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World J Gastroenterol 2002;8(3):571-574
World Journal of Gastroenterology
 ISSN 1007-9327

• CLINICAL RESEARCH •

Epidemiological and histopathological study of relevance of Guizhou Maotai liquor and liver diseases

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Supported by The primary sciences and technology project of Guizhou province., No. 19992015
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Received 2002-05-02 **Accepted** 2002-05-25

Abstract

AIM: **To explore the relevance of Maotai liquor and liver diseases.**

METHODS: **Epidemiological study was conducted on groups of subjects, each consisting of 3 subjects from the Maotai liquor group consisting of 99 individuals and one from the non-alcoholic control group consisting of 33 individuals. Liver biopsy was performed on 23 volunteers from Guizhou Maotai Distillery who had a constant and long history of drinking Maotai liquor. Experimental histopathological study was conducted as follows: sixty male Wistar rats were divided into 3 groups randomly and fed with Maotai liquor, ordinary white wine, and physiological saline respectively for a period of 8 and 12 weeks. The rats were sacrificed in batches, then serum ALT, AST, TBil, and AKP were measured. Rat livers were harvested to measure the liver indexes, GSH, and MDA. Histopathological examinations were also performed. Another eighty mice were randomly divided into 4 groups and fed with Maotai (at different dosages of 10 ml·kg^{-1} and 20 ml·kg^{-1}), ethanol, and physiological saline. The animals were sacrificed after 4 weeks and serum ALT was determined. Then the livers were harvested and liver indexes and MDA were measured.**

RESULTS: **The incidence rate of hepatic symptoms, splenomegaly, liver function impairment, reversal of Albumin/Globulin and increased diameter of portal veins in the Maotai liquor group were 1.0%(1/99), 1.0%(1/99), 1.0%(1/99), 1.0%(1/99), 0(0/99)and 0(0/99), 0(0/99),0(0/99), 0(0/99), 0(0/99), respectively. There was no significant difference between the Maotai group and the non-alcoholic control group(P>0.05). Various degree of fatty infiltration of hepatocytes was found in the 23 volunteers receiving liver biopsy, but there was no obvious hepatic fibrosis or cirrhosis. A comparison was made between the Maotai liquor group and the ordinary white wine group. It was found that hepatic MDA in rats and mice were 0.33±0.10 and 0.49±0.23 respectively in Maotai group and 0.61±0.22 and 0.66±0.32 in the ordinary white wine group; MDA had an obvious decrease in the Maotai liquor group (P<0.05); hepatic GSH were 0.12mg·g^{-1}±0.06mg·g^{-1} in rats of the Maotai liquor group and (0.08±0.02)mg.g^{-1} in white wine group, it was obviously increased in the Maotai liquor group (P<0.05). After the 20 rats had been fed with ordinary white wine for 8 weeks consecutively, disarranged hepatocyte cords, fatty infiltration of hepatocytes, and fibrous septa of varying widths due to hepatic connective tissues proliferation were observed; after 12 weeks, the fibrous tissue proliferation continued and early cirrhosis appeared. Compared with the ordinary white wine group, fatty infiltration was observed in the 8-week and 12-week groups, but no necrosis or fibrosis or cirrhosis was found in the Maotai liquor group (P<0.05).**

CONCLUSION: **Maotai liquor may cause fatty liver but not hepatic fibrosis or cirrhosis, and it can strengthen lipid peroxidation in the liver.**

Wu J, Cheng ML, Zhang GH, Zhai RW, Huang NH, Li CX, Luo TY, Lu S, Yu ZQ, Yao YM, Zhang YY, Ren LZ, Ye L, Li L, Zhang HN. Epidemiological and histopathological study of relevance of Guizhou Maotai liquor and liver diseases. *World J Gastroenterol* 2002;8(3):571-574

INTRODUCTION

Alcohol can cause fatty liver, hepatitis and liver cirrhosis[1-5]. A lot of research showed that there was a close relationship between liver diseases and alcohol content of liquor. If one drinks ardent spirits 80 to 150g daily for more than ten years, he will get alcoholic hepatitis, hepatic fibrosis, liver cirrhosis and even hepatocellular carcinoma[6-13]. Maotai liquor has a very unique brewing technique which is different from that for ordinary white wine, and there are multiple microorganisms in the special geographical situations which are able to absorb abundant amino acids, vitamins and many essential microelements[14], so the taste of Maotai is very sweet and pure. A study on 40 individuals reported by Li et al showed that drinking Maotai liquor more than 150g daily for ten years would not cause liver injury. Our previous study showed that Maotai liquor was able to induce increase of metallothioneins(MT), inhibit the proliferation of hepatic stellate cell (HSC) and generation of collagen and enhance the effect of antioxidation[13]. Our study has initially interpreted the anti-fibrosis mechanism of Maotai liquor. A study was conducted on groups of subjects from Guizhou Maotai Distillery, each consisting of 3 subjects from the Maotai liquor group of 99 individuals who had a constant and long history of drinking Maotai liquor and one from the non-alcoholic control group of 33 individuals. Liver biopsy was performed on some of them and liver biopsy and serological tests in rats and mice fed with Maotai liquor were performed in order to explore the effect of Maotai liquor has on the liver.

MATERIALS AND METHODS

Materials

One hundred and thirty two individuals from Guizhou Maotai Distillery, aged from 30 to 60 years old, were divided into Maotai

liquor group consisting of 99 individuals and non-alcoholic control group consisting of 33 individuals. All members of the Maotai liquor group have the capacity for liquor of more than 250g.d^{-1} and drinking history of longer than ten years. There was no significant difference between the two groups in sex and age. The male Wistar rats weighing (300±20)g were employed in this study (Provided by Experimental Animal center, the Third Military Medical University); the mice of Kunming species weighing (22±2)g (Provided by Experimental Animal center,Guiyang Medical College) were also used. Maotai liquor of (530±2)g·L^{-1} was produced by Guizhou Maotai Distillery, China, whose code bar was 6902952880026; the ordinary white wine of (530±2)g·L^{-1} was provided by the Fourth Department of Guizhou Food Export and Import Company.

Methods

Questionnaires were prepared in advance including sex, age, drinking history (capacity and duration), history of liver diseases, and history of gastropathy, etc. Physical examination, abdominal B ultrasound (the size of liver and spleen, diameter of portal vein), serum ALT,A/G,LN and HA were also done at the same time. Liver biopsy was performed in twenty three volunteers from the Maotai group, the liver tissue was fixed by formalin of 40g·L^{-1}, embedded by paraffin, sectioned and stained by HE. Sixty male Wistar rats were randomly divided into Maotai liquor group, ordinary white wine group and normal control group. Rats in the wine group were fed with 2mL·kg^{-1} Maotai liquor and 2mL·kg^{-1} ordinary white wine both of which were diluted one fold by distilled water, and in the control group were fed with the same volume of saline. All rats were fed once everyday continuously for 8 weeks, and after the last feed, 15 rats were fasted for 20 hours and then sacrificed to get blood for testing serum ALT, AST, TBil and AKP were tested, and Rat livers were harvested to measure the liver indexes, GSH, MDA, the liver tissue was embedded, sectioned and stained with HE. The remaining 5 rats in each group were sacrificed for histopathological examination after fed for 12 weeks. Eighty mice (40male,40female) were randomly divided into 4 groups [two Maotai groups (at different dosage of 1 ml·kg^{-1} and 2 ml·kg^{-1}), ethanol group, and physiological saline group].Mice were fed with dosage of 1 ml·kg^{-1} and 2 ml·kg^{-1} Maotai in the two Maotai groups respectively. In the ethanol group, mice were fed (with) 53 g·L^{-1} ethanol at dosage of 1 ml·kg^{-1}, they were fed once everyday for 4 weeks. After the last feed, the mice were killed to measure serum ALT, the liver indexes and MDA. Questionnaires were done by special messenger, and were examined and verified by persons in charge of the study.

Statistic analysis

The data were analyzed by t test and χ^2 test.

RESULTS

Epidemiologic analysis

The 98 individuals who had a long history of wine drinking had no symptoms abnormal, signs or liver function; only one who had a history of hepatitis showing cirrhosis of liver. He had symptoms of liver disease, splenomegaly, lightly increase of ALT and reversed A/G. There was no increase in the diameter of portal vein trunk in those individuals, compared with that of the control group, (P>0.05). None of those who had a history of drinking Maotai for more than 30 years died of liver diseases. (See Table 1). There was a slight increase of serum LN, but a significant increase of HA, (P<0.05) (See Table 2). All twenty three individuals in the Maotai group who had biopsy had varying degrees of fatty infiltration of hepatocytes. One of them had slight necrosis, but none had obvious hepatic fibrosis or cirrhosis of liver, in compared with that of the white wine group (P<0.05).

Experimental study in rats

Neither Ordinary white wine nor Maotai liquor had obvious effect on ALT, AST, TBil, ALP and liver indexes in rats fed for 8 weeks, compared to the normal control group. There was no significant difference between them (P>0.05) (See Table 3); but Maotai liquor was able to increase the level of GSH and decrease the level of liver MDA (See Table 4). There were no obvious pathologic changes in rat liver of the normal control group (See figure 1). In the ordinary white wine group, all twenty rats had disarrangement of hepatocyte cords, fatty infiltration and hyperplasia of fibrous tissue which formed the fibrous septa (See Figure 2) after they had been fed for 8 weeks. The fibrous tissue had further hyperplasia in the rats of the 12-week group and there were also signs of early cirrhosis of liver (See figure 3).But after the Maotai group had been fed for 8 weeks and 12 weeks, all rats had fatty infiltration, 17 were mild degree and 3 were moderate degree; all were devoided of necrosis of hepatocytes, hepatic fibrosis and cirrhosis (P<0.05) (See figure 4,5) when compared with that of the white wine group.

Table 1 Epidemiologic study of the Relationship between Guizhou Maotai liquor and Liver Diseases

	Group	Number	Positive	
			Number	Rate (%)
History of	Maotai Group	99	1	1.0
liver disease	Control Group	33	0	0
Symptoms of	Maotai Group	99	1	1.0
liver disease	Control Group	33	0	0
Hepatomegaly	Maotai Group	99	15	15.2
	Control Group	33	3	9.1
Splenomegaly	Maotai Group	99	1	1.0
	Control Group	33	0	0
Abnormal ALT	Maotai Group	99	1	1.0
	Control Group	33	0	0
Reversed A/G	Maotai Group	99	1	1.0
	Control Group	33	0	0
Portal trunk	Maotai Group	99	0	0
widening	Control Group	33	0	0

Table 2 Serum ALT, LN, and HA in Maotai Group ($\bar{x}$±s)

Group	n	ALT(nkat·L^{-1})	LN(μg·L^{-1})	HA(μg·L^{-1})
Control Group	33	683±150	88±24	106±32
Maotai Group	99	650±100	132±71[a]	293±194[a]

[a]P<0.05,*vs* control group

Table 3 The effect of Maotai wine to the liver function of rats (n=20, $\bar{x}$±s)

Group	Serum biochemical index				Liver index and biochemical index		
	ALT (nkat·L^{-1})	AST (nkat·L^{-1})	Tbil (nmol·L^{-1})	AKP (nkat·L^{-1})	Index (g·kg^{-1})	GSH (mg·g^{-1})	MDA (A)
Saline	517±233	300±150	12.68±6.47	350±217	27.93±8.64	0.06±0.01	0.66±0.24
Ordinary white wine	567±167	300±67	13.54±2.50	433±67	29.19±4.41	0.08±0.02	0.61±0.22
Maotai wine	533±183	283±133	11.54±6.20	450±133	27.81±2.11	0.12±0.06[a]	0.33±0.10[a]

[a]P<0.05,*vs* control group

Table 4 The effect of Maotai wine to the liver function of mice (n=20,$\bar{x}$±s)

Group	Dosage (ml.kg^{-1})	ALT (nkat.L^{-1})	liver index (mg.g^{-1})	MDA (A)
Saline	10	316±83	40.34±7.54	0.69±0.21
Alcohol	10	333±83	40.96±7.21	0.66±0.32
Maotai wine	10	383±133	43.8±8.79	0.49±0.23[a]
Maotai wine	20	250±100	39.48±3.84	0.46±0.12[a]

[a]P<0.05,*vs* control group

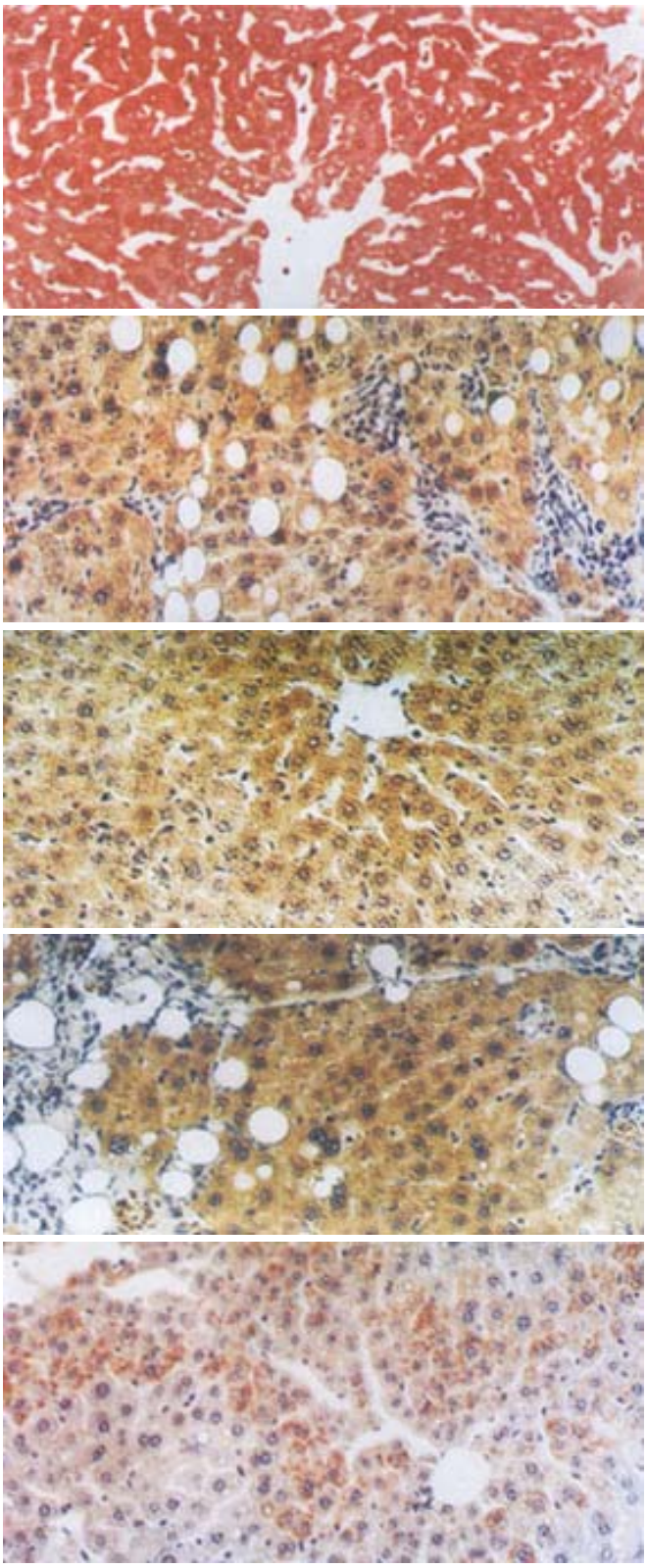

Figure 1 Normal liver tissue, hepatic cords were well arranged, the structure of hepatic lobule were intact. (HE.×200)

Figure 2 After fed with alcohol for 8 weeks, disoraganized hepatic cords, fatty infiltration, mesenchymal hyperplasia, and fibrosis could be seen. (HE.×200)

Figure 3 After fed with alcohol for 12 weeks, there were fatty infiltration of hepatocytes, hyperplasia of connective tissue, and early manifestation of cirrhosis. (HE.×200)

Figure 4 After fed with Maotai liquor for 8 weeks, there were a few infiltrating inflammatory cells, normally arranged hepatic cords, liver sinusoids were dilatated and lipid droplet could be seen in hepatocytes . (HE.×200)

Figure 5 After fed with alcohol for 12 weeks, brown changes and lipid droplet could be obviously seen in hepatocytes. (HE.×200)p

DISCUSSION

About 40% of those who drank 150g ardent spirits every day would have fatty liver and alcoholic hepatitis[15]. Alcoholic fatty liver diseases can be manifested as fatty liver, alcoholic hepatitis, hepatic fibrosis and cirrhosis of liver, frequently they are overlapping. And fatty liver can proceed to hepatic fibrosis via inflammatory progress. The varying pathology of alcoholic fatty liver diseases have similar pathogenesis in hepatic fibrosis and cirrhosis[16-22]. They are mainly caused by activation of HSC, resulting in excessive deposition and relatively insufficient degradation of ECM. Alcohol, acetaldehyde, lipid, fatty acids, etc can activate and stimulate Kupffer cells to secrete many cytokines which activate HSC to produce various components of ECM, as collagen, laminin, and hyaluronic acid, etc.

In recent years, studies have shown alcohol has direct damage effect to the hepatcytes[23-32]. Alcohol can be oxidized to acetaldehyde with elimination hydroxy free radicals, which causes lipid peroxidation, hepatocytic damage and apoptosis. Lipid peroxidation and its metabolic products malondialdehyde (MDA) and free radicals have strong cellular toxicity[33-37]. GSH[38-44] being an antioxidant is able to inhibit lipid peroxidation, and is also involved in hepatic detoxication, thus it can process cytoprotective effect. MT is a low molecular weight metal-binding protein rich in thioaminopropionic acid. Studies in recent years showed that MT has the effects of eliminating hydroxy free radicals and other oxidation products, being also protective[45-49].

Hepatic hatholigcal changes of human being and animals show that Maotai liquor can lead to fatty infiltration in the liver, but no obvious hepatic fibrosis or cirrhosis of liver. Similar findings were shown in epidemiological study, B mode ultrasound and liver biopsy in the Maotai liquor group One died of liver diseases in this group.

By contrast, various widths of fibrous septa and cirrhosis can be seen in the liver of rats fed ordinary white wine. Why is fatty infiltration in which Maotai liquor group dose not lead to liver fibrosis and cirrhosis of liver? The answer is that Maotai liquor was able to lower the MDA level in the rats and mice liver and increase the level of liver GSH as comparing with those in the. ordinary white wine group, $P<0.05$. It also showed that Maotai liquor had the inhibitory effect on lipid peroxidation and was cytoprotective. Moreover, study in vitro showed that Maotai liquor could increase MT which was the most powerful bioactive substance of scavenging free radicals and antagonizing liver damage. Maotai liquor may also inhibit the activation of hepatic stellate cells and generation of collagen.

Our study revealed that Maotai liquor was able to elevate the level of workers' serum LN and HA in Maotai liquor group vs. ordinary white wine group, ($P<0.05$). It may be related with the activation of hepatic stellate cells (HSC) by ethanol, acetaldehyde, lipid and fatty acid. The elevation of LN was mild, but HA was very obvious whose reason may be that the serum level of HA was related with the ability of hepatic secretion and the renal excretion. In the acute hepatic injury caused by alcohol, inflammation and drugs, the level of serum HA may be very high, but it only points at a possible hepatic fibrosis or the early stage of hepatic fibrosis because researches show that the level of HA is not very high, even normal, in the late stage of hepatic fibrosis[50].

As one of the three most famous distilled sprits of the world, Maotai liquor was brewed with open solid state fermentation in a special geographical environment, so it has formed its own style which is hard for others to imitate. According to the analysis of the nutritive component in Maotai liquor in recent years, in every 100 ml Maotai Liquor, there were 18mg protein, 6667 u superoxide dismutase (SOD), 0.06 mg vitamin B_1, 0.1 mg vitamin B_2 and 1.19 mg vitamin C, 0.022 mg manganese (Mn), 0.026 mg copper (Cu), 0.42 mg iron (Fe), 1.0 mg potassium (Ka), and <0.005 mg zinc (Zn); 4.7 mg methanol, 4.7 mg aldehyde compounds. And there were also 18 kinds of amino acids and 6 kinds of essential amino acids

except acimetion and tryptophan which were never found in the Jiang flavor white wine before Maotai. There were SOD and multiple human essential microelements in Maotai liquor, especially rich in Fe, Mn and Cu. The components of methanol, benzene and aldehyde were much lower than it was ruled in the international standard.

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Edited by Wu XN