Research Article

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Hypocholesterolaemic and antioxidant effects of kombucha tea in high-cholesterol fed mice

Zhi-Wei Yang, Bao-Ping Ji,* Feng Zhou,* Bo Li, Yangchao Luo, Li Yang and Tao Li

Abstract

BACKGROUND: Traditional kombucha tea (TKT) is produced by mixed tea fungus. We previously proposed *Gluconacetobacter* sp. A4 as the key functional strain in kombucha culture, because it had strong ability to produce D-saccharic acid-1,4-lactone (DSL, a crucial functional component in KT). This study investigated the hypocholesterolaemic and antioxidant activities of TKT and modified KT (MKT, tea broth fermented by single *Gluconacetobacter* sp. A4).

RESULTS: *In vitro*, TKT and MKT, but not DSL equally increased the radical scavenging effects and inhibited low density lipoprotein (LDL) oxidation. *In vivo*, the total cholesterol and LDL-cholesterol (LDL-C) lowering effects were not different between MKT and TKT. Compared with TKT, MKT showed a significantly elevated effect on the increase of antioxidantive enzymes activities (total antioxidant capacity and superoxide dismutase) and the decrease of malondialdehyde. Meanwhile DSL demonstrated an enhanced activity in lipid profile and antioxidant activities.

CONCLUSION: KT had the hypocholesterolaemic and antioxidant effects. These effects were largely attributed to DSL. MKT was similar to or even more powerful than TKT in antioxidant and hypocholesterolaemic effects. Thus, *Gluconacetobacter* sp. A4 was further established as the main functional microorganism in kombucha culture. Moreover, KT may be useful in treating obesity.

Keywords: kombucha; *Gluconacetobacter* sp.; hypocholesterolaemic; antioxidant; D-saccharic acid-1,4-lactone; phenolic compounds

INTRODUCTION

Atherosclerosis is a major risk factor for development and progression of coronary heart disease (CHD). Lipoprotein oxidation and elevated levels of plasma cholesterol play an important role in the pathogenesis of atherosclerosis.[1] Therefore, it has been proposed that inhibition of low density lipoprotein (LDL) oxidation, and reductions in the level of triglycerides, total cholesterol and LDL would result in retardation of atherosclerotic lesion development. Substances which combine antioxidant and hypocholesterolaemic activities are expected to be effective.[2–4]

Kombucha tea (KT) is a popular health beverage among many traditional fermented foods across the world. It has been claimed that KT can regulate cell proliferation, increase detoxification, and have antioxidant and anti-carcinogenic effects, especially for hormone-dependent tumours.[5–8] Since the scientific data on the hypolipidaemic efficacy of KT were scarce, this was examined in the current study using a mouse model.

KT also contains various antioxidant constituents. Compounds such as polyphenols, and volatile flavour compounds such as theaflavins and thearubigins are found to be higher when compared to black tea.[9] A higher the polyphenol leads to stronger the antioxidant activity.[10] It has been demonstrated that a venous injection of antioxidant or a combined treatment of some antioxidants, inhibits the development of atherosclerotic lesions in rabbits fed atherogenic diets. It is generally assumed that some antioxidants can prevent atherosclerosis by protecting LDL from oxidation and are also associated with an antihypercholesterolaemic effect.[11]

KT samples from various areas are different in microbial composition. Due to the complexity and diversity of these microorganisms, the final quality of this beverage is difficult to reproduce and predict. Thus, industrial production of KT is difficult. Therefore, single culture would be a better choice for KT fermentation. In our previous study, we found a functional strain, *Gluconacetobacter* sp. A4 , in kombucha culture which had the ability to readily produce much more D-saccharic acid-1,4-lactone (DSL, the main functional component in KT, 2.70–3.80 mg mL^{-1}). *Gluconacetobacter* sp. A4 was identified according to morphological, biochemical, physiological and phylogenic characteristics. The present study was designed to investigate the hypolipidaemic effects as well as the antioxidant effects of traditional KT (TKT, tea broth fermented by mixed tea fungus) and modified KT (MKT, tea broth fermented by single *Gluconacetobacter* sp. A4), and to further establish *Gluconacetobacter* sp. A4 as the main functional strain in kombucha culture.

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MATERIALS AND METHODS

Bacterial strains and KT preparation

Sweetened black tea (SBT) was prepared by the following steps: 5 g black tea (Yunnan Dianhong, China) was added into 700 mL fresh boiling water and infused for 15 min. After filtration, 100 g glucose was added to the tea extract as well as some water to make the final volume of 1 L, and then the mixture was autoclaved at 121 °C for 15 min.

The tea fungus was obtained from a local family in Beijing, China. TKT was prepared by fermenting 500 mL SBT with 100 mL fermentation broth from the previous KT fermentation. The vessels were covered with cheesecloth and the contents were left to incubate at constant temperature of 30 °C for 8 days.

Gluconacetobacter sp. A4 was maintained on glucose yeast extract agar (100 g L^{-1} glucose, 10 g L^{-1} yeast extract, 20 g L^{-1} $CaCO_3$, 15 g L^{-1} agar). MKT was prepared by fermenting 500 mL SBT with 50 mL *Gluconacetobacter* sp. A4 suspension. The inoculation density was 6×10^7 cells mL^{-1}. Samples were then incubated at 30 °C for 8 days.

Chemical compositions of TKT and MKT

Chemical compositions were determined by gas chromatography (GC) and high-performance liquid chromatography (HPLC) (Zhi-Wei Yang, unpublished).

Antioxidant activity *in vitro*

Total polyphenols were measured by the Folin–Ciocalteu method.[12] The results were expressed as gallic acid. All samples were analysed in triplicate. The free radical scavenging activity was determined using the methods of Sang *et al*.[13] and Lu and Foo.[14] The hydroxyl radical scavenging activity was determined using the method of de Avellar *et al*.[15] The superoxide radical scavenging activity was determined using the method of Stewar and Beewley.[16]

The inhibitory effect on LDL oxidation was determined by the following steps. Plasma samples were obtained from healthy volunteers after fasting overnight. LDL was prepared by a previously described method.[17] The dialysed LDL was diluted with PBS to a final concentration of 250 mg protein mL^{-1}, and 200 µL of the appropriately diluted samples was then added. Oxidation was initiated by the addition of freshly prepared $CuSO_4$ solution (the final concentration was 2.5 µmol L^{-1}), and the final volume of preparation was adjusted to 2 mL using PBS. The resultant mixture was incubated at 37 °C. Formation of conjugated dienes was monitored continuously by measuring the absorbance at 234 nm using an ultraviolet spectrometer (GBC, Melbourne, Australia) with intervals of 15 min up to 360 min.

Hypolipidaemic and antioxidant activity *in vivo*

Animals and experimental protocol

Institute of Cancer Research (ICR) mice weighing between 20 and 22 g were assigned to five groups (Table 1) after 1 week of adaptation. The mice in the control group were fed a chow diet. The other groups received various samples and a hypercholestrolaemic diet (HCD). HCD was enriched in cholesterol (10 g kg^{-1}), lard oil (100 g kg^{-1}) and cholate (1 g kg^{-1}). Samples were fed orally. Food and water were supplied *ad libitum*. The mice were housed in cages under a 12-h light and 12-h dark cycle. The mice were fed for 12 weeks.

Table 1. Experimental diet groups

Group	Diet/treatment
Control	Control (chow diet)
HCD	HCD
MKT	HCD + MKT (66 mL kg^{-1} BW)
TKT	HCD + TKT (66 mL kg^{-1} BW)
DSL	HCD + DSL (60 mg kg^{-1} BW)

$n = 8$ for all groups.
DSL, D-saccharic acid-1,4-lactone; HCD, hypercholestrolaemic diet; MKT, modified kombucha tea; TKT, traditional kombucha tea.

Body weight and food intake

The body weights were recorded once per week. The food intake efficiency in each group was evaluated by monitoring the food consumption (in grams) in each cage for two consecutive days, and was calculated on per animal per day basis.

Collection of the serum and liver

Blood samples were collected by retro-orbital puncture after 12 weeks. Serum was obtained by centrifugation at 3000 × *g* for 10 min. At the end of experiment, animals were sacrificed. Liver, spleen, kidney and retroperitoneal adipose tissue were removed immediately and weighed.

Lipids and antioxidant status in serum

Lipids concentrations [TC, LDL-C and high density lipoprotein-cholesterol (HDL-C)] and antioxidant activities [total antioxidant capacity (TAOC), superoxide dismutase (SOD) and malondialdehyde (MDA)] in serum were determined with enzymatic methods, and corresponding diagnostic kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) were used according to the manufacturer's instructions.

Histological analysis

Histological slides from the liver were prepared, and the presence of fat was determined by using oil red O fat stain.[18]

Data analysis

Values were expressed as mean ± standard deviation (SD). Differences in mean values between groups were analysed by one-way ANOVA followed by Dunnet's *t*-test. Statistical significance was considered to exist at $P < 0.05$. Analysis was done with SPSS 13.0 (SPSS, Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

KT is a popular health beverage among many traditional fermented foods across the world. There has been much attention regarding its possible benefits. The antioxidant and antimicrobial effects of KT have already been validated by many researchers.[19–23] For the hypocholesterolaemic effect, scientific data on its efficacy is scarce. Meanwhile, we need more evidence to further illustrate that *Gluconacetobacter* sp. A4 was the main functional strain in kombucha.

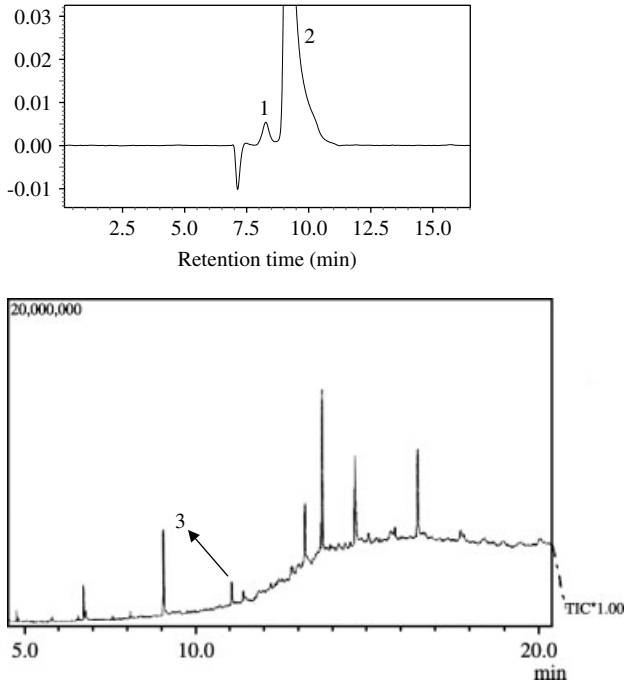

Figure 1. Chemical composition of kombucha tea. Peak 1: 2-keto gluconic acid; peak 2; gluconic acid, peak 3: D-saccharic acid-1,4-lactone.

Chemical compositions of MKT and TKT

According to the HPLC and GC method used, there was no significant difference between MKT and TKT in chemical compositions (Fig. 1). The main organic acids were gluconic acid and 2-ketogluconic acid. But MKT contained more DSL than TKT, which was 3.51 mg mL^{-1} and 2.30 mg mL^{-1}, respectively. The chemical composition of KT has been widely studied,[24–27] but results were different from each other. That was because KT from various areas was different in microbial composition, which resulted in different chemical compositions. Generally, acetic acid, gluconic acid, ketogluconic acid and fructose were the primary constituents of KT.

Antioxidant activity *in vitro*

Unbalanced oxidative stress had been known to be an inducer of various diseases.[28] Antioxidants have been reported to have protective effects on diverse diseases, such as ischaemia–reperfusion injury and hypercholesterolaemic atherosclerosis, in which reactive species of oxygen and nitrogen were involved. It was well known that the radical system used for antioxidant evaluation may influence the experimental results, and two or more radical systems were required to investigate the radical-scavenging capacities of a selected antioxidant.[29] Since many of the claimed beneficial effects of KT may be associated with its antioxidant activities[30] and the antioxidant ability is a major factor for antihyperlipidaemia, in the present study, various systems were adopted to value the antioxidant effect of MKT and TKT.

Phenolic compounds are called high-level antioxidants because of their ability to scavenge free radical and active oxygen species such as singlet oxygen, superoxide free radicals and hydroxyl radicals, and there is a correlation between phenolic compounds and scavenging activity. As shown in Fig. 2, the amount of total phenolic compounds was increased after the fermentation process. The amount of total polyphenols in MKT and TKT accumulated up to 0.34 mg mL^{-1} and 0.33 mg mL^{-1} gallic acid equivalent at the eighth day of fermentation, while it was only about 0.15 mg mL^{-1} in SBT. These observations were in accordance with those reported by Ponmurugan *et al.*[9] Jayabalan *et al.*[21] considered complex phenolic compounds in KT might be depolymerised in an acidic environment and by the enzymes liberated by bacteria and yeasts in the tea fungus consortium, which in turn resulted in the increase of total phenolic compounds. They also observed the degradation of epigallocatechin gallate (EGCG) and epicatechin gallate (ECG) during KT fermentation.[31] Both were assumed to be converted to their corresponding catechin epigallocatechin (EGC) and epicatechin (EC) by enzymes excreted by micro-organisms in the kombucha culture. Duenas *et al.*[32] demonstrated that bioactive polyphenolic compounds of lentils could be modified due to exogenous application of enzymes like phytase, α-galactosidase and tannase. They also demonstrated the increased antioxidant activity of enzyme-treated lentils.

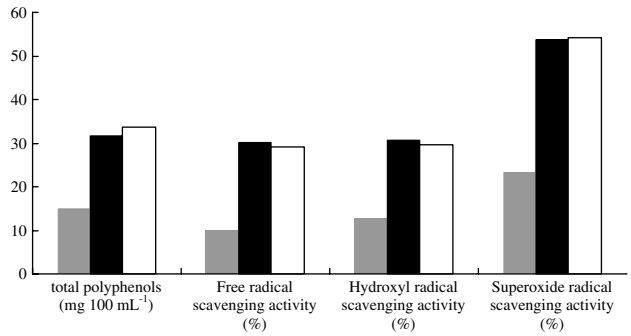

Figure 2. Total polyphenol contents and free, hydroxyl and superoxide radicals scavenging activities of sweetened black tea (grey bars), traditional kombucha tea (black bars) and modified kombucha tea (white bars).

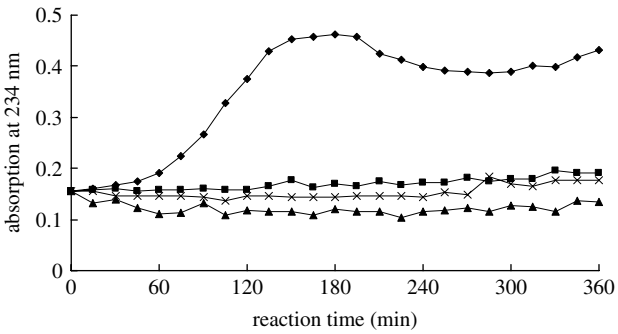

Figure 3. Influence of sweetened black tea (×), traditional kombucha tea (■) and modified kombucha tea (▲) on copper-induced formation of conjugated dienes in low-density lipoprotein (LDL) (♦, LDL control without copper).

Because of the high content of phenolic compounds, MKT and TKT significantly ($P < 0.01$) elevated the free, hydroxyl and superoxide radical scavenging activity of SBT (Fig. 2).

As reported, Cu^{2+}-induced LDL peroxidation was more relevant to the *in vivo* situation than other peroxidation [e.g., 1-(2,6-dimethylphenoxy)-2-(3,4-dimethoxyphenylethylamino) propane hydrochloride (DDPH), 2,2′ -azobis (2-amidinopropane) dihydrochloride (AAPH)], since the former system most likely involved a site-specific attack of the apolipoprotein B in LDL, whereas the latter produced more or less random attack of free radicals.[33] Therefore, protection effect of SBT, MKT and TKT on human LDL

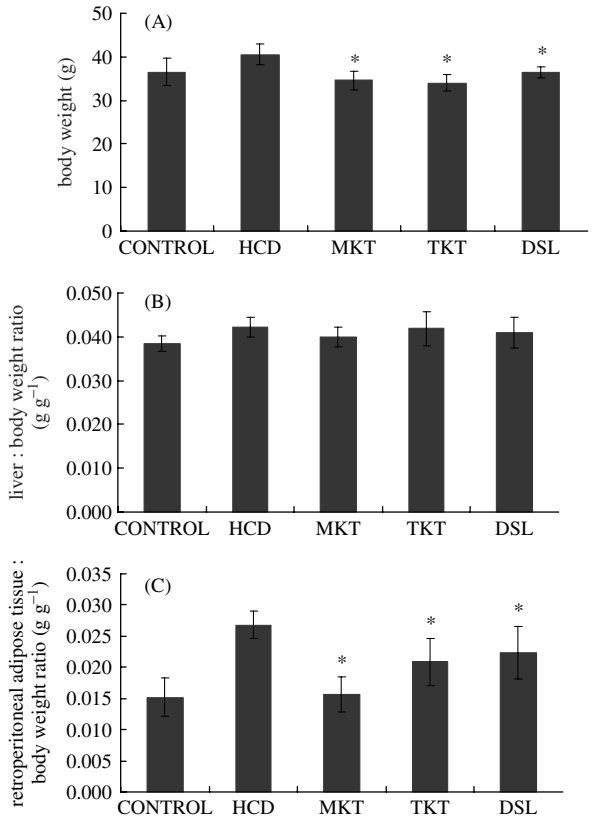

Figure 4. Body weight (g) (A); liver : body weight ratio (g g^{-1}) (B); and retroperitoneal adipose tissue : body weight ratio (g g^{-1}) (C). Values are expressed as mean ± SD ($n = 8$). *Statistically different at $P < 0.05$. DSL, D-saccharic acid-1,4-lactone; HCD, hypercholestrolaemic diet; MKT, modified kombucha tea; TKT, traditional kombucha tea.

was initially investigated in present study. As can be seen from Fig. 3, all the SBT, MKT and TKT contributed to LDL protection from oxidation by significantly prolonging the lag phase ($P < 0.01$).

Various studies have demonstrated that KT had *in vivo* antioxidant activity, but the cause of this remains unclear.[21] The increased potential against radicals might explain the phenomenon that feeding KT significantly reversed chromate(IV)- or lead-induced oxidative injury in rats.[19,20] Therefore, KT may possess some other curative effects such as reduction of atherosclerosis, arthritis and inflammation, which might be related to its antioxidative activity compared to black tea broth,[30] although this has not been validated scientifically.

In addition, Figs 2 and 3 show that there was no significant difference between MKT and TKT in total polyphenol content, radical scavenging activity and LDL oxidation inhibitory effect. That means that MKT and TKT have equal antioxidant activities *in vitro*. DSL had no antioxidant activity *in vitro* (data not shown). The following experiments were carried out to evaluate their hypocholesterolaemic and antioxidant effects *in vivo*.

Hypocholesterolaemic effect *in vivo*

Body growth and organ weights

All mice in each cage consumed the diet provided. Although initial body weights did not differ among the groups, the final body weights in the MKT group, TKT group and DSL group were lowered compared with that in the HCD group ($P < 0.05$). The HCD supplemented with MKT and TKT reduced the body growth by approximately 16.09% and 14.69% of that of the HCD group ($P < 0.05$) (Fig. 4A). Meanwhile, DSL also inhibited body growth by about 9.87%. Food consumption in KT-treated animals showed a slight decline (data not shown), which might be statistically important to body weight change.[34] Since the restriction in food intake also correlates with a reduction in blood cholesterol,[35] it is quite possible that KT has affected cholesterol homeostasis by a simple but efficient method of suppressing food intake.

Although no differences in the liver, spleen and kidney : body weight ratios were observed among the groups (data not shown), a tendency toward a decrease in the liver : body weight ratio was found in the MKT group, TKT group and DSL group, especially in the group treated with MKT (Fig. 4B). Meanwhile, oral administration of KT to hypercholesterolaemic ICR mice for 12 weeks resulted in a significant reduction in the retroperitoneal adipose tissue : body weight ratio ($P < 0.05$ and 0.01). Treatment by MKT and TKT in the test animals yielded reductions in this ratio of 41.70% and 22.02%, respectively, as compared to the high cholesterol control (Fig. 4C). Meanwhile, DSL also reduced the retroperitoneal adipose tissue : body weight ratio by about 9.87%.

These effects have not been observed by other researchers. It is known that obesity is closely associated with hypertension and stroke.[36] Obesity is the single most significant risk factor for the development of fatty liver, both in children and in adults, and is also predictive of the presence of fibrosis, potentially progressing to advanced liver diseases.[37] There were many personal reports about the functional effects of KT, and some advocates believed that KT has an anti-obesity effect,[24] although there is no scientific validation. The observations of the present study suggest a possible anti-obesity effect of KT. MKT showed enhanced influence on the gain of body and organ weights compared to TKT. The possible underlying mechanisms for this finding, however, need further investigations, and the high DSL content would be proposed as a primary reason.

Lipids and antioxidant status in serum

Lipid profiles in serum and liver are main risk factors in the pathogenesis of hyperlipidaemia.[38] As shown in Table 2, the HCD group, which received a high cholesterol diet, showed elevated serum total cholesterol, LDL-C and HDL-C, suggesting that effective induction of hypercholesterolaemia by supplementation of cholesterol in the diet was effectively established in ICR mice. MKT, TKT and DSL administration lowered elevated total cholesterol levels to 82.21%, 82.03% and 59.89%, respectively, of that in the HCD group. Furthermore, the concentrations of LDL-C declined by 33.33%, 31.25% and 45.83%, respectively, of that in the HCD group. The concentrations of HDL-C in most of the treated animals, however, were not significantly altered.

Data in Table 2 show that the antioxidant parameters TAOC and SOD in the HCD group were decreased by 45.40% and 52.60%, respectively, and there was a concomitant 189.80% increase of MDA compared to the control group at week 12 of study. Compared with the HCD group, the TAOC and SOD activity and MDA content in serum of each treatment group were significantly different ($P < 0.05$). MKT, TKT and DSL administration elevated the lowered TAOC levels to 154.05%, 130.33% and 130.42%, respectively, of the HCD group. They also elevated the lowered SOD levels to 185.66%, 152.55% and 199.63%, respectively, of the HCD group. Furthermore, the concentrations of MDA declined to 66.67%, 68.75% and 54.17%, respectively, of the HCD group.

Table 2. Serum cholesterol and antioxidant status after 12 weeks of treatment

Treatment group	Control	HCD	MKT	TKT	DSL
TC (mmol L^{-1})	5.09 ± 0.48	11.02 ± 0.71	9.06 ± 1.21*	9.04 ± 1.34*	6.60 ± 0.86*
LDL-C (mmol L^{-1})	0.35 ± 0.05	0.48 ± 0.09	0.32 ± 0.16*	0.33 ± 0.22*	0.26 ± 0.04*
HDL-C (mmol L^{-1})	4.23 ± 0.48	4.87 ± 0.38	5.19 ± 1.12	5.21 ± 1.39	3.79 ± 1.00*
TAOC (U mg^{-1} protein)	19.69 ± 1.56	10.75 ± 1.67	16.56 ± 2.44*	14.01 ± 1.43*	14.02 ± 2.15*
SOD (U mg^{-1} protein)	194.34 ± 15.11	92.12 ± 5.18	171.03 ± 9.58*	140.53 ± 25.44*	183.90 ± 6.95*
MDA (nmol mg^{-1} protein)	10.00 ± 0.96	28.98 ± 2.49	11.91 ± 1.22*	11.70 ± 1.13*	12.23 ± 1.16*

* Statistically different at $P < 0.05$.
DSL, D-saccharic acid-1,4-lactone; HCD, hypercholestrolaemic diet; MKT, modified kombucha tea; TKT, traditional kombucha tea.

Histological analysis

Histological examination showed that hepatic lipid deposits appeared as many small fatty droplets within the cytoplasm of liver cells in mice fed HCD. However, the treatment of gastric infusion with MKT, TKT and DSL markedly reduced the number of these fatty droplets in mice (Fig. 5).

The present study explored the effect of MKT and TKT on the serum lipid profile in hyperlipidaemia mice induced by HCD. MKT and TKT possessed similar total cholesterol and LDL-C lowering activity *in vivo*, showing a significant difference ($P < 0.05$) compared to the HCD group. It was interesting to note that the total cholesterol and LDL-C contents in the DSL group were significantly lower ($P < 0.05$) compared with the other groups. Compared with the HCD group, the results showed no effect on the HDL-C content in both the MKT and TKT groups. It is possible that the hypocholesterol effect of MKT and TKT may not affect the reverse cholesterol transport of HDL-C. The cholesterol-lowering effect is, in fact, exclusively attributed to the decline of LDL-C. However, KT demonstrated an increasing tendency on the value of HDL-C. Further studies are needed to confirm the HDL-C regulating effect by KT at different doses. The DSL group demonstrated a significant effect on decreasing HDL-C, which suggested a one-way regulating effect by DSL. These observations suggested that there was no significant difference in the hypocholesterol effect between MKT and TKT, and this effect was largely attributed to DSL. There is no reasonable explanation for this finding.

LDL is considered as 'bad cholesterol' as it transfers cholesterol from the liver to the circulation. The results in this study suggested that KT might affecting upregulation at the LDL receptor or gene transcription level and thereby facilitate removal of cholesterol from the circulation.[39] The results of the Lipid Research Clinics Primary Prevention Trial indicated that there was a positive correlation between plasma concentrations of LDL-C and risk of coronary artery disease.[40,41] This work showed that a 20% drug-induced reduction in LDL-cholesterol concentrations resulted in the reduction of newly positive exercise tests (indicative of myocardial ischaemia), angina pectoris, and coronary bypass surgery in the treated group by 25, 20, and 21%, respectively. This work also showed that a 25% reduction of the total cholesterol in plasma would reduce the incidence of coronary events by nearly 50%.[41] Considering the level of serum cholesterol and LDL-C lowering effect achieved of MKT (17.79%, 33.33%), TKT (17.97%, 31.25%) and DSL (40.11%, 45.83%), it can be concluded that KT is a potent hypocholesterolaemic agent.

A high cholesterol diet such as 10 g kg^{-1} would produce large amounts of oxygen radicals, followed by oxidative stress and hypercholesterolaemia. Reactive oxygen species are known to produce endothelial cell injury,[42] which represents a critical initiating event in the development of atherosclerosis.[43] Therefore it is of great importance not only to determine the scavenging effect of free radicals *in vitro*, but also of antioxidant enzymes *in vivo*. Thus TOAC, SOD and MDA in serum were selected to determine the antioxidant effect *in vivo* by administration of MKT, TKT and DSL. Free radicals are the source of lipid peroxidation derived from oxygen, and the first line of defence against them is SOD. Hence, the increased SOD activity in serum suggests that the absence of accumulation of superoxide anion radical might be responsible for decreased lipid peroxidation. MDA formation results from the lipid peroxidation of cell membranes, and increased concentration of MDA possibly leading to tissue damage. It was interesting to note the TAOC and SOD activity in the MKT group showed a significant increase ($P < 0.05$) compared with TKT, which was 18.20% and 21.70% higher, respectively.

The polyphenolic compounds contained in KT may be involved in its *in vivo* antioxidant effect, probably through a direct antioxidant effect on lipoprotein particles. Cartron[44] reported that protocatechuic acid has a direct effect on LDL protection against oxidation, thus decreasing pro-inflammatory lysophosphatidylcholine production. Wang *et al*.[45] found that hibiscus anthocyanin, a pigment extract from the calyx of roselle, significantly reduced oxidative stress in rat hepatocytes *in vivo*. The reduction in MDA levels could also be due to decreases in the cholesterol levels because hypercholesterolaemia increases the levels of oxygen radicals.[46,47]

The above affirmation is supported by the antioxidant activity *in vitro*. Additionally, we also noted that DSL, which showed no antioxidant activity *in vitro*, demonstrated antioxidant activity *in vivo* and showed the highest SOD activity, which was 30.86% higher than TKT. However, further investigation is needed to discover the antioxidant mechanism of DSL.

Hepatic oxidative stress from lipid peroxidation has been identified as playing a pathogenic role in liver disease.[48] Since the mice fed HCD suffered oxidative damage in the liver, there is a need to evaluate the protective effect against fatty liver induced by HCD. From a histopathological diagnosis (Fig. 5), treatment by KT played a remarkable role in inhibiting the accumulation of triglycerides and total cholesterol within the hepatocytes.

CONCLUSION

Our results showed that black tea, MKT and TKT were potent antioxidants to radicals and inhibitors of LDL oxidation. There was no difference between MKT and TKT, but both were more powerful than black tea in antioxidant effect. Moreover, the *in vivo* study demonstrated that MKT and TKT could equally prevent hyperlipidaemia by decreasing serum total cholesterol

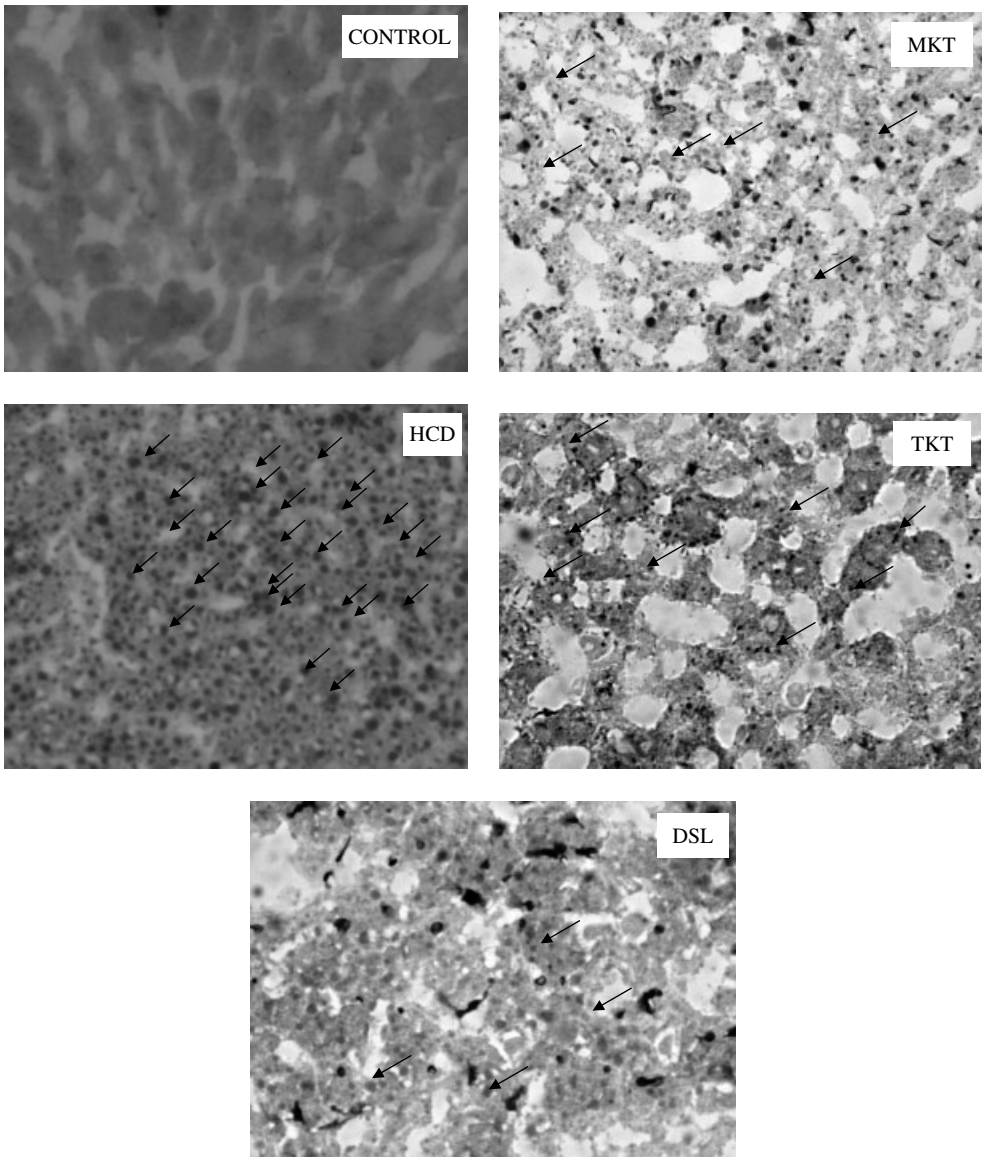

Figure 5. Histological structure of liver in each group. (Stain: oil red O; original magnification: ×400). DSL, D-saccharic acid-1,4-lactone; HCD, hypercholestrolaemic diet; MKT, modified kombucha tea; TKT, traditional kombucha tea.

and LDL-C, significantly improving the antioxidant status in serum, and reducing the number of fatty droplets in mice. DSL, as the main functional component, was partly responsible for the hypocholesterolaemic effect of KT. In conclusion, in the present study, *Gluconacetobacter* sp. A4 was further established as the main functional micro-organism in kombucha culture, and MKT was similar to or even more powerful than TKT in antioxidant and hypocholesterolaemic effects. Therefore, a single culture was a better choice for KT fermentation instead of traditional tea fungus.

Another observation in this study was the weight-losing effect caused by KT. Thus, the extract could be useful in treating obesity. The possible underlying mechanisms for this finding, however, need further investigation. In addition, we must emphasise that these results do not necessarily translate into therapeutic effects in human patients and more human studies are needed before such conclusions are possible.

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