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Protective Effect of Kombucha Tea on Liver Damage Induced by Thioacetamide in Rats

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Abstract: The aim of this study is to evaluate the possible protective effects of Kombucha tea against thioacetamide induced liver damage in rats. A total of 24 male Wistar rats were divided into four groups: Control, treated with thioacetamide (TAA) treated with TAA and then Kombucha tea, treated with Kombucha tea and then TAA; following 3 weeks of treatment. All the animals were killed and liver tissue samples were obtained for histopathological investigation. The data showed that TAA significantly increased aspartate aminotransferase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) but not bilirubin. The treatment by Kombucha tea promoted a significant reduction in serum enzyme levels AST, ALT, ALP, LDH and reduction in bilirubin content. The results show that the Kombucha tea has protective effects against the thioacetamide induced hepatotoxicity that might be due to antioxidant activities of these plants.

Key words: Hepatoprotective, thioacetamide, 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 functions in human metabolism. Therefore, any damage to the liver induced by hepatotoxic agents is of grave consequences. Liver cirrhosis associated with various pathological processes, is characterized by progressive fibrosis producing liver injury, portal hypertension and carcinoma (Shahani, 1999). Thioacetamide (TAA) is a commonly used chemical compound to induce liver fibrosis that mimics human liver cirrhosis (Aydin *et al.*, 2010). TAA is a typical hepatotoxin, causing centrilobular necrosis. It induces apoptosis in the rat liver based on histochemical observations (Ledda-Columbano *et al.*, 1991).

Medicinal plants have been used from ancient times for wide variety diseases (Khosravi-Boroujeni *et al.*, 2012; Shirzad *et al.*, 2011; Shamsi *et al.*, 2011) as well as for hepatotoxicity (Kazemi *et al.*, 2010; Heidarian and Rafieian-Kopaei, 2012) and renal toxicity (Rafieian-Kopaei and Nasri, 2013; Baradaran *et al.*, 2013; Nasri *et al.*, 2013) induced by other drugs. Recent studies have also shown promising effects on different complications such as hypoglycemic (Behradmanesh *et al.*, 2012), antibacterial (Bahmani *et al.*, 2013; Sharafati-Chaleshtori *et al.*, 2011), lipid peroxidation (Madihi *et al.*, 2013; Heidarian *et al.*,

2013) and cancer (Shirzad *et al.*, 2009) prevention and these effects have mostly been attributed to their antioxidants and radical scavenging properties (Rafieian-Kopaei *et al.*, 2013a; Rafieian-Kopaei, 2012; Setorki *et al.*, 2012).

Kombucha tea is a traditional fermented beverage lightly sweetened effervescent drink of black tea that is produced by fermenting the tea using a symbiotic colony of yeast and bacteria (Teoh *et al.*, 2004). Kombucha is with a history of several thousand years in the East and yet is quite popular today in the West (Teoh *et al.*, 2004). Numerous studies refer to Kombucha's antimicrobial properties suggests that; it might influence the gastro-intestinal microbial flora of human body (Sreeramulu *et al.*, 2001). The beneficial properties of Kombucha tea is attributed to the presence of tea polyphenols, gluconic acid, glucuronic acid, lactic acid, vitamins, amino-acids, antibiotics and variety of micronutrients produced during fermentation. This beverage is reported to have medicinal effects against metabolic diseases, arthritis, indigestion and various types of cancer (Sreeramulu *et al.*, 2001). Recent studies suggest that Kombucha tea prevents paracetamol induced hepatotoxicity and chromate (VI) induced oxidative stress in rats (Pauline *et al.*, 2001). In this study the hepatoprotective effects of Kombucha tea were evaluated against TAA induced liver toxicity in Wistar rats.

MATERIALS AND METHODS

One hundred grams of sugar was added to 1 L of distilled water and the solution was boiled for 15 min in a sterile conical flask. Black tea powder (Lipton is a brand of tea) was added to the flask (12 g L^{-1}), which was then left to cool down at room temperature for 1 h. The mixture was filtered using a sterile nylon mesh and the filtrate was used as black tea. The kombucha cultures were purchased from a Exxon Company Isfahan. The cultures were stored at 4°C prior to fermentation. Black tea was poured into 1 L glass jars, which were sterilized and inoculated with 2.5% (w/v) of freshly grown kombucha mat that had been grown and maintained in the same medium. The fermentation, kept under aseptic conditions, was carried out by incubating the kombucha culture at $28\pm 1^\circ\text{C}$ for 12 days. The flask was covered with clean cheese cloth and fixed with rubber bands. The medium (brew) was then centrifuged aseptically at $1500\times g$ for 30 min and stored in polypropylene vials at -20°C for further use. New kombucha mat developed from the mother culture.

Wistar male rats (250-200 g b.wt.), with free access in bread and tap water in the animal house in Isfahan University of Medical Sciences were used. Animals were kept under controlled temperature ($23\pm 2^\circ\text{C}$) and 12:12-h light-dark cycle conditions. Twenty four male Wistar rats were randomly selected to designate six equal groups as follows:

Group 1: Control group

Group 2: Injected (i.p.) with TAA (400 mg kg^{-1} b.wt.) for 2 weeks

Group 3: Injected with TAA (400 mg kg^{-1} b.wt.) and then Kombucha tea (50 mL kg^{-1} each rat, for 3 weeks)

Group 4: Kombucha tea (each rat for 3 weeks) and then TAA (400 mg kg^{-1} b.wt.)

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

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

Histopathological examinations: 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 to three micron thick tissues were cut and sectioned. The sectioned tissues were routinely stained with Hematoxylin and Eosin for quantitative assessment 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 \pm SD. Significant differences among the groups were determined by one-way ANOVA using the SPSS 13.0 software package program. $p < 0.05$ was considered as statistically significant.

RESULTS

Results are shown in Table 1. Significant decrease in body weight was observed in thioacetamide group in comparison to control group which restored in the group 4 (which received Kombucha tea and then TAA) in comparison to the group treated with thioacetamide (Table 1).

Rats treated with TAA developed significant hepatic impairment as observed from elevated serum levels of hepatospecific enzymes AST, ALT, ALP and LDH (Table 1). Serum bilirubin level was also enhanced by TAA treatment. Serum total cholesterol (Ch), HDL-cholesterol (HDL-C) and 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 group 3 and 4 treated with Kombucha tea (respectively before and after TAA administration), compared to thioacetamide treated group and were even comparable with the control group regarding a reduction in bilirubin content (Table 1).

Histopathological observations: Histology of the liver sections of normal control animals (Group 1) showed normal hepatic cells with well preserved cytoplasm, prominent nucleus and nucleolus and well brought out central vein (Fig. 1). Histopathological studies demonstrated that animals in group 2 which were exposed to TAA showed focal necrosis, increased mitosis at cells, apoptosis, abnormally mitosis, inflammation at portal space and enlarged nucleus (Fig. 1). The prevention groups of kombucha tea+TAA and TAA+Kombucha tea, showed normal histology of liver sections.

Table 1: Effect of TAA, Kombucha tea treatment on rat body weight, liver weight and biochemical parameters

Parameters	Groups				
	Control	TAA	Kombucha+TAA	TAA+Kombucha	TAA+Silymarin
ALT (U L ⁻¹)	148±26.058	767±16.971*	106.75±208.290**	126.67±3.055**	275±14.863**
AST (U L ⁻¹)	184.33±62.324	653±53.033*	130.75±81.2**	130.60±14.572**	354±11.899**
ALP (U L ⁻¹)	614.67±196.398	1593.50±214.253*	1261.50±261.439**	947.33±7.506**	836.50±19.122**
LDH (U L ⁻¹)	1116.33±74.272	1269.50±6.364	671.75±127.241**	808±191.909**	819.50±46.837**
TG (mg dL ⁻¹)	29.33±16.563	11±1.414	31.75±9.878	26.33±1.528**	39.50±4.123
cho (mg dL ⁻¹)	88.67±13.051	64.50±3.536*	83±21.556	113.33±1.528	89.5±2.517**
LDL (mg dL ⁻¹)	10±2.646	16±0.0	9.80±10.863	25.67±8.145	28±1.708**
HDL (mg dL ⁻¹)	42.33±5.859	30±5.657	41±9.933	59.33±16.258	56.25±6.551**
Bilirubin (mg dL ⁻¹)	0.4833±0.028	1.05±0.63	0.45±0.04	0.5167±0.028	0.45±0.108
BW (g)	244.76±32.712	191.93±10.909*	208.55±26.344**	186.59±18.763	191.09±17.666
LW (g)	8.47±0.32	7.38±0.72	9.24±0.88	7.87±0.52	7.4±0.98
LW/BW (%)	3.46±0.2	3.84±0.1	4.43±0.3	4.24±0.3	3.87±0.1

Group 1: Control group, Group 2: Injected (i.p.) with TAA (400 mg kg⁻¹ b.wt.) for 2 weeks, Group 3: Injected with TAA (400 mg kg⁻¹ b.wt.) and then kombucha tea (for 3 weeks), Group 4: Kombucha tea (50 mL each rat, for 3 weeks) and then with TAA (400 mg kg⁻¹ b.wt.), Body and liver weights (BW and LW) were recorded at the time of killing. ALT: Alanin aminotransferase, AST: Aspartataminotransferase, ALP: Alkaliphosphatase, LDH: Lactate dehydrogenase, Cho: Total cholesterol, LDL-C: LDL-cholesterol, HDL-C: HDL-cholesterol and TG: Triglyceride, Data is expressed as the Mean±SD, BW, Body weight, LW: Liver weight, *p>0.05 vs. controls **p>0.05 vs. TAA

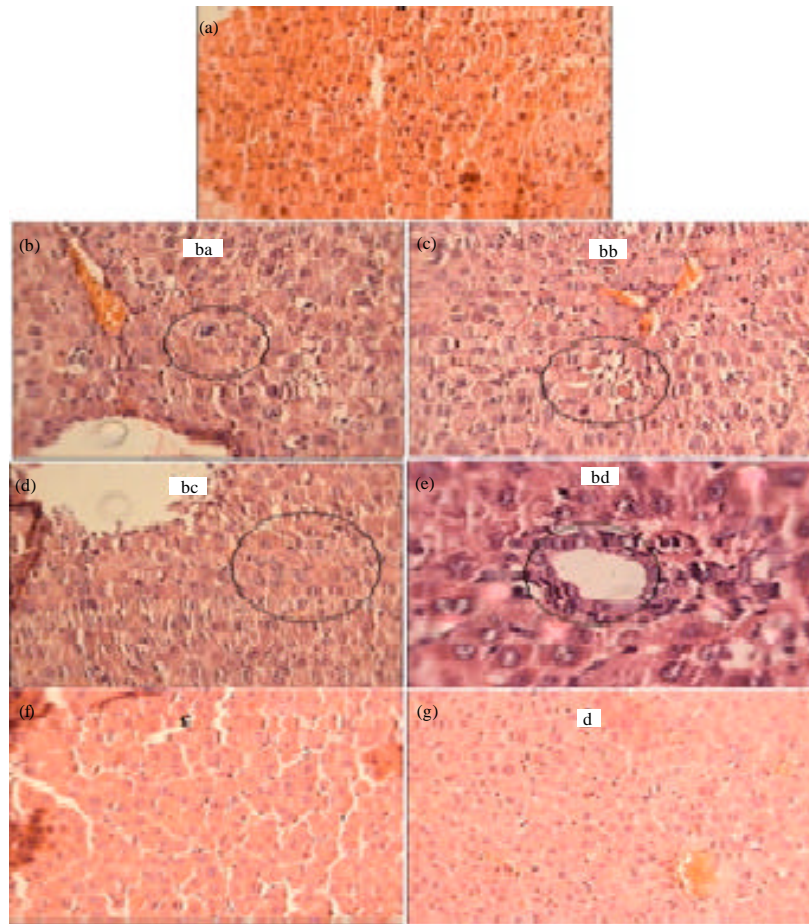


Fig. 1(a-g): Hepatoprotective effect Kombucha tea against Thioacetamide induced acute hepatotoxicity in rats. Liver sections were stained with H and E, (a) Normal, (b and c) TTA, (d and e) TAA and then kombucha tea and (f and g) kombucha tea and then TAA (Magnification 40), ba: Abnormal mitosis, bb: Cells with apoptosis, bc: Mitotic cells, bd: Inflammation around the port area

DISCUSSION

In this study the hepatoprotective effects of Kombucha tea were evaluated against TAA induced liver toxicity. TAA significantly increased AST, ALT, ALP, LDH but not bilirubin. The treatment by Kombucha tea promoted a significant reduction in serum enzyme levels (AST, ALT, ALP and LDH) and reduction in bilirubin content.

Thioacetamide is a classic hepatotoxic reagent used for liver cirrhosis induction. TAA is hepatotoxic and affects DNA, RNA, protein synthesis and glutathione content, which, in turn, induces intrahepatic metabolic changes (Ledda-Columbano *et al.*, 1991). In this study TAA administration to rats for 10 days, other than biochemical changes, caused histopathologic changes such as necrosis, increase mitosis at cells, apoptosis, abnormally mitosis, enlarge nucleus as well as inflammation.

Free radicals are believed to play a major role in the development of TAA induced liver cirrhosis (Ledda-Columbano *et al.*, 1991). TAA induced 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 (Nicotinamide adenine dinucleotide) and lactate and diminishing 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 (Ledda-Columbano *et al.*, 1991). It has been shown that apoptosis caused by thioacetamide is involved the activation of caspase 3 along with extensive necrosis (Perez *et al.*, 2004).

Different antioxidants are shown to prevent liver fibrosis and cirrhosis (Heidarian and Rafieian-Kopaei, 2013; Kazemi *et al.*, 2011; Rafieian-Kopaei *et al.*, 2013a). Studies have shown that diets rich in fruits and vegetables are associated with lower risk of cancer, suggesting that cancer risk can be modified/prevented by making appropriate dietary manipulations (Rafieian-Kopaei *et al.*, 2013b).

The beneficial effects of kombucha tea are attributed to the presence of tea polyphenols, gluconic acid, glucuronic acid, lactic acid vitamins, aminoacids, antibiotics and a variety of micronutrients produced during fermentation (Vijayaraghava *et al.*, 2000). Kombucha tea treatment, however, counteracts to the changes in mitochondrial membrane potential and prevented apoptotic cell death of the hepatocytes. 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 (Bhattacharya *et al.*, 2011). Kombucha

is a potent antioxidant proved to reduce the damage induced by oxidative stress (Bhattacharya *et al.*, 2011). The antioxidant properties of Kombucha constituents and the protective effects of Kombucha on oxidative stress induced nephrotoxicity of Trichloroethylene treated rats have previously been documented. Kombucha tea could be used as a preventive and curative agent against carbon tetrachloride (CCl₄-induced) hepatotoxicity (Gharib, 2007). Kombucha tea is rich in compounds known to be strong antioxidants and has been shown to ameliorate liver damage induced by CCl₄ (Gharib, 2007). Recent studies have suggested that kombucha tea prevents paracetamol induced hepatotoxicity and chromate (VI) induced oxidative stress in rats (Pauline *et al.*, 2001). Gluconic acid has been considered by several researchers to be the main therapeutic agent in kombucha (Loncar *et al.*, 2000).

In this context a rise were observed in the levels of AST, ALT, ALP, LDH and bilirubin in thioacetamide treated rats. The Kombucha tea used in this study showed liver protection and maintained the structural integrity of hepatic cells. 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 proved a better antihepatotoxic activity against thioacetamide induced hepatic damage. More elaborate works are required to establish the exact mechanisms involved in Kombucha tea potent antihepatotoxic activities. Further experimental work is necessary to isolate and identify the active properties in the Kombucha tea which are responsible for the antihepatotoxic activities.

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