Protective Effects of Kombucha Tea and Silimarín Against Thioacetamide Induced Hepatic Injuries in Wistar Rats

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Abstract: Plants consumed by human contain thousands of phenolic compounds. The effects of dietary polyphenols is of great interest due to their antioxidative and possible anticarcinogenic activities. Liver is one of the organ in the body exposed to many of oxidant and carcinogen agents; therefore; the antioxidant compounds are beneficial for liver health. In this study the hepatoprotective effects of Kombucha tea and silimarín and thioacetamide (TAA) induced liver toxicity in wistar rats are investigated and compared. In this study we used 36 male white wistar rats groups of six: (control group, group of treated with thioacetamide (TAA) for 3 weeks, group treated with TAA and Kombucha tea (for 3 weeks), groups treated with Kombucha tea (for 3 weeks) and TAA, group of treated with TAA and silimarín (for 3 weeks), group of treated with TAA, silimarín and Kombucha tea (for 3 weeks). The level of serum aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP) Lactate dehydrogenase(LDH), total bilirubin monitored to assess hepatotoxicity and hepatoprotection. The data here showed that TAA significantly has increase AST, ALT, ALP, LDH but not the same for bilirubin. The treatment by silimarín and Kombucha tea produced a significant reduction in serum enzyme levels (AST, ALT, ALP, LDH ) and reduction in bilirubin content. The results show that the protective effects of silimarín and Kombucha tea against the thioacetamide induced hepatotoxicity that may be due to the existence of polyphenol substances in the plants, these substances have an antioxidative function.

Key words: Hepatoprotective · Thioacetamide · Silimarín · Kombucha tea · Rat

INTRODUCTION

Liver is the most important organ, which plays a pivotal role in regulating various physiological processes in the body. The liver is involved in several vital function in human metabolism. Therefore any damage to the liver indicated by hepatotoxic agents is of grave consequences [1, 2]. Liver cirrhosis associated with various pathological processes, is characterized by progressive fibrosis producing liver injury, portal hypertension and carcinoma [3]. TAA is a commonly used chemical compound that induces experimental liver fibrosis that mimics human liver cirrhosis. 4. Thioacetamide is a typical hepatotoxin causing centrilobular necrosis [5-7]. Ledda-Columbano et al. (1991) reported that thioacetamide induced apoptosis in the rat liver based on histochemical observations. 8 Some plants are known to possess antitumor, [9, 10] hypoglycaemic, [11] antibacterial. [12, 13] free radical scavenging and lipid peroxidation [14] propertice. Silybum marianum belongs to, Family Asteraceae and is known as herbal medicine with hepatoprotective effects, even documented in ancient literature regarding liver disease [15, 16] Chemically.

Active constituent of milk thistle extract is basically a flavolignan, silymarin, which in itself represents the mixture of four isomeric flavonoids such as silibinin, isosilibinin, silydianin and silychirstin. [17] Silibinin is
the major and most active component in silymarin, [8] A number of other flavonolignans have also been found in majority of seeds. [17] Silymarin is reported to have antifibrotic, antiinflammatory, immunomodulating and other properties. [19-22] Kombucha is a traditional fermented beverage with a history of several thousand years in the East and yet is quite popular today in the West. [23-25] Numerous studies refer to Kombucha’s antimicrobial propertice and suggest that it might influence the gastro-intestinal microbial flora of human. [25-28] The beneficial propertice of kombaucha tea is attributed to the presence of tea polyphenols, gluconic acid, glucuronic acid, lactic acid, vitamins, aminoacids, antibiotics and a variety of micronutrients produced during fermentation. [29] This beverage is reported to have medicinal effects against metabolic diseases, arthritis, indigestion and various types of cancer. [27] Recent studies suggest that kombucha tea prevents paracetamol induced hepatotoxicity [30] and chromate (VI) induced oxidative stress in albino rats. [31] In this study the attempt is made to evaluate the iver injury in rats induced by thioacetamide.

MATERIALS AND METHODS

Wistar male rats (250-200 g of body weight), bred and rised in the animal house in Isfahan University were used. Animals were kept under controlled teperture (23± 2 °C) and 12:12 –h light-dark cycle conditions.

The rats were then randomly divided into 6 groups (6 animals per group).

Group 1: Control group

Group 2: Injected (i.p.) with TAA (400 mg/kg b.w) for 2 weeks.

Group 3: Injectd with TAA (400 mg/kg b.w) and then kombucha tea (50 ml each rat, for 3 weeks)

Group 4: Kombucha tea (each rat for 3 weeks) and then injectd with TAA (400 mg/kg b.w)

Group 5: Injectd with TAA (400 mg/kg b.w) and then silimarin(200 mg/kg b.w) (for 3 weeks).

Group 6: Injectd with TAA (400 mg/kg b.w) and then kombucha(50 ml each rat)+ silimarin (400 mg/kg b.w) for 3 weeks.

At the end of experiment, the rats are killed in order to collecte samples of serum and liver. The study was reviewed and approved by the Ethics Committee of Isfahan University, Medical Sciences.

Biochemical Examination: Serum samples from rats in groups 1 to 4 were analyzed for alanin aminotransferase (ALT), aspartataminotransferase (AST), alkalinphosphatase (ALP), Lactate dehydrogenase (LDH), total cholesterol (Ch), LDL-cholesterol (LDL-C), HDL-cholesterol (HDLC) and triglyceride (TG) and were measured applying special kits (DiaSys, Germany) which utilized the colorimetric method, in an autoanalyzer (Hitachi autoanalyzer, Hitachi Co., Tokyo) here the bilirubin was measured as well.

Histopatological Examination: Animals in each group were killed at the end of third week. The liver was removed rapidly and fixed in 10 % formalin; they were then dehydrated and paraffin embedded. Two-or-three-micron-thick tissues sectioned were cut. The sectioned tissues were routinely stained with hematoxilen and eosin for quantitative assement of liver injuries. This assessment was performed a numerical scoring system based on injuries induced by TAA.

Statistical Analysis: All values were expressed as mean±SD. Significant differences among the groups were determined by one-way ANOVA using the SPSS 13.0 software package program. Values of p< 0.05 were considered as statistically significant.

RESULTS

Daily access to food and water was observed to be the same during the test period and significant deference in body weight gain was observed in control group, groups 4 and 6 in compared to the group of treated with thioacetamide at the end (Table1).

Rats treated with Thioacetamide (TAA) developed significant hepatic impairment as observed from elevated serum levels of hepatospecific enzymes AST, ALT, ALP and LDH (Table 1). Serum bilirubin level also enhanced by TAA treatment. Serum total cholesterol (Ch), HDL-cholesterol (HDLC) and triglyceride(TG) levels were considerably reduced and LDL-cholesterol (LDL-C) increased by TAA treatment (Table 1). Plasma AST, ALT, ALP and LDH were decreased in groups 3and 4 treated with Kombucha tea compared to the group of treated with
Table 1: Effect of TAA, Kombucha tea and silimaril treatment on rat body weight, liver weight and biochemical parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>control</th>
<th>TAA</th>
<th>Kombucha+TAA</th>
<th>TAA+Kombucha</th>
<th>TAA+Silimaril</th>
<th>TAA+Kombucha+Silimaril</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT(U/L)</td>
<td>148±26.058</td>
<td>767±16.971*</td>
<td>106.75±208.290**</td>
<td>126.67±3.055**</td>
<td>275±14.863**</td>
<td>125±29.103**</td>
</tr>
<tr>
<td>AST(U/L)</td>
<td>184.33±62.324</td>
<td>653±53.033*</td>
<td>130.75±81.2**</td>
<td>130.6±14.572**</td>
<td>354±11.899**</td>
<td>203.67±41.429**</td>
</tr>
<tr>
<td>ALP(U/L)</td>
<td>614.67±196.398</td>
<td>1593.50±214.253*</td>
<td>1261.50±261.439**</td>
<td>947.33±7.506**</td>
<td>836.50±19.122**</td>
<td>613.33±1.528**</td>
</tr>
<tr>
<td>LDH(U/L)</td>
<td>1116.33±74.272</td>
<td>1269.50±6.364*</td>
<td>671.75±127.241**</td>
<td>808±191.909**</td>
<td>819.50±66.837**</td>
<td>741.67±133.717**</td>
</tr>
<tr>
<td>TG(mg/dl)</td>
<td>29.33±16.563</td>
<td>11±1.414</td>
<td>31.75±9.878</td>
<td>26.33±1.528**</td>
<td>39.50±4.123</td>
<td>25.67±1.155**</td>
</tr>
<tr>
<td>cho(mg/dl)</td>
<td>88.67±13.051</td>
<td>64.50±3.536*</td>
<td>83±21.556</td>
<td>113.33±1.528</td>
<td>89.5±2.517**</td>
<td>113±19.165**</td>
</tr>
<tr>
<td>LDL(mg/dl)</td>
<td>10±2.646</td>
<td>16±0.0</td>
<td>9.80±10.863</td>
<td>25.67±8.145</td>
<td>28±1.708**</td>
<td>20.33±0.577**</td>
</tr>
<tr>
<td>HDL(mg/dl)</td>
<td>42.33±5.859</td>
<td>30±5.657</td>
<td>41±9.933</td>
<td>59.33±16.258</td>
<td>56.25±6.551**</td>
<td>74.67±3.786**</td>
</tr>
<tr>
<td>Bilirubin(mg/dl)</td>
<td>0.483±0.028</td>
<td>1.05±0.63</td>
<td>0.45±0.04</td>
<td>0.5167±0.028</td>
<td>0.45±0.108</td>
<td>0.4767±0.025</td>
</tr>
<tr>
<td>BW (g)</td>
<td>244.76±32.712</td>
<td>191.93±10.909*</td>
<td>208.55±26.344**</td>
<td>186.59±18.763</td>
<td>191.09±17.666</td>
<td>230.08±17.910**</td>
</tr>
<tr>
<td>LW (g)</td>
<td>8.47±0.32</td>
<td>7.38±0.72</td>
<td>9.24±0.88</td>
<td>7.87±0.52</td>
<td>7.4±0.98</td>
<td>8.04±0.27</td>
</tr>
<tr>
<td>LW/BW (%)</td>
<td>3.46±0.2</td>
<td>3.84±0.1</td>
<td>4.43±0.3</td>
<td>4.24±0.3</td>
<td>3.87±0.1</td>
<td>3.49±0.4</td>
</tr>
</tbody>
</table>

Fig. 1: Hepatoprotective effect Kombucha tea and silimaril against Thioacetamide induced acute hepatotoxicity in rats. Liver sections were stained with H&E: (a) normal; (b) TTA; (c) TAA and then kombucha tea (d) : kombucha tea and then TAA (e): TAA and then silimaril(200 mg/kg b.w) (f): TAA and then kombucha+ silimaril, Magnification 40.

ba: Abnormal mitosis, bb: Cells with apoptosis, bc: Mitotic cells bd:Inflammation around the port area

** P > 0.05 vs. TAA
thioacetamide and were even comparable with the control group regarding a reduction in bilirubin content (Table 1). Treatment with silymarin for 3 weeks offered protection against TAA induced elevation in levels of in liver enzymes, bilirubin in serum as evidenced by a fall in its levels. The treatment by silimar reduced serum enzyme levels (AST, ALT, ALP, LDH) and the bilirubin content (Table 1).

Histopathological Observations: Histology of the liver sections of normal control animals (Group I) showed normal hepatic cells with well preserved cytoplasm, prominent nucleus and nucleolus and well brought out central vein (Fig. 1). Histopathological studies demonstrated that that group 2 exposed to TAA, compared to the normal group causes focal necrosis, increases mitosis at cells, apoptosis, abnormally mitosis, inflammation at portal space and enlarged nucleus (Fig. 1). Necrosis which is a more severe form of injury is markedly prevented by silimar in especially by 200 mg:kg body weight. In the group, treated by silimar, the number of cells still have large nucleus but apoptosis and mitosis are not observed apoptosis and mitosis (Fig.1) and prevention groups of Kombucha tea +TTA and TTA+ Kombucha tea, showed normal histology of liver sections.

DISCUSSION

Thioacetamide (TAA) is a classic hepatotoxic reagent used for liver cirrhosis induction. [32] TAA is hepatotoxic and effects DNA, RNA, protein synthesis and glutathione content, which, in turn, induces intrahepatic metabolic changes. [33, 34] In this study TAA administration to rats for 10 days has been observed to cause necrosis, increase mitosis at cells, apoptosis, abnormally mitosis, inflammation at portal space and enlarge nucleus as assessed histopathologically. Liver injury was also determined by biochemical parameters (plasma AST, ALT, ALP, LDH levels). Free radicals are believed to play a major role in the development of TAA induced liver cirrhosis. [35-38] TAA in reduction of the number and function of mitochondria in hepatocytes of cirrhotic rats is considered to cause uncoupling in oxidative phosphorylation leading to accumulation of NADH and lactate and diminish energy synthesis rate. It is also suggested to decrease hepatic protein synthesis in cirrhosis, since most of the cell energy is used in the process. [39] Steinkraus et al and Dhanalakshmi et al reported that apoptosis caused by thioacetamide involving the activation of caspase-3 along with extensive necrosis. [38, 40] Different antioxidants are shown to prevent liver fibrosis and cirrhosis. [36, 41-45] Studies have shown that diets rich in fruits and vegetables are associated with lower risk of cancer, suggesting that cancer risk could be modified/prevented by making appropriate dietary manipulations. [12, 46-48] Silymarin is a popular herbal product marketed to treat liver disorders. [49] Silymarin and quercetin are polyphenolic flavonoids that increase cell resistance to lipid peroxidation. [50-52] Silymarin has been in extensive use for its hepatoprotective effects since ancient times, though other health beneficial effects of it are being recognized in recent years. Most of these effects are attributed to direct and/or indirect antioxidant property of silymarin, such as being a scavenger of reactive oxygen species, a scavenger of phenylglyoxylic ketyl radicals and a chain breaking antioxidant. [53] Silymarin is one such agent, which has been in extensive use for ages for liver treatment and thus has possibly the greatest patient acceptability. In recent years increasing body of evidence has underscored the cancer preventive efficacy of silymarin both in vitro and in vivo animal models of various epithelial cancers. [54] Posttreatment with the silymarin (50 mg/kg) orally for 30 days significantly reversed the diethylnitrosamine induced alterations in the liver tissue and offered almost complete protection. The results obtained this study indicate that silymarin exhibits good hepatoprotective and antioxidant potential against diethylnitrosamine induced hepatocellular damage in rats. [55] In recent years, findings of the studies have in part shade more light on certain aspects of hepatoprotective mechanism of silymarin. Silibinin, an active constituent of silymarin, also affords protection against T cell-dependent hepatitis through the modulation of immune response. [56] There are sufficient evidences that silymarin/silibinin possesses anticancer efficacy and additionally, as discussed further, preventive efficacy in various in vivo models of epithelial cancers. [57] The Results of the study conducted by Singh et al (2006) revealed the antiangiogenic potential of silibinin in animal model of lung tumorigenesis. [58] The in vivo model established that at the examined dose, silymarin exhibits a protective influence on the heart and liver tissue against toxicity induced by doxorubicin. [59] In animal experiments, silymarin and silibinin are shown to protect rat or mouse liver against hepatotoxicity induced by acute ethanol intoxication, [60] thioacetamide, [61] thallium, [62] D-galactosamine, 63 and acetaminophen, [64-66] as well as preventing a wide range of carcinogen and tumor promoter-induced cancers. [67] Silybin,
isolated from the milk thistle plant (Silybum marianum), is widely used as a therapeutic agent for a variety of disease, including human colon cancer, liver disease and prostate cancer. [68-70] Previous studies have indicated that silybin suppresses the growth of human prostate carcinoma PC-3. [71] Silybin inhibits cell proliferation, survival and angiogenesis in established prostate tumor it also, suppresses the levels of angiogenic growth factors and receptor and molecular events driving invasion and EMT in prostate tumor. [72, 73] Kombucha converts sucrose to glucose and fructose and further to ethanol, acetic acid, lactic acid and a large number of other compounds. [74] In conducted studies it is indicated that the daily consumption of kombucha is related to an extremely high resistance to cancer. [23] The beneficial effects of kombucha tea is attributed to the presence of tea polyphenols, gluconic acid, glucuronic acid, lactic acid vitamins, aminoacids, antibiotics and a variety of micronutrients produced during fermentation. [29] Kombucha tea treatment, however, counteract to the changes in mitochondrial membrane potential and prevented apoptotic cell death of the hepatocytes. Study Bhattacharya et al (2011) showed that kombucha tea has the potential to ameliorate tertiary butyl hydroperoxide induced oxidative insult and cell death in murine hepatocytes more effectively than fermented black tea. [75] Kombucha is a potent antioxidant proved to reduce the damage induced by oxidative stress. [31, 76-78] The antioxidant properties of Kombucha constituents and the protective effects of Kombucha on oxidative stress induced nephrotoxicity of Trichloroethylene treated rats (Effects of Kombucha on oxidative stress induced nephrotoxicity in rats. [77] Kombucha tea prepared by fermentation of black tea with tea fungus could be used as a preventive and urative agent against CCl4-induced hepatotoxicity. As kombucha tea is rich in compounds known to be strong antioxidants and is expected to ameliorate liver damage induced by CCl4. [79] Recent studies have suggested that kombucha tea prevents paracetamol induced hepatotoxicity [30] and chromate (VI) induced oxidative stress in albino rats. [31] Gluconic acid is considered by several reseachers to be the main therapeutic agent in kombucha, is considered as its function in the liver is considered as a detoxification agent [34, 80-81].

In this context a rise is observed in the levels of AST, ALT, ALP, LDH and bilirubin in thioacetamide treated rats. The Kombucha tea and silimar stock used in this study seems to offer protection and maintain the structural integrity of hepatic cells. The protective effects are more pronounced and much better when the rats are treated with Kombucha tea and silimar stock. This was evident from the significant reduction in serum AST, ALT, ALP, LDH and bilirubin content. Based on the above findings it could be concluded that Kombucha tea and silimar stock proved a better antihapatotoxic activity against thioacetamide induced hepatic damage. More elaborate work is required to establish the efficiency of Kombucha tea and silimar stock potent antihapatotoxic drug. Further experimental work is necessary to isolate and identify the active properties in the Kombucha tea and silimar stock which are responsible for the antihapatotoxic activity.

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