Effect of camellia sinensis on liver enzymes and electrolyte homeostasis in liver cirrhosis

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Abstract

The present study was designed to evaluate the effects of green tea on different biochemical parameters in thioacetamide induced cirrhotic rats. For this purpose 24 male Sprague-Dawley rats were divided into four groups (n=6). Group I, remained healthy control rats Group II, received thioacetamide at a dose of (200mg/kg b.w,i.p, for 8 weeks, twice a week). Group III, received thioacetamide at a dose of (200mg/kg b.w. i.p,for 8 weeks, twice a week) and green tea at a dose of (500mg/kg b.w. orally, per day for 8 weeks). Group IV, received green tea at a dose of (500mg/kg b.w. orally, per day for 8 weeks). Biochemical analysis were evaluated by serum total and direct bilirubin, serum ALT, serum ALP and GGT activity. Serum electrolyte disturbances were evaluated by serum Na⁺, serum K⁺ and serum Ca²⁺. Marked increase in serum total and direct bilirubin and serum ALT, ALP, and GGT activity was the indicative markers of liver cirrhosis and the disturbance in serum electrolytes activity was also observed in cirrhotic rats. Green tea supplementation markedly reduced serum total and direct bilirubin, serum ALT, ALP and GGT activity and maintained the electrolyte homeostasis. These results indicate that green tea successively attenuates the thioacetamide induced liver cirrhosis.

Key words: Thioacetamide, Electrolytes, Green Tea, Liver Cirrhosis.

INTRODUCTION

Tea is included in the most popular drinks because of its perceived health effects and a pleasant taste. Since the beginning of its history, health benefits have been attributed to tea consumption. Scientific investigation of this beverage and its constituents has been under way for about 30 years (McKay and Blumberg.,2002; Gardner et al.,2007). Tea consumption, particularly green tea (GT), has been correlated with low incidence of chronic pathologies in which oxidative stress has been reported to be involved, such as cancer (Chung et al.,2003; Butt and Sultan.,2009) and cardiovascular diseases (Stangl et al.,2007; Babu and Liu.,2008). The health benefits described for the consumption of teas may be related to the high content of bioactive ingredients such as polyphenols. Polyphenols contain anti-inflammatory, antiviral and antioxidant activities, decrease platelets aggregation, stimulate immune function and modulate detoxification of enzymes (Lampe.,2003; Frankel and Finley.,2008). Epigallocatechin gallate (EGCG) is responsible for much of the health promoting ability of green tea among all tea polyphenols (Khan et al.,2006). The anticarcinogenic properties of green tea polyphenols, mainly EGCG, are likely a result of inhibition of tumor initiation and promotion, inhibition of cell replication rates and induction of apoptosis, thereby restricting the growth and development of neoplasm (Nihal and Hasan.,1999;Ahmad et al.,1886). Antioxidant potential of green tea polyphenols is directly related to the combination of hydroxyl groups and aromatic rings that build up their structure, and is a result of binding and neutralization of free radicals by the hydroxyl groups which prevents the progression of disease process (Serafini et al.,1996;Ichihashi et al.,2000). Green tea as an antioxidant is a popular neutraceutical. Cells are protected against the damaging effects of reactive
oxygen species, such as singlet oxygen, hydroxyl radicals, superoxide, peroxo radicals and peroxynitrite by antioxidants. An imbalance between reactive oxygen species and antioxidants causes oxidative stress which results in cellular damage (Halliwell and Gutteridge.,1985). Catechins are used to prevent these diseases with antioxidant vitamins (i.e., vitamins C and E) and enzymes (i.e., superoxide dismutase and catalase), to the total antioxidant defense system (Abdel-Raheim et al.,2009). Total plasma antioxidant activity is increases by green tea catechins (Yokozawa et al., 2002; Skrzydlewska et al.,2002). The expression of catalase in the aorta and the activity of superoxide dismutase in serum is also increases by consumption of green tea, these enzymes are involved in cellular protection against reactive oxygen species (Skrzydlewska et al.,2002; Negishi et al.,2004). After intake of green tea Malondialdialdehyde, a marker of oxidative stress is also decreases (Yokozawa et al.,1999). In low density lipoprotein green tea catechins increases vitamin E concentration (Tijburg et al.,1997), and thus protect low density-lipoprotein against peroxidation (Yokozawa et al.,2002). The role of green tea is very important in antioxidant defense mechanism as well as in generation of damaged cells. In views of above mentioned previous studies it is hypothesized that cirrhosis of the liver could be prevented by the supplementation of green tea. The present study was designed to examine the protective role of green tea in thioacetamide induced liver cirrhosis in experimental rat’s model.

MATERIALS AND METHODS

Total 24 male Sprague-Dawley rats weighing 200-250gm were purchased from the animal house of Aga Khan University Hospital, Karachi, Pakistan for the study . Animals were acclimatized to the laboratory conditions before the start of experiment and caged in a quiet temperature controlled animal room (23±4°C). Rats had free access to water and standard rat diet throughout the experimental period except 24 hours prior to decapitation.

Ethical guidelines: The experiments were conducted with ethical guidelines of ERB (Ethical Review Board) and internationally accepted principles for laboratory use and care in animal research (Health research extension Act of 1985).

Study Design: 24 Male Sprague-Dawley rats were randomly divided into four groups, each of six rats. The duration of the study was 8 weeks. Each group received following treatment:
- Group I: the control (remained untreated).
- Group II: TAA-treated
- Group III: TAA+Green tea treated
- Group IV: Green tea treated

Group I was the control group and remained untreated and was weighed every week. Group II was the TAA treated group, received thioacetamide at a dose of 200gm/kg b.w, intraperitoneally, twice a week, for 8 weeks. Group III was the TAA+ Green tea treated group, received thioacetamide at a dose of 200mg/kg b.w, twice a week, for 8 weeks and received green tea 500mg/kg b.w, orally, for 8 weeks daily. Group IV received only green tea at a dose of 500mg/kg b.w, orally for 8 weeks and weighed every week. After 24 hours of last dose of treated groups, rats were decapitated and the blood was collected from the neck wound in the lithium heparin coated tubes. The collected blood was mixed gently and then transferred to centrifuged glass tubes and then centrifuged at 2000 rpm for 20 minutes. Serum was separated and collected in eppindroff tubes and stored at -70°C until analysis. Liver was excised, trimmed of connective tissues, rinsed with saline to eliminate blood contamination dried by blotting with filter paper and weighed. The remaining tissues then kept in freezer at -70°C until analysis.

Estimation of Serum ALT, ALP, GGT and total and direct bilirubin: Serum ALT, ALP, GGT and total and direct bilirubin were analyzed using commercially prepared reagent kits from Randox.

Assessment of electrolyte homeostasis: Estimation of serum sodium and potassium: From the main calibrating solution (containing 143mM sodium, 3.8mM potassium), working standard solutions (containing 0.5-3.0mg sodium and potassium) were prepared by diluting main calibration solution with 0.1N HCl in varying proportion. Serum was diluted 1:100 with 0.1 N HCl and was used for simultaneous determination of sodium and potassium. The emission intensities of standards and samples were recorded against the respective blank solutions. The composition of the blank solution was same as that of main calibration except that it did not contain the element to be tested. The emission intensities of sodium, potassium were recorded at 589, 768nm respectively.

Estimation of serum calcium ion by ISE Method Jenway (Ion Meter 3345): Serum Calcium is estimated by ion selective electrode (ISE) using ion meter 3346 (Jenway). The method is followed by Jenway’s available manual operating procedure. A series of standard (concentration range 10 ppm and 100 ppm), from stock solution (1000 ppm) was prepared by adding ISAB (2M KCl) (ionic strength adjusting buffer). The electrode was rinsed with deionized water and blotted dry. The electrode was then placed in the standard, and the value was estimated by ion selective electrode (ISE) using ion meter 3345 (Jenway). The method is followed by Jenway’s available manual operating procedure. A series of standard (concentration range 10 ppm and 100 ppm), from stock solution (1000 ppm) was prepared by adding ISAB (2M KCl) (ionic strength adjusting buffer). The electrode was rinsed with deionized water and blotted dry. The electrode was then placed in the standard, and the value was
obtained until the reading was stable and then recorded, the electrode was then rinsed and blotted again and proceeded it for subsequent standards from lower to higher concentration. For sample preparation 1.7ml of deionized water, 0.2ml of ISAB and 0.1 ml of serum was taken in a clean glass tube, mixed well. The electrode was placed in sample and the value was observed until the reading was stable. The value was recorded as mg/ml.

**Statistical Analysis:** Results are presented as mean ± SD. Statistical significance and difference from control and test values were evaluated by student’s t-test. *P-values of P<0.01 and *P<0.05 were considered significant.

**RESULTS**

**Effect of thioacetamide and green tea treatment on liver weight and liver to body weight ratio in control and treated rats:** Increased liver weight and relative liver weight was observed in TAA group after 8 week administration of TAA as compare to control (5.01±0.1, P<0.01) (0.033±0.001 P< 0.01) (Table 1) where as reduction in the liver weight and relative liver weight was observed in TAA + Green tea group as compare to control (4.6±0.15 P<0.01) (0.036±0.001 P<0.001) respectively. An increase in liver weight was observed in green tea treated rats (5.0±0.34 P<0.01) as compare to control where as relative liver weight was almost normal (0.036±0.001 P<0.01) as compare to control.

**Effect of thioacetamide and Green tea treatment on serum total bilirubin in control and treated rats:** Table 2(figure 1) shows a marked increase in total bilirubin level in TAA-treated group as compare to control (946±21, P<0.01). While green tea supplementation decreased the levels of ALP in TAA + Green tea treated group (854±13, P<0.01). Alone green tea had no significant effect on serum ALT activity. Table 2(Figure 3).

**Effect of thioacetamide and green tea treatment on serum ALP activity in control and treated rats:** Serum Alkaline phosphatase activity was increased in TAA treated group as compare to control (946±21, P<0.01). While green tea supplementatio decreased the levels of ALP in TAA + Green tea treated group as compare to control (854±13, P<0.01). Alone green tea had no significant effect on ALP activity. Table 2(figure 4).

**Effect of thioacetamide and green tea treatment on serum GGT activity in control and treated rats:** Gamma glutamyl transferase activity was markedly increased in TAA-treated group as compare to control (820.3±57.18, P<0.01). Alkaline amino tranferase levels was significantly decreased in TAA + Green tea treated group as compare to control (212.7±14.5, P<0.01). Alone green tea had no significant effect on serum ALT activity. Table 2(Figure 4).

**Effect of thioacetamide and green tea treatment on serum Na+ levels in control and treated rats:** Table 3 (figure1) shows a marked decrease in serum sodium in TAA treated group as compare to control (124±1.0, P<0.01). Serum sodium level was also decreased in TAAA+ Green tea treated group as compare to control (109±0.7, P<0.01), and in green tea group (104±0.8, P<0.01) as compare to control.

**Effect of thioacetamide and green tea treatment on serum K+ levels in control and treated rats**

Serum potassium level was decreased in TAA treated group as compare to control (5.2±0.2, P<0.01). Green tea supplementation increased those decreased levels to the normal levels as compare to control (6.0±0.2, P<0.01) in TAA + Green tea group. Serum potassium levels were normal in green tea treated group. Table 3(Figure 2).

**Effect of thioacetamide and green tea treatment on serum Ca++ levels in control and treated rats:** Serum calcium levels was increased in TAA treated group as compare to control (0.5±0.01, P<0.01). Green tea supplementation decreased those levels almost to the normal level (0.4±0.01,
DISCUSSION

The present study describes the long term administration of TAA resulted in the development of severe liver injury in rats. Our study indicates an altered liver enzyme activity (ALT, ALP, GGT), and Total and Direct bilirubin levels, which strongly indicates liver tissue injury. This may be due to the damaged structural integrity of the liver, because they are cytoplasmic in location and are released into the circulation after cellular damage (Recknagel et al., 1989). The concentration of direct bilirubin was increased in thioacetamide treated group which may be due to decreased secretion from the liver or blockage of bile ducts, increased production, decreased uptake by the liver, or decreased conjugation (Bun et al., 2006). These results may be attributed to the effect of thioacetamide which interferes with the movement of RNA from the nucleus to the cytoplasm which may cause membrane injury resulting in a rise in serum liver markers (Saraswat et al., 1996). Dashti reported that thioacetamide administration is easy and reliable for the induction of liver cirrhosis in experimental animal models and Muller reported that resulting disease resembles the human cirrhosis. Previous studies showed that thioacetamide induced liver cirrhosis can be prevented by the use of radical scavengers and antioxidants. For this purpose we chose green tea for the treatment. The health benefits described for the consumption of green tea may be related to the high contents of bioactive ingredients such as polyphenols which contain anti-inflammatory, antiviral and antioxidant activities. It also decreases platelet aggregation, stimulate immune function and modulate detoxification of enzymes (Lampe., 2003; Frankel and Finely., 2008). Administration of green tea extracts in cirrhotic rats causes a significant decrease in the level of serum bilirubin. These results are in agreement with the studies of (Pyo et al., 2004), who reported that green tea increase the biliary flow and bile helps to eliminate the bile salts, fat toxins from the body as an antioxidant and peroxidant by scavenging reactive oxygen species via enzymatic and non enzymatic reactions. The increased level of liver enzymes was also decreased by green tea extracts in cirrhotic rats. In 2007 Khorsandi et al, showed that the oral consumption of green tea extracts affects severe poisoning of liver due to acetaminophen, improves liver necrosis and decreases serum transaminases, which agrees with the results of this study. Our study also showed decreased levels of serum Na+ and serum K+. The importance of serum ionic Na+ and K+ is correlated with their involvement in many vital activities of cells and tissues where they are actively transported through cell membranes, beside their role in muscle contraction and nerve impulse conduction. Kattab et al., 2003, correlated these changes in Na+ and K+ content with cell membrane damage which lead to disturbance in Na+ and K+ pumping and disorder in membrane permeability. Serum Ca++ levels was increased in our study. The increased level of intracellular Ca++ shutdown the Na+ – K+ ATPase, leading to a disturbance in serum Na+ and K+ levels (Joachim et al., 1999). Green tea administration in cirrhotic rats has no significant effect on serum Na+ levels, while it decreased serum K+ levels to the normal level and also decreases the Ca++ levels significantly in our study. The restorage of the serum Total and Direct bilirubin level, ALT, ALP and GGT activity (Table 2) and maintenance of electrolytes (Table 3), indicates that green tea may play an important role in treatment of liver cirrhosis.

Table 1: Liver weight, liver to body weight ratio in control and treated rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Liver Weights</th>
<th>Relative Liver Weights</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.21±0.31*</td>
<td>0.033±0.001*</td>
</tr>
<tr>
<td>TAA-treated</td>
<td>5.01±0.2*</td>
<td>0.04±0.001*</td>
</tr>
<tr>
<td>TAA + Green tea treated</td>
<td>4.6±0.15*</td>
<td>0.036±0.001*</td>
</tr>
<tr>
<td>Green tea treated</td>
<td>5.0±0.34*</td>
<td>0.031±0.003*</td>
</tr>
</tbody>
</table>

n=6; Values are mean ± SD. Significant difference among control, thioacetamide, thioacetamide + green tea-treated and thioacetamide-treated groups by t-test **P<0.05, *P<0.01.
Zaidi et al., World J Pharm Sci 2014; 2(9): xxxx

Table 2: Effects of thioacetamide and green tea treatment on serum bilirubin ALT, ALP and GGT activity in control and treated rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>TAA</th>
<th>TAA+Green tea</th>
<th>Green tea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total bilirubin (U/L)</td>
<td>0.47 ± 0.04</td>
<td>2.17 ± 0.01</td>
<td>0.54 ± 0.01</td>
<td>0.76 ± 0.2</td>
</tr>
<tr>
<td>Direct bilirubin (U/L)</td>
<td>1.40 ± 0.2</td>
<td>2.30 ± 0.2</td>
<td>1.1 ± 0.02</td>
<td>1.3 ± 0.02</td>
</tr>
<tr>
<td>Alanine amino transferase (U/L)</td>
<td>199.6 ± 10.6</td>
<td>820.3 ± 57.18</td>
<td>212.71 ± 14.5</td>
<td>200.41 ± 10.1</td>
</tr>
<tr>
<td>Alkaline phosphatase (U/L)</td>
<td>946 ± 21</td>
<td>966 ± 21</td>
<td>854 ± 13</td>
<td>519 ± 10</td>
</tr>
<tr>
<td>Gamma Glutamyl Transferase (U/L)</td>
<td>08 ± 1.2</td>
<td>09 ± 1.1</td>
<td>06 ± 0.6</td>
<td>05 ± 0.3</td>
</tr>
</tbody>
</table>

n=6; Values are mean ± SD. Significant difference among control, thioacetamide, thioacetamide + green tea-treated and thioacetamide-treated groups by t-test **P<0.05, *P<0.01.

Table 3: Effect of thioacetamide and green tea on serum electrolytes activity in control and treated rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>TAA</th>
<th>TAA + Green tea</th>
<th>Green tea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum Na+</td>
<td>139.7 ± 1.2</td>
<td>124 ± 1.0</td>
<td>109 ± 0.7</td>
<td>104 ± 0.8</td>
</tr>
<tr>
<td>Serum K+</td>
<td>6.15 ± 0.3</td>
<td>5.2 ± 0.2</td>
<td>6.01 ± 0.2</td>
<td>4.9 ± 0.09</td>
</tr>
<tr>
<td>Serum Ca++</td>
<td>0.3 ± 0.1</td>
<td>0.5 ± 0.1</td>
<td>0.4 ± 0.01</td>
<td>0.37 ± 0.01</td>
</tr>
</tbody>
</table>

n=6; Values are mean ± SD. Significant difference among control, thioacetamide, thioacetamide + green tea-treated and thioacetamide-treated groups by t-test **P<0.05, *P<0.01.

REFERENCES
