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The Hepatoprotective Effect of Ethanolic Bark Extract of *Terminalia arjun*a on Paracetamol Induced Liver Damage

S.P. Sangamithira, J. Revathy, S. Sheik Abdullah* and P. Sampath Kumar

Biochemistry Faculty, Department of Chemistry and Biosciences, SASTRA University, Srinivasa Ramanujan Centre, Kumbakonam – 612 001 (India).

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The present study is used to investigate the hepatoprotective activity of ethanolic bark extract of *Terminalia arjuna* on paracetamol induced liver damage. In hepatotoxic rats, liver damage was studied by assessing the levels of the enzymes Alanine Transaminase (ALT), Aspartate Transaminase (AST), Alkaline Phosphatase (ACP) and Total Bilirubin in serum. The phytoconstituents and the antioxidant activity are also determined. The Albino rats were divided into four groups. Group I served as normal control; Group II received paracetamol (200mg/kg body weight/rat/day) as disease control; Group III treated with ethanolic extract of *Terminalia arjuna* bark; Group IV treated with reference drug, silymarin. The result suggests that the ethanolic bark extract of *Terminalia arjuna* showed a significant hepatoprotective effect.

Key words: Medicinal plants, *Terminalia arjuna*, Hepatoprotective activity, Paracetamol.

Hepatic damage associated with distortion of many metabolic functions. The number of cases of liver diseases remains one of the serious health problems. In spite of tremendous strides in the modern medicine, there is not much drugs available for the treatment of liver disorders. However, there are number of drugs employed in traditional system of medicine for liver diseases. Many formulations containing herbal extracts are sold in Indian market for liver disorders[1]. Liver diseases are mainly caused by toxic chemicals, over doses of drugs (paracetamol, carbon tetra chloride, anti cancer drugs, antibiotic and oral contraceptives), excessive consumption of alcohol, infections and auto immune disorders. Most of the hepatotoxic chemicals damage the liver cells mainly by inducing lipid peroxidation and other oxidative damages[2]. 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 diseases to replace currently used drugs of doubtful efficacy and safety[3]. In view of severe undesirable side effects of synthetic agents, there is growing focus to follow systemic research methodology and to scientifically evaluate the basis for traditional herbal medicines, which are claimed to possess hepatoprotective activity[4]. *Terminalia arjuna* is an important medicinal plant widely used in the preparation of Ayurvedic formulations used against several ailments[5]. The herb *Terminalia arjuna* has potent hepatoprotective effect. The bark of this tree has been commonly used in Ayurvedic preparations to bring hepatoprotective effect in a

* To whom all correspondence should be addressed.
E-mail: sheikhaneef@yahoo.co.in

drug[6]. The present investigation is aimed to evaluate phytochemical constituents, antioxidant activity and the hepatoprotective activity of *Terminalia arjuna*.

MATERIAL AND METHODS

Collection and extraction of plant material

The plant material namely *Terminalia arjuna* bark for the present investigation were collected from the commercial medicinal shop at Kumbakonam. The shade dried and coarsely powdered stem bark was extracted with ethanol using soxhelt apparatus. Then the evaporated final content was used for the phytochemical work and for animal treatment.

Phytochemical analysis

Phytochemical analysis was carried out qualitatively to identify the presence of various secondary metabolites such as alkaloids, flavanoids, tannins, phenols, steroids, glycosides, carbohydrates, aminoacids, proteins, saponins, terpenoids, ascorbic acid, coumarin, quinones, sulphur[7].

Determination of antioxidant

The antioxidants are determined by using hydrogen peroxide scavenging assay and phosphomolybdate method[8].

Animal management

Albino rats (150-180gms) were procured from Periyar Pharmaceutical Institute, Trichy. A total of 24 animals were equally divided into four groups of six each. The rats were used after an acclimatization period of 7 days to the laboratory environment. The animals were maintained under standard experimental conditions. They were provided with food and water *ad libitum*.

Source of chemicals

Paracetamol analgesic drug analytical grade was purchased from SD fine Chemicals Pvt. Ltd., Biosar. All other chemicals used, were obtained from Ranbaxy Research Laboratories, Glaxo Laboratories and Nice Pharmaceutical Company, India.

Induction of hepatotoxicity by paracetamol

Hepatotoxicity was induced by oral administration of paracetamol (SD fine Chemicals Pvt. Ltd., Biosar) with a dose 100 mg /kg, to overnight fasted rats. Control rats received 0.5% carboxy methyl cellulose (CMC) (1ml/kg body weight) alone. Animals that did not develop hepatotoxicity after 48 hr of paracetamol administration were rejected and new animals were used. Immediately after confirmation of hepatotoxicity, rats were classified into four groups of six rats each.

Experimental protocol

The animals were divided into four groups and each consists of six animals.

Group 1- served as normal control received 0.5% carboxy methyl cellulose (CMC) solution (1mg/kg) once daily for 5 days.

Group 2- served as paracetamol control, administrated with paracetamol (1gm/kg) as single dose on the day 5.

Group 3- received, ethanolic extract of *Terminalia arjuna* (200mg/kg) once daily for 7 days.

Group 4- served as reference control, received silymarin (25mg/kg) once daily for 3 days.

Group 3 and 4 received paracetamol (1gm/kg) as single dose on day 3, thirty minutes after the administration of ethanolic extract of *Terminalia arjuna* and silymarin respectively. All the test drugs and paracetamol were administered orally by suspending in 0.5% CMC solution. After 48h of paracetamol feeding, the blood was collected by direct cardiac puncture under light ether anesthesia and serum was separated for the estimations of transaminases, phosphatases, bilirubin and antioxidant enzymes.

RESULTS AND DISCUSSION

The ethanolic extract of *Terminalia arjuna* revealed the presence of various phytoconstituents like alkaloids, flavonoids, tannins, lignins, glycosides, phenols, sterols, saponins, quinines, coumarin, Vitamins, amino acids and terpenoids. The phytoconstituents present in the extract favours its medicinal properties. The major nutritional compounds like carbohydrate, proteins and ascorbic acid are also present in the ethanolic extract of *Terminalia arjuna*.

Table 1 showed the antioxidant activity of ethanolic extract of *Terminalia arjuna*. They were determined by hydrogen peroxide and phosphomolybdate methods. The maximum concentration of ethanolic fraction has potent antioxidant activity. The antioxidant activity or the inhibition of the generation of free radicals is

important in providing protection against hepatic damage. The antioxidant nature of *Terminalia arjuna* have been shown to posses hepatoprotective property by improving antioxidant status. Thus the efficacy of the plant would be preventive and passive for defending against liver damage[9].

Table 2 revealed the effect of *Terminalia arjuna* against paracetamol induced alterations in serum transaminases, phosphatases and total bilirubin.The AST and ALT were markedly elevated in paracetamol intoxicated rats, indicating liver damage. Oral administration of the ethanolic extract of *Terminalia arjuna* at the dose of 200mg/kg remarkably prevented the hepatotoxicity. Analysis of AST and ALT showed a significant increase (129.6 U/I, 152.73 U/I) in the paracetamol treated rats. Treatment with *Terminalia arjuna* (200mg/kg) significantly (82.3, 45.9 U/I) prevented the increase in AST, ALT level which was brought to near normal. The effect of *Terminalia arjuna* was compared with that of reference drug silymarin (25mg/kg).

Table 1. The inhibitory effect of ethanolic extract of *Terminalia arjuna* on hydrogen peroxide and phosphomolybdate

S. No.	Concentration of Extract (µg/ml)	Inhibition on hydrogen peroxide (%)	Inhibition on phospho molybdate (%)
1	50	65.39	62.61
2	100	71.92	67.22
3	150	77.45	72.41
4	200	83.26	80.06

Table 2. Hepatoprotective efficacy of *Terminalia arjuna* against paracetamol induced alterations in serum transaminases, phosphatases and total bilirubin

S. No.	Treatment	AST (U/I)	ALT (U/I)	ALP (U/L)	ACP (U/L)	Total bilirubin (mg/dl)
1	Normal	39.7±1.38	53.39 ±3.3	15.71±0.91	10.5 ±0.08	0.155±0.031
2	Paracetamol (1g/kg oral)	129.6± 1.61	152.73± 1.66	97.9± 6.8	33.1± 1.08	1.67±0.03
3	*Terminalia arjuna* (200mg/kg oral)	82.3** ±7.85	45.9** ±3.7	39.4** ±2.7	18.4* ±0.12	0.52 ±0.04
4	Silymarin (25mg/kg)	85.3** ±4.3	47.4** ±3.6	34.8** ±2.9	16.2* ±1.2	0.24* ±0.03

Data are expressed as mean ±S.E., n=6
*p>0.01 Vs Control, **P>0.001 Vs Control.

Table 3. Levels of serum Glutathione Peroxidase, Super Oxide Dismutase and Catalase in *Terminalia arjuna* treated rats

S. No	Treatment	GPx (mg/liver protein)	SOD (mg/liver protein)	Catalase (mg/liver protein)
1	Normal	0.992±0.05	75.81±1.94	296.83±10.05
2	Paracetamol (1g/kg oral)	0.704± 0.041	51.57± 4.5	177.83± 6.51
3	*Terminalia arjuna* (200mg/kg oral)	0.82* ±0.06	65.74* ±0.58	261.25* ±8.79
4	Silymarin (25mg/kg)	0.96*±0.07	84.25*±0.84	283.38*±9.46

Data are expressed as mean ±S.E., n=6
*p>0.01 Vs Control.

The changes in transaminases levels reflect in hepatic structural integrity. The rise in AST is usually accompanied by an elevation in the levels of ALT which play a vital role in the conversion of amino acids to keto acids. This plant has potent activity against cellular leakage and loss of functional integrity of the cell membrane in the hepatocytes[10]. The increased level of AST and ALT indicated deterioration in the hepatorenal functions and cellular damage due to the toxic effects of paracetamol. The presence of GSH in the cells helps in detoxification of xenobiotics or scavenging free radicals[11]. Thus administration of paracetamol at high doses will produce marked liver damage[12].

Increased elevation of ALP and ACP levels seen in paracetamol treated rats. In this study ALP ($p>0.001$) and ACP ($p>0.01$) levels are significantly decreased due to the administration of *Terminalia arjuna* extract (200mg/kg oral).

ALP and ACP are generally attributed to injury caused to the hepatocytes by paracetamol which affects the normal functions of the liver, since these enzymes are indisputably, marker of liver injury. The phosphatases are localized in the cytoplasm under normal conditions and are released into the circulation under abnormal conditions. Increase in the serum level of ALP is due to increased synthesis in presence of biliary pressure.

The levels of total bilirubin in the serum of the control and experimental groups of albino rats shown in the table2. Abnormal level (1.67mg/ 100ml of blood) of bilirubin seen in rats fed with paracetamol alone. The administration of plant extract (200mg/kg oral) showed the bilirubin level (0.52mg/100ml of blood) near normal.

Bilirubin is an endogenous organic anion, which binds reversibly to albumin and is transported to the liver, where it is conjugated to glucuronic acid and excreted in bile. Bilirubin assay is a sensitive test to substantiate the functional integrity of the liver and severity of necrosis. Bilirubin also measures the binding, conjugating and excretory capacity of hepatocytes and is proportional to the erythrocyte degradation rate[12]. Determination of serum bilirubin serves as an index for the assessment of hepatic function and any abnormal increase in level of bilirubin in the serum indicate hepatobilary disease and serve disturbance of hepatocellular function[13].

Table 3 shown the activity of the antioxidant enzymes GPx, SOD and catalase. The level of GPx was increased (0.704mg/liver protein) in paracetamol treated rats. Whereas decreased (0.82mg/liver protein) when treated with plant extract. Statistically significant decreases (51.57 and 177.83mg/liver protein) in SOD and CAT were observed in paracetamol treated groups, which was increased (65.74 and 261.25mg/liverprotein) with the treatment of ethanolic extract of *Terminalia arjuna.*

GPx is a selenium dependent enzyme has high potency in scavenging reactive free radicals. When GPx activity in liver increased, the glutathione level is decreased. Inhibition of GPx by goldthioglucose (GTG) has been found to increase the susceptibility of hepatocytes to paracetamol toxicity, indicating that a component of paracetamol's toxic effect involves formation of species that are detoxified by GPx enzymes[14]. So the level of GPx reduced to normal by the plant extract.

SOD is the major attractive metalloprotein in the antioxidant family. The increased synthesis of SOD against super oxide anion radical production is an adaptive response of the cell to synthesis increased mitochondrial SOD through the stimulation of gene transcription. The enzyme SOD was decreased due to low level of Zinc (a metal constituent of the enzyme SOD) in plasma and liver tissues. The protective activities of plants against paracetamol-induced decrease in the hepatic SOD suggest that their hepatoprotective activity can be mediated at least in part via the preservation of normal levels of antioxidant activities[15].

Catalase is a crucial enzyme in cellular antioxidative defense mechanisms and efficiently degrades endogenously produced hydrogen peroxide and converts into water and oxygen. Catalase activity was found to be significantly decreased after a toxic paracetamol dose[16]. The decreased activity of catalase may be due to the increased generation of reactive free radicals, which can create an oxidative stress in the cells. So the level must be increased to protect liver from free radical induced oxidative stress[17]. This result suggests the involvement of ethanolic extract of *Terminalia arjuna*'s antioxidant constituents in

facilitating the rapid and efficient consumption of reactive oxygen species generated by paracetamol P_{450} bioactivation.

The experimental results depicted that the ethanolic extract of *Terminalia arjuna* protect the liver from paracetamol induced hepatotoxicity. The curative effect of this plant proved by monitoring the various liver marker enzyme levels and the antioxidant activity.

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