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Ethanol Toxicity Protection on Rat Liver Using Herbal Combination

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Abstract

The aim of this study is to know the toxicity of ethanol and to observe the hepatoprotective role of selected herbs against ethanol induced histological and enzymological changes in rats. Volatile organic compounds and their reaction products are increasingly posing unacceptable risk to public and occupational health. The metabolism of most xenobiotics takes place in the liver, which makes this organ very vulnerable to numerous chemical substances present in the environment. Ethanol is the most common chemical responsible for hepatotoxicity, causing fatty liver, alcoholic hepatitis, Oxidative stress to lipid, protein and DNA. Ethanol increases the risk for hepato-cellular carcinoma and cirrhosis of liver. Ethanol disturbed the liver function like lipid peroxidation, glutathione, collagen, serum bilirubin, alkaline phosphatase and serum transaminase level (SGOT). The liver is the marvelously sophisticated chemical laboratory, capable of carrying out thousand of chemical transformations on which the body depends. The liver produces important chemicals, modifies others to allow the body to use them better, and neutralizes an enormous range of toxins.

Keywords: Ethanol, Herbal Antioxidant, Hepatotoxicity, Hepatoprotective, Oxidative Stress.

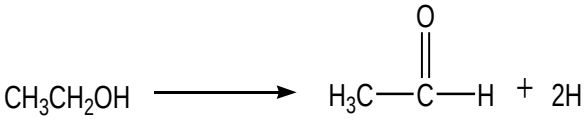
Introduction

Ethanol (CH_3CH_2OH), (ethyl alcohol, grain alcohol) is a clear, colorless liquid with a characteristic, agreeable odor. In dilute aqueous solution, it has a somewhat sweet flavor, but in more concentrated solution it has a burning taste. Ethanol (CH_3CH_2OH), is an alcohol, a group of chemical compounds whose molecules contain a hydroxyl group, -OH, bonded to a carbon atom. Ethanol melts at -114.1 C, boils at 78.5 C and has a density of 0.789 g/ml at 20 C. Its low freezing point has made it useful as the fluid thermometers for temperature below-40 C, the freezing point of mercury and as antifreeze in automobile radiators. Ethanol is the chief industrial organic solvents. It is clear colorless, liquid with a characteristic pleasant odor. Ethanol is metabolized primarily in the liver to a variety of hydroxylated products through cytochrome P-450 and their metabolites play significant role in toxicity. Cytochrome P450 is the terminal oxidase component of an electron transport chain. It consists of a family of closely related 50 isomers. Six of them metabolize 90% of toxic chemicals and drugs. CYP2E1 is of the interest since it metabolizes and activates a wide array of toxicologically important substances, including acetaminophen (APAP), acetone, ethanol, carbon tetra chloride and carcinogens such as the low molecular weight nitrosamines (Kessova and Cederbaum 2003). Ethanol is an alcohol, made through the fermentation of plant sugars from agricultural crops and biomass resources. Although the use of ethanol dates back to the mid 1800, there has been a recent resurgence in its production and use. In 2000, U.S. ethanol production reached an all-time production high of 1.65 billion gallons.

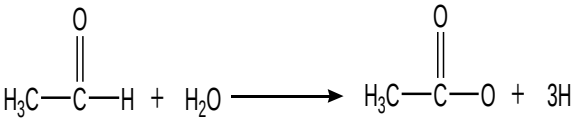
$$C_6H_{12}O_6 \xrightarrow{\text{Zymase}} 2CH_3CH_2OH + 2CO_2$$

Chronic ethanol consumption causes injury to almost all organ systems including liver and gastro-intestine and has serious medical and public health implications. Oxidative damage to lipid, protein and DNA after ethanol intoxication of the liver is very well described (Nordmann et. al. 1992). Alcohol increases the risk for hepatocellular carcinoma and colon cancer, our knowledge on the mechanism of actions of ethanol at the sub cellular and cellular levels is poor. It has further been observed that most of the consumed alcohol is eventually broken down by the liver and the products generated and accumulated during alcohol metabolism (e.g. acetaldehyde) are more toxic than alcohol itself (Kurose et. al. 1996).

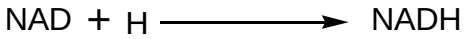
Therapeutic tools to control or reverse the ethanol induced cellular damages, such as alcoholic liver injury, are also lacking. In the liver, the alcohol dehydrogenase enzyme converts ethanol into acetaldehyde, which is itself toxic.



Acetaldehyde is destroyed almost immediately by the Aldehyde dehydrogenase enzyme, which converts it to acetate ions.



The hydrogen ion represented by these equations are not unattached, but are picked up by another biologically important compound, nicotinamide adenine dinucleotide (NAD), whose function is to carry hydrogen atoms. NAD is involved in both of the above process, being converted into NADH.



NADH must be recycled to NAD for the disposal of ethanol. As concentration of ethanol increases, further suppression of brain function produces the classic symptoms of intoxication: slurred speech, unsteady walk, disturbed sensory perceptions and inability to react quickly.

The body makes enough antioxidants to neutralize free radicals generates by normal metabolism. These include superoxide dismutase (SOD), catalase, glutathione peroxidase, certain vitamins, minerals, herbs and other nutritional substances also perform as antioxidants. Ayurveda emphasizes use of herbs, neutraceuticals or life style changes for controlling age related neurodegenerative disorders.

Review of Literature

Several hundred plants have been examined for use in a wide variety of liver disorders (Saleem, 2001). Several Indian medicinal plants have been extensively used in the Indian traditional system of medicine for the management of liver disorder. Some of these plants have already been reported to posses strong antioxidant activity (Achuthan *et al*., 2003; Aniya, 2002; Ramamurthy and Raveendran, 2014; Ramamurthy and Sagaya Giri, 2013). Drug-induced liver injury is a major health problem that challenges not only health care professionals but also the pharmaceutical industry and drug regulatory agencies. Herbal medicines have recently attracted much attention as alternative medicines useful for treating or preventing life style related disorders and relatively very little knowledge is available about their mode of action. *Aegel marmelos* commonly known as *Bael*, has great medicinal significance, is also knows for its great properties keeping blood sugar level under control, maintains healthy cholesterol and increase the activity of peroxidase (Kumar and Sanjeev 2012). Treatment of Aegel *marmelos* had significantly improved the level of glutathione both in plasma and liver tissue and show hepatoprotective property (Singanan et.al, 2007). *Arctium lappa* commony called Burdock, significantly improve pathological and biological parameters which were disturbed by ethanol and carbon tetra chloride induced damage (Lin et.al. 2002). *Phyllanthus niruri* commonly known as Stone Breaker, are originated in India, usually occurring as a winter weed throughout the hotter parts. The *Phyllanthus* genus contains over 600 species of shrubs, trees, and annual or biennial herbs distributed throughout the tropical and subtropical regions of hot hemispheres. *Phyllanthus niruri* is used in the treatment of various ailments like jaundice, diabetes, kidney stones, and liver disorders and for treatment of Hepatitis B viral infection and oxidative stress in the liver of rats (Ramamurthy and Abarna 2014). *Andrographis paniculata* commonly called Green Chireta, show hepatoprotective activity in ethanol induced hepatotoxicity in albino wistar rats (Subramaniyam et.al, 2011). Picrorhiza kurroa (Kutki) is a well known herb in the ayurvedic system of medicine and has traditionally been used to treat disorders of the liver and upper respiratory track, reduce fevers and to treat dyspepsia and chronic diarrhea. Kutkin is the active principal constituent of *Picrorhiza kurroa* and is comprised of kutkoside and the iridoid glycoside picrosides, apocyanin, drosin and nine cucurbitacin glycosides. Current research on *Picrorhiza kurroa* has focused on its hepatoprotective, anticholestatic, antioxidant and immunomodulating activity (Chandra et. al. 2006).

Aims of the study

Now a day there is a need to manage the toxicity of chemicals with the use of plant products. The present investigations describe the antioxidative and hepatoprotective role of herbal complex. Most of the Ayurvedic drugs and herbo-mineral compound contain different parts of the herbs and are use for the manufacturing of the drugs. There is a lack of information on mechanism of chemical toxicity and protective role of herbs and their combination. Thus hepatoprotective and antioxidative role of mixture of selected herbs as *Aegle marmelos, Picrorrhiza kurroa, Andrographis paniculata, Arctium lappa and Phyllanthus niruri,* has been performed in the ethanol intoxicated rats in this communication.

Material

Twenty male Charles foster laboratory bred rats (150±20 g) were divided into four groups at random, each containing five rats. Each rat was housed individually and feed on commercial pellets and kept in laboratory conditions (room Temp. = 25 ± 5 ^{0}C, relative humidity = 60 ± 10%). After acclimatization to laboratory condition rats were treated with sub lethal dose of ethanol. Group one was kept as control. Group two treated with ethanol 1ml/kg / body weight orally on alternate days. Group three treated with ethanol and herbal complex. Group four is treated with herbal complex. Herbs were collected from local field, herbal expo and Dehradun market. Herbal mixture contains the mixture of five herbs like *Aegle marmelos, Andrographis paniculata,*

Arctium lappa, Picrorrhiza kurroa, and Phyllanthus niruri. Herbs were given in powder form on every day with the dose of 100 mg/kg body weight. Treatment was given for thirty days.

Experiment

After completion of treatment on thirty first day rats were sacrificed by using ether anesthesia. Blood samples from each rat were drawn through cardiac puncture and liver homogenate was prepared. Lipid peroxidation in liver was measured by thiobarbituric acid (Wako, Japan) method (Smith and Anderson 1987). Reduced glutathione (GSH) was estimated in the liver by the method of Ellman (1959). Glutathione peroxidase was estimated by the method of Wendel (1980). Glutathione reductase was estimated by the method of Carlberg and Mannervik (1980). Catalase activity in the liver was determined by the titration method of Von Euler and Josephson as modified by Takahara et. al.(1960). Liver collagen was extracted from dried sample by the method suggested by Fitch et. al (1955). Serum was collected and bilirubin was estimated according to the method suggested by White et. al. (1958). Alkaline phosphatase was determined following the standard method of Ree (1972). Serum transaminase was determined following the procedure of Reitman and Frankel (1957).

Values reported are mean and standard error, inter group comparisons were made using student's test (Fisher, 1950). P Value <0.05, 0.01 and 0.001 were considered as significant. NS denotes non significant.

Results and Discussion

Toxic effect of ethanol is due to the generation of free radicals, causing heptotoxicity thus altering the permeability of liver cell membranes (Kumar and Singh 2008). After chronic ethanol consumption, the activity of MEOS increases which is associated with rise in cytochrome P450, specially shown in alcohol dehydrogenase in mice (Lieber 2004). Further increase in serum enzymes level in present study suggested disturbance in the transport function hepatocytes resulting in leakage of enzyme from cell. Treatment of ethanol cause hepatic dysfunction and collagenesis. Treatment of herbal complex show protective role against ethanol induced hepatotoxicity. Result on depletion on glutathione and concomitantly increase in malondialdehyde level corresponding to each other. The higher level of ethanol intake develops cirrhosis and liver damage by enhancing lipid peroxidation in liver. The GSH depletion in hepatic mitochondria is considered the most important sensitizing mechanism in the pathogenesis of alcoholic liver injury. Reduction of GSH may results in alteration of membrane integrity and cell injury. Decrease in the GSH level is indicator of cell injury and cell death. Ethanol treatment decrease the GSH content but herbal treatment protects the cell injury as indicated by improvement in lipid peroxidation, catalase and glutathione content. Result on liver lipid peroxidation, collagen and glutathione after ethanol treatment individually and with the combination of herbal complex indicates that the level of lipid peroxidation and collagen increased in comparison to control rats (Table-1). Treatment of herbal complex protects ethanol induced cell injury and oxidative stress as indicated by improvement in above mention parameters. Treatment of ethanol with herbal complex cause enzymatic improvement as observed by decrease in aldehyde dehydrogenase, catalase and glutathione reductase level.

Table-1: Lipid peroxidation mg/g tissue, Glutathione mg/g tissue, glutathione peroxidase nmole/min./mg, glutathione reductase n mole NADPH/min and catalase mg/100g tissue in liver of rats treated with ethanol and herbal complex.

Group	Treatment	Lipid peroxidation	Glutathione	Glutathione reductase	Glutathione peroxidase	Catalase
1.	Control	134 ± 4.20	1475 ± 11.42	18 ± 0.90	40 ± 1.30	40 ± 1.10
2.	Ethanol	280 ± 3.18**	1250 ± 10.60*	26 ± 0.80*	33 ± 0.80*	30 ± 1.70*
3.	Ethanol+herbal complex	200 ± 2.25**	1356 ± 8.40*	22 ± 1.20	39 ± 1.20**	34 ± 1.72*
4.	Herbal complex	150 ± 4.28*	1490 ± 7.40 ^{Ns}	15 ± 1.50*	39 ± 1.40*	43 ± 1.30^{Ns}

Results are mean ± S.E. for five observations in each group of rats.
P value = * <0.01, ** <0.001(between control and experimental rats).

Ethanol disturbed the liver function by increasing serum bilirubin, alkaline phosphatase and serum transaminase level (SGOT) (Table-2). Our results are supported by the studies that the herbs significantly improve the level of glutathione, activation of superoxidedismutase (SOD) and catalase in the ethanol injured rat hepatocytes (Kim et. al. 2003). From these results it was suggested that *Arctium lappa* could protect the liver cell from ethanol induced liver damage with same mechanism (Song et. al. 2003). *Andrographis paniculata* shows pronounced protective effect against liver damage caused by various hepatotoxins like paracetamol and carbon tetra chloride in rats. *Andrographis paniculata* and *Embilica* extract significantly reduce the biochemical alterations caused by cadmium through its antioxidant, antilipoperoxidative and membrane stabilizing nature (Prabu, 2008). Herbal treatment protected ethanol induced hepatotoxicity in all the parameters and shown the dose dependent protective effect. Antioxidants act as free radicals quencher. Thus antioxidants, which naturally occurring in plants has potential protective role against oxidative stress induced by free radicals. Polyphenols and flavonoids are usually recognized as medicinal plants compounds, responsible for antioxidative activity. Herbs contain glutathione and antioxidants, which might be quenching the oxidative radicals and give the protection.

Table-2. Bilirubin mg/100ml, alkaline phosphatase IU/1000ml and SGOT K.A.unit/litre level in liver of rats treated with ethanol and herbal complex.

Group	Treatment	Bilirubin	A. L. P.	SGOT
1.	Control	0.51 ± 0.020	11 ± 0.95	30 ± 2.08
2.	Ethanol	1.85 ± 0.016*	32 ± 1.70**	82 ± 2.51**
3.	Ethanol+herbal complex	1.26 ± 0.018*	27 ± 1.10*	55 ± 2.34*
4.	Herbal complex	0.72 ± 0.026^{NS}	10 ± 1.14*	30 ± 2.45^{NS}

Results are mean ± SE. for five observations in each group of rats.
P value=*<0.01, ** <0.001, (Between control and experimental rats).

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References

Aniya, Y. (2002). Free radical scavenging action of the medicinal herb Limonium wrightii from the Okinawa islands. Phytomedicine, 9: 239-244.

Achuthan, C.R. (2003). Antioxidant and Hepatoprotective effects of Rosa damascene. Pharmaceut. Biol., 41: 357-361.

Carlberg I. and Mannervik B.; In "methodos in enzymology ", Academic press New York (1985), 484.

Chandra B., Palni L. M. S. and Nandi S. K. (2006), Propagation and conservation of Picrorhiza Kurrooa Royle ex Benth: An endangered Himalayan medicinal herb of high commercial value. Biodiversity and conservation. 15, (7), 2325.

Ellman CL (1959) Tissue sulphahydroxyl groups. Arch. Biochem. Biophys. 82:70-73

Fitch SM, Harness MLR, Harness RD (1955) Nature 163:4473-4476.

Fisher RA (1950) Statistical methods for research work. Oliver and Byol. London.

Kessova I. and Cederbaum A. I(2003), CYP2E1: biochemistry, toxicology, regulation and function in ethanol induced liver injury. Curr Mol Med. 3, 509.

Kurose I., Higuchi H., Kato S., Miura S. and Ishii H. (1996), Ethanol-induced oxidative stress in the liver. Alcohol Clin Exp Res. 20, 77.

Kim JS, Shukla SD (2006) Acute in vivo effect of ethanol on histone H3 modification in rat tissues. Alcohol Alcohol 41:126-132.

Kumar S, Sing J, Singh L (2008) Hepatoprotective activity of Bacopa monnieri, Terminalia chebula and Picrorhiza kurrooa herbs against CCl_4 induced toxicity in rats. Biochem. Cell. Arch. 8:51-54.

Kumar S.V, Sanjeev T, Ajay S, Kumar P.S, Anil S and Seth G, (2012), A review on hepatoprotective activity of medicinal plants. IJARPB, 2(1),31-38.

Lin S. C., Lin C. H., Lin C. C., Lin Y. H., Chen C. E., Chen C. and Wang L. Y. (2002), Hepatoprotective effect of Arctium lappa linne on liver injuries induced by chronic ethanol consumption and potentiated by carbon tetra chloride. J of Biomedic Scie. 9, (5).

Lieber CS (2004) The discovery of the microsomal ethanol oxidizing system and its physiologic and pathologic role. Drug Metab. Rev. 36:511-529.

Nordmann R., Ribiere C. and Rouach H. (1992): Implication of Free radical mechanisms in ethanol induced cellular injury. Free Rad Biol and Medic. 12, (3), 219.

Prabu SM, Selvarajan N, Hemalatha S, Ramesh kumar T (2008) Hepatoprotective effect of Andrographis paniculata against cadmium induced toxicity in male Wistar rats. Toxicology International 15: 21-25.

Ramamurthy V and Abarna T (2014) Hepatoprotective activity of phyllanthus niruri whole plant extract against staphylococcus aureus intoxicated albino rats. Global Journal Of Biology, Agriculture and Health Science 3(3):256-260.

Ramamurthy V., Raveendran, S. and Anil Kumar, H. V. (2014). Hepatoprotective Activity of the methanolic extract of Aegle marmelos Leaves in Paracetamol Intoxicated Albino Rats. Int. J. Universal Pharmacy and Bio Sciences. 3 (2): 1 - 10.

Ramamurthy, V. and Sagaya Giri, R. (2013). Hepatoprotective Activity of Acorus calamus Linn in Paracetamol Intoxicated Albino Rats. Inter. J. Pharmacy and Drug Res., 2(1): 11-18

Ree G, (1972) Colorimetric method for measurement of alkaline phosphate. Clin. Biochem. 10:182-186

Reitman S, Frankel S (1957) Colorimeteric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminase. Am. J. Clin. Pathol. 28:56-63.

Subramaniyam et.al. (2011) Hepatoprotetive ativity of Andrographis paniculata in ethanol induced hepatotoxicity in albino wistar rats.Pharm. Glob. Intern. Jour.(2).

Saleem, Ahotupa M. and Pihlaja K. (2001), Total phenolics concentration and antioxidant potential of extracts of medicinal plants of Pakistan. Z Naturforsch [c]. 56, (11-12), 973.

Singanan V, Singanan J and Begum N, (2007), The hepatoproective effect of Bael leaves(Aegle marmelos) in alcohol induced liver in Albino Rats. Int. Jour. Of Sci. and tech.(2), 83-92.

Smith CV, Anderson RE (1984) Method for determination of lipid peroxidation in biological samples. Free. Rad. Biol. Med. 3:341-347.

Song E. K., Cho H. and Kim J. S. (2003), Diarylheptanoids with free radical scavenging and hepatoprotective activity in vitro from Curcuma longa. Planta Med. 67, 876.

Takahara S, Hamilton HB, Neal JV, Kobara TY, Ogura Y, Nishimera ET (1960) Hypocatalasemica: A new genetic carrier state. J. Clin. Invest. 39:610-615

Wendel A. (1980), In enzymatic basis of detoxification. (W. B. Jacoby. ed.) Academic Press, New York 1.

White D, Haider GA, Reinold JG (1958) Spectrophotometeric measurement of bilirubin concentration in the serum of the new born by the use of the microcapillary method. Clin. Chem. 4: 211-214.