

Protective Effect of aqueous bark extract of *Terminalia Arjuna* against Alcohol-Induced Hepato and Nephrotoxicity in Rats

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Abstract

Present study is an attempt to forward a locally available aqueous bark powder extract of *Terminalia arjuna* (AETA) as potential therapeutic agent against alcohol-induced oxidative/nitrosative stress mediated hepato and nephrotoxicity in rats. Alcohol administration significantly raised the plasma concentrations of nitrogenous compounds and increased activities of alcoholic marker enzymes, gamma glutamyl transferase (γ GT), plasma transaminases (AST and ALT), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH). Besides, we found abnormalities in the levels of plasma lipids, lipoproteins in alcohol administered rats along with increased lipid peroxidation and nitric oxide (NOx) levels. Moreover, significantly decreased hepatic and kidney antioxidant enzymes, superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and the content of reduced glutathione (GSH) in alcohol administered rats were noticed. Administration of AETA to alcoholic rats significantly brought these alterations in plasma to normal and also significantly reduced the levels of lipid peroxidation and restored the enzymic and nonenzymic antioxidants in liver. These findings were further confirmed by hepatic and kidney histopathological studies. Co-administration of alcohol along with AETA offers protective effect against alcohol-induced stress and these protective effects are due to its multiple actions of its bioactive compounds.

Keywords: Alcohol; Hepatotoxicity; Nephrotoxicity; Oxidative stress, Terminalia Arjuna

Introduction

Long term and excessive alcohol consumption leads to alcoholic liver disease (ALD) and renal disorders [1,2]. Liver is a primary organ affected by alcohol and its metabolites [3]. Studies confirmed that oxidative stress and excessively produced nitric oxide (NO) leading to nitrosative stress significantly contribute to the adverse and toxic effects of alcohol in the liver [4,5]. There has been a great demand for remedies and therapeutic agents to treat alcoholism, alcoholic damage and alcohol related diseases [6]. Over the past decade locally available phytoextracts/herbal medicines have attracted much attention as potential therapeutic agents in prevention and treatment of various diseases including ALD due to their multiple targets and minimal toxic side effects in several countries [7]. Many medicinal plants exhibit different bioactive compounds including tannins, flavonoids and other active compounds. These compounds typically present in small amounts have more subtle effects than nutrients and can influence cellular activities that modify the risk of diseases. Thorough screening of ethno botanical and scientific literature revealed that *Terminalia arjuna* (Combretaceae) with its multiple phytoconstituents has great potential to provide several health benefits [8].

T. arjuna, a deciduous tree, is distributed throughout the world, in particular more in India than elsewhere. Its bark has been an ingredient in several formulations of Indian traditional medicine [9]. Since *T. arjuna* contain several potent phytoprinciples, it has been used to treat diseases ranging from simple illnesses to complex degenerative diseases [10]. Bark extract of *T. arjuna* was reported to contain many specific phytochemicals including Triterpene glycosides - arjunetin, arjunoglucoside - I/II/III, arjunoside I/II/III/IV, arjunolitin and terminolitin; triterpene saponins - arjunic acid, arjunolic acid, arjungenin; flavonoids – arjunone and arjunolone and also it contains non-specific phytochemicals including phytosterols i.e., β -sitosterol, proanthocyanidins and minerals - Ca, Mg, Zn and Cu [8, 11]. These specific and non-specific phytochemicals possess antioxidant [12], anti-hyperlipidemic [13] antibacterial [14], anticarcinogenic [15,16], anti-inflammatory [17, 18] properties and known to confer the bark extract of *T. arjuna* with cardioprotective [19, 20], hepatoprotective [21, 22], renal protective [23], gastroprotective [24] and wound healing effects [25, 26]. Despite the fact that *T. arjuna* possesses many novel therapeutic phytochemicals it was never tested to treat alcohol-induced liver and nephrotoxicity/damage. Therefore, this study chiefly investigates the protective role of aqueous extract of bark powder of *T. arjuna* (AETA) against chronic alcohol-induced hepato and nephrotoxicity in rats.

Materials and methods

Chemicals

All the chemicals used in the present study were of analytical grade procured from Sigma (St.Louis, USA) and SRL Chemicals (Mumbai, India). Alcohol used for administration to the rats was obtained by redistillation. Bark of *Terminalia arjuna* (Roxb.) Wight & Arn. was purchased from a local vendor and authenticated at National Ayurveda Dietetics Research Institute, Jayanagar, Bangalore, India.

Preparation of aqueous extract

Aqueous bark powder extract of *Terminalia arjuna* (AETA) was prepared by soaking pulverized bark in distilled water. Overnight extraction was performed on an orbital shaker set at room temperature and the procedure was repeated twice and the pooled filtrate was concentrated under reduced pressure and further air dried in a dessicator for 24-48 hrs until they become moist free. The resultant dry flakes and scrapings were finely powdered and stored in air tight containers (Percentage yield - 35.07%). The powder was resuspended in appropriate volumes of distilled water to prepare AETA for animal experimentation.

Experimental design

Two month old male albino Wistar rats each weighing 120-140g, were obtained and acclimatized to the facility for one week before experimentation. Commercial rodent chow (Hindustan Lever Ltd, Mumbai, India) and tap water were made available *ad libitum*, and rats were maintained on a 12-h light/dark cycle in a temperature-regulated (20°C ± 1°C) room. Animals were then categorized into six groups of eight rats in each group as shown below.

Group I (Control rats) received glucose as a caloric equivalent of alcohol;

Group II (Alcoholic rats) received a forced oral administration of alcohol through a gastric tube (20% alcohol v/v; 5g alcohol/kg b.wt/day);

Group III (Therapeutic) received a co-administration of AETA (250mg of AETA/kg b.wt/day along with alcohol (20% alcohol v/v; 5g alcohol/kg b.wt/day);

Group IV (Therapeutic) received a co-administration of AETA (500mg of AETA/kg b.wt/day) along with alcohol (20% alcohol v/v; 5g alcohol/kg b.wt/day);

Group V received 250mg of AETA/kg b.wt/day

Group VI received 500mg of AETA/kg b.wt/day.

All the animals received treatments together with food and water *ad libitum* for a period of 60 days. Selection of alcohol dose was based on our earlier studies [27] and the dosage of AETA was fixed based on earlier studies [28, 29] with minor modifications for a better understanding of the dose response relationship. Rats in all groups were killed by cervical dislocation after completion of the experimental period. Blood was immediately collected into heparinized tubes by cardiac puncture and plasma was separated

for assessing different biochemical parameters. Liver and kidneys were removed, washed 2 to 3 times in ice cold saline and stored at -80°C until further analysis. Small section of liver and kidney was fixed in 10% neutral formalin solution for histological analysis. The present study was taken with prior approval of departmental and institutional ethics committee.

Plasma biochemical analysis

Plasma nitrogenous compounds, urea [30], uric acid [31], total bilirubin [32], creatinine [33] and marker enzymes, AST / ALT [34], ALP [35], LDH [35], γ GT [36] were analyzed as described previously. Moreover plasma lipids, total cholesterol [37], HDL-C [38], triglycerides [39] were analyzed using the commercially available ERBA diagnostic kits (Mannheim, Germany). LDL and VLDL cholesterol levels were determined using the formula of Friedewald *et al.*, 1972 [40]. Atherogenic index, the ratio of total cholesterol and HDL cholesterol was calculated as described in Kumari *et al.*, 1995 [41].

Measurement of TBARS and nitrate/nitrite levels

Thiobarbituric acid reactive species (TBARS) were measured by the formation of malondialdehyde [42]. Nitrate/nitrite levels were estimated as described by the method of Sastry *et al.*, 2002 [43].

Determination of liver and kidney antioxidant status

Liver and kidney tissues were homogenized (10% w/v) in Tris-HCl buffer (0.1M, pH 7.4), centrifuged (10,000xg for 20 minutes at 4°C) and supernatant used for all the biochemical parameters. Catalase (CAT) was assayed as reported in Aebi, 1984 [44]. The CAT activity was expressed as nmol H₂O₂ decreased/mg protein/min. Superoxide dismutase (SOD) was assayed [45]. A single unit of enzyme was expressed as 50% inhibition of NBT (nitro blue tetrazolium) reduction/min/mg protein. Glutathione peroxidase (GPx) activity was measured as described [46]. GPx activity was expressed as μ mol GSH oxidized/min/mg protein. Total reduced glutathione (GSH) content was also measured [46]. Protein concentration was estimated by the method of Lowry *et al.*, 1951 [47].

Histopathological examination

Portions of liver and kidney were dissected and fixed in 10% neutral buffered formalin solution for 24 h. The fixed tissues were processed routinely, and then embedded in paraffin, sectioned to 3–5 inch thickness, deparaffinized, and rehydrated using standard techniques. Alcohol-induced morphological changes in liver and kidney sections were observed by staining with hematoxylin and eosin (H&E).

Statistical analysis

Results were reported as means ± standard deviation (SD). Significant differences for comparisons were determined by a one-way analysis of variance (ANOVA) followed by student "t" test were performed to determine significant difference among the test



groups. Results with 5% level of confidence ($p < 0.05$) were considered statistically significant.

Results and Discussion

Alcohol-induced nitrosative and oxidative stress causes multiple organ injury amongst which liver and kidney are understood to be the chief targets for alcoholic damage [2, 48]. Present study, emphasizes the specific role of AETA in protecting the system against alcohol-induced hepato and nephrotoxicity. Chronic alcohol administration increased the plasma creatinine, urea and bilirubin levels and also decreased the plasma uric acid levels (Table 1) suggesting an enhanced nitrosative stress in the system by a biochemical, functional and possible morphological impairment in the kidney which is in agreement with earlier reports [49, 50]. Alcohol-induced increase in oxidative stress and deficiency of antioxidant machinery may lead to interstitial inflammation and reduction of number of nephron units which limits sodium filtration and causes uremia. As a consequence of diminished renal catabolism and function, uremic oxidant mediators accumulate urea and creatinine in blood. Studies also revealed that alcohol increases breakdown/degradation of proteins in muscle along with impairment in glomerular filtration with rhabdomyolysis and uremia [51]. In this study, increased nitrogenous compounds in plasma are due to glomerular filtration impairment with rhabdomyolysis. Administration of AETA along with alcohol resulted in a significant decrease ($p < 0.05$) in plasma levels of these nitrogenous compounds with increase in uric acid. However, administration of AETA at 500mg/kg b.wt/day showed maximum restoration. Phytoprinciples present in AETA exhibited a potential ability to counteract and offer protection against alcohol-induced changes in liver and kidney.

Literature suggests that alcohol administration to rats significantly increases lipid peroxidation leading to liver and kidney damage resulting in leakage of cellular contents into the circulation [5]. In the present study, elevated plasma aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) levels in alcohol treated animals is an indicative of cellular breakage and loss of functional integrity in hepato and renal cell membranes (Table 2). LDH, found in all major tissues is considered a good marker for diagnosing myocardial infarction, hepatitis, renal damage and anemia [52] and γ GT is clinically useful in detecting obstructive jaundice, cholangitis and choecystitis and is found to elevate when drugs viz., alcohol, sedatives, anticonvulsants and tranquilizers are used and infact considered as an early and sensitive marker in detecting liver injuries during chronic ethanol intoxication [53]. Increased activity of γ GT in the alcohol intoxicated rats in the present investigation may be due to progression towards hepatobiliary disease and AETA administration to alcohol receiving rats effectively restored the enzyme levels close to normal values. The phytochemicals present in AETA might have maintained structural integrity of the

cell membrane by scavenging ROS and thereby could protect the cells from alcohol-induced cellular damage.

Observed hypercholesterolemia, triglyceridemia and hyperlipidemia with increased LDL-C and VLDL-C followed by decreased HDL-C in the present study are characteristic biochemical features in chronic alcohol consumption, and were reported by earlier researchers [48, 54, 55, 56]. It is well known that modifications of lipids are integral components for initiation and progression of atherosclerosis impart by its effects on lipid profile. Additionally LDL, VLDL and HDL are considered to be powerful markers and risk factors for CVD and the accumulation of fat in liver upon chronic alcohol consumption acts as a stimulus for secretion of lipoproteins into blood stream and the development of hyperlipidaemia. Moreover, decreased fatty acid synthesis would increase the availability of substrate for lipoprotein synthesis. Lipoprotein lipase (LPL) is an important enzyme which catalyzes the reactions of hydrolysis of triglycerides present in chylomicrons and VLDL. Low activity of LPL in alcoholic rats causes accumulation of triglycerides and hydrolysis of VLDL. HDL-C helps in scavenging cholesterol from extra hepatic tissues in presence of L-CAT (lecithin cholesterol acetyl transferase) and brings it to liver. Moreover increased NO production was observed in alcoholic rats in present study and is in agreement with earlier reports [5]. NO mediated regulation in hepatic production (or) secretion of apolipoprotein particles increasing triglyceride lipases and decreasing the circulating HDL-C was observed. AETA supplementation to alcoholic rats restored the alcohol induced changes in lipid and lipoprotein profile of alcoholic rats to normal. Several earlier reports revealed the hypolipidaemic actions of *T.Arjuna* [28, 57] and it is known for its cardioprotective functions by regulating and mitigating the changes in cardiovascular system and lipid abnormalities, thereby exerting beneficiary role [19]. Further the cardioprotective effects of AETA were even reported to be due to the presence of active compound arjunolic acid [11]. Effects of AETA administration on alcohol-induced changes in plasma lipid profile of rats belonging to different treatment groups is presented in Table 3. We noticed significant increase ($p < 0.05$) in total cholesterol, LDL-C, VLDL-C and triglycerides levels and a decrease in HDL-C level of alcohol receiving rats compared to controls. This study also revealed that upon AETA administration there was rectification of the above said parameters by restoring the levels to normal. Therapeutic administration of both the doses of AETA rectified the levels of the parameters significantly by decreasing the levels of total cholesterol, LDL-C, VLDL-C and triglycerides with increasing HDL-C levels at the two different doses of AETA, however, 500mg/kg b.wt/day showed maximum restoration.

Lipid peroxidation has been reported to be a marker of oxidative stress [58]. Free radicals induce lipid peroxidation, resulting in reactive molecules such as MDA and 4-hydroxy-3-nonenal (HNE). Both of these can react with proteins to form MDA-protein and HNE-protein adducts which have adverse effects on cell functions and biochemistry [59, 60]. Increased lipid peroxidation (Figure 1) and overproduction of NO (Figure 2) were recorded from plasma



samples of alcoholic rats in present study which indicates alcohol – induced increase in oxidative stress and nitrosative stress, resulting in functional impairment of several biomolecules, organelles and derangement in metabolic pathways. Increased lipid peroxidation was found to be ameliorated in alcoholic rats supplemented with AETA. Flavonoids present in AETA act as antioxidants by chelating redox-active metals and by scavenging free radicals. Chelation of both iron and copper by the carbonyl and hydroxyl groups of flavonoids prevents peroxy radical and lipid peroxidation. Flavonoids function as terminators of free radicals by donation of electrons to form stable products. Hike in lipid peroxidation and NO production observed in the alcoholic rats in the present study is in agreement with earlier reports [4, 5]. Furthermore, results of this study demonstrated that AETA is effective in modulating NO production as NO generation was maximally restored to that of normal level upon AETA supplementation to alcohol treated rats. Similarly AETA administration was also found to be effective in reducing the extent of lipid peroxidation in liver and kidney tissues of alcohol treated rats (Figures 3 and 4 respectively). A significant increase ($\rho < 0.05$) in TBARS of liver and kidney was observed in alcohol treated rats and co-administration of AETA at 250 and 500mg/kg b.wt/day along with alcohol resulted in a significant decrease ($\rho < 0.05$) in the extent of lipid peroxidation in liver and kidney.

Protective efficacy of AETA against alcohol-induced oxidative stress is more specifically understood by investigating the concentrations of GSH, which is a non-protein thiol, which plays a central role in coordinating the antioxidant defense processes, and

maintains normal cell structure and function through its redox and detoxification reactions [5]. In addition, activities of antioxidant enzymes viz., SOD, GPx and Catalase are being investigated to understand the antioxidant status of the hepatic and kidney tissue. Since, reactive oxygen species (ROS) viz., superoxide and hydroxyl radicals are known to cause injuries to the tissues and organs, natural compounds with antioxidant properties may help to alleviate the damage totally or partially [61, 62]. Reduced activities of the mentioned antioxidant enzymes in the hepatic and kidney tissues of the alcohol intoxicated rats indicate the excess accumulation of reactive free radicals which results in the loss of membrane integrity and function. Therefore, scavenging the ROS would probably be the most effective defense mechanisms conferred by any therapeutic agent and AETA by virtue of its rich phytoconstituent profile could effectively rectify the condition by scavenging hydroxyl and peroxy radicals as well as quenching superoxide radicals and singlet oxygen species. Alcohol-induced effects on the liver and kidney antioxidant enzymes viz., GSH, GPx, SOD and Catalase and effect of AETA administration in rats are depicted in Tables 4 and 5. Alcohol treated rats showed a significant ($\rho < 0.05$) decline in the activities of all the antioxidant enzymes and the content of GSH in the liver and kidney tissue when compared with control group. Co-administration of alcohol along with AETA resulted in a significant rise ($\rho < 0.05$) in the antioxidant enzyme activities, with maximum restoration at 500mg/kg b.wt/day of AETA.

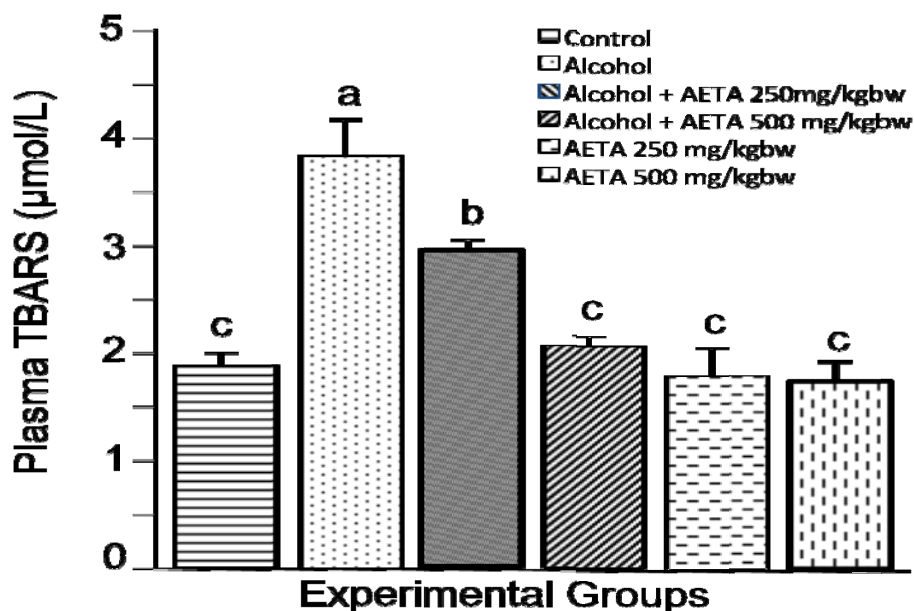


Figure 1. Effect of AETA administration on plasma TBARS in control and experimental rats. Values are Mean \pm SD (n=8). Means in a row not sharing a common superscript are significantly different ($P < 0.05$) among groups.



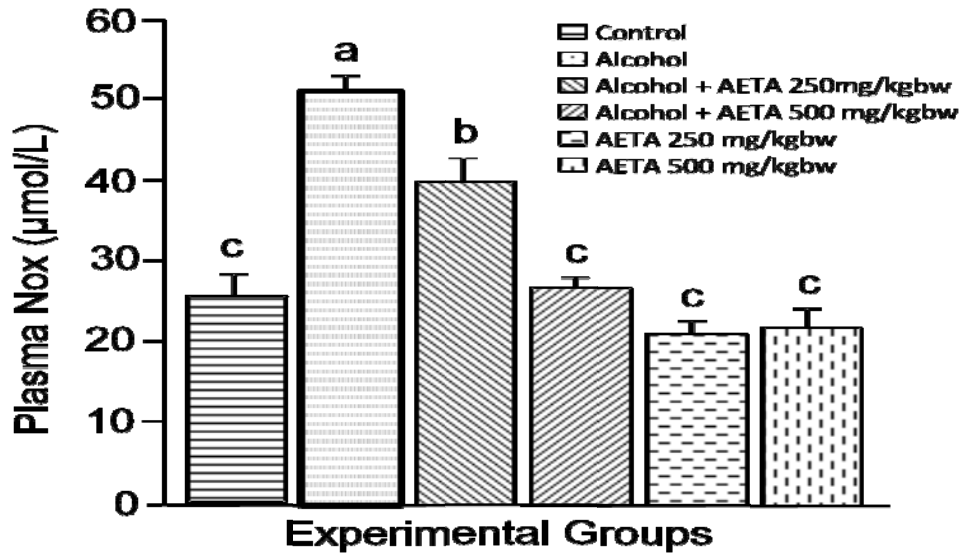


Figure 2. Effect of AETA administration on plasma NOx levels in control and experimental rats. Values are Mean ± SD (n=8). Means in a row not sharing a common superscript are significantly different ($P<0.05$) among groups.

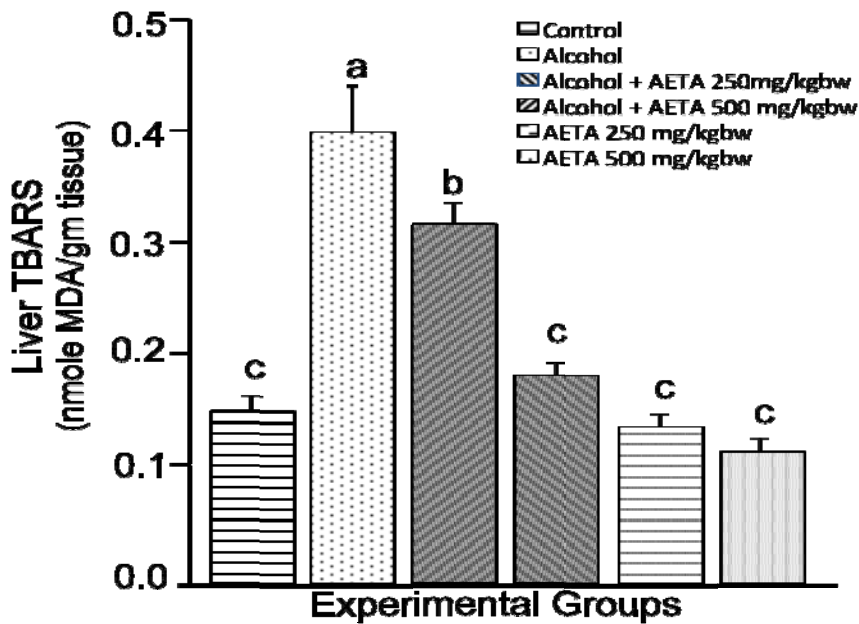


Figure 3. Effect of AETA administration on liver TBARS in control and experimental rats. Values are Mean ± SD (n=8). Means in a row not sharing a common superscript are significantly different ($P<0.05$) among groups.



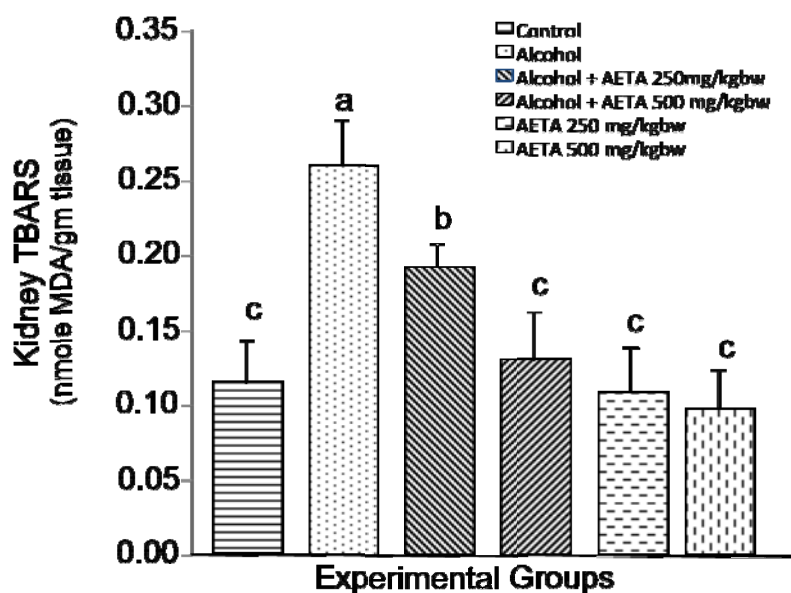


Figure 4. Effect of AETA administration on kidney TBARS in control and experimental rats. Values are Mean \pm SD (n=8). Means in a row not sharing a common superscript are significantly different ($P < 0.05$) among groups.

Table 1: Effect of administration of AETA on plasma biochemical parameters in chronic alcoholic rats.

Parameter (mg/dL)	Control	Alcohol	Alcohol + AETA 250 mg/kg b.wt	Alcohol + AETA 500 mg/kg b.wt	AETA 250 mg/kg b.wt	AETA 500 mg/kg b.wt
Urea	25.2 \pm 1.7 ^c	34.0 \pm 2.7 ^a	29.5 \pm 3.2 ^b	24.8 \pm 1.8 ^c	25.7 \pm 3.5 ^c	23.8 \pm 2.9 ^c
Uric acid	2.32 \pm 0.05 ^a	1.52 \pm 0.05 ^b	1.95 \pm 0.05 ^a	2.11 \pm 0.04 ^a	2.24 \pm 0.2 ^a	2.38 \pm 0.05 ^a
Bilirubin (Total)	0.59 \pm 0.04 ^c	1.19 \pm 0.2 ^a	0.75 \pm 0.05 ^b	0.61 \pm 0.03 ^c	0.54 \pm 0.03 ^c	0.52 \pm 0.03 ^c
Creatinine	0.42 \pm 0.06 ^c	0.95 \pm 0.09 ^a	0.72 \pm 0.07 ^b	0.49 \pm 0.07 ^c	0.40 \pm 0.12 ^c	0.42 \pm 0.06 ^c

Values are Mean \pm SD of eight rats in each group. Means in a row not sharing a common superscript are significantly different ($P < 0.05$) among groups.

Table 2: Effect of administration of AETA on plasma enzymes in chronic alcoholic rats.

Parameter (IU/L)	Control	Alcohol	Alcohol + AETA 250 mg/kg b.wt	Alcohol + AETA 500 mg/kg b.wt	AETA 250 mg/kg b.wt	AETA 500 mg/kg b.wt
AST	53.9 \pm 2.5 ^c	121.9 \pm 2.9 ^a	88.7 \pm 3.4 ^b	56.5 \pm 2.4 ^c	53.7 \pm 2.3 ^c	52.2 \pm 1.4 ^c
ALT	25.0 \pm 1.8 ^c	72.1 \pm 1.4 ^a	44.4 \pm 2.5 ^b	26.8 \pm 2.9 ^c	25.1 \pm 2.0 ^c	24.7 \pm 1.8 ^c
ALP	62.1 \pm 2.5 ^c	139.1 \pm 2.7 ^a	82.8 \pm 2.4 ^b	68.2 \pm 1.3 ^c	62.5 \pm 2.5 ^c	61.3 \pm 1.8 ^c
LDH	375 \pm 20 ^c	588 \pm 22 ^a	513 \pm 17 ^b	409 \pm 16 ^c	368 \pm 18 ^c	350 \pm 17 ^c
GGT	2.9 \pm 0.1 ^c	10.6 \pm 0.7 ^a	6.3 \pm 0.6 ^b	3.4 \pm 0.5 ^c	2.8 \pm 0.1 ^c	2.8 \pm 0.2 ^c

Values are Mean \pm SD of eight rats in each group. Means in a row not sharing a common superscript are significantly different ($P < 0.05$) among groups.



Table 3: Effect of administration of AETA on plasma lipid profile in chronic alcoholic rats.

Parameter (mg/dL)	Control	Alcohol	Alcohol + AETA 250 mg/kg b.wt	Alcohol + AETA 500 mg/kg b.wt	AETA 250 mg/kg b.wt	AETA 500 mg/kg b.wt
Total Cholesterol	67.9 ± 2.4 ^c	103.1 ± 4.6 ^a	87.3 ± 1.7 ^b	69.5 ± 3.5 ^c	68.7 ± 3.9 ^c	65.5 ± 2.1 ^c
Triglycerides	67.1 ± 2.7 ^c	89.7 ± 5.0 ^a	77.2 ± 3.2 ^b	68.2 ± 2.9 ^c	65.5 ± 2.3 ^c	64.5 ± 1.8 ^c
HDL-C	40.2 ± 2.0 ^a	23.0 ± 1.9 ^c	31.7 ± 1.3 ^b	39.0 ± 2.9 ^a	38.3 ± 2.7 ^a	38.7 ± 2.0 ^a
LDL-C	14.3 ± 0.5 ^c	62.2 ± 5.0 ^a	40.2 ± 2.0 ^b	16.9 ± 3.0 ^c	16.5 ± 4.7 ^c	13.9 ± 2.9 ^c
VLDL-C	13.4 ± 0.5 ^c	17.9 ± 1.0 ^a	15.4 ± 0.6 ^b	13.6 ± 0.6 ^c	13.1 ± 0.5 ^c	12.9 ± 0.4 ^c

Values are Mean ± SD of eight rats in each group. Means in a row not sharing a common superscript are significantly different ($P < 0.05$) among groups.

Table 4: Effect of administration of AETA on liver antioxidant enzyme activity in chronic alcoholic rats.

Parameter	Control	Alcohol	Alcohol + AETA 250 mg/kg b.wt	Alcohol + AETA 500 mg/kg b.wt	AETA 250 mg/kg b.wt	AETA 500 mg/kg b.wt
GSH (μmol/mg P)	7.12 ± 0.5 ^a	3.61 ± 0.6 ^c	5.63 ± 1.0 ^b	7.18 ± 0.7 ^a	7.06 ± 0.1 ^a	7.95 ± 0.9 ^a
GPx (μmol GSH oxid/ min)	10.82 ± 1.7 ^a	5.74 ± 0.8 ^c	8.14 ± 0.9 ^b	10.06 ± 0.1 ^a	11.09 ± 0.6 ^a	11.55 ± 1.6 ^a
SOD (U/mg P/min)	21.3 ± 1.2 ^a	14.2 ± 0.8 ^c	18.1 ± 1.6 ^b	21.6 ± 2.0 ^a	21.2 ± 1.7 ^a	21.9 ± 1.6 ^a
Catalase (U/mg P/min)	42.2 ± 3.8 ^a	29.0 ± 1.9 ^c	35.7 ± 1.5 ^b	40.6 ± 2.8 ^a	41.8 ± 2.7 ^a	42.7 ± 2.3 ^a

Values are Mean ± SD (n=8), Means in a row not sharing a common superscript are significantly different ($P < 0.05$) among groups.

Table 5: Effect of administration of AETA on kidney antioxidant enzyme activity in chronic alcoholic rats.

Parameter	Control	Alcohol	Alcohol + AETA 250 mg/kg b.wt	Alcohol + AETA 500 mg/kg b.wt	AETA 250 mg/kg b.wt	AETA 500 mg/kg b.wt
GSH (μmol/mg P)	6.71 ± 0.4 ^a	3.94 ± 0.5 ^c	4.85 ± 0.4 ^b	6.92 ± 0.2 ^a	6.88 ± 0.8 ^a	6.94 ± 0.6 ^a
GPx (μmol GSH oxid/ min)	8.20 ± 0.7 ^a	5.24 ± 0.4 ^c	6.13 ± 0.4 ^b	8.02 ± 0.4 ^a	8.21 ± 0.5 ^a	8.25 ± 0.9 ^a
SOD (U/mg/min)	15.5 ± 1.4 ^a	5.81 ± 1.2 ^c	7.18 ± 0.7 ^b	14.8 ± 1.7 ^a	15.8 ± 2.7 ^a	16.1 ± 1.9 ^a
Catalase (U/mg P/min)	33.4 ± 2.7 ^a	19.0 ± 2.0 ^c	21.5 ± 1.4 ^b	32.5 ± 2.8 ^a	34.1 ± 2.5 ^a	35.3 ± 2.4 ^a

Values are Mean ± SD (n=8), Means in a row not sharing a common superscript are significantly different ($P < 0.05$) among groups.

Protective efficacy of AETA was further supported by the histopathological evidences of liver (Figure 5) and kidney tissues (Figure 6) from the rats of different treatment groups compared with control group rats. Figure 5 represents the histopathological micrographs of liver tissue from alcohol treated rats in comparison with other test groups. Compared to the intact architecture of parenchyma, sinusoids and normal appearance of central veins in

the hepatocytes of control group of rats (Figure 5a), alcohol-administered rat hepatocytes appears to have developed areas of fibrosis and atrophy with degenerated nucleus (Figure 5b). Alcohol treated rats coadministered with AETA appears to have a restored the hepatocyte morphology to normal at both the doses (250 and 500 mg/kg b.wt/day) of AETA (Figure 5c & 5d) and a similar morphology of hepatocytes was observed even in rats receiving

either 250 mg/kg b.wt/day or 500 mg/kg b.wt/day of AETA alone (Figure 5e & 5f). Figure 6 represents the histopathological micrographs of kidney tissue from rats belonging to different groups. Hypercellularity with decreased Bowman's space in the glomerulus and periglomerular inflammatory infiltration is evident in alcohol intoxicated rats (Figure 6b) when compared to the normal cellularity and intact glomerular space in control group rats (Figure 6a). Alcohol treated rats fed with AETA (250 mg/kg b.wt/day) showed a remarkable protection against alcohol induced pathological changes in the kidney tissue as evidenced by a slight increase in Bowman's space and reduced inflammatory infiltration (Figure 6c); whereas rats treated with alcohol and 500 mg/kg b.wt/day of AETA (Figure 6d) showed an enhanced protection by maintaining intact structural features of glomerulus and blood vessels than 250 mg/kg b.wt/day of AETA almost comparable to that of control group of rats. The extract alone at the doses 250 and 500 mg/kg b.wt/day was found to cause no damage to the kidney tissue (Figures 6e & 6f).

Conclusion

In conclusion, our study revealed that AETA has shown protective action against alcohol – induced hepatic and renal injury by ameliorating both the nitrosative and oxidative stress basically by enhancing the antioxidant status of the system. The results are supported by histopathological observations of both the tissues where in AETA administration was found to be effective in maintaining the normal physiological and morphological functioning of both the tissues. Phytoprinciples of *Terminalia arjuna* could have functioned in a synergistic fashion conferring an enhanced medicinal property to the tree and also, this would have been the reason behind the observed amelioration of alcohol – induced organ and biochemical pejection in the present study.

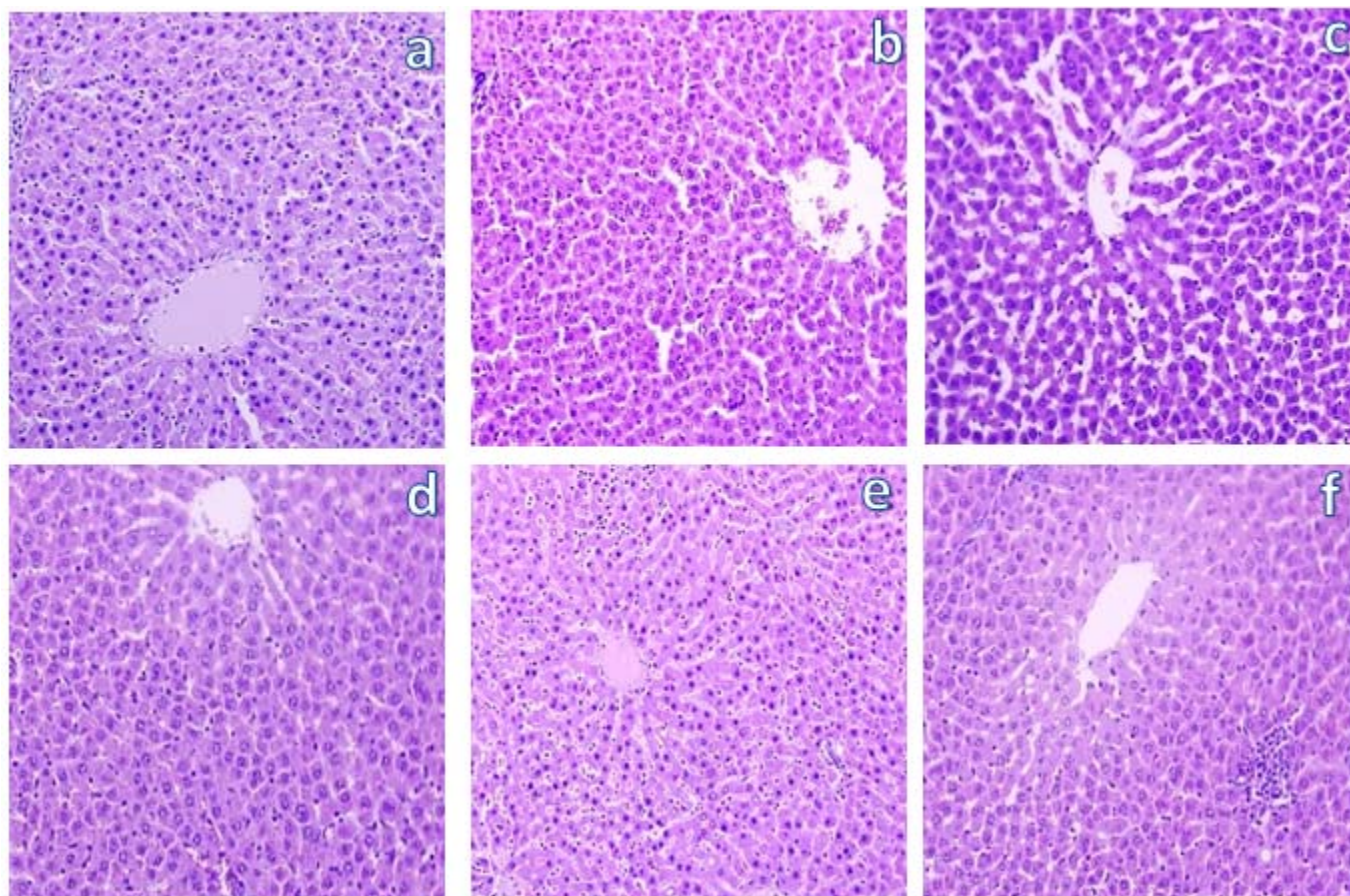


Figure 5. Representative photomicrographs of livers in different experimental groups. Liver sections were fixed in 10% buffered formalin, embedded in paraffin, sectioned and stained with hematoxylin–eosin. a) Liver sections from control rats, b) alcohol administered rat liver section, c) alcohol and AETA (250mg/kg b.wt/day) administered rat liver section, d) alcohol and AETA (500mg/kg b.wt/day) administered rat liver section, e) AETA alone (250mg/kg b.wt/day) administered rat liver section, f) alcohol and AETA (500mg/kg b.wt/day) administered rat liver section(originalmagnification,X100).



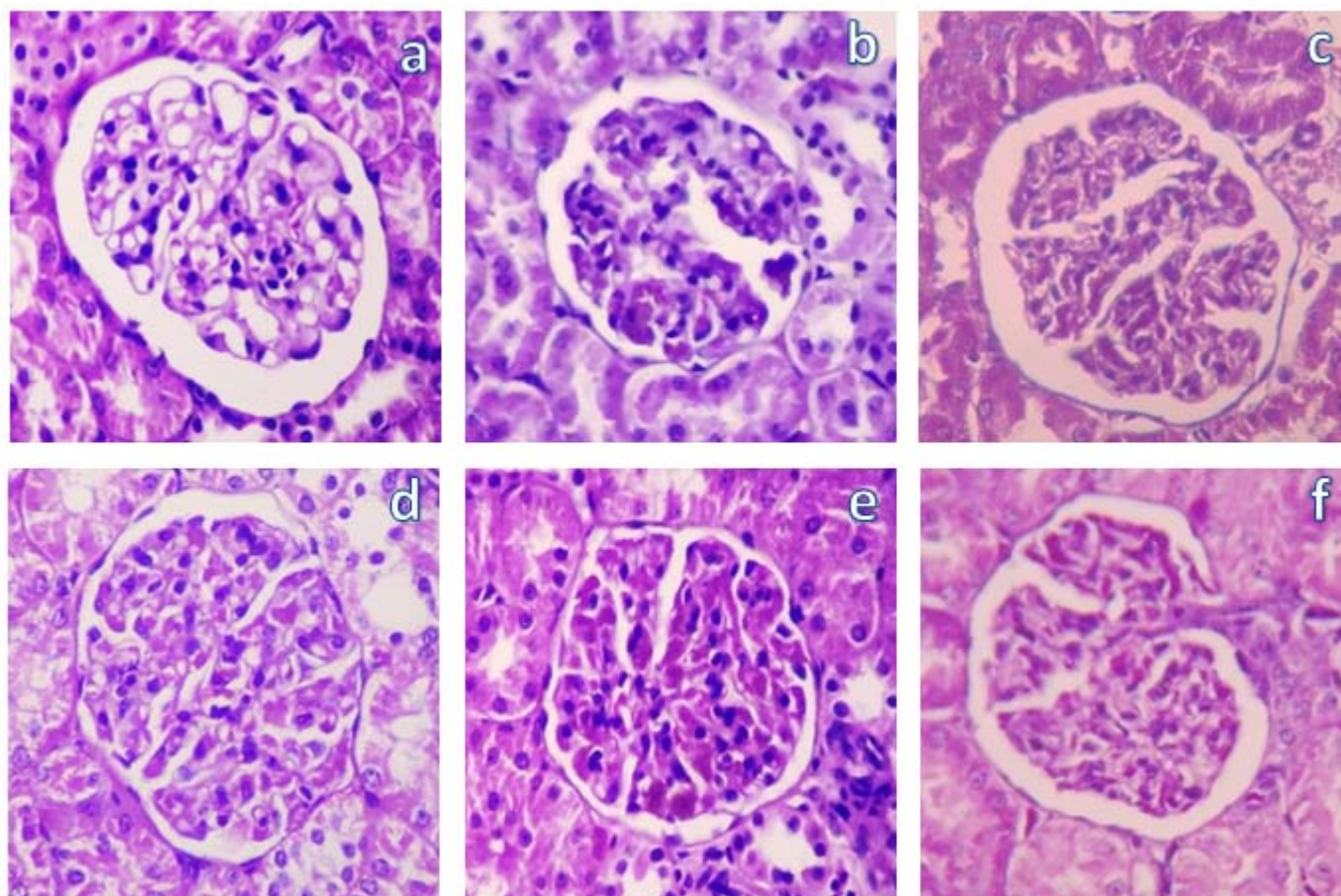


Figure 6. Representative photomicrographs of kidney in different experimental groups. Kidney sections were fixed in 10% buffered formalin, embedded in paraffin, sectioned and stained with hematoxylin–eosin. a) Kidney section from control rat, b) alcohol administered rat kidney section, c) alcohol and AETA (250mg/kg b.wt/day) administered rat kidney section, d) alcohol and AETA (500mg/kg b.wt/day) administered rat kidney section, e) AETA alone (250mg/kg b.wt/day) administered rat kidney section, f) alcohol and AETA (500mg/kg b.wt/day) administered rat kidney section (original magnification, X100).

Authors Contribution

Ananda Vardhan Hebbani is the research fellow who performed the experiments and wrote the paper which was planned and scrutinized by Dr. Vaddi Damodara Reddy.

Dr.N.Ch.Varadacharyulu is the research supervisor who gave his advices and inputs by editing the manuscript.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

References

- [1]. Singal K, Ashwani, and Sarat C Jampana. Current Management of Alcoholic Liver Disease. Current drug abuse reviews 2014; 7(1): 18-28.
- [2]. Kumar SD and Vasudevan DM. Alcohol induced effects on kidney. Indian J Clin Biochem 2008; 23: 4-9.
- [3]. Ponnappa BC, Rubin E. Modeling alcohol's effects on organs in animal models. Alcohol Res Health 2000; 24: 93-104.
- [4]. Zelickson B R, Benavides G A, Johnson M S, Chacko B K, Venkatraman A, Landar A. and Darley-Usmar, V. M.. Nitric oxide and hypoxia

- exacerbate alcohol-induced mitochondrial dysfunction in hepatocytes. *Biochimica et Biophysica Acta (BBA)-Bioenergetics* 2011; 1807(12): 1573-1582.
- [5]. Reddy VD, Padmavathi P, Kavitha G, Saradamma B and Varadacharyulu N. Alcohol-induced oxidative/nitrosative stress alters brain mitochondrial membrane properties. *Molecular and cellular biochemistry* 2013; 375(1-2): 39-47.
- [6]. McCarty MF. Nutraceutical strategies for ameliorating the toxic effects of alcohol. *Medical hypotheses* 2013; 80: 456-462.
- [7]. Seeff Leonard B, Karen L, Lindsay Bruce R, Bacon Thomas F, Kresina and Jay H, Hoofnagle. Complementary and alternative medicine in chronic liver disease. *Hepatology* 2001; 34(3): 595-603.
- [8]. Dwivedi S. *Terminalia arjuna* Wight & Arn. A useful drug for cardiovascular disorders. *J Ethnopharmacol* 2007; 114: 114-129.
- [9]. Khare CP Ed. Indian medicinal plants: an illustrated dictionary. Springer, New Delhi. 2007.
- [10]. Tyagi P, Khan HA. Amelioration of oxidative stress in the joint tissue may be the basis for the antiarthritic activity of *Terminalia arjuna* bark extract. *Int J Rheum Dis* 2014- In Press.
- [11]. Ghosh J, Sil PC. Arjunolic acid: A new multifunctional therapeutic promise of alternative medicine. *Biochimie* 2013; 95: 1098-1109.
- [12]. Sultana B, Anwar F, Przybylski R. Antioxidant activity of phenolic components present in barks of *Azadirachta indica*, *Terminalia arjuna*, *Acacia nilotica*, and *Eugenia jambolana* Lam. trees. *Food Chem* 2007; 104:1106-1114.
- [13]. Subramaniam S, Ramachandran S, Uthrapathi S, Gnamanickam V R, and Dubey G P. Anti-hyperlipidemic and antioxidant potential of different fractions of *Terminalia arjuna* Roxb. bark against PX-407 induced hyperlipidemia. *Indian journal of experimental biology* 2011; 49(4), 282-288.
- [14]. Kumar S, Singh AK, Verma SK. Antibacterial and phyto-chemical analysis of some medicinal plants and their efficacy on multidrug resistant bacteria. *J Pure Applied Microbiol* 2013; 7: 2191-2204.
- [15]. Ahmad M S, Ahmad S, Gautam B, Arshad M, and Afzal M. *Terminalia arjuna*, a herbal remedy against environmental carcinogenicity: An *in vitro* and *in vivo* study. *Egyptian Journal of Medical Human Genetics* 2014; 15(1): 61-67.
- [16]. Sivalokanathan S, Ilayaraja M, Balasubramanian MP. Antioxidant activity of *Terminalia arjuna* bark extract on N-nitrosodiethylamine induced hepatocellular carcinoma in rats. *Mol Cell Biochem* 2006; 281: 87-93.
- [17]. Shukla SK, Sharma SB, Singh UR. Pre-treatment with -tocopherol and *Terminalia arjuna* ameliorates, markers of inflammation, cardiac and apoptotic markers in experimental myocardial ischemic rats. *Redox Report* 2014- In Press
- [18]. Halder S, Bharal N, Mediratta P K, Kaur I, & Sharma K K. Anti-inflammatory, immunomodulatory and antinociceptive activity of *Terminalia arjuna* Roxb bark powder in mice and rats. *Indian journal of experimental biology* 2009; 47(7): 577.
- [19]. Kapoor D, Vijayvergiya R, Dhawan V. *Terminalia arjuna* in coronary artery disease: Ethnopharmacology, pre-clinical, clinical & safety evaluation. *J Ethnopharmacol* 2014; 155: 1029-1045.
- [20]. Gupta Amit K, Manoj K. Rai Mahendra Phulwaria, Tanvi Agarwal, and Shekhawat N. S. "In vitro propagation, encapsulation, and genetic fidelity analysis of *Terminalia arjuna*: a cardioprotective medicinal tree. *Applied biochemistry and biotechnology* 2014; 173(6): 1481-1494.
- [21]. Sinha M, Manna P, Sil PC. Aqueous extract of the bark of *Terminalia arjuna* plays a protective role against sodium-fluoride-induced hepatic and renal oxidative stress. *J Nat Med* 2007; 61: 251-260.
- [22]. Manna P, Sinha M, and Sil P C. Aqueous extract of *Terminalia arjuna* prevents carbon tetrachloride induced hepatic and renal disorders. *BMC complementary and alternative medicine* 2006; 6(1): 33.
- [23]. Vasanthi P, Parameswari CS. Aqueous extract of *Terminalia arjuna* prevents cyclosporine-induced renal disorders. *Comp Clin Pathol* 2014; 23: 583-588.
- [24]. Devi R S, Narayan S, Vani G and Shyamala Devi C S. Gastroprotective effect of *Terminalia arjuna* bark on diclofenac sodium induced gastric ulcer. *Chemico-biological interactions* 2007; 167(1): 71-83.
- [25]. Kumar B, Vijayakumar M, Govindarajan R, & Pushpangadan P. Ethnopharmacological approaches to wound healing—exploring medicinal plants of India. *Journal of Ethnopharmacology* 2007; 114(2): 103-113.
- [26]. Chaudhari M, Mengi S. Evaluation of phytoconstituents of *Terminalia arjuna* for wound healing activity in rats. *Phytother Res* 2006; 20: 799-805.
- [27]. Reddy VD, Padmavathi P, Paramahamsa M and Varadacharyulu, N. Modulatory role of *Embluca officinalis* against alcohol induced biochemical and biophysical changes in rat erythrocyte membranes. *Food Chem Toxicol* 2009; 47: 1958-1963.
- [28]. Raghavan B, Krishna Kumari S. Effect of *Terminalia arjuna* stem bark on antioxidant status in liver and kidney of



- alloxan diabetic rats. *Ind J Physiol Pharmacol* 2006; 50: 133-142.
- [29]. Das K, Chakraborty P P, Ghosh D, and Nandi D K. Protective effect of aqueous extract of *Terminalia arjuna* against dehydrating induced oxidative stress and uremia in male rat. *Iranian journal of pharmaceutical research* 2010; 9(2): 153.
- [30]. Fawcett JK, Scott J. A rapid and precise method for the determination of urea. *J Clin Pathol* 1960; 13: 156-159.
- [31]. Caraway WT. Uric acid. *Standard methods of clinical chemistry*. 1963; 4: 239-247.
- [32]. Laurence KM, Abbott AL. A micromethod for the estimation of serum bilirubin. *J Clin Pathol* 1956; 9: 270-273.
- [33]. Owen J A, Betty Iggo F J, Scandrett and C P Stewart. The determination of creatinine in plasma or serum, and in urine; a critical examination. *Biochemical Journal* 1954; 58(3): 426.
- [34]. Reitman S, Frankel S. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. *Am J Clin Pathol* 1957; 28: 56-63.
- [35]. Tietz NW. *Fundamentals of clinical chemistry*, WB Saunders, Philadelphia 1976; 518-19.
- [36]. Rosalki SB, Tarlow D. Optimized determination of γ -glutamyltransferase by reaction-rate analysis. *Clin Chem* 1974; 20: 1121-1124.
- [37]. Allian CC, Poon LS, Chan CSG, Richmand W and Fu P. Enzymatic determination of total serum cholesterol. *Clin Chem* 1974; 20: 470-475.
- [38]. Zlatkis A, Zak B, Boyle AJ. A new method for the direct determination of serum cholesterol. *J Lab Clin Med* 1953; 41: 486.
- [39]. Fossati P, Prencipe L. Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. *Clin Chem* 1982; 28: 2077-2080.
- [40]. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 1972; 18: 499-502.
- [41]. Kumari K, Mathew BC, Augusti KT. Antidiabetic and hypolipidemic effects of S-methyl cysteine sulfoxide isolated from *Allium cepa* Linn. *Ind J Biochem Biophys* 1995; 32: 49-54.
- [42]. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem* 1979; 95: 351-358.
- [43]. Sastry KVH, Modgal RP, Mohan J, Tyagi JS and Rao GS. Spectrophotometric determination of serum nitrite and nitrate by Copper - Cadmium alloy. *Anal Biochem* 2002; 306: 79-82.
- [44]. Aebi H. Catalase in vitro. *Methods Enzymology* 1984; 105: 121-126.
- [45]. Kakkar P, Das B, Viswanathan PN. A modified spectrophotometric assay of superoxide dismutase. *Ind J Biochem Biophys* 1984; 21: 130-132.
- [46]. Ellman GL. Tissue sulfhydryl groups. *Arch Biochem Biophys* 1959; 82: 70-77.
- [47]. Lowry OH, Roseberough NJ, Farr AL and Ramdall R. Protein measurement with the Folin-phenol reagent. *J Biol Chem* 1951; 193: 263-275.
- [48]. Wang Z, Yao T, Song Z. Chronic Alcohol Consumption Disrupted Cholesterol Homeostasis in Rats: Down-Regulation of Low-Density Lipoprotein Receptor and Enhancement of Cholesterol Biosynthesis Pathway in the Liver. *Alcohol Clin Exp Res* 2010; 34: 471-478.
- [49]. Das SK, Dhanya L, Varadhan S, Mukherjee S and Vasudevan DM. Effects of chronic ethanol consumption in blood: a time dependent study on rat. *Indian Journal of Clinical Biochemistry* 2009; 24(3): 301-306.
- [50]. Amanvermez R, Ankarali S, Tuncel OK, Tomak L and Alvur M. Effect of chronic high dose-alcohol consumption on the general biochemical parameters. *Turkish Journal of Biochemistry* 2009; 34(3): 113-120.
- [51]. Rodrigo Ramón, Cleofina Bosco, Patricia Herrera, and Gonzalo Rivera. Amelioration of myoglobinuric renal damage in rats by chronic exposure to flavonol-rich red wine. *Nephrology Dialysis Transplantation* 2004; 19(9): 2237-2244.
- [52]. Kwiterovich Jr PO. The metabolic pathways of high-density lipoprotein, low-density lipoprotein, and triglycerides: a current review. *Am J Cardiol* 2000; 86: 5-10.
- [53]. Kim E, Yang J, Lee H, Park JR, Hong S H, Woo H M and Yang S R. γ -Glutamyl Transferase as an Early and Sensitive Marker in Ethanol-Induced Liver Injury of Rats. In *Transplantation proceedings* 2014; 46(4): 1180-1185.
- [54]. Yuan G, Al-Shali K Z, & Hegele R A. Hypertriglyceridemia: its etiology, effects and treatment. *Canadian Medical Association Journal* 2007; 176(8): 1113-1120.
- [55]. Grauvogel J, Daemmrich T D, Ryschich E, Gebhard M M, & Werner J. Chronic alcohol intake increases the severity of pancreatitis induced by acute alcohol administration, hyperlipidemia and pancreatic duct obstruction in rats. *Pancreatology* 2010; 10(5): 603-612.
- [56]. Brinton EA, & Nanjee M N. Effects of ethanol intake on high density lipoprotein metabolism in humans. In *High Density Lipoproteins, Dyslipidemia, and Coronary Heart Disease* 2010: (129-138). Springer New York.



- [57]. Shaila HP, Udupa SL, Udupa AL. Hypolipidemic activity of three indigenous drugs in experimentally induced atherosclerosis. *International journal of cardiology* 1998; 67(2): 119-124.
- [58]. Del Rio D, Stewart AJ and Pellegrini N. A review of recent studies on malondialdehyde as toxic molecule and biological marker of oxidative stress. *Nutr Metabol Cardiovascu Dis* 2005; 15: 316-328.
- [59]. Niemela O. Distribution of ethanol-induced protein adducts in vivo: relationship to tissue injury. *Free Radic Biol Med* 2001; 31: 1533-1538.
- [60]. Tuma J and Casey CA. Dangerous byproducts of alcohol breakdown-focus on adducts. *Alcohol Research and Health* 2003; 27: 285-290.
- [61]. Zhang A, Sun H, Wang X. Recent advances in natural products from plants for treatment of liver diseases. *European journal of medicinal chemistry* 2013; 63: 570-577.
- [62]. Masella Roberta Roberta Di Benedetto, Rosaria Vari, Carmela Filesi, and Claudio Giovannini. Novel mechanisms of natural antioxidant compounds in biological systems: involvement of glutathione and glutathione-related enzymes. *The Journal of nutritional biochemistry* 2005; 16(10): 577-586.

