Advanced Journal of Microbiology Research Vol. 2007
Available online at http://internationalscholarsjournals.org/journal/ajmr

Full Length Research Paper

Effect of *Emblica officinalis* against alcohol-induced biochemical changes in plasma and red blood cells of rats

Damodara Reddy. V, Saayi Krushna G, Padmavathi P and Varadacharyulu. N.Ch*

Department of Biochemistry, Sri Krishnadevaraya University, Anantapur - 515 003, India.

Accepted 19 January, 2007

Alcohol is widely abused as a psychoactive drug. *Emblica officinalis* is known for its therapeutic effects and the present study is aimed at investigating therapeutic efficacy of aqueous extract of *Emblica officinalis* (AEEO) against alcohol-induced damage. Thirty three percent (v/v) alcohol (10 g/kg body weight) and AEEO (250 mg/kg body weight/day) was orally administered once a day for 60 days. Chronic alcohol feeding resulted in higher activities of the alcoholic plasma marker enzyme gamma glutamyl transferase (GT) and abnormalities in plasma lipid and lipoproteins, minerals as well as activities of plasma transaminases (AST and ALT), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH). Chronic alcohol administration significantly lowered the activities of superoxide dismutase (SOD), catalase (CAT) glutathione peroxidase (GPx) and the content of reduced glutathione (GSH) in erythrocytes. The AEEO supplementation to chronically alcohol fed rats beneficially modulated plasma lipids and lipoprotein patterns, minerals, and vitamin C and also corrected zigzags in plasma enzyme activities and improved antioxidant and defense enzyme machinery status. However, AEEO supplementation resulted in a significant increase in GSH content and the activities of SOD, CAT and GPx. AEEO can contribute to the alleviation of alcohol induced adverse effects by enhancing the status of antioxidant defense system and by regulating lipid and mineral metabolism.

Key words: Alcohol; Antioxidant enzymes; *Emblica officinalis*; Lipid profile.

INTRODUCTION

Chronic excessive alcohol consumption is more prevalent all over the world and is associated with tissue and organ damage leading to coronary heart disease, alcohol liver disease and several other manifestations including neurological disorders for which therapeutic approaches are sought (Lee, 2006; Pramyothin et al., 2006). Enhanced oxidative stress and decreased antioxidant status induced by ethanol metabolism play a major role in the causation of alcohol toxicity and damage (Dey and Cederbaum, 2006). Chronic alcohol consumption has major effects on the absorption, elimination, and serum concentrations of many physiologically important electrolytes and minerals (McClain et al., 1986). The research work that has been conducted for the past fifty years is mainly to understand the metabolism, pathologies and characteristic physiological as well as biochemical actions/mechanisms related to alcohol. This facilitated us to understand new concepts and theories successfully from alcohol toxicity to treatment (Seitz et al., 2005).

Due to several undesirable effects of synthetic drugs, the world population is turned towards medicines derived from locally available plants or native extracts of edible plant parts. The developments of alcoholism remedies have medical, social and economical significance. In view of the pitfalls of psychological dependence and adverse effects of synthetic drugs, the development of low toxicity and high efficiency medicines derived from natural products exhibit expansive market prospects (Xu et al., 2005). *Emblica officinalis* is a medium to large deciduous tree belonging to a small subgenus of trees of the Euphorbiaceae widely growing in different parts of India, Srilanka, Pakistan, Uzbekistan, S.E. Asia and China. *Emblica officinalis* is known for its antioxidant properties and for its therapeutic effects, and is a component in more than hundred herbal formulations that are widely used in India

*Corresponding author. E-mail: vdp_1975@yahoo.co.in. Tel. +91 8554 -255761(0) / 273337 (R). Fax : +91 8554 -255244.

and other countries. The fruits of *Emblica* are widely consumed raw, cooked or pickled, but they are also principle constituents of Ayurvedic preparations (Scartezzini et al., 2006). The wide use of *Emblica officinalis* in India for various purposes prompted us to select the same to treat alcohol toxicity which has not been done so far. Under these circumstances *Emblica officinalis* opens up a new avenue to treat alcohol related problems. Hence present study is aimed at investigating the therapeutic efficacy of *Emblica* i.e. alleviation of alcohol toxicity and alcohol induced adverse effects in rats.

MATERIALS AND METHODS

Chemicals

The chemicals used in the present study were procured from Sigma Chemical Co. (St. Louis, MO, USA) and SISCO Research Laboratories (Maharashtra, India). Ethanol used for administration to rats was obtained by re-distillation. An aqueous *Emblica officinalis* fruit extract was obtained from Chemiloids Ltd, Vijayawada, Andhra Pradesh, India (Manufacturers and exporters of herbal extracts). AEEO 5% was prepared in distilled water freshly prior to use.

Animals and experimental design

Two month old male albino Wistar rats, weighing about 120-140g, were procured from Sri Venkateswara Enterprises (Bangalore, India), acclimatized for seven days in our animal house and maintained at standard conditions. The animals were divided in to four groups of eight each, group I – controls, group II – alcohol, group III – alcohol plus AEEO (A+AEEO) and group IV- AEEO alone. The dose of the AEEO in the present study was based on the earlier reports (Jeena and Kuttan, 2000). Controls rats received iso-caloric amounts of glucose equals that of alcohol, 33% alcohol was administered orally at a dose of 10g /kg body weight/day, AEEO were administered orally (250 mg/ kg body weight/day) between two successive alcohol treatments to provide a gap of 12 h between the treatment of alcohol. Animals were fed on standard pellet diet with water *ad libitum* and all animals received human care continuously for 60 days. At the end of experimental period, the rats in each group were fasted overnight, and sacrificed by cervical decapitation. Blood was collected by cardiac puncture into heparinized tubes was separated into plasma and formed elements and analyzed immediately. The present work was undertaken with prior approval by our departmental as well as institutional ethical committee.

Plasma analysis

The plasma total cholesterol (Allian et al., 1974), triglycerides (Fossati and Principe, 1982), HDL-cholesterol (Zlatkis et al., 1953), LDL-cholesterol, VLDL-cholesterol (Friedwold et al., 1972) levels as well as activities of AST and ALT (Reitman and Frankel, 1957), alkaline phosphatase and lactate dehydrogenase (Teitz, 1976) were determined using commercial kits (Span diagnosis Ltd, Surat, India) . Plasma -glutamyl transferase (Rosalki and Tarlow, 1974) vitamin C (Roe and Kuther, 1961), calcium (Carl and Edward, 1996), phosphorous (Fiske and Subbarow, 1925), iron (Ramsay, 1958) sodium (Trinder, 1951), potassium (Jacobs and Hoffman, 1931) and chloride (Schales and Schales, 1941) were determined.

Erythrocyte antioxidant enzyme activities

Erythrocytes separated from whole blood by the method adopted by Beutler et al., (1975). Erythrocytes were washed thrice with 0.9% NaCl and suspend in 1 volume of 0.9% NaCl. The packed cell volume was adjusted to 5% with PBS- pH7.5 (10mM phosphate buffer saline). Hemoglobin in erythrocytes was determined by the method of Samuel (1989). Reduced glutathione (GSH) content was estimated according to the method of Beutler et al., (1963) and expressed as μmoles/gHb. The superoxide dismutase (SOD) activity was measured based on the ability of the enzyme to inhibit the autoxidation of adrenaline and was assayed by using the method described by Mishra and Fridovich (1972) from hemoglobin free hemolysate. SOD activity was expressed as Units/mg Hb/min. The Catalase (CAT) activity in hemolysate was estimated by the method of Chance (1954) . The activity of the enzyme was calculated using the extinction coefficient of H_2O_2 as 0.071 cm^{-1} mol^{-1} and expressed as IU x 10^4/g Hb at 37^0C. The glutathione peroxidase (GPx) activity was measured using the method of Rotruck et al., (1973). The activity of GPx was expressed as μmoles of glutathione oxidized/min/ mg Hb.

Statistical analysis

Mean and standard deviation values of all the parameters were determined for each group. Duncan's multiple range test (Bennet and Franklin, 1967) was performed to determine significant difference among the groups. A $p<0.05$ was considered statistically significant.

RESULTS

Levels of cholesterol, triglycerides and lipoprotein patterns in plasma of different experimental rat groups are presented in Table 1. Increased cholesterol, triglycerides, LDL-C, VLDL-C in plasma followed by a decrease in HDL-C in alcoholic rats is evident from the data. AEEO administration to alcoholic group not only decreased plasma cholesterol, triglycerides but also lipoprotein patterns viz. LDL-C, VLDL-C with no change in HDL-C when compared with alcoholic rats. No adverse changes were observed in rats administered with AEEO alone, when compared with control rats.

A significant decrease in the activities of erythrocyte CAT, SOD and GPx with a fall in GSH content in alcohol group was observed (Table 2). AEEO administration to alcohol treated rats showed relatively increased activities of these enzymes and GSH content. Treatment with AEEO alone to control rats did not alter the GSH content and these enzyme activities.

Enhanced activities of the plasma enzymes viz., AST, ALT, ALP and LDH in alcoholic group are depicted in Table 3. AEEO administration to rats resulted in significant decrease the activities of the above enzymes in alcohol fed group. Further, the activity of the marker enzyme GT in alcohol treated rats, are shown in Table 3. As expected values of the enzyme were higher in rats that received alcohol alone. Though alcoholic rats that received AEEO showed a significant elevation in the enzyme activity and the increase is more pronounced in alcohol alone administered rats. The two other non alcoholic groups viz., control and AEEO alone administered group showed normal activities of the enzyme.

Table 4 shows minerals (calcium, phosphorous and iron), electrolytes (Na^+, K^+ and Cl^-) and vitamin C levels in

Table 1. Effect of AEEO administration on plasma lipid profile in chronic alcoholic rats.

Parameter	Control	Group		
		Alcohol	A+AEEO	AEEO
Cholesterol	76.80 ± 5.03^{b}	99.42 ± 5.27^{a}	80.90 ± 5.86^{b}	74.03 ± 5.61^{b}
Triglycerides	75.61 ± 5.12^{b}	97.10 ± 3.11^{a}	77.10 ± 8.14^{b}	70.83 ± 7.90^{b}
HDL-Cholesterol	38.60 ± 3.48^{a}	26.41 ± 3.71^{c}	34.15 ± 3.12^{b}	32.90 ± 1.62^{b}
LDL-Cholesterol	21.14 ± 1.97^{c}	51.20 ± 1.43^{a}	31.11 ± 1.67^{b}	23.97 ± 1.42^{c}
VLDL-Cholesterol	15.12 ± 1.22^{b}	19.42 ± 1.62^{a}	15.42 ± 1.42^{b}	14.16 ± 1.60^{b}
Atherogenic index $^{\#}$	0.98 ± 0.02^{c}	2.76 ± 0.05^{a}	1.36 ± 0.01^{b}	1.15 ± 0.01^{c}

All the values (mg/dL) are mean ± SD of eight rats in each group. ^{abc} means in the same column not sharing a common superscript are significantly different (p< 0.05) between groups.
$^{\#}$ Atherogenic index = Total cholesterol – HDL-cholsterol/HDL-cholesterol.

Table 2. Effect of AEEO administration on erythrocyte GSH content and antioxidant enzymes in chronic alcoholic rats.

Parameter	Control	Group		
		Alcohol	A+AEEO	AEEO
GSH (µmol/ g Hb)	3.42 ± 0.14^{a}	3.08 ± 0.19^{c}	3.20 ± 0.24^{b}	3.40 ± 0.16^{a}
Catalase (IUx10^{4}/ g Hb)	8.64 ± 0.14^{a}	6.48 ± 0.41^{c}	8.11 ± 0.15^{b}	8.51 ± 0.13^{a}
SOD (Units/mg Hb/min)	6.02 ± 0.16^{a}	4.14 ± 0.04^{c}	5.27 ± 0.07^{b}	6.15 ± 0.06^{a}
GPX (mols of GSH oxidized /min /mg Hb)	15.84 ± 0.22^{a}	11.41 ± 0.21^{c}	13.58 ± 0.16^{b}	15.97 ± 0.17^{a}

Values are mean ± SD of eight rats in each group. ^{abc} means in the same column not sharing a common superscript are significantly different (p< 0.05) between groups.

Table 3. Effect of AEEO administration on plasma SGOT, SGPT, ALP, GT and LDH in chronic alcoholic rats.

Parameter	Control	Group		
		Alcohol	A+AEEO	AEEO
SGOT (IU/L)	26.53 ± 3.16^{c}	78.02 ± 3.31^{a}	59.70 ± 3.76^{b}	25.85 ± 2.95^{c}
SGPT (IU/L)	26.30 ± 3.84^{c}	84.02 ± 11.38^{a}	55.44 ± 5.80^{b}	26.58 ± 3.67^{c}
ALP (IU/L)	69.21 ± 9.04^{c}	315.54 ± 28.19^{a}	159.77 ± 25.85^{b}	50.16 ± 3.29^{c}
LDH (IU/L)	360.90 ± 4.16^{c}	481.12 ± 15.09^{a}	391.77 ± 3.90^{b}	354.60 ± 14.16^{c}
GT (U/L)	5.51 ± 0.60^{c}	25.36 ± 1.46^{a}	12.65 ± 1.25^{b}	5.37 ± 0.82^{c}

Values are mean ± SD of eight rats in each group. ^{abc} means in the same column not sharing a common superscript are significantly different (p< 0.05) between groups.

plasma of different groups of rats. Alcohol administration to rats significantly decreased levels of plasma vitamin C, electrolytes and minerals was observed when compared to control group. Rats administered AEEO as well as alcohol showed significantly augmented the levels of the vitamin C, electrolytes and minerals near to control values. AEEO alone administered rats did not showed any alterations.

DISCUSSION

Present study suggested a therapeutic strategy for alcoholic injury by the use of *Emblica officinalis* fruit extract. Ethanol induced free radicals generation during the metabolism leading to oxidative stress are largely responsible for alcohol induced adverse effects causing damage (Wu and Cederbaum, 2003). Since alcoholism remedies with promising novel therapeutic strategies involving phytochemicals, the use of natural extracts from plant foods aiming to reduce the risk of oxidative stress (Xu et al., 2005).

Our work evidenced the therapeutic efficacy of AEEO *in vivo* not only by reducing alcohol induced oxidative damage but also by ameliorating the characteristic changes observed in alcohol group in lipids (cholesterol and triglycerides), lipoproteins (LDL, VLDL and HDL) upon *Emblica* supplementation. Reduced plasma cholesterol

Table 4. Effect of AEEO on plasma vitamin C and mineral content in alcohol-treated rats.

Parameter	Control	Group		
		Alcohol	A+AEEO	AEEO
Vitamin C (mg/dL)	1.55 ± 0.07^{b}	1.10 ± 0.09^{c}	1.46 ± 0.05^{b}	1.73 ± 0.08^{a}
Sodium (mM/L)	140.31 ± 3.79^{a}	106.72 ± 9.44^{c}	122.33 ± 8.11^{b}	141.88 ± 4.43^{a}
Potassium (mM/L)	5.47 ± 0.37^{a}	3.63 ± 0.31^{c}	4.57 ± 0.33^{b}	5.20 ± 0.43^{a}
Chloride (mM/L)	145.89 ± 2.50^{a}	117.37 ± 6.02^{c}	139.51 ± 4.37^{b}	148.03 ± 2.96^{a}
Calcium (mg/dL)	10.91 ± 0.33^{a}	8.92 ± 0.41^{b}	10.51 ± 0.72^{a}	10.82 ± 0.43^{a}
Phosphorus (mg/dL)	5.67 ± 0.24^{a}	4.45 ± 0.32^{c}	5.12 ± 0.34^{b}	5.91 ± 0.21^{a}
Iron (µg/dL)	175.28 ± 3.24^{a}	151.47 ± 4.16^{b}	174.66 ± 4.37^{a}	176.69 ± 3.05^{a}

Values are mean ± SD of eight rats in each group. ^{abc} means in the same column not sharing a common superscript are significantly different ($p< 0.05$) between groups.

content in AEEO supplemented alcoholic group in the present study can be attributed to the hepatic increased cholesterol catabolism induced by AEEO as observed by Anila and Vijayalakshmi (2002) and Kim et al., (2005). Besides decreased cholesterol synthesis by regulating / inhibiting the activity of the rate limiting enzyme HMG-CoA reductase was reported earlier (Saravanan et al., 2007). However the possibility of regulation of HMG-CoA reductase by the principles of AEEO at transcription or translation levels can not be ruled out. Further the reduction in LDL, VLDL, cholesterol and triglycerides in alcoholic rats receiving AEEO suggests the cardio protective effects of *Emblica* by inhibiting NF-kB transcriptional pathways through modulating hepatic PPAR expression, suggesting that *Emblica* may be beneficial in preventing atherosclerotic cardiovascular diseases as reported by Yakozawa et al. (2007).

Chronic consumption of ethanol causes injury to the liver cells. The activities of AST and ALT are the most sensitive tests employed in the diagnosis of hepatic diseases. The increased plasma GT activity in alcoholic rats, a marker enzyme for alcoholism was observed in the present study, but in alcoholic rats which received AEEO shows maximum depletion. The results of the present study reveal that increased activities of AST, ALT and ALP in alcoholic rats. Administering AEEO to alcoholic rats significantly decreased AST, ALT and ALP activities. Hepato protective activity of AEEO against alcohol induced adverse changes was evident from decreased activities of the above plasma enzymes and AEEO can preserve the structural integrity of the liver from the adverse effects of ethanol. Superoxide (O_2^-) and hydroxyl radicals are known to cause marked injuries to the surrounding tissues and organs. Natural and synthetic compounds with antioxidant properties may help to alleviate the liver damage totally or partially. Therefore, removing superoxide ion and hydroxyl radical is probably one of the most effective defense mechanisms against a variety of diseases (Sheela and Angusti, 1995).

Red blood cells are more prone to oxidative damage due to high content of polyunsaturated fatty acids, iron and oxygen (Hebbel, 1986). Alcohol induced adverse changes were evident from decreased red blood cell defense enzyme activities viz., catalase, SOD and GP $_X$ followed by GSH, plasma vitamin C depletion. In the present study upon *Emblica* supplementation the above enzymes were restored to normal in red blood cells. Moreover, as reported by Bhattacharya et al., (2000) the tannoid principles emblicanin A, emblicanin B, punigluconin and pedunculagin may have contributed largely for the observed elevation in the activities of antioxidant machinery as these principles are capable of enhancing the concentrations of SOD, CAT and GPx and the content of GSH and vitamin C. The antioxidant activity of *Emblica* does not depend only upon vitamin C content. In particular, the antioxidant activity of processed fruit is due to ascorbic acid for only 60% or less, while in the other products this percentage increases as reported by Scartezzini et al., (2006).

Electrolytes and minerals are involved in most cellular activities and assume a major role in metabolism. They have multiple functions such as holding fluids in compartments of the body and maintaining normal acid-base balance. Chronic alcohol consumption has major effects on the absorption, elimination, and serum concentrations of many physiologically important electrolytes and minerals, including sodium, potassium, phosphorus, calcium, iron. Electrolyte disturbances may lead to severe and even life-threatening metabolic abnormalities such as liver disease, frequently have abnormal sodium serum concentrations, with hyponatremia as the most common alteration. Sodium together with potassium assists in the maintenance of the body's electrolyte and water balance. In addition, potassium and sodium play an important role in nerve conduction, muscle contraction, and the trans-port of substances across membranes. Animal studies involving chronic alcohol intake have shown significant retention of water, sodium, potassium, and chloride after the first week of daily alcohol ingestion (Marsano and McClain 1989). Supplementation of AEEO to alcoholic rats significantly maintained acid-base balance by increasing the absorption of electrolytes and minerals from intestine and

inhibited electrolytes elimination through urine.

In this study *Emblica officinalis* fruit extract has shown protective action against alcohol induced oxidative stress to the cells as evidenced by the lowered plasma transaminases, ALP, LDH and GT enzyme activities and elevated levels of the enzymic and non-enzymic antioxidants. In addition lowered cholesterol level and elevated HDL level demonstrate that *Emblica officinalis* fruit extract offers protection against cardiovascular risk. The active tannoid principles, poly phenolic compounds and vitamin C present in AEEO could be contributed for the above mechanism.

REFERENCES

Allian CC, Poon LS, Chen CSG, Richmond W, Fu PC (1974). Enzymatic determination of serum cholesterol. Clin. Chem. 20: 470-475.

Anila L, Vijayalakshmi NR (2002). Flavonoids from Emblica officinalis and *Mangifera indica*-effectiveness for dislipidemia. J. Ethnopharmacol. 79: 81-87.

Bennet CA, Franklin NL (1967). Statistical Analysis of Chemistry and the Chemical industry. John Wiley and Sons Inc: London. pp. 208-227.

Beutler E, Dixon O, Kelly BM (1963). Improved method for the determination of blood glutathione. J. Lab. Clin. Med. 61: 882-890.

Beutler E (1975). In: Red cell metabolism. A manual of biochemical methods 2nd edn, Grune and Stratton publishers, New York. pp. 8-18.

Bhattacharya A, Ghosal S, Bhattacharya SK (2000). Antioxidant activity of tannoid principles of *Emblica officinalis* (amla) in chronic stress induced changes in rat brain. Ind. J. Exp. Biol. 38: 877-880.

Carl AB, Edward RA (1996). "Mineral and Bone metabolism" in Tietz fundamentals of clinical chemistry, W.B. Saunders and company, Philadelphia, PA. pp. 685-703.

Chance B (1954). Catalase and peroxidases. Part II. Special methods. In: Methods of biochemical analysis. 1: 408-412.

Dey A, Cederbaum AI (2006). Alcohol and Oxidative Liver injury. Hepatol. 43: S63-S74.

Fiske CH, Subbarow Y (1925). The colorimetric determination of inorganic phosphorous. J. Biol. Chem. 66: 375-404.

Fossati P, Principe L (1982). Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. Clin. Chem. 28: 2077-2080.

Friedwold WT, Levy RI, Fredrickson DS (1972). Estimation of the concentration of low-density lipoprotein cholesterol in plasma without use of the preparative ultracentrifuge. Clin. Chem. 18: 499-502.

Hebbel RP (1986). Erythrocyte antioxidants and membrane vulnerability. J. Lab. Chem. Med. 107: 401-405.

Jeena KJ, Kuttan R (2000). Hepatoprotective activity of *Emblica officenalis* and Chyavanaprash. J. Ethnopharmacol. 72: 135-140.

Kim HJ, Yokozawa T, Kim HY, Tohda C, Rao TP, Juneja LR (2005). Influence of amla (*Emblica officinalis* Gaertn) on hypercholestero-lemia and lipid peroxidation in cholesterol-fed rats. J. Nutr. Sci. Vitaminol. (Tokyo). 51: 413-418.

Lee JS (2006). Supplementation of *Pueraria radix* water extract on changes of antioxidant enzymes and lipid profile in ethanol -treated rats. Clin. Chim. Acta. 347: 121-128.

Marsano L, McClain CJ (1989). Effects of alcohol on electrolytes and minerals. Alcohol. Health Res. World. 13(3): 255-260.

Mishra PH, Fridovich I (1992). The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J. Biol. Chem. 247: 3170-3175.

Pramyothin P, Samosorn P, Poungshompoo S, Chaichantipyuth C (2006). The protective effects of *Phyllanthus emblica* Linn. Extract on ethanol induced rat hepatic injury. J. Ethnopharmacol. 107: 361-364.

Ramsay WNM (1958). Advances in Clinical Chemistry. Sobotka H and Stewart CP (edn), Academic Press, New York. pp.1-5.

Reitman S, Frankel S (1957). A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminase. Am. J. Clin. Pathol. 28: 8-15.

Roe JH (1961). Standard Methods in Clinical Chemistry Vol III, Seligson D (ed), Academic Press, New York. pp. 35-37.

Rosalki SB, Tarlow D (1974). Optimized Determination of - Glutamyl transferase by reaction -Rate Analysis. Clin. Chem. 20**:** 1121-1124.

Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, Hoekstra WG (1973). Selenium: biochemical role as a component of glutathione peroxidase. Science. 179: 588- 590.

Samuel KM (1989). In: Fundamentals of clinical chemistry, WB Saunders and company, Philadelphia. pp. 602-603.

Saravanan S, Srikumar R, Manikandan S, Jeya Parthasarathy N, Sheela Devi R (2007). Hypolipidemic effect of Triphala in experimentally induced hypercholesteremic rats. Yakugaku. Zasshi. 127: 385-388.

Scartezzini P, Antognoni F, Raggi MA, Poli F, Sabbioni C (2006). Vitamin C content and antioxidant activity of the fruit and of the Ayurvedic preparation *Emblica officinalis* Gaertn. J. Ethnopharmacol. 104: 113-118.

Schales O, Schales S (1941). A simple and accurate method for the determination of chloride in biological fluids. J. Biol. Chem. 140: 879-884.

Seitz HK, Salaspuro M, Savolainen M, Haber P, Ishii H, Teschke R, Moshage H, Lieber CS (2005). From Alcohol Toxicity to Treatment. Alcohol. Clin. Exp. Res. 29: 1341-1350.

Sheela CG, Augusti KT (1995). Antiperoxide effects of S-allyl cysteine sulphoxide isolated from *Allium sativum* Linn and gugulipid in cholesterol diet fed rats. Ind. J. Exp. Biol. 33: 337-341.

Teitz RW (1976). In: Fundamentals of clinical chemistry, WB Saunders and company, Philadelphia. pp. 602-603.

Trinder P (1951). A rapid method for the determination of sodium in serum. Analyst 76: 596-599.

Wu D, Cederbaum AI (2003). Alcohol, Oxidative stress, and Free Radical Damage. Alcohol. Res. Health. 27(4): 277-284.

Xu BJ, Zheng YN, Sung CK (2005). Natural medicines for alcoholism treatment: a review. Drug Alcohol Rev. 24: 525-536.

Yokozawa T, Kim HY, Kim HJ, Okubo T, Chu DC, Juneja LR (2007). Amla (*Emblica officinalis* Gaertn.) prevents dyslipidaemia and oxidative stress in the ageing process. Br. J. Nutr. 97(6): 1187-1195.

Zlatkis A, Zak B, Boyle AJ (1953). A new method for the direct determination of serum cholesterol. J. Lab. Clin. Med. 4: 486-492.