Remediation of hepatic antioxidant defense system during ethanol withdrawal stress with dietary ginger

M. Swaroopa

Division of Molecular Biology and Exercise Physiology, Department of Zoology, Sri Venkateswara University, Tirupati, Andhra Pradesh, India

ABSTRACT

This analysis has been conducted to investigate the influence of ginger on hepatic antioxidant enzymes system in rats withdrawn from ethanol treatment. Ethanol withdrawal showed a marked decrease in the superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase and glutathione content while an increase of malondialdehyde (MDA) levels were estimated in the hepatic tissue. This effect was reversed by treatment with 1% dietary ginger for 4 weeks in rats that showed improved antioxidant status which suggest that treatment of ginger may have protective role against both the ethanol and withdrawal induced hepatotoxicity.

Keywords: Ethanol withdrawal; Antioxidant system; Hepatic tissue; Free Radicals; Ginger.

INTRODUCTION

Alcoholic liver disease (ALD), a common consequence of prolonged and heavy alcohol intake, is a major health problem. Alcohol-induced liver injury causes grave medical, financial and social problems. The disease progresses from fatty infiltration, followed by a pernicious course of inflammation leading to irreversible damage; liver transplantation being the only cure. Chronic consumption of ethanol significantly increases the steady-state levels of ROS in hepatocytes isolated from animals fed the Lieber-De Carli ethanol liquid diets [1,2,3]. While ethanol itself is pro-oxidant because it directly generates reactive oxygen species during its metabolism [4]. Unmanaged sudden withdrawal from the excessive consumption of alcohol (ethanol), ethanol withdrawal (EW) provokes intense generation of reactive oxygen species (ROS) and an increase in the levels of various peroxidation products. The oxidative stress was more severe during EW than ethanol exposure per se and was accompanied with cell death.

Antioxidant agents of natural origin have always been of special interest because they can protect human body from free radicals [5]. Numerous medicinal plants and their formulations are used in diverse models of liver injury and cholestasis in ethnomedical practices as well as in traditional systems of medicine in India [6]. Ginger rhizome (Zingiber officinale Rosc, Zingiberaceae), commonly known as ginger, is consumed worldwide in cooking as a spice and flavoring agent. Various in vitro studies have shown that water and organic solvent extracts of ginger possess antioxidant property [7,8]. Oral consumption of ginger, dried and powdered, has been shown to result in relief of pain and swelling in patients with rheumatoid arthritis, osteoarthritis or muscular discomfort [4]. Ginger oil has been found to be an inhibitor of cyclooxygenase and lipoxygenase activities [9]. Previously Sharma et al. [10] have...
suggested that ginger oil possess anti-inflammatory properties. It is also reported that dietary ginger protected the tissues from oxidative stress induced by organophosphate pesticide (Malathion) in rats [11]. The ethanolic extract of the rhizome of the plant has been reported to possess significant hepatoprotective activity by some authors [12].

Given that the above mentioned significance of ginger it is of much interest and of profound practical importance to study the influence of long term dietary ginger supplementation on anti-oxidative potential with reference to alcohol withdrawal. Keeping in view of medicinal value of ginger, the present study was designed to explore a possible new strategy to improve recovery from alcoholic liver stress and injury in rats. Hence it is thought worthwhile to investigate, the effects of dietary ginger in alcoholics and withdrawal subjects by analyzing the activities of antioxidant enzymes in the liver tissue of male albino rats. Also presented in this article is evidence that increased ROS production, combined with decreases in activities associated with the antioxidant system in liver tissue is greater during withdrawal compared to chronic ethanol treatment alone.

MATERIALS AND METHODS

2.1. Animals
Pathogen free Wistar male albino rats weighing 170±10 g were used in the current investigation. The study is reviewed and approved by the Institutional Animal Ethics committee in its resolution number 438 / 01/a / CPCSEA / IAEC / SVU / KSR-1 /dt: 11.09.2008. The rats were housed in clean polypropylene cages having 6 rats per cage and maintained under temperature controlled room (27 ± 2°C) with a photoperiod of 12 h light and 12 h dark cycle. The rats were fed with a standard rat pellet diet and water ad libitum.

2.2. Effect of ginger on alcohol and withdrawal-induced liver damage in rats
The rats were divided into six groups and treated as described below.

Group I. Normal control (NC): Six rats received normal (0.9%) saline orally via orogastric tube for equivalent handling.

Group II. Ethanol treatment (Et): six rats received absolute EtOH orally at the dose of 2.0 g / kg via orogastric tube for 4 weeks.

Group III. Ginger treatment (Gt): rats were fed with dietary ginger (w/w) along with pulverized feed and thoroughly mixed so as to get a diet containing 1% of ginger.

Group IV. Ethanol plus ginger treatment (Et + Gt). This group of rats received both EtOH and ginger as described in group II and group III for a period of 4 weeks.

Group V. Ethanol withdrawal treatment (EW): six rats received absolute EtOH orally at the dose of 2.0 g / kg via orogastric tube for 4 weeks followed by 72 hours of withdrawal after last dose of ethanol.

Group VI. Ethanol withdrawal plus ginger treatment (EW + Gt). This group of rats received both EtOH and ginger as described in group II and group III for a period of 4 weeks and allowed for 72 hours of withdrawal after last dose of ethanol.

Animals are killed after the last treatment except for withdrawal groups which are sacrificed 72 hours after the last dose of ethanol treatment and liver tissue was excised. The tissue is washed with ice cold saline, immediately immersed in liquid nitrogen and stored at -80°C for further biochemical analysis. The selected antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), glutathione reductase (GR) and the content of glutathione (GSH) and MDA (lipid peroxidation) levels are estimated by employing the methods of Misra and Fridovich [13], Aebi [14], Flohe and Gunzler [15], Carlberg and Mannervik [16], Theodorus [17] and Ohkawa [18] respectively.

2.3. Statistical analysis
Statistical significance of the data was determined by Student’s- t test and analysis of variance (ANOVA) was done by using the statistical package ‘SPSS’ with 6 replications. The values of significance were evaluated with ‘P’ values. The difference is considered significant at P<0.05.
RESULTS

Chronic intake of ethanol as well as ethanol withdrawal is associated with marked alterations in antioxidant defense system of mammals and leads to oxidative stress. All the antioxidant enzymes evaluated are significantly decreased by ethanol administration and rather intense during withdrawal. This effect was completely reversed by treatment with ginger which restore the normal value observed in normal controls (Tables 1-3).

Table 1: Effect of ginger (1% w/w) on ethanol (2g/kg b.w.)- and withdrawal induced reduction of superoxide dismutase (SOD) and catalase (CAT) in rat liver

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Normal control (NC)</th>
<th>Ethanol treated (Et)</th>
<th>Ginger treated (Gt)</th>
<th>Combination of ethanol plus Ginger (Et+Gt)</th>
<th>Withdrawal group (EW)</th>
<th>Combination of ethanol withdrawal plus Ginger (EW+Gt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOD</td>
<td>12.66 ± 0.47</td>
<td>8.526 ± 0.501*</td>
<td>17.707</td>
<td>15.409 ± 0.351*</td>
<td>7.775 ± 0.274**</td>
<td>14.891 ± 0.316*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(-32.68)</td>
<td>(439.80)</td>
<td>(-583.16)</td>
<td>(-38.61)</td>
<td>(+17.575)</td>
</tr>
<tr>
<td>CAT</td>
<td>0.384 ± 0.023</td>
<td>0.287 ± 0.051**</td>
<td>0.566</td>
<td>0.036**</td>
<td>0.201 ± 0.087***</td>
<td>0.417 ± 0.006*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(-25.66)</td>
<td>(+47.395)</td>
<td>(-53.61)</td>
<td>(+35.410)</td>
<td>(+8.593)</td>
</tr>
</tbody>
</table>

All the values are mean ± SD of six individual observations
*Values are expressed in units of superoxide anion reduced/mg protein/minute
**Values in the parenthesis denote percent change over sedentary control
*Significant at P< 0.001, ** significant at P< 0.01.

Table 2: Effect of ginger (1% w/w) on ethanol (2g/kg b.w.)- and withdrawal induced reduction of glutathione peroxidase (GSH-Px) and glutathione reductase (GR) activities in rat liver

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Normal control (NC)</th>
<th>Ethanol treated (Et)</th>
<th>Ginger treated (Gt)</th>
<th>Combination of ethanol plus Ginger (Et+Gt)</th>
<th>Withdrawal group (EW)</th>
<th>Combination of ethanol withdrawal plus Ginger (EW+Gt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSH-Px</td>
<td>0.81 ± 0.041</td>
<td>0.513 ± 0.032**</td>
<td>1.144</td>
<td>0.982 ± 0.095*</td>
<td>0.446 ± 0.009*</td>
<td>1.367 ± 0.098***</td>
</tr>
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<td></td>
<td></td>
<td>(-36.977)</td>
<td>(+40.50)</td>
<td>(-32.97)</td>
<td>(-45.208)</td>
<td>(+69.936)</td>
</tr>
<tr>
<td>GR</td>
<td>0.671 ± 0.048</td>
<td>0.30 ± 0.020**</td>
<td>0.866</td>
<td>0.907 ± 0.030*</td>
<td>0.258 ± 0.010*</td>
<td>1.112 ± 0.174*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(-55.29)</td>
<td>(+29.061)</td>
<td>(-30.87)</td>
<td>(-45.14)</td>
<td>(+65.722)</td>
</tr>
</tbody>
</table>

All the values are mean ± SD of six individual observations
*Values are expressed in µ moles of NADPH oxidized/mg protein/minute
**Values in the parenthesis denote percent change over sedentary control
*Significant at P< 0.001.

Table 3: Effect of ginger (1% w/w) on ethanol (2g/kg b.w.)- and withdrawal induced reduction of the levels of glutathione (GSH) and malondialdehyde (MDA) in rats liver

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Normal control (NC)</th>
<th>Ethanol treated (Et)</th>
<th>Ginger treated (Gt)</th>
<th>Combination of ethanol plus Ginger (Et+Gt)</th>
<th>Withdrawal group (EW)</th>
<th>Combination of ethanol withdrawal plus Ginger (EW+Gt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSH</td>
<td>108.925 ± 1.28</td>
<td>51.081 ± 2.533**</td>
<td>170.859</td>
<td>114.317 ± 1.533*</td>
<td>78.348 ± 1.457**</td>
<td>158.588 ± 3.573*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(-53.104)</td>
<td>(+56.859)</td>
<td>(+56.859)</td>
<td>(+56.859)</td>
<td>(+65.593)</td>
</tr>
<tr>
<td>LP</td>
<td>60.625 ± 2.005</td>
<td>117.84 ± 3.809**</td>
<td>46.25</td>
<td>99.916 ± 3.878*</td>
<td>108.865 ± 1.138**</td>
<td>51.472 ± 2.911***</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(+94.375)</td>
<td>(+23.711)</td>
<td>(+94.375)</td>
<td>(+94.375)</td>
<td>(+79.571)</td>
</tr>
</tbody>
</table>

All the values are mean ± SD of six individual observations
*Values are expressed in µ moles of glutathione / gram wet weight of the tissue
**Values are expressed in µ moles of malondialdehyde / gram wet weight of the tissue
Values in the parenthesis denote percent change over sedentary control
* Significant at P< 0.001.

DISCUSSION

Liver being a vital metabolic site is the major target of ethanol toxicity and the role of oxidative stress in the pathogenesis of alcohol related diseases, particularly in liver, has been repeatedly confirmed [19]. Hepatocellular enzymes (SOD, CAT) serve as biomarkers of hepatocellular injury due to alcohol and drug toxicity [20]. So the
studies on antioxidant enzymes (SOD, CAT) have been found to be of great importance in assessment of liver damage. The antioxidant activity of *Z. officinale* is shown to be as effective as vitamin C in lowering lipid peroxidation in rats by influencing the enzymatic blood level of superoxide dismutase, catalase and glutathione peroxidase [21].

In the present study the rats which received 2.0 g of EtOH for a period of 4 weeks showed a significant decrease in the hepatic SOD activity and the decrease is much higher in withdrawal group. Similar decrease in SOD activity in plasma and liver [22] and in hepatic tissue [23] has also been reported during EtOH intoxication. The significant decrease in SOD activity due to ethanol and EW indicates inefficient scavenging of reactive oxygen species which might be implicated to oxidative inactivation of enzymes [24]. The SOD activity was elevated in rats of groups III, IV and VI which received ginger. Khanom et al. [25] reported the presence of phenolic compounds such as, flavonoids and ferulic acid in ginger rhizome [26] which may be responsible in scavenging the superoxide anion radicals and thereby maintain the high activity of SOD even in alcoholics and after withdrawal.

Liver CAT activity is also found to be decreased due to administration of EtOH followed by withdrawal. Ethanol enhances the production of oxygen derived free radicals and decreases the CAT activity in the hepatic tissue. Bindu [27] reported a significant decrease in CAT activity with 4g/kg EtOH treatment for a period of 50 days in rats. Das and Vasudevan [28] reported a significant decrease in CAT activity the hepatic tissue of rats treated with 2g/kg EtOH for a period of 4 weeks. The decreased CAT activity with EtOH treatment results in inefficient scavenging of hydrogen peroxide due to oxidative inactivation of enzyme [29]. The groups of rats which received ginger for a period of 4 weeks showed significant elevation in CAT activity in the hepatic tissues which indicates the antioxidant property of dietary ginger. This result presents further evidence for the hepatoprotective effect of dietary ginger. These results are in agreement with that of a previous investigations by Ahmed [21] that reported the antioxidant property of ginger in wistar rats in a comparative study of with renowned antioxidant ascorbic acid.

The present study predicted a significant decrease in the activity of GSH-Px in withdrawal rats compared to EtOH treated rats, which suggests that disturbance in the glutathione homeostasis in the liver cells that ultimately leads to the damage of hepatocytes. Decrease in GSH-Px activity may be implicated to either free radical dependent inactivation of enzyme [24] or depletion of its co-substrate i.e., GSH and NADPH in the ethanol treatments [30], this has further decreased effect in depleting the free radicals formed further increasing the oxidative stress. The reduced GSH-Px activity may also be due to reduced availability of GSH as observed in the current investigation. The activity of GSH-Px was significantly increased with ginger and also with ethanol and ginger and withdrawal and ginger combination treatment groups which indicates that ginger could inhibit and / or scavenge the free radicals in rat hepatic tissue.

The activity of hepatic GR is considerably decreased with ethanol treatment in the rats. Similar decrease in GR activity with EtOH treatment (1.6g/kg) in hepatic tissue of rats is reported by Husain [22]. Das [28] showed a significant decrease in GR activity in their alcohol dose dependent studies in the hepatic tissue of rats. The decrease in GR activity after ethanol intoxication reflects the impaired conversion of glutathione of oxidized form to reduced form [31] thus alters the GSH/GSSG ratio. The increase in GSH/GSSG ratio in the liver of EtOH fed rats and inhibition of GR activity are indicative of ethanol induced oxidative stress in the liver [22] leading to the decreased antioxidant enzyme capacity. The rats received 1% of dietary ginger for a period of 4 weeks showed a remarkable elevation in hepatic GR activity. Similarly the decreased GR activity with ethanol treatment was also recovered with ginger supplementation. Due to its efficient antioxidant property, the ginger could improve the antioxidant status and may enhance the hepatic GSH concentration.

The present results also showed significant elevation in MDA not only during EtOH intoxication but more profoundly after withdrawal in the hepatic tissue of rats. Das [28] reported increased lipid peroxidation with ethanol in their dose dependant studies. Ostrowska [32] reported a threefold higher concentration of lipid hydroperoxides in ethanol treated rats than that of the control groups. During ethanol metabolism a potentially dangerous byproducts are generated including reactive oxygen species (ROS) [33,2] which react with membrane lipids and cause lipid peroxidation leading to cell death [28]. Ethanol also increases the activity of cytochrome P450 in the hepatic tissue and causes oxidative stress by enhancing the production of free radicals. EW induces a deleterious interaction between oxidative pathways and P38 activation, mitogen-activated protein kinases that mediate signaling cascades...
and regulate cell fate in response to cellular stress (34). Rats received 1% of dietary ginger for a period of 4 weeks clearly showed a significant reduction in liver tissue MDA levels increased by ethanol and after withdrawal.

In summary, we demonstrate that ginger prevents withdrawal-induced oxidative stress and hepatic injury. Since these models of hepatic damage in the rat simulate many of the features of human liver pathology, we suggest that natural antioxidants and scavenging agents in ginger might be effective as plant hepatoprotective agents and thus may have some obvious therapeutic implications. Hence, it seems logical to conclude that ginger, because of its antioxidant property, might be capable of protecting the hepatic tissue from ethanol-induced injury, withdrawal related stress and inflammatory changes (35).

CONCLUSION

In conclusion, the data obtained in the present investigation confirmed the well known effect of ethanol in decreasing the antioxidant enzymes in liver tissue. These effect may be due to the production of high amount of reactive oxygen species (ROS) which is further exacerbated by withdrawal. However withdrawal from alcohol also aids in the restoration of antioxidant enzyme levels of liver to a considerable level. However this phenomenon becomes more pronounced with supplementation of 1 % of dietary ginger suggesting that the dietary ginger exerts antioxidative effect by activating antioxidant enzyme status and reducing MDA in liver tissue.

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REFERENCES