GINGER EXTRACT AIDS IN REINSTATING THE ALTERED ACTIVITIES OF HEPATIC AND RENAL ENZYME SYSTEM DUE TO CHRONIC ETHANOL CONSUMPTION DURING ETHANOL WITHDRAWAL

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Abstract

This study investigate the possible antioxidant properties of ginger in preventing damages to the liver and kidney of albino rats. To investigate this, 20% ethanol was injected into rats in order to induce liver and kidney damage. The rats were simultaneously given oral dose (200mg/kg body weight) of aqueous extract of ginger. The effects of these treatments on some liver and kidney enzymes were evaluated. Administration of ethanol alone to rats significantly ($P<0.05$) reduced the activities of liver ALT and AST by 71% and 60% respectively. The values for kidney AST and ALT are 47% and 80% respectively. Significant reversal in the activity of the enzymes under study is found by withdrawal from ethanol ($P<0.05$). Simultaneous treatment of ethanol injection with oral administration of 200mg/kg of the aqueous extract significantly reversed ($P<0.05$) these changes in both the liver and kidney. The activities of liver and kidney GGT were considerably reduced by ethanol administration and were found to be reversed by the plant extract. The activity of XOD and ALP were also found to be significantly different ($P<0.05$) in the ethanol group compared to control, withdrawal and extract treated groups. The present findings suggest that the extract may probably possess components that are hepatoprotective and anti-nephrotoxic.

Keywords: Peroxidative damage; Liver; Kidney; Ginger; Antioxidant; Rat.

Introduction

Products of plant origin have been shown to be effective sources of therapeutic agents without much side effects (Neetu and Meenakshi, 2003). In recent times, a number of practitioners claim the efficacy of medicinal plants in treating a
The majority of ailments are on the increase (Kabir et al., 2005). Ginger has been used for thousands of years for the treatment of a large number of maladies, such as antibacterial, antiviral, gastroprotective, antidiabetic, anti-hypertensive, cardioprotective, anticancer, chemopreventive and immunomodulatory effects. Several studies have extensively reviewed the medicinal, chemical, and pharmacological properties of ginger (Surh, Lee and Lee 1998; Ernst and Pittler 2000; Bode and Dong 2004; Boone and Shields 2005; Borrelli et al. 2005; Chrubasik, Pittler and Rousfogails 2005; Grzanna, Lindmark and Frondoza 2005; Thompson and potter 2006; Eliopoulos 2007; Shuka and Singh 2007; White 2007; Ali et al. 2008; Nicoll and Henein 2009). Previous studies have revealed that other plants with polyphenols exhibit clear hepatoprotective properties (Lima et al., 2006), and that flavonoids could protect human hepatocytes against oxidative injury induced by H$_2$O$_2$ or CCl$_4$ in vitro (Zhao and Zhang, 2009). Although many other plants have been reported to possess hepatoprotective properties, the scientific authentication of most of these which are used traditionally to treat liver disorder and several other diseases during ethanol withdrawal is unavailable. Z. officinale grows predominantly in almost all regions round the world. Rhizome from this plant serve as culinary herb and commonly consumed dietary condiment. Many reviews (Barrett, Kiefer and Rabago 1999; Ness, Sherman and Pan 1999; Talalay and Talalay 2001) have emphasized the significance of meticulous scientific research in establishing the safety and efficacy of potential therapeutic plant remedies and emphasized on defining the risks and benefits of herbal medicine. The aim of this work is to provide some experimental support for the health benefit of ginger in condition of withdrawal from chronic alcohol addiction. To achieve this, studies were carried out to investigate the phytochemical component of Z. officinale and to evaluate the hepatoprotective and nephroprotective activities of aqueous extract of the ginger roots against oxidative damage induced by ethanol withdrawal in rats.

**Material and Methods**

**Animals**

The study involved young (2–3 months old; 200 - 220g) Wistar strain male albino rats purchased from Sri Venkateswara Traders Pvt. Limited, in Bangalore and were maintained in the animal house of the department in polypropylene cages. Standard conditions of humidity (50% relative humidity), room temperature (25 - 28°C) and 12 h light/dark cycle (6:00 A.M. to 6:00 P.M.) were maintained. A standard rodent diet (M/s Hindustan Lever Ltd., Mumbai), and water was provided *ad libitum*. All experimental procedures were approved by the CPCSEA on Animal Care, Govt. of India.
Dr. Swaroopa, Maralla* et al. International Journal Of Pharmacy & Technology bearing the CPCSEA No. 438 / 01/a / CPCSEA / IAEC / SVU / KSR-1 (dt: 11.09.2008).

Chemicals:
All reagent kits used for enzyme assay were obtained from Randox Company, United Kingdom. All other reagents used were of analytical grade.

Plant material and extraction:
Aqueous ginger extract was prepared from locally available ginger roots. Ginger rhizomes were purchased fresh from the local market of Tirupati and were authenticated by staff in the Department of Botany at Sri Venkateswara University, Tirupati in India. Whole rhizome of ginger was thoroughly washed, sliced, grated, and ground to a fine paste. A weighed quantity (30gm) of the paste was subjected to continuous hot extraction in a Soxhlet apparatus using double distilled water. The extract was evaporated under reduced pressure using a rotary evaporator and then lyophilized until all of the solvent was removed. This aqueous ginger extract (AGE) was stored at 4°C.

Experimental design: The rats were randomly grouped into four (n = 4) as follows:

(i) Group I Control received normal saline daily (p.o.).
(ii) Group II received 20% Ethanol (2 gm/kg body wt (p.o.) per day)
(iii) Group III received 20% Ethanol and 200 mg/kg body weight of ginger extract (orally) simultaneously daily.
(iv) Group IV received 20% Ethanol (2 gm/kg body wt (p.o.) per day) for 6 weeks and subjected to ethanol withdrawal for 72 hrs
(v) Group V were subjected 72 hrs withdrawal after receiving 20% Ethanol (2 gm/kg body wt (p.o.) per day) for 6 weeks along with simultaneous administration of 200 mg/kg body weight of ginger extract (orally) daily.

Biochemical studies: The activities of liver and kidney aminotransferases (ALT, AST, and GGT) were assayed basically by the method of Reitman and Frankel 1957. ALP activity was determined by the method of Martins et al., 2001. XOD (EC 1.1.3.22) activity was determined by using xanthine as substrate, and by following the rate of reduction of nitroblue tetrazolium at 560 nm (Fried and Fried 1974). The ADH (EC 1.1.1.1) activity was assayed using the method described by Kim et al., 2001. Hepatic cytochrome P450 2E1 (EC 1.14.14.1) activity was measured according to the method of Imai et al., 1966 as modified by Nanji 1997.

Statistical analysis
The statistical analysis was carried out by one way Analysis of variance and Duncan Multiple Range test. $P < 0.05$ was considered significant.

Results

Table 1 shows the results obtained for the phytochemical screening of hot water extract of ginger. Tables 2 shows the effect of the extract of ginger on the activities of liver and kidney AST in ethanol-induced liver damage in rats. The activities of liver and kidney AST were significantly reduced ($P<0.05$) by ethanol administration alone on the rats when compared with the control. It was observed that simultaneous administration of 200mg/kg extract with ethanol to rats significantly increased ($P< 0.05$) the activities of the enzymes when compared with administration of ethanol alone to rats. The activities of the liver and kidney ALT as shown in Table 3 indicates that the treatment of ethanol injection with oral administration of 200mg/kg extract significantly reversed ($P< 0.05$) the enzyme activity in the kidney when compared with the administration of ethanol alone to rats. The activities of liver GGT were considerably reduced by ethanol administration when compared with the control and were significantly reversed ($P< 0.05$) by the administration of 200mg/kg extract (Table 4). Changes in the activities of ALP, XOD, ADH and CYP 450 were shown in tables 5,6,7 and 8 respectively.

Table- 1: Phytochemical screening of aqueous extract of ginger rhizome.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Phytochemical component</th>
<th>Method</th>
<th>Availability in the extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Alkaloids</td>
<td>Sofowara, 1993</td>
<td>++</td>
</tr>
<tr>
<td>2.</td>
<td>Terpenoids</td>
<td>Sofowara, 1993</td>
<td>+++</td>
</tr>
<tr>
<td>3.</td>
<td>Steroids</td>
<td>Sofowara, 1993</td>
<td>+++</td>
</tr>
<tr>
<td>4.</td>
<td>Flavonoids</td>
<td>Brain and Turner, 1975</td>
<td>++++</td>
</tr>
<tr>
<td>5.</td>
<td>Phenols</td>
<td>Evans WC, 1989</td>
<td>++++</td>
</tr>
<tr>
<td>6.</td>
<td>Glycosides</td>
<td>Legal test</td>
<td>-</td>
</tr>
<tr>
<td>7.</td>
<td>Tannins</td>
<td>Sofowara, 1993</td>
<td>-</td>
</tr>
<tr>
<td>8.</td>
<td>Anthraquinones</td>
<td>Sofowara, 1993</td>
<td>+++</td>
</tr>
<tr>
<td>9.</td>
<td>Saponins</td>
<td>Sofowara, 1993</td>
<td>-</td>
</tr>
<tr>
<td>10.</td>
<td>Carbohydrate</td>
<td>Molisch’s and fehling test</td>
<td>++++</td>
</tr>
<tr>
<td>11.</td>
<td>Resins</td>
<td>Trease and Evans 1983</td>
<td>+++</td>
</tr>
<tr>
<td>12.</td>
<td>Fats and oil</td>
<td>Saponification test</td>
<td>+</td>
</tr>
</tbody>
</table>
Proteins and amino acids | Biuret and Ninhydrin test | +

**Key:**

“-” Not present.

“+” Present in low concentration.

“++” Present in moderately high concentration.

“+++” Present in very high concentration.

“++++” Abundantly present.

**Table- 2:** Effects of aqueous extract of ginger rhizome on the activities of liver and kidney AST in ethanol and ethanol withdrawal-induced liver and kidney damage in rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Asparte Aminotransferase Activity</th>
<th>µmoles of pyruvate formed/mg protein / min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liver</td>
<td>Kidney</td>
</tr>
<tr>
<td>CONTROL</td>
<td>30.90±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>38.50±0.07&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>A</td>
<td>10.80±0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>22.00±0.09&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>B</td>
<td>15.40±0.07&lt;sup&gt;c&lt;/sup&gt;</td>
<td>24.00±0.02&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>C</td>
<td>10.25±0.01&lt;sup&gt;d&lt;/sup&gt;</td>
<td>17.34±0.01&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>D</td>
<td>27.93±0.11</td>
<td>34.08±0.93</td>
</tr>
</tbody>
</table>

n= 6 in each group,*

- Each value is the mean of six different determinations ± SD
- Values with different superscript across a row are significantly different (P<0.05) from control

CONTROL - Normal Control rats; A - Ethanol Control rats; B - Ethanol + Extract treated rats; C - Ethanol withdrawal rats; D - Extract treated rats + Ethanol withdrawal.

**Table- 3:** Effects of aqueous extract of ginger rhizome on the activities of liver and kidney ALT in ethanol and ethanol withdrawal-induced liver and kidney damage in rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Alanine Aminotransferase Activity</th>
<th>µmoles of pyruvate formed/mg protein / min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liver</td>
<td>Kidney</td>
</tr>
<tr>
<td>CONTROL</td>
<td>44.86±1.95&lt;sup&gt;a&lt;/sup&gt;</td>
<td>50.90±0.73&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>A</td>
<td>41.97±0.06&lt;sup&gt;b&lt;/sup&gt;</td>
<td>21.91±0.55&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>B</td>
<td>11.51±0.57&lt;sup&gt;c&lt;/sup&gt;</td>
<td>27.10±0.71&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>C</td>
<td>30.32±.82&lt;sup&gt;d&lt;/sup&gt;</td>
<td>22.5±1.04&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>D</td>
<td>40.80±1.95</td>
<td>47.90±0.73</td>
</tr>
</tbody>
</table>

n= 6 in each group,*

- Each value is the mean of six different determinations ± SD
- Values with different superscript across a row are significantly different (P<0.05) from control

CONTROL - Normal Control rats; A - Ethanol Control rats; B - Ethanol + Extract treated rats; C - Ethanol withdrawal rats; D - Extract treated rats + Ethanol withdrawal.

**Table- 4:** Effects of aqueous extract of ginger rhizome on the activities of liver and kidney GGT in ethanol and ethanol withdrawal-induced liver and kidney damage in rats.
Groups | Gamma Glutamyl Transferase Activity
| µmoles of p-nitroanilide/mg of protein/min |
|---|---|
| **Liver** | **Kidney** |
| CONTROL | 1.18±0.02<sup>a</sup> | 3.87±1.95<sup>a</sup> |
| A | 1.14±0.01<sup>b</sup> | 4.93±0.06<sup>b</sup> |
| B | 1.16±0.02<sup>c</sup> | 1.55±0.57<sup>c</sup> |
| C | 1.17±0.08<sup>d</sup> | 3.24±0.82<sup>d</sup> |
| D | 1.09±0.59 | 3.56±1.03 |

n= 6 in each group,<sup>*</sup>

- Each value is the mean of six different determinations ± SD
- Values with different superscript across a row are significantly different (P<0.05) from control

CONTROL - Normal Control rats; A - Ethanol Control rats; B - Ethanol + Extract treated rats; C - Ethanol withdrawal rats; D - Extract treated rats + Ethanol withdrawal.

Table- 5: Effects of aqueous extract of ginger rhizome on the activities of liver and kidney ALP in ethanol and ethanol withdrawal-induced liver and kidney damage in rats.

Groups | Alkaline Phosphatase Activity
| µmoles of phenol liberated/ mg protein/ min |
|---|---|
| **Liver** | **Kidney** |
| CONTROL | 0.021±0. 63<sup>a</sup> | 0.158±0.95<sup>a</sup> |
| A | 0.023±0.4 8<sup>b</sup> | 0.197±0.26<sup>b</sup> |
| B | 0.016±0.62<sup>c</sup> | 0.150±0.17<sup>c</sup> |
| C | 0.019±0.56<sup>c</sup> | 0.320±0.81<sup>d</sup> |
| D | 0.023±0. 11 | 0.161±0.42 |

n= 6 in each group,<sup>*</sup>

- Each value is the mean of six different determinations ± SD
- Values with different superscript across a row are significantly different (P<0.05) from control

CONTROL - Normal Control rats; A - Ethanol Control rats; B - Ethanol + Extract treated rats; C - Ethanol withdrawal rats; D - Extract treated rats + Ethanol withdrawal.

Table- 6: Effects of aqueous extract of ginger rhizome on the activities of liver and kidney XOD in ethanol and ethanol withdrawal-induced liver and kidney damage in rats.

Groups | Xanthine Oxidase Activity
| (µmol/mg protein/min) |
|---|---|
| **Liver** | **Kidney** |
| CONTROL | 0.185±0.018<sup>a</sup> | 0.096±0.019<sup>a</sup> |
| A | 0.273±0.040<sup>b</sup> | 0.208±0.015<sup>b</sup> |
| B | 0.191±0.017<sup>c</sup> | 0.193±0.017<sup>c</sup> |
| C | 0.215±0.035<sup>d</sup> | 0.127±0.018<sup>d</sup> |
| D | 0.179±0.053 | 0.109±0.031 |

n= 6 in each group,<sup>*</sup>

- Each value is the mean of six different determinations ± SD
- Values with different superscript across a row are significantly different (P<0.05) from control
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CONTROL - Normal Control rats; A - Ethanol Control rats; B - Ethanol + Extract treated rats; C - Ethanol withdrawal rats; D - Extract treated rats + Ethanol withdrawal.

Table- 7: Effects of aqueous extract of ginger rhizome on the activities of liver and kidney ADH in ethanol and ethanol withdrawal-induced liver and kidney damage in rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Alcohol Dehydrogenase Activity</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µmoles of NADH formed/mg protein / min</td>
<td>Liver</td>
<td>Kidney</td>
</tr>
<tr>
<td>CONTROL</td>
<td>1.175±0.32&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.259±0.51&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>0.704±0.13&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.090±0.88&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>1.016±0.47&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.150±0.19&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>1.101±0.78&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.225±0.56&lt;sup&gt;d&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>1.139±0.25</td>
<td>0.271±0.33</td>
<td></td>
</tr>
</tbody>
</table>

n= 6 in each group,*

- Each value is the mean of six different determinations ± SD
- Values with different superscript across a row are significantly different (P<0.05) from control

Table-8: Effects of aqueous extract of ginger rhizome on the activity of liver and kidney Cyp 450 in ethanol and ethanol withdrawal-induced liver and kidney damage in rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Cytochrome P450 Activity</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>nmols /mg of protein / hr</td>
<td>Liver</td>
<td>Kidney</td>
</tr>
<tr>
<td>CONTROL</td>
<td>0.075±0.54&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.039±0.51&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>1.704±0.34&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.250±0.88&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>1.016±0.21&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.090±0.19&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>0.101±0.97&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.125±0.56&lt;sup&gt;d&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>0.073±0.88</td>
<td>0.041±0.13</td>
<td></td>
</tr>
</tbody>
</table>

n= 6 in each group,*

- Each value is the mean of six different determinations ± SD
- Values with different superscript across a row are significantly different (P<0.05) from control

Discussion

The effect of aqueous extract of ginger rhizome on ethanol-induced hepatotoxicity as well as renal impairment in albino rats was evaluated. The significant reduction (P< 0.05) in activities of liver and kidney AST and ALT in the group treated with only ethanol when compared with controls suggests hepatotoxicity. This may be as a result of leakage from the cells through peroxidative damage of the membrane (Nanjial et al., 1994). The toxic effects are due to the active metabolite acetaldehyde formed by the action of alcohol dehydrogenase on alcohol, acetaldehyde dehydrogenase (ALDH) converts acetaldehyde to acetate. (Lieber 1998). Ethanol has been known to be toxic on cells and was often
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used to induce oxidative stress in cell systems. It has also been reported that treatment of rats with ethanol over long period is capable of generating free radicals that trigger a cascade of events resulting in alcoholic hepatitis and hepatic cirrhosis (Bondy 1992; Cederbaum 2001). Acetaldehyde, the toxic product of ethanol metabolism in the liver, covalently binds to a variety of proteins. Acetaldehyde adduct formation also impairs some enzyme activities (Lieber. 1998). The mechanism by which ethanol causes cell oxidative injury involves that cytochrome P-450 system transforms ethanol into acetaldehyde. There is also a concomitant 4- to 10-fold induction of cytochrome P 450 (2E1) both in rats and in humans. Indeed, 2E1 is rather "leaky" and its operation results in a significant release of free radicals. In addition, induction of this microsomal system results in enhanced acetaldehyde production, which in turn impairs defense systems against oxidative stress (Lieber, 1997) and causes lipid peroxidation, disturbs Ca2+ homeostasis and eventually kills cells. Earlier reports revealed that compounds from plants such as Silybum marianum (milkthistle), Picrorhiza kurroa (kutkin), Curcuma longa (turmeric), Camellia sinensis (green tea), Glycyrrhiza glabra (licorice) have confirmed the therapeutic effectiveness for use in a wide variety of disorders due to oxidative stress in liver and kidney (Scott-Luper 1998, 1999). Also extract such as bilberry displayed protective effect against primary rat hepatocytes oxidative stress induced by tertbutylhydroperoxide or allyl alcohol by protecting the leakage of the organ (Valentova, et al., 2007). The monitoring of the leakage of liver and kidney enzymes into the serum has proven to be very useful tool in assessing liver damage (Nelson and Cox 2000). The leakage of the organ leads to the decrease in the activity of the enzyme. The significant increase showed in liver and kidney AST and ALT activities after administration of 200mg/kg body weight of the extract (Tables 2,3 and 4) when compared with that administered with only ethanol suggest that the extract must have reverse the injurious effect of chronic ethanol consumption on these organs. This property might be as a result of antioxidant activity of the extracts which might be attributed to the presence of phenolics and flavonoids (Table1). The antioxidant activity of some extracts has been attributed to the presence of phenolics and flavonoids (Osawa 1994; Ferrali et al., 1997; Elliott et al., 1992; Hirano et al., 2001; Cos et al., 2001). The protective effect of the extract on liver especially after administration of 200mg/kg was further supported in the significant increases showed in the liver GGT (Table 4). Thus hot water extract of ginger has hepatoprotective properties against ethanol induced liver damage. Hence it is possible that a probable mechanism of hepatoprotection and anti-renal toxic capabilities of ginger extract against ethanol induced damage is the antioxidant activity.
Alkaline (ALP) is a ‘marker’ enzyme for the plasma membrane and endoplasmic reticulum (Wright and Plummer, 1974), being an ectoenzyme of the plasma membrane (Shahjahan et al., 2004). It is often used to assess the integrity of the plasma membrane (Akanji et al., 1993). The reduction in alkaline phosphatase activities in the male rat liver might be adduced to either loss of membrane components (including ALP) into the extracellular fluid, the serum (Malbica and L.G. Hart, 1971), inactivation of the enzyme molecule in situ (Umezawa and Hooper 1982), or inhibition of the enzyme activity at the cellular/molecular level (Yakubu et al., 2003). It may also be due to a reduction in concentration or total absence of specific phospholipids required by this membrane-bound enzyme to express its full activity (Yakubu et al., 2002). The corresponding increase in the rat serum ALP activity (Table 1) confirmed that damage has been inflicted on the plasma membrane, which might lead to compromise of its integrity (Yakubu et al., 2003). Such reduction in the activities of ALP from the tissues could be attributed to disruption of the ordered lipid-bilayer of the membrane structure leading to escape of detectable quantity of ALP out of the cell into the extracellular fluid (Yakubu 2006; Yakubu et al., 2003). The reduction in the liver ALP activities would hinder adequate transportation of required ions or molecules across their cell membrane and this may lead to starvation of the cells (Akanji et al., 1993). On the contrary, the significant increase in the ALP activity of rat kidney following the administration of the extract may be adduced to induction in the enzyme synthesis probably by de novo (Umezawa and Hooper, 1982). Such increase in alkaline phosphatase activity of the kidney may lead to indiscriminate hydrolysis of phosphate esters in the organs and other cells requiring these essential molecules (Butterworth and Moss 1966).

Gamma glutamyl transferase is the most sensitive enzymatic indicator of hepatobiliary disease (Mayne 1998). It is a membrane-localized enzyme that plays a major role in glutathione metabolism and resorption of amino acids from the glomerular filtrate and intestinal lumen (Kaplan 1972). GGT is used to distinguish whether increased serum alkaline phosphatase activity is from the bone or the liver (Lum and Gambino 1972). The increase in the GGT activity of the liver and the kidney may be attributed to the induction of the enzyme synthesis in both tissues. The increase in the liver enzyme may also be due to the blockage to the flow of bile, leading to the raised level of this enzyme and consequent increase in serum levels (Ruppine et al., 1982). Therefore, the concurrent increase in the serum enzymes of alkaline phosphatase and gamma glutamyl transferase activities following the administration of the plant extract may be as a result of damage to the liver. The alterations in the liver and kidney GGT activity by the extract might adversely affect...
the metabolism of glutathione and resorption of amino acids from the glomerular filtrate and intestinal lumen (Yakubu 2006). Nevertheless, the reduction of the enzyme activity in the liver to control level might be an attempt to recover from the assault inflicted on it.

To investigate a possible role of XOD in free radicals production involved in ethanol-induced pathogenesis, the activity of this enzyme was examined. Alcohol-induced oxidative stress is linked to the metabolism of ethanol. Three metabolic pathways of ethanol have been described in the human body so far. They involve the following enzymes: alcohol dehydrogenase, microsomal ethanol oxidation system (MEOS) and catalase. Each of these pathways could produce free radicals which affect the antioxidant system. Ethanol per se, hyperlactacidemia and elevated NADH increase xanthine oxidase activity, which results in the production of superoxide. Lipid peroxidation and superoxide production correlate with the amount of cytochrome P450 2E1. MEOS aggravates the oxidative stress directly as well as indirectly by impairing the defense systems. Hydroxyethyl radicals are probably involved in the alkylation of proteins. XOD activities were unmodified after exposure to ethanol. The data suggest that this enzyme is not a major source of oxidative stress in rat kidney under long-term ethanol exposure. A different mechanism has been observed in the liver by Kato et al. 2001. They have reported increased levels of XOD that might have contributed to enhance free radical production and lipid peroxidation in the liver following ethanol administration. Among various enzymes known to generate ROS, xanthine oxidase has recently been documented as a biological source to produce superoxide radicals which play important roles in the development of various complications. The clinical significance of XO in the oxidative stress of alcoholics has been emphasized by a study in which Alcohol consumption promote the conversion of xanthine dehydrogenase to xanthine oxidase (Sultatos 1988), which can generate ROS, thereby enhancing oxidative stress that increase the plasma lipid peroxide levels in alcoholics. More importantly, it has also been reported that the vascular xanthine oxidase originates in the liver of alcoholic rats. In fact, the liver and intestine are the tissues that express the highest activity of the enzyme (Parks and Granger 1986). Taken together, our results that predict that ginger extract significantly reduced xanthine oxidase activity and type conversion of the enzyme in alcoholic rat liver (Table 2) further suggest the clinical significance of ginger extract in the prevention of late-onset vascular complications of alcohol withdrawal. Since hepatic xanthine oxidase has been known as a major source of reactive oxygen species (ROS) generation in the pathogenesis of alcoholic complications, we also examined whether the preventive effect of ginger extract on ethanol-induced
withdrawal and oxidative stress is mediated through inhibition of XD/XO activity and type conversion. The increased activity of XO is an important factor of oxidant load in kidney tissue, since it is the main producer of the superoxide radical in vivo (Joannidis et al., 1990). The flavonoids in ginger might exhibit this antioxidant activity as flavonoids are proved as inhibitors of xanthine oxidase and serve as scavengers of the superoxide radical, produced by the action of the enzyme xanthine oxidase, was established (Cos et al., 1998).

Alcohol dehydrogenase (ADH) (E.C. 1.1.1.1) is a cytoplasmic NAD⁺ dependent zinc metalloenzyme that catalyzes the reaction oxidizing an alcohol to an aldehyde and reduces NAD⁺ to NADH. Kidney ADH has been studied in several species (Moser et al., 1968). Because proximal tubule cells contain cytochrome P450 and ADH, the cells have the potential to oxidize a variety of compounds to aldehydes that are potential cytotoxins. And ADH-2 is anodic isoenzymes, while ADH-3 represents the group of cathodic forms. The oxidation of ethanol to acetaldehyde proceeds via ADH and is associated with the reduction of NAD to NADH which produces a striking redox change with various associated metabolic disorders. NADH also supports microsomal oxidations via transhydrogenation to NADPH (Boleda et al., 1989). The specific localization and the kinetic properties of rat ADH isoenzymes suggest that ADH-1 and ADH-3 may act as metabolic barriers to external alcohols, whereas ADH-2 may have a vital function in the metabolism of the endogenous long-chain alcohols and aldehydes (Julia et al., 1987). Despite an increased ADH activity in long-term ethanol administration, the activity of the kidney ADH in ginger extract treated groups is apparently not altered. Further the activity of the enzyme during withdrawal from ethanol tends towards restoration to normal, a property that is still enhanced in the extract supplemented withdrawal group.

The Cytochrome P450 enzymes are membrane-bound (mostly to the endoplasmic reticulum) existing in a multienzyme system including a flavin-containing NADPH-CYP reductase. Besides producing radicals, P450 may also inactivate fatty acid hydroperoxides (Zangar et al., 2004). Fatty acid hydroperoxides may act as direct oxidizing agents to P450 leading to substrate oxidation and monohydroxylated fatty acids; in the absence of substrate co-metabolism, P450 can catalyse the isomerization of fatty acid hydroperoxides to the dihydroxylated fatty acids. Alcohol was shown to induce P450 isozyme 3a (CYP2E1) in the kidney using antibodies to the liver protein. This increased expression accompanied a seven-fold increase in the isozyme 3a dependent rate of aniline and butanol metabolism in kidney microsomes (Ding et al., 1986). Ethanol has been found to induce specifically isozyme CYP2E1 in the kidney by 50 to 80% (Ueng et al.,
Dr. Swaroopa. Maralla* et al. International Journal Of Pharmacy & Technology 1987) and 500% (Ding et al., 1986). Cyp2e-1 (the mouse ortholog of CYP2E1) is present in kidney and induced by alcohol (Thomas et al., 1987; Sewer et al., 1997). However ginger extract administration to chronic ethanol treated and withdrawal groups shows a decrease towards normal (Table 8).

**Conclusion**

Generally, there were significant changes in liver and kidney AST, ALT, ALP and GGT activities in ethanol test group (Group II) and ethanol test group simultaneously treated with 200mg/kg b.w. when compared to their controls: an indication that the extract is likely to prevent liver and kidney pathology in experimental animals particularly at dose of 200mg/kg b.w. These results suggest further strongly that ethanol withdrawal tends to reverse the activity of the metabolic enzymes like XOD, ADH and CYP 450, a process which is further accelerated by treatment with ginger extract, whereas such renal and hepatic pathology developed in ethanol and withdrawal experimental rats, treatment with ginger extract up to 200mg/kg b.w. provided alleviation and protection to the kidney and liver under condition of oxidative stress due to chronic ethanol and ethanol withdrawal.

**Conflict of Interest:** None Declared.

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**References**


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