

ANTIHYPERLIPIDEMIC EFFECT OF WHEATGRASS ON ALCOHOL AND Δ PUFA INDUCED LIVER TOXICITY IN MALE ALBINO WISTAR RATS

VARALAKSHMI DURAIRAJ, GARIMA SHAKYA, RUKKUMANI RAJAGOPALAN*

Department of Biochemistry and Molecular Biology, School of Life Sciences, Pondicherry University, Puducherry, India.
Email: ruks2k2@yahoo.co.in

Received: 10 Dec 2013, Revised and Accepted: 09 Feb 2014

ABSTRACT

Objective: Alcohol is one of the widely used and abused drugs in the society. Chronic alcohol consumption causes various hepatic lesions that are grouped under common term, alcoholic liver diseases. Dietary intake of fats, especially repeatedly heated polyunsaturated fatty acid (Δ PUFA) produces various lipid peroxidative end products which can aggravate the pathological changes produced by ethanol. In the present study, we have investigated the effect of wheatgrass on alcohol and Δ PUFA induced hyperlipidemia in male albino wistar rats.

Methods: In our study, we analysed the liver marker enzymes AST and ALT in plasma and lipid profiles: free fatty acids, triglycerides, cholesterol and phospholipids in liver.

Results: Our results showed that there was an increase in the activities of AST and ALT in plasma of alcohol and Δ PUFA rats. Treatment with the wheatgrass effectively prevented the release of these marker enzymes. The levels of cholesterol, triglycerides and free fatty acids were increased whereas the levels of phospholipids were decreased in alcohol and Δ PUFA groups compared to normal. This was reversed significantly on treatment with wheatgrass. Histopathological observations were also in correlation with the biochemical parameters.

Conclusion: Results obtained in the study proves that wheatgrass can effectively protect the liver and has hypolipidemic effect in alcohol and Δ PUFA induced liver toxicity.

Keywords: Alcohol, Hyperlipidemia, Lipids, Wheatgrass, Δ PUFA.

INTRODUCTION

Alcohol related liver diseases are one of the current challenging health problems in the world. Ethanol is known to promote accumulation of hepatic fat, leading to fibrosis and cirrhosis [1, 2, 3]. Alcohol's metabolism via alcohol dehydrogenase in liver, generates high NADH/NAD⁺ ratio, alters the oxidation-reduction potential of the hepatic cell, which in turn inhibits the fatty acid oxidation and enhances triglyceride synthesis resulting in fatty liver [4].

Polyunsaturated fatty acid (PUFA) is highly recommended nowadays due to its hypocholesterolemic effect [5]. But some studies have shown that PUFA increases the oxidative stress status of the hepatic cells [3]. Alcoholics generally eat fried food made up of PUFA along with alcohol. The combined ingestion of alcohol and fried food aggravates the alcohol related liver diseases [6, 7].

Wheatgrass refers to the young grass of wheat (*Triticum aestivum*) germinated for a period of 6 to 10 days. It contains vitamin C and E, β carotene, ferulic acid, vanilic acid and phenols, especially flavonoids. Wheatgrass juice is found to have healing properties in various degenerative diseases and is known to benefit blood cells, bones, glands, kidney and other parts of the body [8, 9, 10]. Since little or no work has been done on the antihyperlipidemic effects of wheat grass, the present study was designed to analyse its role on alcohol and Δ PUFA induced hyperlipidemia.

MATERIALS AND METHODS

Animals

Male albino rats, Wistar strain, of body weight 140-160g bred in central animal house, Pondicherry University were used in this study. The animals were fed on the standard pellet diet (Hindustan Lever Limited, Mumbai, India). Water was given *ad libitum*. The standard pellet diet comprised 21% protein, 5% lipids, 4% crude fiber, 8% ash, 1% calcium, 0.6% phosphorus, 3.4% glucose, 2% vitamins and 55% nitrogen free extract (carbohydrates). It produces a metabolisable energy of 3600 K Cal. The animals were housed in plastic cages under controlled condition of 12h light / 12h dark cycles, 50% humidity and at 30 \pm 2°C.

The animals used in the present study were maintained in accordance with the guidelines of the National Institute of Nutrition, Indian Council for Medical Research, Hyderabad, India and approved by the Institutional Animal Ethical Committee (PU/IAEC/11/06), Pondicherry University.

Materials Used

Absolute ethanol was obtained from Hayman Private Limited, England. Sunflower oil marketed by Gold Winner was purchased from local market, Puducherry, India. Sunflower oil was subjected to heating at 180°C for 30 minutes, twice. The oil was analyzed by gas chromatography and found to contain altered fatty acid composition [11]. All chemicals and solvents used were of the highest purity and analytical grade.

Experimental design

The animals were divided into 4 groups of 6 rats each.

Group 1 normal (N) - Rats were given standard pellet diet

Group 2 (alcohol+ Δ PUFA) - Rats were given 20% ethanol orally, using intragastric tube + 15% heated sunflower oil mixed with standard pellet diet once daily.

Group 3 (alcohol+ Δ PUFA+WG) - Rats were given 20% ethanol orally, using intragastric tube + 15% heated sunflower oil mixed with standard pellet diet + wheatgrass (75 mg/Kg b. Wt.) orally as a suspension once daily.

Group 4 (WG) - Rats were given standard pellet diet + wheatgrass (75mg/Kg b. Wt.) orally as a suspension once daily.

At the end of the experimental period (45 days), rats were sacrificed after an overnight fast by cervical dislocation

Sample Preparation

Blood was collected in heparinised tubes and plasma was separated by centrifugation at 1000g for 15 minutes. Liver tissues were removed, cleared off blood and immediately transferred to ice-cold containers containing 0.9% NaCl. A known amount of tissue was weighed and homogenized in appropriate buffer (10%) for the

estimation of various parameters. Tissue lipids were extracted according to the method of Folch et al., [12].

Biochemical estimation

The activity of aspartate transaminase (AST) and alanine transaminase (ALT) in plasma were estimated by the procedure of Reitman and Frankel [13]. Antihyperlipidemic action of wheatgrass extract was assessed by analyzing the levels of free fatty acids by the method of Falholt et al., [14], triglycerides by the method of Foster and Dunn [15], cholesterol by the CHOD-PAP method of Allain et al., [16] by using AGAPPE reagent kit and phospholipids by the method of Zilversmit and Davis [17].

For histopathological studies, two animals from each group were perfused with 10% formalin and the liver tissues were separated and stored in 10% formalin. They were later sectioned using a microtome, dehydrated in graded alcohol, embedded in paraffin section, and stained with hematoxylin and eosin (H&E).

Statistical Analysis

Statistical analysis was done by analysis of variance (ANOVA) followed by Tukey's test. $p \leq 0.05$ was considered to be statistically significant.

RESULTS

Figure 1 and 2 shows the changes in the activities of plasma AST and ALT, respectively. The activities of these liver markers were increased significantly in plasma of alcohol and Δ PUFA groups and reduced on treatment with wheatgrass.

ANOVA followed by Tukey's test. Values are mean \pm SD from 6 rats in each group. Bars not sharing a common superscript differ significantly at $P \leq 0.05$.

ANOVA followed by Tukey's test. Values are mean \pm SD from 6 rats in each group. Bars not sharing a common superscript differ significantly at $P \leq 0.05$.

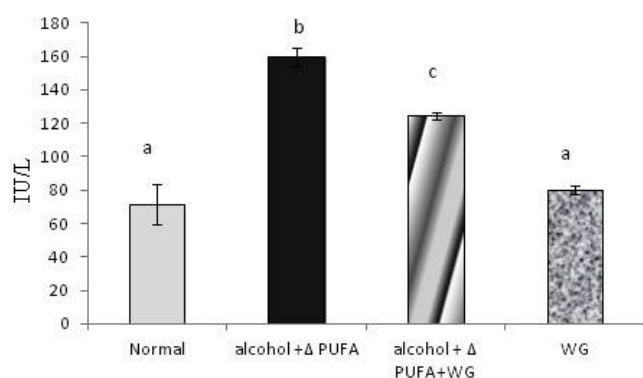


Fig. 1: Activities of AST in plasma

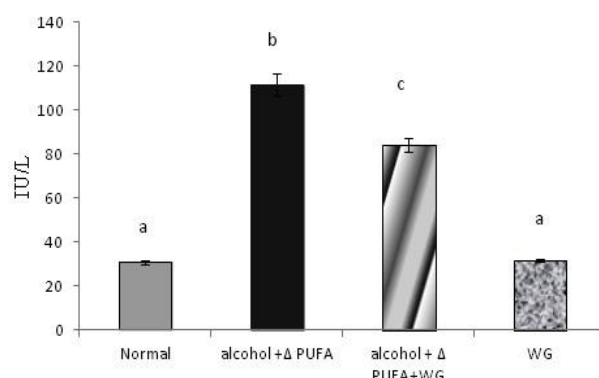


Fig. 2: Activities of ALT in plasma

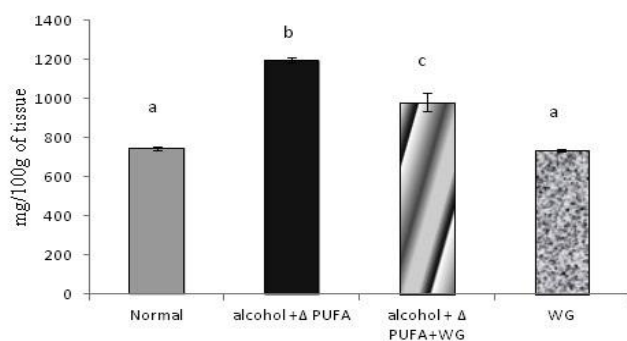


Fig. 3: Levels of free fatty acids in liver

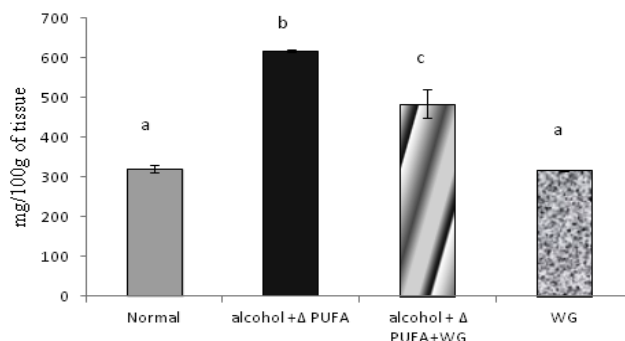


Fig. 4: Levels of triglycerides in liver

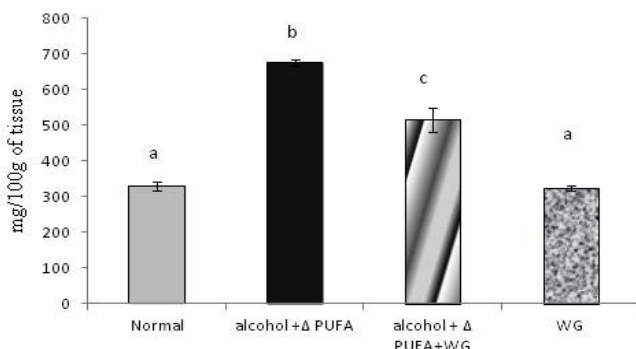


Fig. 5: Levels of cholesterol in liver

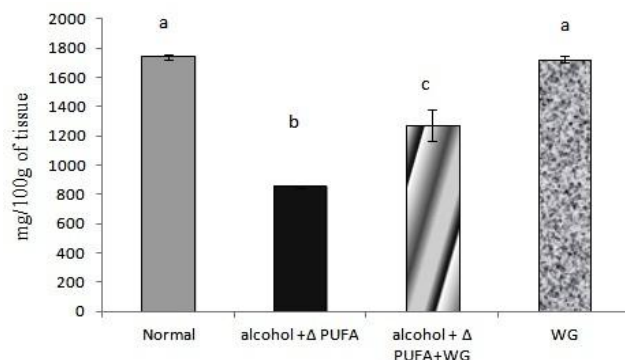


Fig. 6: Levels of phospholipids in liver

Figure 3 and 4 shows the changes in the levels of free fatty acids and triglycerides in liver, respectively. The levels were significantly increased in alcohol and Δ PUFA groups, which were decreased after wheatgrass treatment.

ANOVA followed by Tukey's test. Values are mean \pm SD from 6 rats in each group. Bars not sharing a common superscript differ significantly at $P \leq 0.05$.

ANOVA followed by Tukey's test. Values are mean \pm SD from 6 rats in each group. Bars not sharing a common superscript differ significantly at $P \leq 0.05$.

Figure 5 shows the cholesterol levels in liver. The levels were high in alcohol and Δ PUFA treated rats which were significantly reduced after wheatgrass treatment.

ANOVA followed by Tukey's test. Values are mean \pm SD from 6 rats in each group. Bars not sharing a common superscript differ significantly at $P \leq 0.05$.

Figure 6 depicts the phospholipids levels in liver. The levels of phospholipids were decreased in alcohol and Δ PUFA treated rats, which were significantly increased after wheatgrass treatment.

ANOVA followed by Tukey's test. Values are mean \pm SD from 6 rats in each group. Bars not sharing a common superscript differ significantly at $P \leq 0.05$.

Figure 7 shows the histopathology of liver. Mild portal inflammation and increased microvesicular fatty acid changes were seen in alcohol and Δ PUFA group (Fig. 7B). Treatment with wheatgrass effectively

reduced the pathological abnormalities and only inflammatory cell infiltrations were seen in the treated liver (Fig. 7C). Normal histology was unaltered in wheatgrass control rats (Fig. 7D).

DISCUSSION

Worldwide unhealthy alcohol consumption remains a major health problem [18]. Heavy alcohol consumption primarily affects the liver [1]. Liver plays an important role in lipid metabolism and therefore, it is expected to have abnormal lipid profile with severe liver dysfunction [19].

Liver marker enzymes like AST and ALT are hallmark for the hepatic injury due to alcohol [20, 21]. Increases in the levels of these enzymes are indices of increased permeability, damage and necrosis of hepatocytes [20]. Moreover, the membranes are made up of lipid conjugates particularly, PUFA, which are highly susceptible for lipid peroxidation which also contribute to the leak of liver marker enzymes [1].

Thermal oxidation of fat, especially PUFA, modifies the nutritional property and exacerbates the alcoholic liver injury [5]. Thus in our study the combined ingestion of alcohol and Δ PUFA might have disturbed the normal architecture of the hepatic cells and released the biomarker enzymes in the plasma. Wheatgrass is a good source of phenolics [8], which are known to be powerful antioxidants. The antioxidant activity of phenols may be due to various mechanisms like free radical scavenging, hydrogen donating, singlet oxygen quenching and metal ion chelating ability [22]. Thus the phenols present in the wheatgrass could have effectively prevented the membrane damage and decreased the leak of liver marker enzymes

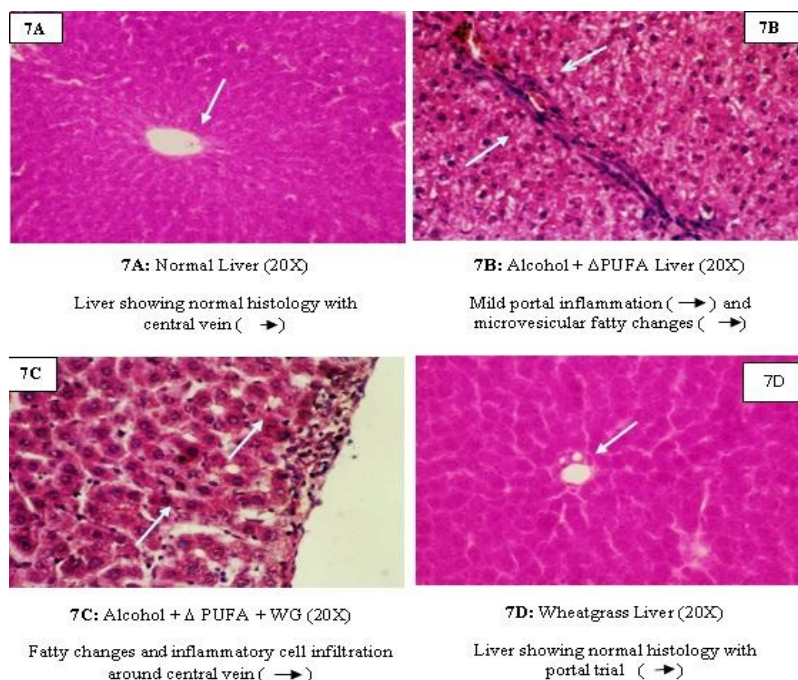


Fig. 7: Histopathology of liver

It is well established that the earliest response to the chronic alcohol consumption is the development of fatty liver [1]. During metabolism of excess ethanol, high NADH is generated which alters the redox status of the hepatic cell. This causes depressed citric acid cycle, enhances hepatic lipogenesis, decreases hepatic release of lipoproteins, increases lipolysis of peripheral fat, increases fat uptake, thus predisposing fatty liver [23].

Triglycerides are transported to peripheral tissues by very low density lipoprotein (VLDL). Heavy ethanol consumption blocks the secretion of VLDL, thus suppressing the transport of triglycerides and release of free fatty acid from lipoprotein [2].

On the other hand high intake of dietary fats leads to increased storage of triglycerides not only in adipose tissues but also in liver [24].

Heavy alcohol consumption causes accumulation of fatty acids and triglycerides in the liver by inducing the expression of key enzyme of lipid synthesis like hepatic glycerol-3-phosphate acyltransferase, fatty acid synthase, and malic enzyme [25]. Several reports have suggested that phenolic antioxidants possess hypocholesterolemic, hypolipidemic, antidiabetic, antihypertensive and anticancer activities [26]. Wheatgrass treatment significantly reduced the lipids levels, which could be due to the presence of phenolics, which might have decreased the expression of key enzymes involved in lipid synthesis.

Alcohol increases the biosynthesis and decreases the catabolism of cholesterol resulting in hypercholesterolemia. Various studies have shown that oil rich in PUFA, increases the circulating cholesterol [27, 28]. These reports are in agreement with our finding that cholesterol levels were increased in alcohol and Δ PUFA treated rats (fig. 5). This may be related with the increase in HMG CoA reductase activity by ethanol, which is a rate limiting enzyme involved in cholesterol biosynthesis [27]. Moreover increased oxidative stress due to alcohol induces the synthesis of cholesterol and fatty acid and decreases their degradation [27]. Fatty acid in turn increases the production of other lipids. As wheatgrass is a good source of antioxidants like vitamin C and E, ferulic acid, vanilic acid and phenolics [8], could have effectively reduced oxidative stress and thus contributed for decreased cholesterol.

Phospholipids are the major constituents of the biomembrane and are the primary targets of peroxidation process and they are altered by ethanol consumption [27]. In our study we found a decrease in the levels of phospholipids in liver (fig. 6), which could be due to increased enzymatic degradation, which modify the composition, structure and stability of the biomembranes thus leading to liver dysfunctioning [27].

Induction of CYP2E1 by ethanol is the central pathway for the generation free radicals [29]. Together with excess PUFA in diet, it causes oxidative damage to cellular membranes and decreases the membrane phospholipids [30]. Wheatgrass by the virtue of its effective antioxidant property decreased the membrane damage and could have preserved the levels of phospholipids.

Thus in our study, wheatgrass effectively modulated the marker enzymes and lipid profiles in the liver. This could be either by enhanced protection against free radical attack, or by augmentation of fatty acid turnover, or perhaps both. Wheatgrass is found to have good antioxidant property.

It is a rich source of vitamin C and E, ferulic acid, vanilic acid and phenolic compounds especially flavonoids [8]. Datta et al., [1] has shown that the most common antioxidants like tocopherol, β carotene and ascorbic acid are potent hepatoprotective agents against ethanol induced oxidative damage. Wheatgrass extract also contains ferulic acid, a natural phenolic antioxidant that was found to modulate the altered lipid profile during alcohol and Δ PUFA induced toxicity [8, 31]. Due to the presence of such potent antioxidants, wheatgrass extract could have effectively reduced the lipid levels.

Histopathological observations were in correlation with our biochemical results. Wheatgrass treatment effectively reduced the pathological abnormalities and showed inflammatory cell infiltration, which is a positive immune response (Fig. 7C). This proves that wheatgrass is very effective in lowering the lipid levels induced by alcohol and Δ PUFA.

Wheatgrass by virtue of its flavonoids and phenolics, effectively protected the liver from alcohol and Δ PUFA induced damage. Hence wheatgrass extract can become an effective therapeutic agent for treatment of hyperlipidemia.

ACKNOWLEDGEMENT

Authors acknowledge DST-FIST, UGC-SAP and DBT-IPLS for providing infrastructural support

Declaration of Interest

The authors report no conflict of interest.

REFERENCES

- Datta G, Basu S, Sen A, Nath P. Role of α -tocopherol, β -carotene and ascorbic acid against alcohol induced hepatotoxicity: A comparative analysis. *J Pharm Res* 2012; 5: 2485-2488.
- Yang HS, Lin HS, Chao JC, Chien YW, Peng HC, Chen JR. Effects of soy protein on alcoholic liver disease in rats undergoing ethanol withdrawal. *J Nutr Biochem* 2012; 23: 679-684.
- Purohit V, Russo D, Coates PM. Role of fatty liver, dietary fatty acid supplements, and obesity in the progression of alcoholic

- liver disease: introduction and summary of the symposium. *Alcohol* 2004; 34: 3-8.
- Garcia-Villafranca J, Guillen A, Castro J. Ethanol consumption impairs regulation of fatty acid metabolism by decreasing the activity of AMP-activated protein kinase in rat liver. *Biochimie* 2008; 90: 460-466.
- Patere SN, Majumdar AS, Saraf MN. Exacerbation of alcohol-induced oxidative stress in rats by polyunsaturated fatty acid and iron load. *Indian J Pharm Sci* 2012; 73: 152-158.
- Leibowitz SF. Overconsumption of dietary fat and alcohol: Mechanisms involving lipids and hypothalamic peptides. *Physiol Behav* 2007; 91: 513-521.
- Alexander JC. Chemical and biological properties related to toxicity of heated fats. *J Toxicol Env Health* 1981; 7: 125-138.
- Kulkarni SD, Tilak JC, Acharya R, Rajurkar NS, Devasagayam TPA, Reddy AVR. Evaluation of the antioxidant activity of wheatgrass (*Triticum aestivum* L.) as a function of growth under different conditions. *Phytother Res* 2006; 20: 218-227.
- Padalia S, Drabu S, Raheja I, Gupta A, Dhamija M. Multiple potential of wheatgrass juice (green Blood): An overview. *Chron Young Scientist* 2010; 1: 23-28.
- Sri jaya M, Gayathri S. Antioxidant activity of wheatgrass and impact of supplementing grass extract anaemics. *Biomed* 2009; 4: 262-268.
- Rukkumani R, Aruna K, Varma PS, Rajasekaran KN, Menon VP. Comparative effects of curcumin and analog of curcumin on alcohol and PUFA induced oxidative stress. *J pharm Pharmaceut Sci* 2004; 7: 274-283.
- Folch J, Lees M, Sloane-Staney GH. A simple method for the isolation and purification of total lipids from animal tissues. *J Biol Chem* 1957; 226: 497-507.
- Reitman S, Frankel S. A colorimetric method for the determination of serum glutamic, oxaloacetic and glutamic pyruvic transaminases. *Am J Clin Pathol* 1957; 28: 56-63.
- Falholt K, Lund B, Falholt W. An easy colorimetric micromethod for routine determination of free fatty acids in plasma. *Clin Chim Acta* 1973; 46: 105-111.
- Foster LB, Dunn RT. Stable reagents for determination of serum triglycerides by a colorimetric Hantzsch condensation method. *Clin Chem* 1973; 19: 338-340.
- Allain CC, Poons LS, Chan CSG, Richmond W, Fu PC. Enzymatic determination of total serum cholesterol. *Clin Chem* 1974; 20: 470-475.
- Zilversmit DB, Davis AK. Microdetermination of plasma phospholipids by trichloroacetic acid precipitation. *J Lab Clin Med* 1950; 35: 155-60.
- Gramenzi A, Caputo F, Biselli M, Kuria F, Loggi E, Andreone P. et al. Review article: alcoholic liver disease – pathophysiological aspects and risk factors. *Aliment Pharmacol Ther* 2006; 24: 1151-1161.
- Ghadir MR, Riahin AA, Havaspour A, Nooranipour M, Habibinejad AA. The relationship between lipid profile and severity of liver damage in cirrhotic patients. *Hepat Mon* 2010; 10: 285-288.
- Hidiroglou N, Madere R. Effect of chronic ethanol dosing on hepatic triglyceride and phospholipid profile and fatty acids in the guinea pig. *Alcohol* 1999; 19: 229-233.
- Tsai J, Ford ES, Li C, Zhao G. Past and current alcohol consumption patterns and elevations in serum hepatic enzymes among US adults. *Addict Behav* 2012; 37: 78-84.
- Barreira JCM, Ferreira ICFR, Oliveira MBPP, Pereira JA. Antioxidant activities of the extracts from chestnut flower, leaf, skins and fruit. *Food Chem* 2008; 107: 1106-1113.
- Maralla S, Kesireddy SR, Wudayagiri R. Effect of ginger consumption on serum makers of general metabolism, liver and kidney functions and lipid profiles in ethanol induced withdrawal rats. *J Pharm Res* 2012; 5: 485-491.
- Perez-Martinez P, Perez-Jimenez F, Lopez-Miranda J. n-3 PUFA and lipotoxicity. *Biochim Biophys Acta* 2010; 1801: 362-366.

25. You M, Crabb DW. Molecular mechanisms of alcoholic fatty liver: role of sterol regulatory element-binding proteins. *Alcohol* 2004; 34: 39-43.
26. Chon SU, Heo BG, Park YS, Kim DK, Gorinstein S. Total phenolics level, antioxidant activities and cytotoxicity of young sprouts of some traditional korean salad plants. *Plant Foods Hum Nutr* 2006; 64: 25-31.
27. Nechifor MT, Dinu D. Ethanol-induced changes of the lipid profile in rat kidney. *Rev Roum Chim* 2010; 55: 573-577.
28. Otunola GA, Oloyede OB, Oladiji AT, Afolayan AA. Effects of diet-induced hypercholesterolemia on the lipid profile and some enzyme activities in female wistar rats. *Afr J Biochem Res* 2010; 4:149-154.
29. Wang X, Cederbaum AI. Acute ethanol pretreatment increases FAS-mediated liver injury in mice: Role of oxidative stress and CYP2E1-dependent and -independent pathways. *Free Radical Bio Med* 2007; 42: 971-984.
30. Shireen KF, Pace RD, Mahboob M, Khan AT. Effects of dietary vitamin E, C and soybean oil supplementation on antioxidant enzyme activities in liver and muscles of rats. *Food Chem Toxicol* 2008; 46: 3290-3294.
31. Rukkumani R, Aruna K, Varma PS, Menon VP. Ferulic Acid, a natural phenolic antioxidant modulates altered lipid profiles during alcohol and thermally oxidized sunflower oil induced toxicity. *J Nutraceut Function Med Food*. 2005; 4: 119 -132.