Curative Effect of Garlic on Alcoholic Liver Diseased Patients

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Abstract

Alcohol is currently recognized as the most prevalent known cause of abnormal human development. Our aim was to investigate the effect of raw garlic on patients suffering from alcoholic liver disease. 20 alcoholic patients and 20 healthy individuals were selected. Both patients and normal individuals were subjected to detailed clinical examination and laboratory investigations. Blood samples were collected and the liver disease was assessed by measuring the activities of liver marker enzymes such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma glutamyl transferase (GGT) and lactate dehydrogenase (LDH) which were elevated in alcoholic patients. Increased lipid peroxidation in alcoholic patients was accompanied by decreased activities of Superoxide dismutase (SOD), Catalase (CAT), Glutathione peroxidase (GPx). Oral supplementation of 2 small sized raw garlic cloves (1 clove = 1.2g) to alcoholic patients for 45 days, significantly lowered the activities of liver marker enzymes, decreased the levels of lipid peroxidation and enhanced the antioxidant status to near normal.  Thus, the data of the present study suggest that raw garlic offers protection against oxidative stress and antioxidant activities in alcoholic liver disease patients.

1. Introduction

Alcoholic liver disease is one of the most serious consequences of chronic alcohol abuse. The disease is often progressive and is considered to be a major cause of morbidity and mortality (Sherlock, 1995). Free radicals and oxidative stress have been implicated in the pathogenesis of ethanol induced liver injury in humans and experimental animals (Lin et al., 1998; Zima et al., 2001). Basically, ethanol is metabolized into cytotoxic acetaldehyde by alcohol dehydrogenase in the liver and acetaldehyde is oxidized to acetate by aldehyde oxidase or xanthine oxidase giving rise to Reactive oxygen species (ROS) via Cytp450 (Fridovich, 1989; Nordmann et al., 1992). Thus, excess intake of alcohol resulted in the production of oxygen radicals which leads to lowering the body’s normal defense mechanism thereby altered enzyme activity, decreased DNA repair and impaired utilization of oxygen, lipid peroxidation and protein oxidation. Some of these alterations induced by oxidative stress can eventually cause necrosis and subsequently leads to oxygen damage (Kurose et al., 1996). In recent years, the popularity of native medicine has increased for various reasons. Since there is no reliable hepatoprotective drug available in modern medicine, alcohol researchers have focused on developing phytotherapeutic medicines which can provide many invaluable drugs to treat alcoholic liver disease. Thus, the research conducted on several natural plant products used as hepatoprotective agents is well-documented (Saravanan et al., 2006).

Allium sativum commonly known as garlic is a bulb-forming herb of liliaceae family. Garlic is the oldest cultivated plant and has been used as a spice, food and folklore medicine for over 4000 years. It has been used as a traditional medicine in the treatment of heart diseases, tumors and headaches and exhibits medicinal properties including immunomodulation, hepatoprotection, antioxidant, antimutagenic, antibacterial and anticarcinogenic effects (Agarwal, 1996). Moreover, it has also been reported to possess antifungal (Halliwell et al., 1992), hypoglycemic (Yoshida et al., 1987), hyperglycemic (Nadkarni, 1976), hypolipidemic (Pushpendran et al., 1982), anti-atherosclerotic properties (Bordia, 1981) and has been claimed to be effective against a number of diseases (Block et al., 1984). The active principle present in garlic is organosulphur compound such as allicin, allin, alliase, S-allyl cystein, diallyl disulphide and allyl methyl trisulphide (Augusti, 1996). These active compounds are mainly responsible for protecting from tissue damage and various disorders. Among many supplements, aged garlic extract has a reproducible array of compounds which have been analyzed and studied extensively for their high antioxidant content and health protective potential. However, the inhibition of lipid peroxidation and free radical scavenging activity has been suggested as a possible mechanism of...
hepatoprotective action. Thus, the present study was under-taken to establish the hepatoprotective effect of raw garlic on alcohol liver disease patients.

2. Patients and Methods

The present study is comprised of 20 newly diagnosed alcoholic patients from Rajah Muthiah Medical College and Hospital, Annamalai University, Tamilnadu, India. An equal number of healthy subjects (volunteers) were also investigated. The subjects were all males with the ages ranging from 48-55 years. Patients suffering from any other diseases other than alcohol intake were excluded from the study. The selected patients were alcoholic for the past 5-6 years and during the treatment, the patients stopped consuming alcohol. Both patients and normal individuals were subjected to detailed clinical examination and laboratory investigations. The ethical committee of Rajah Muthiah Medical College and Hospital Annamalai University, Tamilnadu, India, approved the study protocol in the year 2008.

Alcoholic patients received 2 small sized raw garlic cloves (1 clove = 1.2 g) daily morning under fasting conditions (12-24 hrs) for 45 days. Blood samples were collected from various arm puncture into plain tubes from healthy individuals and alcoholic patients before and after the treatment with raw garlic. Heparinised blood samples containing serum and plasma were separated by centrifugation at 3000rpm for 5min and buffy coat was removed and packed cell washed three times with physiological saline. Biochemical estimations were done in serum and in erythrocyte membranes of alcoholic patients before and after treatment with raw garlic. The results obtained were compared with normal individuals.

2.1. Biochemical Analysis

2.1.1. Estimation of liver marker enzymes

The activities of serum aspartate aminotransferase (AST, E.C.2.6.1.1) and serum alanine aminotransferase (ALT, E.C.2.6.1.2) were assayed by the method of Reitman’s and Frankel (1957). Serum alkaline phosphatase (ALP, E.C.3.1.2.3.1) was estimated using Kind & King’s method (1954), King (1965). The serum gamma glutamyl tranferase (GGT, E.C.2.3.2.2) was assayed according to the method of Rosalki and Rau (1972). The activity of lactate dehydrogenase (LDH, E.C.1.1.27) was estimated by the method of King (1965). Serum total protein, albumin were estimated by Biuret method Reinhold (1953).

2.1.2. Lipid peroxidation and enzyme assays

Lipid peroxidation was measured by estimating the levels of malondialdehyde (MDA) using Thiobarbituric acid reaction method. Thiobarbituric acid (TBARS) in plasma was estimated by the method of Yagi (1978) and TBARS in erythrocyte membrane was estimated by the method of Donnan (1950). The activities of enzymatic antioxidants SOD (E.C.1.15.1.1) was assayed by the method of Kakkar et al., (1984). The activity of CAT (E.C.1.11.1.6) was assayed by the method of Sinha (1972). The activity of GPx was assayed by the method of Rotruck et al., (1973).

2.2. Statistical analysis

The values were expressed as mean ±S.D. Statistical evaluation was done using one way analysis of variance (ANNOVA) which is followed by Duncan’s multiple range test (DMRT). The level of statistical significance was set at $p< 0.05$.

3. Results

Table 1 show that alcoholic patients have severe liver damage which was indicated by the increase in marker enzymes such as AST, ALT, ALP, GGT and LDH. However, administration of raw garlic significantly decreased the activity of these enzymes which was compared to that before treatment.

The levels of serum total protein was increased and the albumin levels were decreased in alcoholic patients, while on treatment with raw garlic it significantly improved both protein levels and albumin levels to near normal which was also compared to that of the normal individuals.

Table 2 shows that the levels of lipid peroxidation indicated by TBARS were significantly higher in plasma and erythrocytes of alcoholic patients as compared with normal subjects. TBARS level was lowered significantly in the plasma and erythrocytes of patients treated with garlic.

Further, the activities of SOD, CAT and GPx in erythrocytes were observed in normal and alcoholic patients. In alcoholic patients, the activity of SOD, CAT and GPx were significantly lower than the normal subjects. Treatment of alcoholic patients with garlic significantly elevated the antioxidant defense activity compared with that before treatment.
Table 1. Effect of raw garlic treatment on hepatic marker enzymes and serum proteins in alcoholic patients.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>I Normal individuals</th>
<th>II Alcoholic patients before garlic treatment</th>
<th>III Alcoholic patients after garlic treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST (IU/L)</td>
<td>19.4±4.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>80±21&lt;sup&gt;b&lt;/sup&gt;</td>
<td>45±12&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>ALT (IU/L)</td>
<td>43.4±7.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>124.2±15.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>55±8.5&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>ALP (IU/L)</td>
<td>89.9±13.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>165.9±21.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>92±13.5&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>GGT (IU/L)</td>
<td>41.7±5.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>226.7±28.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>65±10.5&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>LDH (IU/L)</td>
<td>88.3±20.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>349.8±32&lt;sup&gt;b&lt;/sup&gt;</td>
<td>105±15.2&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>Total protein (g/dl)</td>
<td>7.2±0.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.5±0.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.0±0.2&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>4.1±0.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.4±0.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.0±3.0&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD; n=20. Values not sharing a common superscript letter are significantly different at p<0.05 (DMRT).

Table 2. Effect of raw garlic on lipid peroxidation and enzymatic antioxidants in alcoholic patients.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>I Normal individuals</th>
<th>II Alcoholic patients before garlic treatment</th>
<th>III Alcoholic patients after garlic treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBARS in plasma (nmol/ml)</td>
<td>2.17±0.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.9±0.28&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.42±0.20&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>TBARS in erythrocytes (nmol/ml)</td>
<td>0.30±0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.53±0.11&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.31±0.03&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>SOD (U*/mg Hb)</td>
<td>1.54±0.16&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.01±0.16&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.42±0.15&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>CAT (U*/mg Hb)</td>
<td>17.2±1.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.3±0.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>16.07±1.4&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>GPs (U*/mg Hb)</td>
<td>2.01±0.11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.24±0.11&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.91±0.14&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD; n=20. Values not sharing a common superscript letter are significantly different at p<0.05 (DMRT).

*µ moles of H2O2 utilized per minute.

Enzymes required for 50% inhibition of nitroblue tetrazolium (NBT) reduction per minute.
4. Discussion

Free radical mediated damage to macromolecule plays a crucial role in the pathophysiology of atherosclerosis, inflammation, carcinogenesis, aging, drug reaction and toxicity (Jose et al., 1999). When the liver gets damaged after consumption of alcohol, it leads to leakage of cellular enzymes into the plasma (Baldi et al., 1993). The increased levels of serum enzymes such as (AST), (ALT), (ALP), (GGT) and (LDH) observed in alcoholic patients, resulted in liver damage, increased permeability and necrosis of hepatocytes (Goldberg and Watts, 1965). In our study, administration of raw garlic to alcoholic patients alleviates the increased activities of serum enzymes AST, ALT and ALP to near normal. Serum GGT is a sensitive marker enzyme widely used as a laboratory test for the hepatobiliary diseases especially alcoholic liver disease and alcohol induced liver damage (Nakanishi et al., 2006).

In the present study, we observed that GGT has invariably elevated while AST and ALP are slightly increased in alcoholic patients. Garlic supplementation significantly lowered the activities of GGT demonstrating reduced liver damage following garlic administration.

Albumins and globulins are two key components of serum proteins. As albumin is synthesized in the liver, it can be used as a biomarker to monitor liver function (Friedman et al., 1980). In serum total proteins, albumin contents were reduced in alcoholic patients. Hence a significant decrease in the serum total protein and increase in serum albumin was observed in alcoholic patients treated with raw garlic. This stabilization of serum protein level is a clear indication of garlic being related to an improvement in the functional status of the liver cells.

Lipid peroxidation mediated by free radicals is considered to play a pivotal role in the mechanism by which ethanol may exert its toxic effects on the liver and other extra hepatic tissues (Nordmann, 1994). Increase in the levels of TBARS indicates enhanced lipid peroxidation leading to tissue injury and failure of the antioxidant defense mechanism to prevent the formation of excess free radicals (Comporti, 1985). In our study we observed an increase in TBARS and a decline in antioxidant status in plasma and erythrocytes of alcoholic patients. However, treatment with garlic significantly decreased the levels of lipid peroxidation.

Free radical scavenging enzymes such as SOD, CAT, and GPX are the major defence enzymes against oxidative injury. SOD is a ubiquitous chain breaking antioxidant, plays an important role in protection against deleterious effects of lipid peroxidation (Dinkova–Kostova and Talalay, 1999). It converts the highly reactive superoxide radical to hydrogen peroxide, which in turn either metabolized by catalase or by glutathione peroxidase.

The primary role of catalase is to scavenge H₂O₂ and convert it into H₂O. It plays an important role in the acquisition of tolerance to oxidative stress in adaptive response of cells. Studies have shown that decrease in catalase during alcohol consumption may be due to the decreased protein synthesis. Thus, there is an increased utilization of CAT during alcohol consumption.

Gpx is a selenium dependent enzyme found primarily in the cytoplasm and also found in the mitochondria. It catalyses the detoxification of endogenous metabolic peroxides and hydroperoxides that leads to the oxidation of GSH. It has a high potency in scavenging reactive free radicals in response to oxidative stress.

The antioxidant defense systems SOD, CAT and GPx activity is significantly decreased in alcoholic patients. This decrease could be due to a feedback inhibition or oxidative inactivation of enzyme protein because of excess ROS generation. The generation of α-hydroxyethyl radical may lead to inactivation of these enzymes (Pigeolot et al., 1990) and accumulation of highly reactive free radicals also lead to deleterious effects such as loss of cell membrane integrity & membrane function (Krishnakanth and Lokesh, 1993).

There was a significant increase in the activity of these enzymes after raw garlic administration. It is reported that garlic suppresses the formation of superoxide anion and hydrogen peroxide by increasing the activity of SOD, CAT and GPx (Borek 2001). Therefore, garlic increases antioxidant action by scavenging ROS, enhancing the cellular antioxidant enzymes and increasing glutathione in the cells. Moreover, it has also been reported that garlic modulates the levels of lipid peroxidation (Hussein et al., 2007). Although multiple actions may take place during hepatoprotective activity, modulation of lipid peroxidation and antioxidant status may be one of the important mechanisms by which garlic exerts its toxic inhibitory effect.

Thus, our results suggest that, oral administration of raw garlic protects tissue damage by increasing the antioxidant status against oxidative stress. Hence, garlic plays a promising role in antioxidant and it can be considered as a potent drug for the treatment of alcoholic disorders. Further studies are needed to unravel the mechanism of action of garlic and its active components.

References


Krishnakanth TP and Lokesh BR. 1993. Scavenging of superoxide anions by spice principles. Ind J Biochem Biophys. 3: 133-134.


