid and arjunolic acid and other triterpenoid glycosides.

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