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Antihepatotoxic Activity of Ginger Ethanol Extract in Rats

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Abstract

The effect of an ethanol extract of ginger was studied on country-made liquor (CML)–induced liver injury in rats. Hepatotoxicity was induced by administering CML (3 ml/100 g/day in 2 divided doses) and corn oil (1 ml/100 g/day, in a single dose) orally for 21 days. The administration of ginger ethanolic extract (200 mg/kg) orally from day 15 to day 21 along with CML produced significant ($P < 0.01$) lowering of serum AST, ALT, ALP, γ-GTP and tissue lipid peroxide levels. The results were comparable to silymarin (25 mg/kg, orally). The study shows that the reduction of liver damage by ethanol ginger extract treatment involves several mechanisms.

Keywords: Ginger, country-made liquor, lipid peroxidation, alcoholic liver disease.

Introduction

Alcoholic liver disease, a common consequence of prolonged and heavy alcohol intake, is a leading health problem after cardiovascular disease, cancer and AIDS. The consumption of alcoholic beverages is a common part of the modern way of life, and alcoholism ranks as a major health problem today (Kai, 1995; Smuckler, 1975). Hence, there are numerous animal studies assessing the effect of ethanol on hepatic metabolism and the mechanism of ethanol-induced liver injury (Robert, 1970). Alcohol in the form of country-made liquor (CML) containing 28.5% v/v ethanolic content is commonly consumed in India. It has been reported that there is a correlation between CML consumption and hepatic disorders (Gulati et al., 1995). Hence, this can be used as an experimental model of liver injury.

A wide variety of plants and their active principles have undergone experimental studies in diverse models of liver injury and cholestasis. Pretreatment with extracts of these plants has shown hepatoprotective activity based on biochemical and/or histopathological assessment (Bhatt & Bhatt, 1996).

Ginger (*Zingiber officinale* R, Zingiberaceae) is consumed worldwide in cooking as a spice and flavouring agent. So far, limited preliminary information is available on the hepatoprotective activity of ginger rhizome (Hikino et al., 1985; Shirwaikar et al., 1992; Sohni et al., 1995; Sohni & Bhatt, 1996). However, the mechanism of hepatoprotective action of ginger is not reported in the literature.

The present communication reports for the first time the effect of ethanol extract of ginger rhizome during CML-induced hepatotoxicity in rats and the effect of the test drug was compared with silymarin, a known hepatoprotectant.

Material and methods

Plant material

The fresh ginger rhizomes were locally purchased in the month of January, identified and authenticated by a pharmacognosist, Prof. R. Zafar in our Institute. A voucher specimen was retained in the department (UB#01).

Preparation of the extract

The 100 g fresh ginger was peeled in fine pieces of variable sizes and extracted with 90% ethanol in a Soxhlet apparatus for 72 h. The solvent was removed under reduced pressure to give a dry extract, 9.5% yield w/w (with respect to the crude material) and a dose equivalent to 200 mg of the crude drug per kg body weight was calculated.

Chemicals

All chemicals and reagents used in the study were of analytical grade, commercially available and were obtained from E. Merck (Darmstadt, Germany), Sigma Chemical Company (St. Louis, MO, USA) and Glaxo Labs., India. The kits of AST, ALT and ALP were purchased from Span Diagnostics, Surat, India. Country-made liquor (CML) was purchased

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locally from Govt. Liquor Shop, New Delhi, India. Silymarin tablets (Silybon 140, Microlabs, Bangalore, India) were purchased from a local chemist shop, New Delhi, India.

Animals and treatments

Wistar rats of either sex (120–200 g) were housed in plastic cages in a room with a controlled temperature (22 °C) and maintained on a 12 h light-dark cycle with free access to food and water. All animals received humane care. They were divided into four groups of ten rats each as follows: Group I (normal healthy control): rats treated with 1% gum acacia suspension + corn oil (1 ml/100 g/day) + glucose isocaloric to the amount of alcohol for 21 days; Group II (pathogenic hepatotoxic control): country-made liquor (3 ml/100 g/day in two divided doses) + corn oil (1 ml/100 g in a single dose) for 21 days; Group III: CML (3 ml/100 g/day) + corn oil (1 ml/100 g/day) for 21 days + ethanol extract of ginger (100 mg/kg) from day 15 to day 21 i.e., for seven days; Group IV: CML (3 ml/100 g/day) + corn oil for 21 days + silymarin (25 mg/kg) from day 15 to day 21 i.e., for seven days. All the drug solutions were fed orally by oral feeding tube. Corn oil, gum acacia and glucose were administered in the form of an emulsion to Group I.

Blood collection

On the 21st day, prior to sacrifice, blood samples were withdrawn from the retroorbital plexus of all the rats and analysed for serum aspartate amino transferase (AST) and alanine amino transferase (ALT) (Reitman & Frankel, 1957); alkaline phosphatase (ALP) (King & King, 1971) and γ-glutamyl transpeptidase (γ-GTP) activities (Naftaline et al., 1969).

Measurement of tissue lipid peroxidation

Then, all the animals were sacrificed and the livers were removed for estimation of lipid peroxidation, which can be measured by the formation of malondialdehyde (MDA) after the breakdown of polyunsaturated fatty acids and was measured by assay of thiobarbituric acid reactive substances (TBARS) as described by Ohkawa et al. (1979). Liver protein content was evaluated by the method of Lowery et al. (1951) using bovine serum albumin (BSA) as standard.

Statistical analysis

The data were expressed as mean ± SEM and analyzed using the one-way analysis of the variance test (ANOVA) followed by Dunnett's *t*-test. A probability level of $P < 0.01$ was chosen as the criterion of statistical significance.

Results

As shown in Table 1, CML administration along with corn oil for a period of 21 days resulted in significant ($P < 0.01$) rise in the serum AST, ALT, ALP, γ-GTP and tissue lipid peroxide levels as compared to Group I. Oral administration of ethanol extract of ginger and silymarin to the rats attenuated the CML-induced rise in serum AST, ALT, ALP, and γ-GTP levels. The treatment also reversed the increased levels of tissue lipid peroxides when compared to a pathogenic control group, i.e., Group II ($P < 0.01$).

Discussion

Alcoholic liver disease (ALD) is a common consequence of prolonged and heavy alcohol intake. This disease encompasses a wide spectrum of lesions, the most characteristic being alcoholic steatosis (fatty liver), alcoholic hepatitis, alcoholic fibrosis and cirrhosis (Kai, 1995).

Although research into the mechanism of ALD has identified several potentially important pathogenic factors, no single factor has so far been demonstrated to be more important than others. There is considerable evidence that the amount of fat in the diet is a key determinant in producing the lesions in ALD. Polyunsaturated fatty acids and fats rich in linoleic acid such as corn oil, when given to the rats along with alcohol, showed severe liver injury, the mechanism of which is attributed to the decline in the microsomal level of

Table 1. Effect of ginger and silymarin treatments on various biochemical parameters in rats subjected to country-made liquor (CML) – induced hepatotoxicity.

Groups	AST (U/ml)	ALT (U/ml)	ALP (IU/L)	γ-GTP (IU)	Lipid peroxides (nmoles MDA/mg protein)
I (Normal control)	93.5 ± 0.884	82.0 ± 0.856	97.8 ± 0.575	7.5 ± 0.542	0.532 ± 0.030
II (CML + corn oil)	160.3 ± 3.323*	143.3 ± 3.921*	212.4 ± 0.555*	22.6 ± 1.149*	2.078 ± 0.128*
III (CML + ginger)	98.4 ± 0.374$^{\#}$	88.0 ± 2.143$^{\#}$	102.9 ± 1.188$^{\#}$	9.9 ± 0.367$^{\#}$	0.833 ± 0.043$^{\#}$
IV (CML + silymarin)	121.0 ± 1.316$^{\#}$	103.6 ± 1.171$^{\#}$	105.3 ± 0.506$^{\#}$	8.1 ± 0.987$^{\#}$	0.713 ± 0.020$^{\#}$

Values are mean ± SEM, * $P < 0.01$, when compared with a normal control group (Group I); $^{\#}P < 0.01$, when compared with a pathogenic control group (Group II).

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arachidonic acid and an increased level of thromboxane B_2 (Amin et al., 1996). Furthermore, this induction increases the consumpiton of oxygen by human liver to the point that centrilobular cells become anoxic. These processes ultimately lead to the conversion of fatty acid to lipid peroxides (Nanji et al., 1989). This lipid peroxidative degradation of biomembranes is one of the principal causes of hepatotoxicity (Kapur et al., 1994). This eventually leads to hepatocellular necrosis and is reflected in our experiment by marked changes in various enzymatic parameters of CML-treated rats.

Alcohol in the form of country-made liquor is mainly consumed in India. It contains 28% v/v alcohol plus some unreported ingredients supposed to be the flavouring agents in alcohol. It was reported that there is a correlation between CML consumption and hepatic disorders (Gulati et al., 1995) and because it is more realistic to clinical setting, CML was chosen as a model of hepatic damage in rats.

A drug that protects the liver cells from toxic substances could diminish hepatocellular necrosis and could thereby delay or prevent the occurrence of hepatic failure. There is general revival of interest in an alternative system of medicine based on drugs derived from plants partly because of inadequacies of the modern synthetic approaches and their adverse reactions (Handa et al., 1986).

In the present study, the induction of hepatotoxicity by CML for a period of 21 days resulted in significant ($P < 0.01$) rise in serum AST, ALT, ALP, γ-GTP and tissue lipid peroxide levels in Group II as compared to the normal control group, i.e., Group I.

In alcoholics, γ-GTP is more frequently elevated than either of the transaminases or alkaline phosphatases. It provides a more specific test for hepatic involvement than does aspartate transaminase determination, since γ-GTP is virtually absent from skeletal and cardiac muscle, whereas aspartate transaminase elevation from these non-hepatic sources may occur as a result of alcoholic myopathy or cardiomyopathy. Therefore, γ-GTP determination is useful for diagnostic confirmation in patients in whom excessive drinking is suspected (Rollason et al., 1972).

Significant increased activity of γ-GTP indicates a severe damage to tissue membranes during CML toxicity because γ-GTP is a membrane bound enzyme (Chander et al., 1994). Oral administration of ginger attenuated the CML-induced rise in γ-GTP, thus revealing the membrane-stabilizing activity of ginger. The hepatoprotective properties of silymarin have been related to the inhibition of lipid peroxide formation or scavenging of free radicals (Bindoli et al., 1977). Ginger also inhibited or reversed the increased level of lipid peroxides in CML-treated rats, indicating a potential antioxidant property.

Enzymes such as aspartate aminotransferases (AST), alanine aminotransferases (ALT) and alkaline phosphatases (ALP) are the most frequently utilized indicators of hepatic injury and represent markers of hepatocellular necrosis (Amin et al., 1996). In the present study, the rise in AST, ALT and ALP levels induced by CML was also reduced by ginger treatment, thus indicating further evidence for the hepatoprotective effect of ethanolic ginger extract.

The present study suggests that ginger prevents hepatotoxicity by reducing hepatic injury, exhibiting membrane stabilizing and antioxidant properties. Our results could be the first major step for research on hepatoprotective potential of ginger in liver injury.

References

Amin A, Nanji A, David Z (1996): In: David Z, Thomas B, eds., *Hepatology. A Text Book of Liver Disease 1*, London, WB Saunders Co., p. 891.

Bhatt AD, Bhatt NS (1996): Indigenous drugs and liver disease. *Indian J Gastroenterol 15*: 63–67.

Bindoli A, Cowallini L, Silipandi N (1977): Inhibitory action of silymarin on lipid peroxidation formation in rat liver mitochondria and microsomes. *Biochem Pharmacol 26*: 2405–2409.

Chander R, Kapur NK, Dhawan BN (1994): Picroliv affects γ-glutamyl cycle in liver and brain of Mastomys natalensis infected with *Plasmodium berghei*. *India J Expt Biol 32*: 324–327.

Gulati R, Agarwal S, Agarwal SS (1995): Hepatoprotective studies on *Phyllanthus embelica* Linn. and quercetin. *Indian J Expt Biol 33*: 261–268.

Handa SS, Sharma S, Chakraborty KK (1986): Natural products and plants as liver protecting drugs. *Fitoterapia 57:* 307–357.

Hikino H, Kiso Y, Kato N, Hamada Y, Sankawa U (1985): Antihepatotoxic actions of gingerols and diaryl hepatanoids. *J Ethanopharmacol 14*: 31–39.

Kai OL (1995): Alcoholic liver disease: pathological aspects. *J Hepatol 23(Suppl 1)*: 7–15.

Kapur V, Pillai KK, Hussain SZ, Balani DK (1994): Hepatoprotective activity of jigrine on liver damage caused by alcohol-carbon tetrachloride and paracetamol in rats. *Indian J Pharmacol 26*: 35–40.

King J, King R (1971): A method for determination of alkaline phosphatase in vitro. *J Clin Pathol 7*: 322–328.

Lawrence ST, Paul M, Santiago J, Munoz A (1996): In: David Z, Thomas B, eds., *Hepatology. A Textbook of Liver Disease 1*, London, WB Saunders Co., p. 791.

Lowery OH, Rosenbrough NJ, Forr AL, Ramdall RJ (1951): Protein measurement with the Folin's phenol reagent. *J Biol Chem 193*: 265–275.

Nanji AA, Merdenhall CL, French SW (1989): Beef fat prevents alcoholic liver disease in the rat. *Alcoholism Clin Expt Res 13*: 15–19.

Naftaline L, Margaret S, Whitakar JF, Tracey DA (1969): A routine procedure for estimating serum γ-glutamyl transpeptidase activity. *Clin Chim Acta 26*: 293–296

Ohkawa H, Ohishi N, Yogi K (1979): Assay for lipid peroxides in animal tissue by thiobarbituric acid reaction. *Anal Biochem 95*: 351–358.

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Reitman S, Frankel AS (1957): A colorimetric method for the determinaiton of serum glutamic oxaloacetic acid. *Clin Pathol 28*: 53–56.

Robert S (1970): Effects of ethanol on the liver. *AM J Clin Nutr 31*: 467–473.

Rollason JG, Pincherie G, Robinson D (1972): Serum gamma-glutamyl transpeptidase in relation to alcohol consumption. *Clin Chim Acta 39*: 75–80.

Shirwaikar A, Srinivasan KK, Vasanth Kumar A, Krishnanand BR (1992): Antihepatotoxic activity of Kamilari, a marketed Ayurvedic preparation. *Indian Drugs 29*: 219–224.

Smuckler EA (1975): Alcoholic drink, its production and effects. *Fed Proc 34*: 2039–2044.

Sohni YR, Bhatt RM (1996): Activity of a crude extract formulation in experimental hepatic amoebiasis and in immunomodulation studies. *J Ethnopharmacol 54*: 119–124.

Sohni XR, Kaimal P, Bhatt RM (1995): The antiamoebic effect of a crude drug formulations of herbal extracts against Entamoeba histolytica in vitro and in vivo. *J Ethnopharmacol 45*: 43–52.

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