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Antioxidative role of selected herbs against ethanol induced liver injury in rats

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Abstract: The purpose of this study was to know the hepatotoxicity of ethanol in laboratory rats *Rattus rattus* and to observe the individual and combined phytotherapeutic role of five herbs *viz. Arctium lappa, Curcuma longa, Piper longum, Plumbago zeylanica* and *Terminalia chebula* through biochemical and histopathological parameters. Ethanol is commonly used as solvent, pharmaceutical, drugs and alcohol abuse. Lipidperoxidation, glutathione content, urinary hydroxyproline, collagen and histopathological studies showed hepatotoxicity of 1 ml/kg body weight dose of ethanol and protective role of 100 mg/kg body weight dose of herbs. Histopathological changes observed in the liver of rats after ethanol treatment showed hepatitis, collagenesis, fatty infiltration, sclerosis, perilobular necrosis, cytoplasmic degeneration, enlarged bile canaliculi, hydropic degeneration, focal necrosis, binucleated hepatocytes and nuclear degeneration. Mild cytoplasmic degeneration, necrosis, collagenesis and hepatocytes regenerations were observed in rats treated with same dose of ethanol and herbal combination. Ethanol treatment decreased the glutathione content, increased tissue malondialdehyde and collagen content, thus causing tissue injury and liver collagenesis. Urinary hydroxyproline level and biochemical parameters also showed the protective role of herbs against ethanol induced toxicity. Herbal combination i. e. 100ml/kg body weight from the mixture of five herbs given orally was found more effective than their individual role. Herbs and plants contain aromatic substances, secondary metabolites, alkaloids and polyphenols which act as antioxidant thus showing protective role.

Keywords*:* Hepatotoxicity, Histopathology, Ethanol, Herbal Combination, Antioxidant

INTRODUCTION

Medicinal plants are natural resources and their use in the regulation of chemical induced toxic effects has gained more impetus in recent years. The plants and herbal medicines used generally known as the Rasayan drug in Ayurveda are known to prevent ageing, increase longevity and resistance to disease by augmenting the immune system and quenching the free radicals. Free radicals are generated in the body during normal cellular processes like stress, environment toxins, smoking, alcohol, drugs, pollutants and radiations. Free radicals induced damage accumulates with age. Oxidative stress has been shown to be involved in the development and pathogenesis of hypertension, diabetes, arthritis, aging, immune inflammatory disorders and neurodegenerative disorders (Ammon and Wahl, 1994).

The metabolism of most xenobiotics takes place in the liver, which makes this organ very vulnerable to numerous chemical substances present in the environment (Le Blanc, 1994). 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). 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 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 addition to its direct actions, ethanol induced effects are also mediated by oxidative e.g. acetaldehyde reactive oxygen species (ROS) and non-oxidative e.g. phosphatidyl ethanol, fatty acid ethyl ester, metabolites/ products and impairments in the methylation process. It is the combination of metabolic stress path ways, termed as "ethanol metabolic stress", which contributes to the epigenetic effect of ethanol (Shukla and Aroor, 2006).

Arctium lappa and *Curcuma longa* have anti-inflammatory and free radical scavenging activities (Lin *et al.*, 2002). *Piper longum* Indian long piper commonly called "Pippali". Its principal constituents are piperine and piplartine. *Plumbago zeylanica* commonly known as "Chitrak" is a useful Indian medicinal plant, its constituents are credited with potential therapeutic properties, including anti-atherogenic, cardiotonic and hepatoprotective and neuroprotective properties. *Terminalia chebula* is useful in asthma, piles, bile trouble, burn, bleeding, blood pressure, cooling wash of the eyes,

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cough, dysentry and vomiting. Use and search for drugs and dietary supplement derived from plants have accelerated now days (Chopra *et al.*, 1992 and Yuen *et al.*, 2006). *Terminalia chebula* (Haritaki) is known to have extraordinary powers of healing with a wide spectrum of biological activity (Chattopadhyay and Bhattacharyya, 2008). Thus, a study has been undertaken with the objective to observe the individuals and combined role of certain herbs against ethanol induced liver injury in rats.

MATERIALS AND METHODS

Forty male laboratory bred rats *Rattus rattus* (albino) (150±20 g) were divided into eight 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 conditions, rats were treated with sub lethal dose of ethanol. Group I was kept as control. Group II was treated with ethanol 1ml/kg / body weight i.e. 0.15 ml each rat orally on alternate days. Group III was treated with ethanol + A*rctium lappa*, Group IV was treated with ethanol + *Curcuma longa*, Group V was treated with ethanol + *Piper longum*, Group VI was treated with ethanol + *Plumbago zeylanica*. Group VII was treated with ethanol + *Terminalia chebula* and finally Group VIII was treated with ethanol + herbal combination. Herbs were given in powder form orally on every day with the dose of 100 mg/kg body weight i. e. 15 mg each rat. Herbal complex was prepared by mixing the equal amount of five herbs and given 15mg of this combination to each rat. Herbs were collected from local field and Dehradun market. Treatment was given for thirty days.

After completion of treatment on thirty first day rats were sacrificed by using ether anesthesia. Liver was collected and homogenate was prepared with 0.9% sodium chloride. 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 following the method of Ellman (1959) using dithiobisnitrobenzoic acid. Oxidised glutathione (GSSG) was estimated in the liver following the method of Ohmori *et al.* (1981). Liver collagen was extracted from dried liver sample by the method suggested (Fitch *et al.*, 1955) using 30% trichloroacetic acid. Hydroxyproline a reliable marker of collagen metabolism was estimated in urine samples using colorimetric method of Pondenphent *et al.* (1984). Histopathology of liver was performed through hematoxylin- eosin stain. Values reported are mean and standard error and inter group comparisons were made using students't test (Fisher, 1950).

RESULTS AND DISCUSSION

Results on liver lipid peroxidation, collagen and glutathione after ethanol treatment individually and with the combination of herbal complex indicated that the level of lipid per oxidation and collagen increased and the level of glutathione decrease in all the treated rats. An increase in liver collagen and urinary hydroxyproline level was reported after ethanol treatment. Individual and combination of herbs with ethanol decreased the collagen and hydroxyproline level in comparison to ethanol treatment. Treatment of herbal complex gave more protection in comparison to individual herbal treatment in ethanol treated rats. A non significant decrease in glutathione level in ethanol co-treated with herbal combination was observed indicating its protective role (Tables 1 and 2). Treatment of ethanol caused hepatic dysfunction and collagenesis. Ethanol administration promoted hepatic fibrosis, collagenesis, necrosis, cytoplasmic degeneration and fatty infiltration. Fatty infiltration is the most common pathological manifestation studied in a number of laboratory animals after exposure to ethanol leading to severe histological lesions viz. hepatitis, fibrosis, necrosis and cirrhosis (Ronis *et al.*, 2004). Pathological changes were less severe in rats co-treated with herbs and showed hepatocyties regeneration (Table 3, Figs.1-8).

The present results are supported by the studies that the herbs significantly preserve the level of glutathione,

Table 1. Malondialdehyde, reduced glutathione and oxidized glutathione content in liver of rats treated with ethanol and with herbal complex.

S. No.	Treatment	Malondialdehyde (nano.mole/mg protein)	Reduced glutathione (mg/g of tissue)	Oxidized glutathione (µg/g Liver)
1.	Control	132 ± 4.20	1480 ± 11.62	410 ± 6.78
2.	Ethanol	275 ± 5.18 **	1200 ± 13.60 **	340 ± 8.10 **
3.	Ethanol + *Arctium lappa*	270 ± 0.90*	1275 ± 10.09**	357 ± 8.04*
4.	Ethanol + *Curcuma longa*	268 ± 4.12 *	1315 ± 8.01*	370 ± 3.01*
5.	Ethanol + *Piper longum*	260 ± 2.31*	1339 ± 3.67*	363 ± 1.20*
6.	Ethanol + *Plumbago zeylanica*	271 ± 1.18*	1200 ± 1.03*	344 ± 1.11*
7.	Ethanol + *Terminalia chebula*	270 ± 2.21*	1252 ± 2.03*	362 ± 0.01*
8.	Ethanol + Herbal complex	245 ± 2.28*	1357 ± 7.40*	391 ± 6.68*

Results are mean ± S.E. of 5 observations in each group of rats. P = < *0.1, **0.05 (between control and experimental rats).

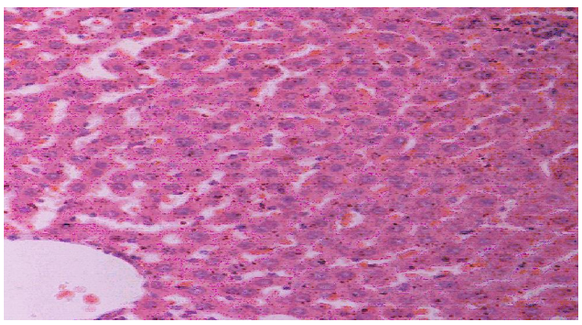

Fig. 1. *T. S. of liver of control rat showing normal hepatocytes and portal canal.*

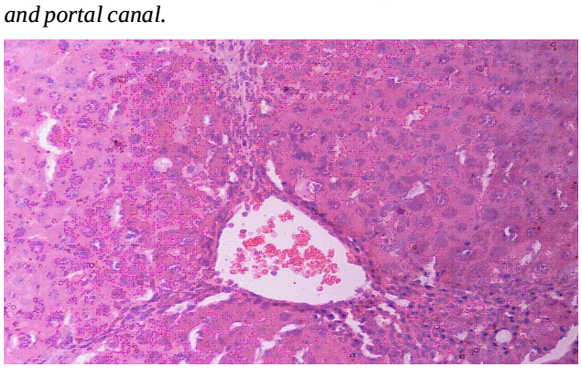

Fig. 2. *T. S. of liver of rat treated with ethanol showing hepatitis, hemorrhage, necrosis, sclerosis and nuclear degeneration.*

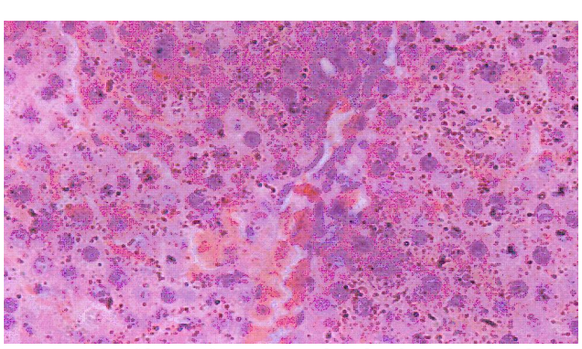

Fig. 3. *T. S. of liver of rat treatced with ethanol and A. lappa showing cytolpasmic degeneration and necrosis.*

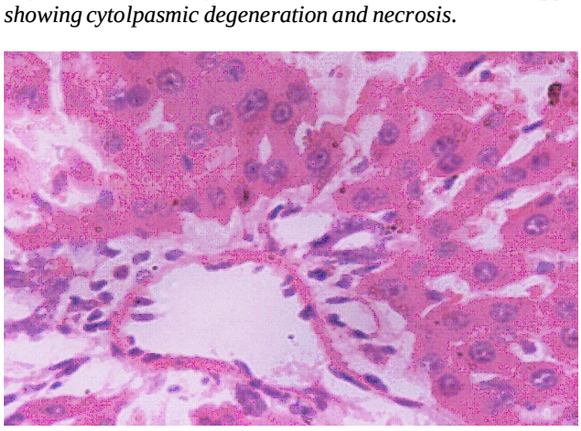

Fig. 4. *T. S. of liver of rat treated with ethanol and C. longa showing collagenesis and degeneration.*

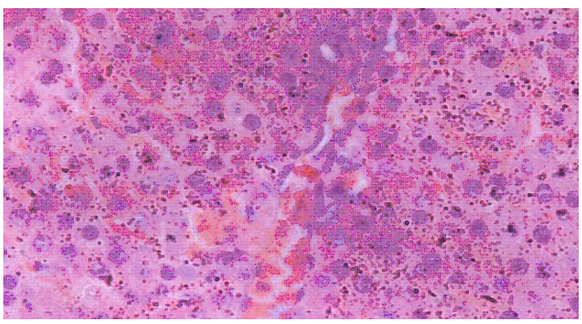

Fig. 5. *T. S. of liver of rat treated with ethanol and P. longum showing degeneration and sclerosis.*

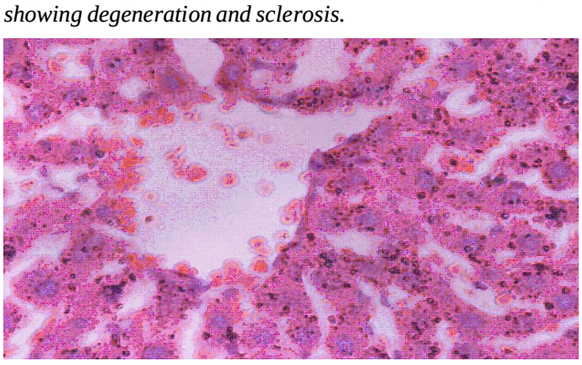

Fig. 6. *T. S. of liver of rat treated with ethanol and P. zeylanica showing hemorrhage, fatty infiltration with portal and cytoplasmic degeneration.*

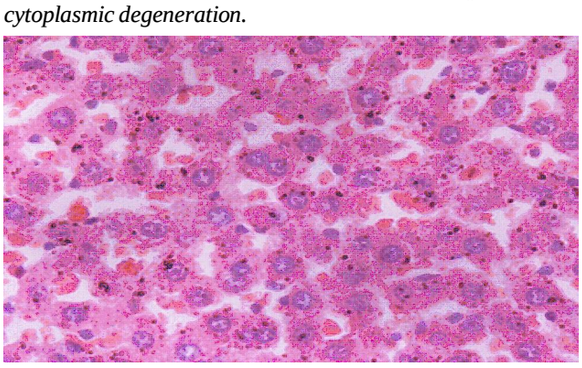

Fig. 7. *T. S. of liver of rat treated with ethanol and T. chebula showing degeneration and necrosis.*

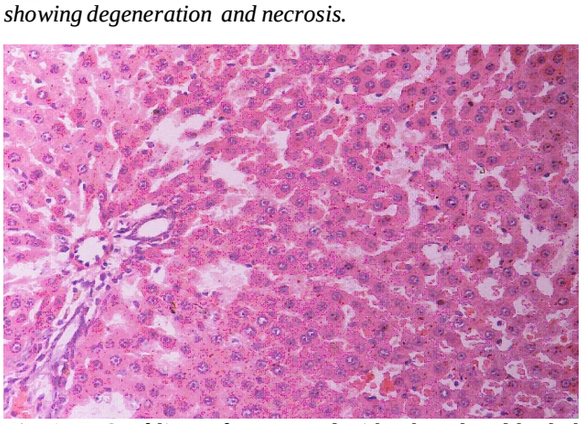

Fig. 8. *T. S. of liver of rat treated with ethanol and herbal complex showing regeneration of hepatocytes and mild cytoplasmic degeneration.*

Table 2. Collagen and urinary hydroxyproline content in liver of rats treated with ethanol and with herbal complex.

S. No.	Treatment	Collagen level (mg/100gm)	Urinary hydroxyproline (µg/24hours)
1.	Control	42 ± 0.29	14±2.10
2.	Ethanol	68 ± 1.17 *	33±0.80**
3.	Ethanol + *Arctium lappa*	60.3 ± 0.16*	28±0.80*
4.	Ethanol + *Curcuma longa*	61.4 ± 0.22*	30±0.80*
5.	Ethanol + *Piper longum*	62± 0.10*	29±2.01*
6.	Ethanol + *Plumbago zeylanica*	58± 1.40*	30±0.80*
7.	Ethanol + *Terminalia chebula*	62± 2.15*	27±1.80*
8.	Ethanol + Herbal complex	55 ± 1.24*	24±0.20*

Results are mean ± S.E. of 5 observations in each group of rats. P = < *0.1, **0.05.

in the ethanol injured rat hepatocytes (Kim and Shukla, 2005; Perez *et al*., 2003). Toxic effect of ethanol is due to free radical generation, causing lipid peroxidation thus altering the permeability of the liver cell membranes. It causes liver necrosis in dose dependent manner *in vivo* (Johnston and Kroening, 1998).Wang *et al.* (2009) states that the presence of ethanol in combination with hypoxia causes greater cellular damage as compared to conditions of ethanol or hypoxia alone. Clinical studies demonstrated that both the apoptotic index and proliferating cell numbers were higher in patients with chronic alcoholic disease than controls (Farias *et al.*, 2008). The mechanism of this protective effect of herbal combination remains to be elucidated. Herbs restore the antioxidant level and quench the oxidative radicals and give protection. Herbal complex treatment further shows the protective role.

Protective effect of burdock (*Arctium lappa*) on oxidation of low-density fat and stress in macrophages is known (Wang *et al.*, 2006). *A. lappa* could protect the cell from ethanol induced liver damage with the same mechanism (Song *et al.*, 2003). *Plumbago zeylanica* is useful Indian medicinal plant and its cardiologic, hepatoprotective and neuroprotective effect has been reported (Tilak *et al.*, 2004). *Piperine* is an active principle of *piper longum* and is known for its hepatoprotective and antioxidative properties as well as its ability to enhance the bioavailability and therapeutic effectiveness of various agents (Khajuria *et al.*, 1998; Nirala *et al*., 2007). *Terminalia chebula* also protected the human lymphocytes from undergoing the gamma radiation induced damage to DNA exposed in vitro (Gandhi and Nayar, 2005). Protective effect of *T. chebula* fruit on the

Table 3. Histopathological changes in liver of rats treated with ethanol and herbs individually and with herbal complex.

Observations	Control	Ethanol	Eth+*A. Lappa*	Eth+*C. longa*	Eth+*P. longum*	Eth+*P. zeylanica*	Eth+*T. chebula.*	Eth+Herbal complex
Binucleated cells	Rare	+	+	+	+	+	+	+
Centrilobular necrosis	-	+	++	++	+	+	+	+
Collagenesis	-	++	+++	+++	++	++	++	+
Sclerosis	-	++	++	++	+	++	+	+
Perilobular necrosis	-	+	++	+	+	+	+	+
Fibrosis	-	++	+++	++	++	++	+	+
Cytoplasmic degeneration	-	++	++	++	+	+	+	+
Multinucleated cells	-	++	+	+	+	++	+	+
Hydropic degeneration	-	++	+++	+++	++	++	++	++
Hepatitis	-	+++	++	+	++	+	++	+
Lipofuschin granules	+	++	+	+	++	++	+	+
Increased cell volume	-	++	+	+	++	++	+	+
Nuclear degeneration	-	+	++	+	++	+	+	+
Kupfer cell hyperplasia	-	+	+	+	+	+	+	+
Fatty infiltration	-	++	++	++	+	++	+	+
Periportal inflammation	+	++	++	+	+	++	++	+

(+) = Mildly Present, (++) = moderately present, (+++) = markedly present and (-) = absent.

tert-butyl hydroperoxide induced oxidative injury observed in cultured rat primary hepatocytes and rat liver (Lee *et al*., 2007). Results are supported by the previous studies on enzymological changes through herbs against chemical induced toxicity in rats (Kumar *et al.*, 2009).

Conclusion

Ethanol treatment causes hepatitis and collagenesis by decreasing glutathione content and increasing malondialdehyde level, which cause lipidperoxidation leading to liver injury. Combination of herbs was found more effective than their individual role. Herbs and plants contain aromatic substances, secondary metabolites, alkaloids, glutathione, flavanoids and polyphenols, which acts as antioxidants and quench the oxidative radicals.

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