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Effect of grape leaf extract on the alcoholic induced lipid profile changes in rats

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Abstract
Alcoholism is a growing medical and public health issue both in adults and in the younger generation. Moderate consumption of alcohol is medically beneficial and socially acceptable. However, chronic consumption of alcohol leads to a complex of health problem to human. The present study was carried to evaluate the effect of grape leaf extract (GLEt) on antioxidant and lipid profile states in liver of alcohol induced toxicity. *In-vivo* administration of ethanol (7.9 g/kg bw/day) for 45 days resulted a significant elevation in the levels of lipid profile in plasma and tissues and significantly lower activity of non enzymic antioxidants (vitamin E, vitamin C and GSH) in liver as compared with control rats. Administration of ethanol along with GLEt significantly decreased elevation in the levels of cholesterol, free fatty acid, triglycerides and phospholipids and improving non enzymatic antioxidants activities in plasma and tissue towards near normal level.

Keywords: Alcoholism, grape leaf extract, liver, lipids and antioxidants

1. Introduction
Alcoholism is a growing serious medical and public health issue both in adults and in particular the younger generation. Moderate consumption of alcohol is medically beneficial and socially acceptable. However, chronic consumption of alcohol leads to a complex of health problem to human (Brick, 2003; Lacoviello and De Gaetano, 2003) [3, 18]. Alcohol consumption is the leading risk factor for disease burden in developing countries and the third largest risk factor in developed countries. Ethanol non-enzymatically reacts with hydroxy radicals (OH•) originated by iron - catalysed degradation of hydrogen peroxide through so-called Fenton reaction and this reaction produced one of the several radicals that may be formed from abstraction of a hydrogen atom from ethanol (Carrasco *et al*., 2002) [4]. Liver is the major site of ethanol oxidation and metabolism and exhibits a crucial role in the detoxification of ethanol. The toxicity of ethanol is mainly due to its metabolism by alcohol dehydrogenase and acetaldehyde, the metabolic product. Enhanced free radical production and acetaldehyde causes the oxidation of DNA proteins, biomembrane, reduced endogenous antioxidants, finally to extent liver damage. Even though great progress made in the field for the development of appropriate drugs for the management of alcoholism leftovers a challenging goal. In common, plants contain various biologically active compounds, which could be responsible for the prevention and detoxification of free radicals thereby protecting themselves from oxidative stress and other consequences. Hence, identification of an effective hepatoprotective agent will be a useful tool for the treatment of liver diseases.
Vitis vinifera L is a common grape, which is also called 'Old World grape' belong to *vitaceae* family (Bombardelli and Morazzoni 1995) [2]. Grapes are one of the world's largest fruit crops, with an annual production of approx 58 million tons. It has been used as a food and beverage. In Ayurvedic (Indian) system, grapes leaves are used as a folk remedy for the treatment of diarrohea and vomiting (Zargari, 1993) [29]. The grape leaves have been used to stop bleeding, to treat inflammatory disorder, pain, hepatitis, free radical related disease (Lardos and Kreuter, 2000) [19]. The leaves are composed of wide range of polyphenoles including anthocyanins, flavonoids and also organic acid (Fan *et al*, 2004) [10]. Therefore, the present study was carried out to analysis the mechanisms by which grape leaf extracts protects against alcohol toxicity by assaying antioxidants and lipid profile in the control and experimental rats.

2. Material and Methods
2.1. Chemical
Ethanol was purchased from E. Merck, Darmstadt, Germany. ascorbic acid, potassium

persulfate, Folin–Ciocalteu's phenol reagent and all other chemicals were of analytical grade and solvents were purchased from Sigma chemical Co. (St. Louis, MO, USA) and the other organic solvents were distilled before use

2.2. Plant authentication and Extract preparation

Grape leaves were collected from Cumbam, Theni (dist), Tamil Nadu, South India. The plant was identified and (voucher specimen No.: 884) was stored in the herbarium of botany directorate, Department of Botany, Annamalai University. The leaves were shade dried, powdered and extract by maceration with 70% (v/v) alcoholic for 72 h in ambient temperature. The extract was filtered and then solvent evaporated to dryness under reduced pressure in a rotary evaporator. The residual extract was used for the study.

2.3. Animals

Adult male albino wistar (150-170 g) rats were obtained from the Central Animal House. Rajah Muthiah Medical College, Annamalai University. The rats were housed in plastic cages under controlled conditions of 12-h light/12-h dark cycle, 50% humidity and 25 ± 3oC. They all received standard pellet diet comprised of 20% crude protein, 5% fat, 4.5% Crude fiber, 8% ash, 2% calcium, 0.6% phosphorus, 3.4% glucose, 2% vitamins and 55% nitrogen free extract (M/S. Pranav Agro Industries Ltd., Bangalore, India) and water ad libitum. This study was approved (Vide. No. 311, 2005) by Institutional Animal Ethics Committee, Annamalai University, Annamalai Nagar. The study was conducted in accordance with the ''Guide for the Care and Use of Laboratory Animals'' (National Institute of Health, 1985) [30].

2.4. Preparation of plasma and tissue homogenate

At the end of the experimental period, the animals were sacrificed by cervical decapitation. Blood was collected and centrifuged for serum separation. For plasma, blood was collected with anticoagulant and centrifuged (2000g for 20 min) to separate plasma. The tissues were dissected out, weighed and washed using ice cold saline solution. Tissues were minced and homogenized (10% w/v) in Tris-HCl buffer (0.1 M; pH 7.4) and centrifuged at 3000g for 20 min at 4˚C. The resulting supernatant was used for various biochemical assays.

2.5. Experimental design

The animals were randomly divided into four groups of six rats in each group. The Extract and alcohol was administered as aqueous solution using intragastric tube daily for 45 days.

Group 1: Control rats (normal rats treated with 30% glucose (isocaloric to ethanol) and 0.1% Carboxyl methylcellulose (CMC).

Group 2: Control rats orally received GLEt (100 mg/kg body weight) suspended in 0.1% CMC.

Group 3: Normal rats orally received 20% Ethanol (3.95g/kg body weight twice a day i.e.7.9g/kg/day) (Rajakrishnan *et al*., 1997) [25].

Group 4: Normal rats orally received 20% Ethanol with GLEt (100 mg/kg body weight). (Pari and Suresh, 2008) [24]

At the end of experimental regimen, the animals in different groups were sacrificed by cervical decapitation. Blood was collected in two different tubes i.e. one with anticoagulant, for plasma separation and another without anticoagulant for serum separation. Plasma and serum were separated by centrifugation at 2000 rpm and used for various biochemical estimations. Liver and kidney were dissected out, washed in ice-cold saline and kept in ice cold container for various biochemical estimations.

2.6. Antioxidants assays

Ascorbic acid (vitamin C) concentration was measured by method discussed elsewhere. To 0.5 ml of sample, 1.5 ml of 6% TCA was added and to 0.5ml of DNPH reagent (2% DNPH and 4% thiourea in 9N sulfuric acid) mixed with and incubated for 3h at room temperature. After incubation, 2.5ml of 85% sulfuric acid and colour developed was read at 530nm after 30 min. Vitamin E estimated by the method of Desai (1984) [7]. Reduced glutathione (GSH) was determined by the method of Ellman (1959) [9]. To the homogenate added 10% TCA, centrifuged. 1.0 ml of supernatant was treated with 0.5 ml of Ellmans reagents (19.8 mg of 5, 50- dithiobisnitro benzoic acid (DTNB) in 100 ml of 0.1% sodium nitrate) and 3.0 ml of phosphate buffer (0.2 M, pH 8.0). The absorbance was read at 412 nm.

2.7. Lipid extraction

Lipids were extracted from tissues by the method of Folch *et al*., 1957) [12] using chloroform - methanol mixture (2:1 v/v). A known weight of tissue was homogenized in 7.0 ml of chloroform-methanol using potter Elvehjam homogeniser. The contents were filtered into a previously weighed side arm flask; residue on the filter paper was scrapped off and homogenized with 14 ml of chloroform- methanol mixture. This was again filtered into the side arm flask and the residue was successively homogenized in chloroform – methanol (2:1 v/v) and each time this extract was filtered. The pooled filtrates in the flask was adjusted to a final volume ratio using chloroform-methanol (2:1 v/v) and evaporated to dryness to a constant weight. The dried residue of lipid was dissolved in 5 ml of chloroform–methanol mixture (2:1 v/v) and transferred into a centrifuge tube; 2 ml of 0.1M potassium chloride was added, shaken well and centrifuged. The upper aqueous layer containing gangliosides was discarded. The chloroform layer was mixed with 1.0 ml of chloroform–methanol–potassium chloride mixture (1:10:10 v/v) and then centrifuged. This washing was repeated thrice and each time, the upper layer was discarded. The lower layer was made up to 5.0 ml and used for the analysis. Cholesterol, triglycerides, free fatty acids, phospholipids and α-tocopherol were estimated as discussed in the literature elsewhere ()

3. Statistical analysis

The data for various biochemical parameters were analyzed by one way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT) using statistics software package (SPSS for Windows, V. 13.0, Chicago, USA). P values <0.05 were considered as statistically significant (Duncan, 1957) [8].

4. Results

Table 1 represents the initial and final body weight, body weight gain of control and experimental rats. Grape leaf extract alone treated rats (Group 2) did not show any statistically significant (P <0.05) difference in the bodyweight as compared to that of the control rats. Ethanol fed rats (Group 3) showed a decrease in the weight gain as compared to the control rats. On co-supplementation of ethanol with Grape leaf extract (Group 4), the weight gain improved significantly as control rats.

Table 1: Effect of GLEt on bodyweight and liver weight in control and experimental animals

Groups	Initial Body weight (g)	Final bodyweight (g)	Net gain (g)	Liver weight (g)
Control	155.90 ± 12.95[a]	220.52 ± 18.11[a]	64.62 ± 5.37[a]	5.62 ± 0.37[a]
Control + GLEt (100 mg/kg)	152.20 ± 10.55[a]	224.62 ± 20.15[a]	72.42 ± 6.44[a]	5.50 ± 0.25[a]
Ethanol	160.82 ± 10.30[a]	172.25 ± 12.15[b]	11.43 ± 1.28[b]	8.20 ± 0.80[b]
Ethanol + GLEt (100 mg/kg)	154.80 ± 12.10[a]	218.38 ± 16.10[a]	63.58 ± 4.72[a]	5.75 ± 0.28[a]

Values are given as mean ± S.D for 6 rats in each group. Values not sharing a common superscript letter differ significantly at p<0.05 (DMRT). GLEt - Grape leaf extract

Tables 2 represent the levels of non-enzymatic antioxidants (vitamin E, vitamin C and GSH) status in plasma. The levels of vitamin E, vitamin C and reduced GSH were significantly decreased in alcohol fed rats when compared with control rats. Administration of GLEt significantly increased the levels of non-enzymatic antioxidants in plasma the levels of serum and tissue free fatty acid, triglycerides, total cholesterol and phospholipids in control and experimental rats were shown in table 3 and 4. Significant elevations in the concentration of these lipids profiles were observed in ethanol fed rats, when compared to control rats. Administration of GLEt decreased the levels of lipid profiles in serum and tissue of alcohol fed rats.

Table 2: Changes in levels of plasma non-enzymatic antioxidants in control and experimental animals

Groups	Vitamin C (mg/dl)	Vitamin E (mg/dl)	GSH (mg/dl)
Control	1.98 ± 0.05[a]	1.52 ± 0.11[a]	25.93 ± 2.37[a]
Control + GLEt (100 mg/kg)	2.04 ± 0.57[a]	1.62 ± 0.15[a]	27.88 ± 2.44[a]
Ethanol	0.82 ± 0.07[b]	0.69 ± 0.03[b]	15.06 ± 1.28[b]
Ethanol + GLEt (100 mg/kg)	1.80 ± 0.10[c]	1.38 ± 0.10[c]	22.20 ± 1.72[c]

Values are given as mean ± S.D for 6 rats in each group. Values not sharing a common superscript letter differ significantly at p<0.05 (DMRT). GLEt - Grape leaf extract

Table 3: Levels of lipid profiles in plasma of control and experimental animals

Groups	Cholesterol (mg/dl)	Free fatty acids (mg/dl)	Triglycerides (mg/dl)	Phospholipids (mg/dl)
Control	77.19 ± 5.89[a]	57.49 ± 4.38[a]	55.85 ± 4.25[a]	87.07± 6.63[a]
Control + GLEt (100 mg/kg)	73.97 ± 6.04[a]	55.95 ± 4.26[a]	53.30 ± 4.06[a]	86.26 ± 6.57[a]
Ethanol	141.43 ± 13.03[b]	104.00 ± 7.96[b]	140.89 ± 10.78[b]	149.32 ± 11.43[b]
Ethanol + GLEt (100 mg/kg)	88.41 ± 8.60[c]	66.74 ± 5.10[c]	67.31 ± 5.14[c]	98.28 ± 7.5[c]

Values are given as mean ± S.D for 6 rats in each group. Values not sharing a common superscript letter differ significantly at p<0.05 (DMRT). GLEt - Grape leaf extract

Table 4: Lipid profiles in the liver of control and experimental animals

Groups	Cholesterol (mg/100g wet tissue)	Free fatty acids (mg/100g wet tissue)	Triglycerides (mg/100g wet tissue)	Phospholipids (g/100g wet tissue)
Control	341.31 ± 25.99[a]	736.03 ± 56.05[a]	361.60 ± 27.53[a]	1.83 ± 0.13[a]
Control + GLEt (100 mg/kg)	335.90 ± 25.58[a]	715.21 ± 54.46[a]	335.35 ± 25.54[a]	1.82 ± 0.13[a]
Ethanol	589.78 ± 45.14[b]	1021.05 ± 78.15[b]	709.89 ± 54.34[b]	2.84 ± 0.21[b]
Ethanol + GLEt (100 mg/kg)	382.07 ± 29.18[c]	798.52 ± 60.98[ac]	423.68 ± 5.14[c]	2.03 ± 0.15[c]

Values are given as mean ± S.D for 6 rats in each group. Values not sharing a common superscript letter differ significantly at p<0.05 (DMRT). GLEt - Grape leaf extract

5. Discussion

Chronic ethanol administration associated with increased alterations in lipid metabolism. The major pathway for hepatic oxidation is ethanol to acetaldehyde through alcohol dehydrogenase, which is associated with the reduction of nicotinamide adenine dinucleotide (NAD) to reduced nicotinamide adenine dinucleotide (NADH) and produces redox change. The reducing equivalents inhibit tricarboxylic acid cycle activity and fatty acid oxidation. Furthermore, interaction of ethanol with biological membranes including lipid and protein components is complex and can cause significant changes in membrane function (Rubin and Rottenberg, 1982) [26]. Fatty acid accumulation in the tissues may be directly due to lipid breakdown and indirectly due to the oxidation of ethanol by the liver to acetate and its conversion to fatty acids (Katbamna *et al*., 1997) [15]. All these changes lead to a marked accumulation of fat in the liver during chronic alcohol consumption. Lowering of tissue lipid levels through dietary or drug therapy seems to be associated with a decrease in the risk of liver disease. The reduced tissue lipids in GLEt co-treated rats might be due to increase in the mobilization and hydrolysis of certain lipoproteins for their selective uptake and metabolism by different tissues. Previous literature has shown that phenolic compounds have the ability to normalize the levels of tissue lipids during diseased conditions (Anbu *et al*., 2016) [1]. The phenolic compound present in the GLEt might be responsible to reduce the lipids in the ethanol fed rats co-treated with GLEt.

Further, alcohol provides calories (7.1Kcal g-1) and devoid of nutrients. Thus, isocaloric exchange of carbohydrates by ethanol is caused in a decreased weight gain, whereas alcohol reduces the absorption of nutrients from the intestine and causes maldigestion, all these changes could lead to reduction in body weight. Since body weight is recognized indicator of health, the increased weight gain in GLEt co-administered suggested the beneficial effect of GLEt against ethanol induced liver toxicity.

Non-enzymatic antioxidants such as GSH, vitamin C and

vitamin E are closely interlinked to each other and play an excellent role in protecting the cell from lipid peroxidation (Sies, 1993) [27]. Non-enzymatic are important free radical scavengers, which protect the cell membrane against toxic agents such as alcohol. Glutathione is a major non-protein thiol in living organisms, which plays a central role in coordinating the antioxidant defense system in our body. It is involved in the maintenance of normal cell structure and function probably through its redox and detoxification reaction (Guerri and Grisolia, 1980) [14]. The depleted level of GSH in alcohol toxicity may due to scavenging of toxic radicals or inhibition of the synthesis and increased rates of turnover (Lieber, 1997) [20]. It has been considered as evidence, which is supporting the hypothesis that reactive oxygen intermediates generated during the metabolism of ethanol lead to glutathione oxidation and lipid peroxidation. In addition to GSH, we have also observed a decrease in the levels of antioxidants such as vitamins C and E in plasma of alcohol treated rats (). Supplementation of GLEt to alcohol treated rats restored the non-enzymic antioxidants levels in plasma. The biologically active components found in grape leaf sparing the antioxidant activity and reduced the consumption of endogenous antioxidants, which could be responsible for the reduction of oxidative stress during ethanol toxicity. The active constituents such as flavanols, flavanol oligomers and proanthrocyanidins were found in *V. vinifera* leaves (Monagas *et al*, 2006) [23] and it has been reported as powerful antioxidants (Fauconneau *et al*., 1997) [11]. Further, the $ABTS^+$ and $DPPH^+$ assay showed that GLEt effectively scavenges the toxic free radicals (Pari and Suresh, 2008) [24]. This could be responsible for the reversal of nonenzymatic antioxidants levels in plasma and tissues of alcohol fed rats treated with GLEt.

Phospholipids are basic components of cell membranes, mainly acting as a regulator of membrane bound enzymes and in membrane transport processes and thus they are important in determining the pathology of alcoholism (Carrasco *et al*., 2002) [5]. The increased phospholipid content in the alcohol rats may be due to the elevated levels of free fatty acids (Frayn, 1993) [31] and cholesterol, which can promote the synthesis of phospholipids (Marsch *et al*., 1996) [21].

Administration of GLEt decreased the levels of free fatty acid, triglycerides, total cholesterol and phospholipid in alcohol treated rats and prevents the toxic complications. The proanthocyanidines found in grape leaf can prevent the production of acetaldehyde in alcohol metabolism and it is well protective agent against alcohol toxicity (Kushnerova *et al*., 2003) [17]. Grape flavonoids such as proanthocyanidines and reservatrol having hypocholesterolemic and hypolipidemic activity (Miura *et al*., 2003; Kravchenko *et al*., 2003) [22, 16], which could be responsible for the suppression of elevation in the levels of lipid in alcohol fed rats. It is well known that proanthocyanidine, anthocyanins, and trans-resveratrol found in grape leaves have the capability to protect the cardiac functional and prevent coronary heart disease (Das *et al*., 1999; St Leger *et al*., 1979) [6, 28]. In agreement with these reports on study suggest that GLEt has the capability to ameliorate the alcohol-induced abnormalities in the levels of lipids and its metabolism.

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