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EFFECT OF VIMLIV ON LIPID PEROXIDES AND ANTIOXIDANTS IN ETHANOL INDUCED HEPATOTOXICITY IN ALBINO WISTER RATS

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ABSTRACT

Keywords:

Hepatoprotective,
Vimliv,
Lipid peroxidation,
Ethanol,
Antioxidant

This study was carried out to investigate the hepatoprotective and antioxidant properties of vimliv in ethanol-induced hepatotoxicity in rats. The liver toxicity was induced by the administration of ethanol to the animals at dose of 3 g/kg orally for 35 days. During the period of vimliv was co-administered to the rats at doses of 25 and 50 mg/kg for 35 days. The levels of lipidperoxidative products significantly increased and the levels of antioxidants decreased in ethanol induced rats. Co-administration of vimliv to ethanol-induced rats were significantly minimized the alterations in the levels of lipidperoxidation and antioxidants. Administration of vimliv protected the liver against ethanol-induced toxicity, possibly by reducing the rate of lipidperoxidation and increasing the antioxidant defense mechanism in rats.

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INTRODUCTION: Alcohol dependency is a major health and socio-economic problem throughout the world. Alcohol administration has been found to cause accumulation of reactive oxygen species, which in turn is the source of lipid peroxidation of cellular membranes and proteins as well as DNA oxidation resulting in hepatocyte injury. Each biological system has certain antioxidant mechanisms against the aggregations of such free radicals. The balance of oxidant-antioxidant system must exist in the cell while the disturbance of antioxidant-prooxidant balance causes oxidative stress¹.

Antioxidants are important endogenous defense mechanism against injury caused by lipid peroxidation and harmful reactions induced by reactive oxygen species (ROS), which are constantly produced in the body during normal metabolic processes².

Antioxidants may act individually or complementary in synergetic action. Many antioxidant enzymes are sequestered in peroxisomes. Repair mechanisms are

also available in the cells as potent mechanisms for removal of oxidized membrane fatty acids³.

Antioxidant vitamins are important elements in protecting many cellular damages, for example vitamin E and C which protect against oxidant-mediated inflammation and inflammatory tissue damage⁴. Vitamin E may protect against liver damage and prevent the fibrosis and cirrhosis progression in metal overload states⁵.

Ethanol produces specific metabolic and toxic disturbances, result in the production of acetaldehyde, a highly toxic. The major pathway for ethanol disposition involves alcohol dehydrogenase (ADH), an enzyme that catalyzes the conversion of ethanol to acetaldehyde. This enzyme might be to rid the body of the small amounts of alcohol produced by fermentation in the gut⁶. Oxidative stress is known to play an important role in the pathogenesis of ethanol-induced liver injury⁷⁻⁸. Oxidative damage correlates with the amount of ethanol consumed⁹.

Recently, it has been demonstrated that nitric oxide (NO) is an important mediator of hepatotoxicity, and the changes in its generation or actions may contribute to pathologic states¹⁰⁻¹¹. It has been proposed that the high production of NO causes injury, perhaps through the generation of potent radicals¹². An increase in NO production has been reported in monocytes of patients with chronic liver disease¹³ and in the livers of rats chronically fed with ethanol¹⁴. However, in some models of inflammation, it has been shown that inhibition of NO increases tissue dysfunction or injury¹².

Alcoholic liver disease is a worldwide health problem¹⁵. The three most widely recognized forms of alcoholic liver diseases are fatty liver/steatosis, alcoholic hepatitis and liver cirrhosis. At least 80% of heavy drinkers have been reported to develop steatosis, 10–35% alcoholic hepatitis, and approximately 10% liver cirrhosis¹⁶⁻¹⁸. Various experimental studies described that ethanol caused accumulation of reactive oxygen species like super oxide, hydroxyl radical¹⁹⁻²⁰ and hydrogen peroxide in hepatocytes that oxidized the reduced glutathione, which in turn lead to lipid peroxidation of cellular membranes, and oxidation of protein and DNA resulting in hepatocytes injury^{21, 22}.

Vimliv fortified is a rationale combination of hepatoprotective herbs mentioned in Ayurvedic scriptures for the treatment of liver ailments (**table 1**).

TABLE 1: COMPOSITION OF VIMLIV

Bhumyamalaki (<i>Phyllanthus niruri</i>)	200 mg
Kasni (<i>Cichorium intybus</i>)	150 mg
Punarnava (<i>Boerhaavia diffusa</i>)	100 mg
Bhringaraj (<i>Eclipta alba</i>)	200 mg
Guduchi (<i>Tinospora cordifolia</i>)	50 mg
Rohitak (<i>Tecomella undulata</i>)	50 mg
Kalmegh (<i>Andrographis paniculata</i>)	25 mg
Daruharidra (<i>Berberis aristata</i>)	50 mg
Kakmachi (<i>Solanum nigrum</i>)	25 mg
Vidanga (<i>Embelia ribes</i>)	50 mg
Katuki (<i>Picrorrhiza kurroa</i>)	10 mg
Parpat (<i>Fumaria parviflora</i>)	25 mg
Sharapunkha (<i>Tephrosia purpurea</i>)	25 mg
<i>Terminalia chebula</i>	16.66 mg
<i>Terminalia bellerica</i>	16.66 mg
<i>Emblica officinalis</i>	16.66 mg
Kumari (<i>Aloe barbadensis</i>)	50 mg
Chitrak(<i>Plumbago zeylanica</i>)	10 mg

The benefits of vimliv reduces inflammation in the liver, facilitates regeneration of hepatocytes & prevents hepatic degeneration, maintains hepatic architecture, eliminates hepatotoxins, improves hepatic antioxidant status, stimulates bile flow, improves appetite and digestion. The present study was aimed to evaluate the role of vimliv on ethanol-induced toxicity in Wistar rats.

MATERIALS AND METHODS:

Experimental Animals: All the experiments were done with female albino Wistar rats weighing 140-150 g, obtained from the Venkateswara Enterprises, Bangalore were used in this study. They were housed in polypropylene cages (47x34x20 cm) lined with husk, renewed every 24 h under a 12:12 h light/dark cycle at around 22°C and had free access to water and food. The rats were fed on a standard pellet diet (Pranav Agro Industries Ltd., Maharashtra, India). The pellet diet consisted of 22.02% crude protein, 4.25% crude oil, 3.02% crude fibre, 7.5% ash, 1.38% sand silica, 0.8% calcium, 0.6% phosphorus, 2.46% glucose, 1.8% vitamins and 56.17% nitrogen free extract (carbohydrates). The diet provided metabolisable energy of 3, 600 kcal. The experiment was carried out in according with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India.

Drugs and Chemicals: Vimliv was purchased from venkataswara medical store in Salem. Ethanol was purchased from Changshu yangyuan chemicals Pvt Lvd, China. Butylated hydroxy toluene (BHT), xylenol orange, dithionitro bis benzoic acid (DTNB), ascorbic acid, 2, 2' dipyridyl, p-phenylene diamine and sodium azide were obtained from Himedia laboratory, Mumbai, India. Thiobarbituric acid were purchased from Himedia Laboratories Pvt Lvd., Mumbai, India. All other chemical used in this study were of analytical grade.

Experimental Induction of Hepatotoxicity: Ethanol (3 gm/kg) was dissolved in water and injected intragastrically, for a period of 35 days²³. Vimliv was given at the concentration of 25 & 50 mg/kg, dissolved in carboxy methyl cellulose and given to rats through orally.

Experimental Design: Vimliv tablets were powdered with the help of mortar and pestle. The Vimliv powder was administered at concentrations of 25 and 50 mg/kg was dissolved in carboxy methyl cellulose (CMC) given to rats through intragastric intubations for a period of 35 days.

In the experiment, a total of 36 rats (18 toxicity surviving rats, 12 control rats) were used in the study. The rats were divided into 5 groups of 6 rats in each group.

- Group 1: Normal control rats
- Group 2: Normal rats treated with Vimliv (50 mg/kg)
- Group 3: Ethanol control rats
- Group 4: Vimliv treated (25 mg/kg) + Ethanol
- Group 5: Vimliv treated (50 mg/kg) + Ethanol

After the last treatment, all the rats were sacrificed by cervical decapitation. Blood was collected for the estimation of blood glucose. Serum and plasma were separated from blood after centrifugation. The liver and kidney tissues were excised immediately from the rats, washed off blood with ice-cold physiological saline. A known weight of the liver and kidney tissues were homogenized in appropriate buffer solution. The homogenate was centrifuged and the supernatant was used for the estimation of various biochemical parameters.

Biochemical Estimation: Plasma thiobarbituric acid reactive substances were estimated by the method of Yagi (1987)²⁴. The concentration of TBARS in the tissue was estimated by the method of Fraga et al., (1988)²⁵. The levels of HP were estimated by the method of Jiang et al. (1992)²⁶. The activity of SOD was assayed according to the procedure of Kakkar et al. (1984)²⁷. The activity of catalase was assayed by the method of Sinha (1972)²⁸. The level of GSH was estimated by the method of Ellman (1959)²⁹. The levels of vitamin C were estimated by the method of Omaye et al., (1979)³⁰. The levels of vitamin E were estimated by the method of Baker et al., (1980)³¹.

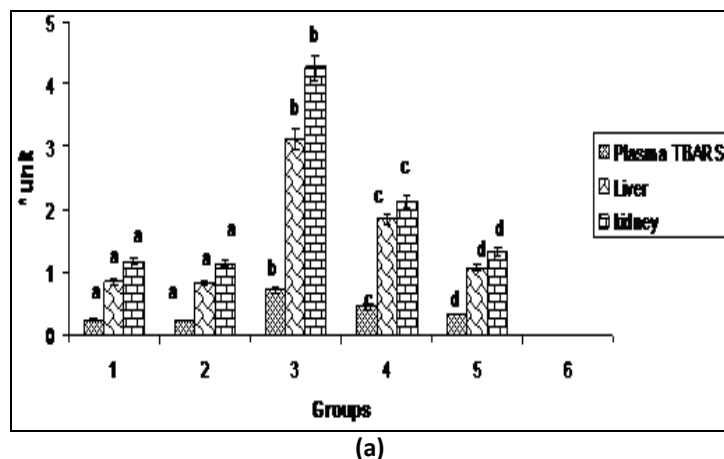
Statistical Analysis: Statistical analysis was performed by one way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT) using Statistical Package for the Social Sciences (SPSS) software

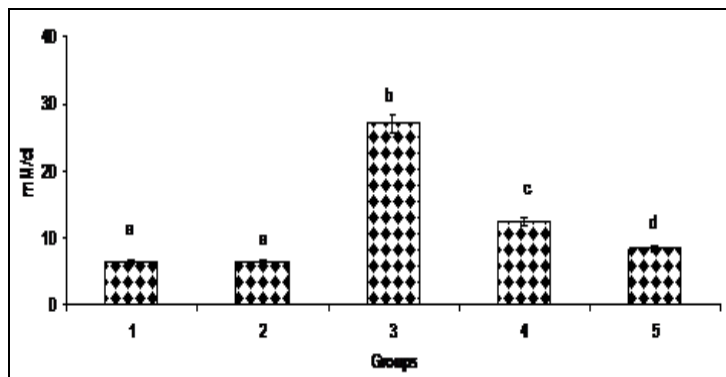
package version 9.05P values <0.05 were considered significant.

RESULTS: Figure 1 (a, b, c) represents the effect of vimliv on the levels of thiobarbituric acid reactive substances (TBARS), and hydroperoxides (HP) in plasma and tissues (liver and kidney) of normal and ethanol induced rats. Rats administered with ethanol, showed a significant increases levels of TBARS and HP as compared to normal control rats. Intragastric incubations of vimliv to ethanol induced rats at the doses of 25 and 50 mg/kg for a period of 35 days were significantly decreased the levels of these lipid peroxidative products.

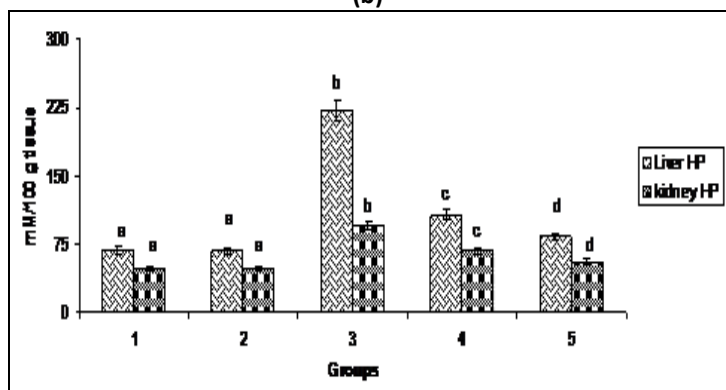
Figure 2 (a,b,c) represents the effect of vimliv on the activities of superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx), in the tissues (liver and kidney) in ethanol induced rats. Rats administered with ethanol, exhibited a significant decrease in the activities of these antioxidant enzyme in the liver and kidney. Vimliv co-treatment to ethanol induced rats significantly increased the activity of these antioxidant enzymes.

The levels of vitamin C & E in plasma and reduced glutathione (GSH) in the tissues (liver and kidney) in normal and ethanol induced rats are shown in Figure 3 (a, b). Rats administered with ethanol exhibited a significantly decrease in the levels of these non-enzymatic antioxidants in liver and kidney. Rats co-treated with Vimliv to ethanol induced rats significantly increased levels of non-enzymatic antioxidants.





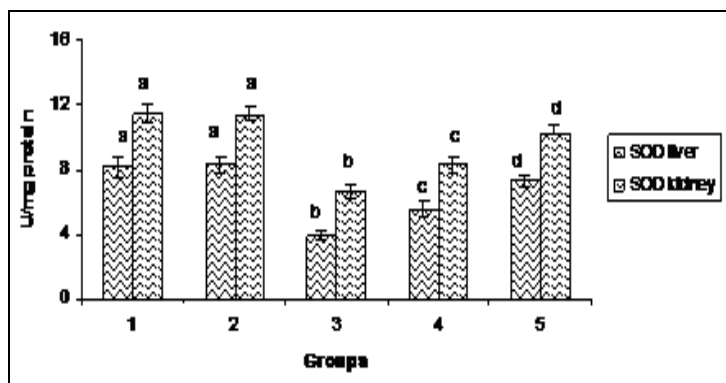
(b)



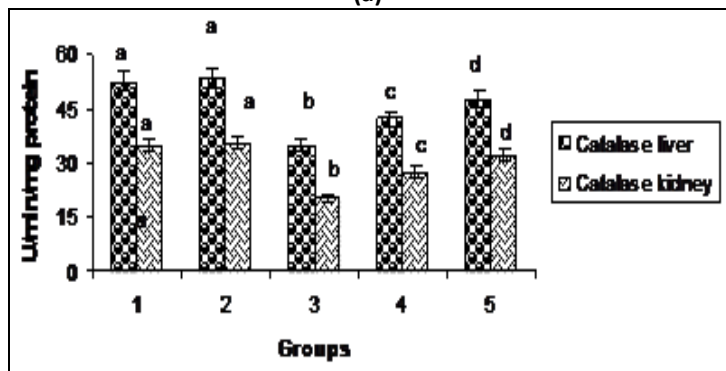
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Figure 1: Effect of vimliv on the levels of TBARS (Fig. 1a) and lipid hydroperoxide (HP) in plasma (Fig. 1b), liver and kidney (Fig. 1c) of normal and ethanol-induced hepatotoxicity in rats

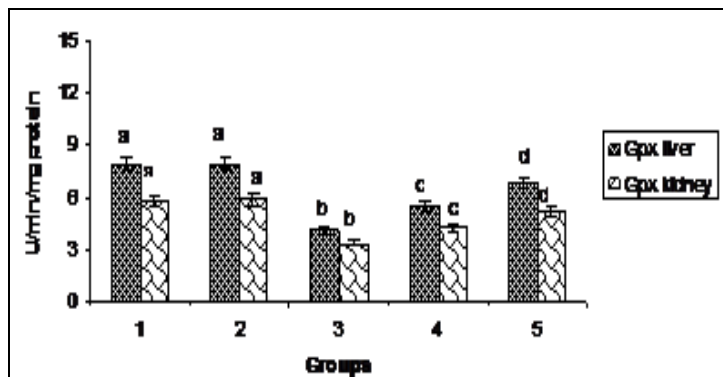
* Plasma TBARS - nmol/ml, *Tissue TBARS – nmol/g tissue. Each value is mean ± S.D. for 6 rats in each group. Values not sharing a common superscript (a-d) differ significantly with each other (P<0.05, DMRT).



(a)



(b)



(c)

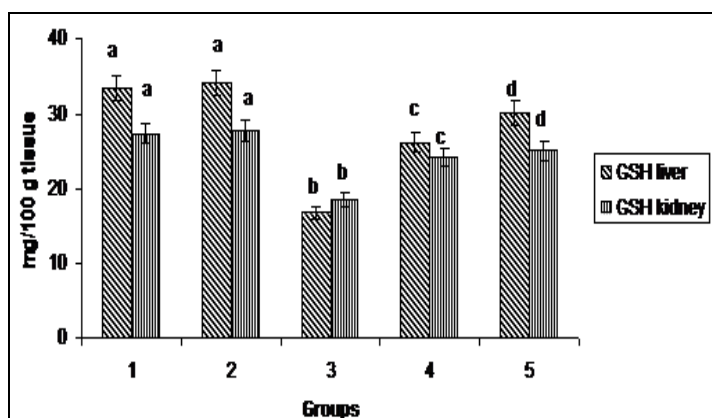
Figure 2: Effect of vimliv on the activities of SOD (Fig. 2a), Catalase (Fig. 2b), and GP_x (Fig. 2c) in liver and kidney of normal and ethanol-induced hepatotoxicity in rats

a) SOD enzyme activity: U-enzyme concentration required to inhibit the chromogen produced by 50% in one minute.

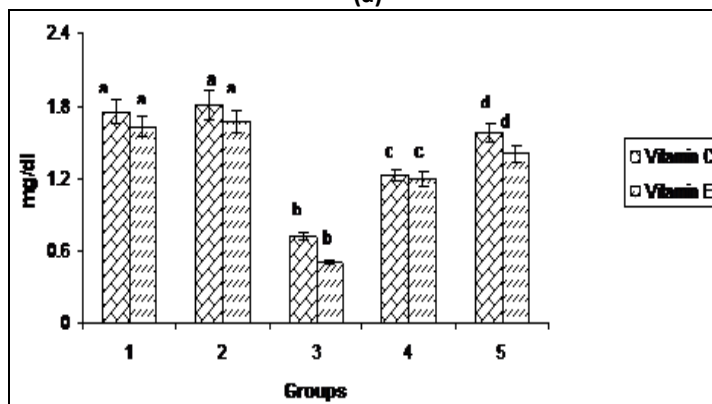
b) Catalase enzyme activity: U- μmoles of H₂O₂ consumed.

c) GP_x enzyme activity: U-μg of GSH consumed.

Each value is mean ± S.D. for 6 rats in each group. Values not sharing a common superscript (a-d) differ significantly with each other (P<0.05, DMRT).



(a)



(b)

Figure 3: Effect of vimliv on the levels of reduced glutathione (GSH) (Fig. 3a) in liver and kidney and plasma vitamin C and E (Fig. 3b) of normal and ethanol-induced hepatotoxicity in rats

Each value is mean ± S.D. for 6 rats in each group. Values not sharing a common superscript (a-d) differ significantly with each other (P<0.05, DMRT).

For all the parameters studied, vimliv at 50 mg/kg to ethanol induced rats showed better effect than 25 mg/kg. Vimliv treatment to normal rats didn't show any significant effect.

DISCUSSION: Reactive oxygen species (ROS) formed *in vivo*, such as superoxide anion, hydroxyl radical and hydrogen peroxide, are highly reactive and potentially damaging transient chemical species. These are continuously produced in the human body, as they are essential for energy supply, detoxification, chemical signaling and immune function. ROS are regulated by endogenous superoxide dismutase (SOD), glutathione peroxidase (GP_x) and catalase, as a result of over-production of ROS, due to the exposure to external oxidant substances or a failure of enzyme regulatory mechanisms leading to damage of cell structures, DNA, lipids and proteins. Excessive generation of ROS can cause oxidative damage to biomolecules resulting in lipid peroxidation³².

The levels of lipid peroxidation in liver tissue were determined by measuring the levels of thiobarbituric acid reactive substances (TBARS) and hydroperoxides (HP). In the present study, significantly elevated levels of lipid peroxidation products such as TBARS and HP observed in ethanol administered rats, which may be excessive formation of free radicals and activation peroxidation system resulting in hepatic and other cellular damage by ethanol³³.

The significant depletion of the levels of TBARS and HP in plasma, liver and kidney of Vimliv administered rats might be due to reduced lipid peroxidation and or elevation of levels of tissue antioxidant defense enzymes. It's well known that, Auyurvedic medicines could reduce the generation of free radicals and increase free radicals scavenging mechanism.

Antioxidant enzymes are important components of the cellular defense system against ROS and reactive nitrogen species (RNS). SOD, catalase, GP_x, and glutathione-S-transferase (GST) are defense against oxidative damage by supplying NADPH, which is needed for the regeneration of GSH. Superoxide dismutase catalysis the reaction of superoxide anion radicals (O₂•) dismutation to hydrogen peroxide (H₂O₂), whereas catalase degrades H₂O₂ into a molecular oxygen and water.

Superoxide ion (O₂•) and hydroxyl radicals are known to cause marked injuries to the surrounding tissues and organs. Therefore removing superoxide ion and hydroxyl radicals is probably one of the most effective defense mechanisms against a variety of diseases. Lowered activities of SOD, catalase and GP_x will result in the accumulation of these highly reactive free radicals leading to deleterious effects such as loss of cell membrane integrity and membrane function³⁴.

Oxidant scavenging at the intracellular level within the cytosol appears to rely on GP_x for elimination of low molecular levels of hydroperoxides and lipid peroxidation. Decreased level of GP_x in the liver of rats that ingested with alcohol could be due to either free radical dependent inactivation of enzyme or depletion of its co-substrates, that is GSH and NADPH. Catalase activity was decreased in alcohol exposed rats, which could possibly be due to loss of NADPH, or generation of superoxide, or increased activity of lipid peroxidation or combination of all^{35,36}.

The decrease in the levels of serum vitamin C & E in alcohol treated group could be as a result of increased utilization of this antioxidant in scavenging the free radicals during alcohol induction³⁷. Treatment with Vimliv, significantly increased the level of antioxidant enzymes and increase the levels of serum vitamin C & E. This could be due to the protective effect of Vimliv on the hepatocytes, which minimized the destruction of hepatocytes and the permeability of liver cells. Various medicinal plants present in vimliv may be responsible for its protective effect.

CONCLUSION: Oral administration of vimliv to ethanol-induced rats significantly decreased the levels of lipoperoxidative products with subsequent increase in the activities/levels of enzymatic/non-enzymatic antioxidants. Thus, our observations suggested that treatment with vimliv exhibited hepatoprotective effect against ethanol-induced hepatic damage in albino rats.

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REFERENCE:

1. Yamamoto Y and Yamashita S: Plasma ratio of ubiquinol and ubiquinone as a marker of oxidative stress. *Molecular Aspects of Medicine* 1999; 18(1):79-84.
2. Krinsky NI: Mechanism of action of biological antioxidants. *Proceedings of the Society for Experimental Biology and Medicine* 1992; 200(2):248-254.
3. Baynes J and Dominiczak M.H: Antioxidant defenses and reactive oxygen species. In: *Medical Biochemistry*, Mosby, 2nd ed, Ch 11, 1999; 10:133-137.
4. Conner E.M and Grisham M.B: Inflammation free radicals and anti-oxidants. *Nutrition* 1996; 12(4):274-277.
5. Sokol RJ: Antioxidant defenses in metal induced liver damaged. *Seminars in liver disease* 1996; 16(1):39-46.
6. Baraona E, Julkunen R, Tannenbaum L: Role of intestinal bacterial overgrowth in ethanol production and metabolism in rats. *Gastroenterology* 1986; 90:103-10.
7. Lindros KO: Alcoholic liver disease pathobiological aspects. *Journal of Hepatology* 1995; 23: 7-15.
8. Balkan J, Dogru -Abbasoglu S, Kanbagli O, Çevikbaş U, Aykaç-Toker G, Uysal M: Taurine has a protective effect against thioacetamide-induced liver cirrhosis by decreasing oxidative stress. *Human and Experimental Toxicology* 2001; 20:251-254.
9. Clot P, Tabone M, Arico S, Albano E: Monitoring oxidative damage in patients with liver cirrhosis and different daily alcohol intake. *Gut* 1994; 35:1637-1643.
10. Gardner CR, Heck DE, Yang CS, Thomas PE, Zhang XJ, DeGoerge GL, Laskin JD, Laskin DL: Role of nitric oxide in acetaminophen-induced hepatotoxicity in rat. *Hepatology* 1998; 27:748-754.
11. Diez-Fernandez C, Sanz N, Alvarez AM, Zaragoza A, Cascales M: Influence of aminoaguanide on parameters liver injury and regeneration induced in rats by a necrogenic dose of thioacetamide. *British Journal of Pharmacology* 1998; 125:102-108.
12. Kubes P, McCafferty DM: Nitric Oxide and intestinal inflammation. *American Journal of Medical Genetics* 2000; 109:150-158.
13. Hunt NC, Goldin RD: Nitric oxide production by monocytes in alcoholic liver disease. *Journal of Hepatology* 1992; 14:146-150.
14. Wang JF, Greenberg SS, Spitzer JJ: Chronic alcohol administration stimulates nitric oxide formation in the rat liver with or without pretreatment by lipopolysaccharide. *Alcoholism, Clinical and Experimental Research* 1995; 19:387-393.
15. Walsh K and Alexander G: Alcoholic liver disease. *Postgraduate Medical Journal* 2000; 76:280-6.
16. Thurman RG: Alcoholic liver injury involves activation of Kupffer cells by endotoxin. *American Journal of Physiology* 1998; 275:605-11.
17. Worman HJ. Alcoholic liver disease [on line] 2002. Cited on 24 August 2006. URL: <http://www.cumc.columbia.edu/dept/gi/alcohol>.
18. Tsukamoto H, Lu SC: Current concepts in the pathogenesis of alcoholic liver injury. *Federation of American Societies for Experimental Biology* 2001; 15:1335-49.
19. Kaplowitz N, Tsukamoto H: Oxidative stress and liver disease. *Progress in liver diseases* 1996; 14:131-59.
20. Younes M and Strubelt O: Alcohol induced hepatotoxicity: A role for oxygen free radicals. *Free Radical Research* 1987; 3:19-26.
21. Cederbaum AI: Role of lipid peroxidation and oxidative stress in alcohol toxicity. *Free Radical Biology and Medicine* 1989; 7:537-9.
22. Nordmann R, Ribiere C, Rouach H: Implication of free radical mechanisms in ethanol-induced cellular injury. *Free Radical Biology and Medicine* 1992; 12:219-40.
23. Rajat sandhir D and Gill KD: Protective effect of Liv.52 in ethanol induced hepatotoxicity in rats. *Indian journal of experimental biology* 1999; 32:762-766.
24. Yagi K: Lipid peroxidase and human disease. *Chemistry and Physics of Lipids* 1972; 45:37-51.
25. Fraga CG, Leibovitz BE, Toppel AL: Lipid peroxidation measured as TBARS in tissue characterization and comparison with homogenates and microsomes. *Free Radical Biology and Medicine* 1988; 4:155-161.
26. Jiang ZJ, Hunt JV, Wolff SD: Ferrous ion oxidation in the presence of xylenol orange for detection of lipid hydroperoxide in low density lipoprotein. *Analytical Biochemistry* 1992; 202:384-389.
27. Kakkar P, Dos B, Vishwanathan PN: A modified spectrophotometric assay of superoxide dismutase. *Indian Journal of Biochemistry and Biophysics* 1984; 21:130-132.
28. Rotruck JT, Pope AI, Ganther HE, Swanson Ab: Selenium: biochemical roles as a component of glutathione peroxidase. *Biological Science* 1973; 779:588-590.
29. Ellman GL: Tissue sulfhydryl groups. *Archives of Biochemistry and Biophysics* 1959; 82:70-77.
30. Omaye ST, Turbull TP, Sauberlich HC: Selected methods for determination of ascorbic acid in cell tissue and fluids. *Methods in enzymology* 1979; 6:3-11.
31. Baker H, Frank O, Angelis B, Feingold S: Plasma α -tocopherol in man at various times after ingesting free or acetylated tocopherol. *Nutrition Reports* 1980; 21:531-536.
32. Valko M, Rhodes CJ, Moncol J, Izakovic M, Mazur M: Free radical metals and antioxidants in oxidative stress-induced cancer. *Chemical Biological Interaction* 2006; 160:1-40.
33. McCord JM, Keele B, Fridovich I: An enzyme based theory of obligate anaerobiosis: the physiological function of SOD. *The Proceedings of the National Academy of Sciences* 2000; 68:1024-7.
34. Krishnakantha S and Lokesh D: *Proceedings of the Hill's European Symposium on Canine and Feline Liver Disease*. Amsterdam 1993; 32:10-19.
35. Chandra A, Chaney AL: Alcohol, oxidative stress and cell injury. *Biochemical and Biophysical Research Community* 2000; 98:122-30.
36. Das and Vasudevan: Lipid peroxidation and human disease. *Chemistry and Physics of Lipids* 2005; 45:337-351.
37. Shen DD, Kunze KL, Thummel KE: Enzyme-catalyzed processes of first-pass hepatic and intestinal drug extraction. *Advanced Drug Delivery* 1997; 27:99-127.
