Journal of Medical Sciences

ISSN 1682-4474

science alert

ANSInet
an open access publisher
http://ansinet.com

Short Communication

JMS (ISSN 1682-4474) is an International, peer-reviewed scientific journal that publishes original article in experimental & clinical medicine and related disciplines such as molecular biology, biochemistry, genetics, biophysics, bio-and medical technology. JMS is issued eight times per year on paper and in electronic format.

For further information about this article or if you need reprints, please contact:

Pavels Semjonovs
Laboratory of Industrial Microbiology and Food Biotechnology,
Institute of Microbiology and Biotechnology,
University of Latvia,
4 Kronvalda Blvd,
LV-1010, Riga, Latvia,

Tel: +37167034887
Fax: +37167034885

J. Med. Sci., 14 (3): 147-152
1st April, 2014
DOI: 10.3923/jms.2014.147.152

Evaluation of Physiological Effects of Acetic Acid Bacteria and Yeast Fermented Non-alchocolic Beverage Consumption in Rat Model

P. Semjonovs, I. Denina and R. Linde

Kombucha is a traditional beverage consumed in various parts of the world. It is made by fermentation of sugared black tea by a consortium of yeasts and acetic acid bacteria. Recent studies have demonstrated that Kombucha possesses antioxidant, antimicrobial, hepatoprotective, nephroprotective and hypocholesterolaemic properties. Microbial composition of Kombucha association is highly variable therefore the chemical composition of obtained beverage can vary a lot and affect products quality. Thus, industrial production of Kombucha beverage is difficult and defined starter culture is strongly needed for obtaining the product with standard characteristics. In the present study individual starter cultures were used to obtain Kombucha like fermented beverage to evaluate its physiological effects in rat model for further development of functional beverage. Low density cholesterol, triglycerides and homocysteine levels elevated by fat diet decreased during intake of fermented beverage (2.5 mL kg^{-1} b.wt.). To evaluate liver cell status alanine aminotransferase (ALAT) and aspartate Transaminase (ASAT) were assayed. It was shown that ASAT and ALAT decreased due to fermented beverage consumption in both-normal and high fat diet group. Zero rat mortality was observed during fermented beverage intake for 60 days (2.5-7.5 mL kg^{-1} b.wt.). This study on the consumption of fermented beverage obtained by use of defined starter suggests that it could be suitable for prevention of some metabolic disorders, like cardiovascular diseases or liver disorders, attributed to unhealthy lifestyle like high-fat diet. However, further research on physiological effects and possible mechanisms of action is needed and human studies would be welcomed.

Key words: Kombucha, fermented beverage, cholesterol, triglycerides, homocysteine, liver health

ANSInet
Asian Network for Scientific Information

Laboratory of Industrial Micorbiology and Food Biotechnology, Institute of Microbiology and Biotechnology, University of Latvia, 4 Kronvalda blvd, LV-1010, Riga, Latvia

INTRODUCTION

Kombucha is a traditional beverage consumed in various parts of the world. It is made by fermentation of sugared black tea with a consortium of yeasts and bacteria (Greenwalt *et al*., 2000). Dominating bacterial species identified in Kombucha symbiotic association are *Gluconacetobacter xylinum*, *Acetobacter aceti*, *Acetobacter pasteurianus* and *Gluconobacter oxydans* (Liu *et al*., 1996; Greenwalt *et al*., 2000; Kurtzman *et al*., 2001; Dayal *et al*., 2013). The dominant yeast species in Kombucha symbiosis are: *Schizosaccharomyces pombe*, *Saccharomycodes ludwigii*, *Saccharomyces cerevisiae*, *Zygosaccharomyces bailii*, *Torulaspora delbrueckii*, *Brettanomyces bruxellensis*, *Torulopsis* sp., *Pichia* sp. (Mayser *et al*., 1995; Liu *et al*., 1996; Greenwalt *et al*., 2000; Kurtzman *et al*., 2001; Markov *et al*., 2001). Acetic acid and gluconic acid are the major components of the beverage (Chu and Chen, 2006). Other minor constituents are other organics acids like glucuronic, folic, usnic acid, amino acids and vitamins (B1, B2, B3, B6, B12 and C) (Blanc, 1996). Kombucha also contains various antioxidant constituents like phenolic compounds and carotenoids (Blanc, 1996).

The beverage has been claimed to be a prophylactic agent and to be beneficial to human health (Dufresne and Farnworth, 2000; Vina *et al*., 2013). Recent studies have demonstrated that Kombucha possesses antioxidant (Dipti *et al*., 2003; Yang *et al*., 2009; Sai Ram *et al*., 2010), antimicrobial (Steinkraus *et al*., 1996; Sreeramulu *et al*., 2001; Battikh *et al*., 2012), hepatoprotective (Murugesan *et al*., 2009; Abshenas *et al*., 2012; Abshenas *et al*., 2012), nephroprotective (Gharib, 2009) and hypocholesterolaemic properties (Yang *et al*., 2009; Adriani *et al*., 2011; Aloulou *et al*., 2012) and provides cure for gastric ulceration (Banerjee *et al*., 2010).

Kombucha samples of various origins are of varied microbial composition (Marsh *et al*., 2014) therefore the final chemical composition of the fermented beverage can vary a lot and affect products quality. Thus, industrial production of Kombucha beverage is difficult and defined starter culture is strongly needed for obtaining the product with standard characteristics. Considering the claimed health effects of Kombucha beverage it could be important to develop defined starter that could be used for industrial production of polyfunctional healthy beverage.

Cardiovascular diseases cause approximately 17 million deaths a year and are a leading cause of global deaths. One of the main risk factors for cardiovascular diseases is elevated blood cholesterol level or hypercholesterolemia. Hypercholesterolemia can promote heart attack, stroke, asthma and cataracts (Grundy *et al*., 1998). Free radicals and excess of low-density lipoprotein-cholesterol (LDL-Ch) in plasma can cause damage to arterial walls and increase probability of atherosclerosis, leading to the blockage of blood vessels. Kombucha has been noted to induce a marked delay in the absorption of LDL-Ch and triglycerides and a significant increase in HDL-Ch in experimental animals (Yang *et al*., 2009; Adriani *et al*., 2011; Aloulou *et al*., 2012). Another independent cardiovascular risk factor is increased concentration of homocysteine. Homocysteine is a sulfur-containing amino acid intermediate in methionine-cysteine metabolism (Finkelstein and Martin, 2000). Plasma homocysteine concentrations are dependent on complex metabolic regulation but it was shown that high intakes of saturated fatty acids could be associated with high plasma concentrations of homocysteine (Berstad *et al*., 2007).

Increased levels of alanine aminotransferase (ALAT) and aspartate transaminase (ASAT) indicate the liver cell damage. Determination and evaluation of these parameters in the serum and tissue samples of experimental animals are used to assess the physiological status of liver. Since consumption of Kombucha beverage for hepatoprotective properties has been assessed (Murugesan *et al*., 2009; Abshenas *et al*., 2012) and taking into consideration growing incidents of hepatic illnesses all over the world (partly connected with unhealthy lifestyle e.g., high-fat diet), ALAT and ASAT were also chosen to be evaluated in our study.

Hence, in the present study individual starter cultures were used to obtain Kombucha like fermented beveraget to evaluate its possible physiological effects in rat model for further development of functional beverage.

MATERIALS AND METHODS

Preparation of fermented beverage: Black tea (English Breakfast, Lipton, UK) was added to boiling water (1.2%) and allowed to infuse for 10 min after which the infusions were filtered through a sterile sieve. Sucrose (7%) was dissolved in hot tea and the preparation was left to cool. The cooled tea was inoculated with 2% (w/v) of starter cultures of acetic acid bacteria LUMBI B11 and yeast LUMBI L55 (3:1) isolated from Kombucha symbiotic association obtained from local household. Fermentation was carried out in laboratory type bioreactor (New Brunswick, USA) at 30±1°C for 24 h. Subsequently, the culture medium was stored in polypropylene vials at -20°C for further use.

Acetic acid bacteria was maintained on Hestrin-Schramm (HS) agar plates (20 g L^{-1} glucose, 5 g L^{-1} peptone, 5 g L^{-1} yeast extract, 2.72 g L^{-1} Na_2HPO_4, 1.15 g L^{-1} citric acid), pH 5.5. Yeast was maintained on Sabouraud agar plates (10 g L^{-1} peptone, 40 g L^{-1} glucose), pH 5.6.

Characteristics of obtained fermented beverage: Dry matter 6.7%, 30 g L^{-1} glucose, 21.4 g L^{-1} gluconic acid, pH 2.7.

Animals: Male albino Wistar rats weighing 180-200 g were used. Rats were fed pellet diet (A/S Tukuma Straume, Tukums, Latvia), tap water was provided *ad libitum*. The animals were maintained in accordance with the guidelines of the Regulations No 52 of 22 January 2013 of Cabinet of Ministers, the Republic of Latvia according to the Law for the Protection of Animals, Republic of Latvia. All experimental procedures were approved by the Food and Veterinary Agency.

Rats were divided into groups of six animals each. Each group was subjected to one of the following treatments described in the section below.

Experimental design: In this experiment, a total of 36 rats were used. The rats were divided into four groups of six each. Group I: Rats receiving normal diet. Group II: Rats receiving 2.5 mL of fermented beverage kg^{-1} b.wt. Group III: Rats receiving high fat diet (butter 82.5%, Smiltenes Piens, Latvia). Group IV: Rats receiving high fat diet (butter 82.5%, Smiltenes Piens, Latvia) and 2.5 mL of fermented beverage/kg b.wt.

Fermented beverage was orally administered to rats using an intragastric tube daily for a period of 60 days. For obtaining fat enriched feed, pellets were saturated with liquefied butter to contain 6 g butter per daily portion.

Analytical determinations: The analysis of Total Cholesterol (TC), High Density Cholesterol (HDC), Low Density Cholesterol (LDC), Triglycerides (TG), Alanine Transaminase (ALAT), Asparagine Transaminase (ASAT) and Homocysteine (HC) were performed in E. Gulbis Laboratory Ltd., (ISO 15189:2008).

Statistics: All the data were expressed as Mean±Standard Deviation (S.D) for six rats in each group (n = 6). Values were considered statistically significant when $p<0.05$.

RESULTS AND DISCUSSION

Recently there has been much attention paid regarding Kombucha health benefits (Dufresne and Farnworth, 2000; Vina *et al*., 2013). The hepatoprotective and hypocholesterolaemic effects of Kombucha have already been evaluated by several studies (Murugesan *et al*., 2009; Yang *et al*., 2009; Adriani *et al*., 2011; Abshenas *et al*., 2012; Aloulou *et al*., 2012). As the fermented beverage used in this study was obtained using microorganisms isolated from Kombucha symbiotic association, it was important to evaluate whether it can provide comparable physiological activity as native Kombucha symbiotic association.

Hypercholesterolemia is one of the main risk factors for cardiovascular diseases. As shown in Table 1 the group which was fed with high fat diet had higher low-density cholesterol (LDCh) concentration in plasma than group receiving normal diet. Fermented beverage administration lowered elevated LDCh levels. The same was observed for triglycerides concentration. Triglycerides concentration in high fat diet group was increased almost double. However, in groups receiving fermented beverage the increase was less pronounced. Triglycerides in plasma are derived from animal fats consumed foods or produced in the body from other energy sources like carbohydrates (Parks and Hellerstein, 2000; Miller *et al*., 2011). Excess of triglycerides in plasma are linked to coronary artery disease (McBride, 2007; Gandotra and Miller, 2008). Slight increase in High-density Cholesterol (HDCh) was observed in groups receiving fermented beverage. HDCh particles remove fats and cholesterol from cells, including within artery wall atheroma and transport it back to the liver for excretion or reutilization (Holven *et al*., 2013).

Different mechanisms of Kombucha anti-hypercholesterolemic action have been suggested by researchers. Fermented beverage under study could reduce cholesterol levels in the blood through the inhibition of cholesterol synthesis enzyme HMG (3-hydroxy 3-metilglutaril CoA reductase) activity in liver and/or through the mechanism of increased excretion of cholesterol (Vina *et al*., 2013). Since Kombucha contains considerable amount of poliphenols as well vitamins E and C, known as potent antioxidants (Jayabalan *et al*., 2008; Yang *et al*., 2009; Kallel *et al*., 2012), suppression of oxidative stress was proposed as a mechanism of hyperholesterolemic atherosclerosis risk reduction (Yang *et al*., 2009).

Elevated levels of homocysteine in the blood may be associated with atherosclerosis as well as an increased risk of heart attacks, strokes, blood clot formation and possibly Alzheimer's disease (Nygard *et al*., 1999; Gursu *et al*., 2002). When combined with hypercholesterolemia elevated levels of homocysteine

Table 1: Serum cholesterol and triglycerides status in rats after 60 days intake of fermented beverage

Group	Total cholesterol (mmol L^{-1})	High density cholesterol (mmol L^{-1})	Low density cholesterol (mmol L^{-1})	Triglycerides
Control	1.71±0.08	0.48±0.02	0.13±0.01	1.70±0.08
Fermented beverage (2.5 mL kg^{-1} b.wt.)	1.63±0.08	0.50±0.03	0.12±0.01	1.51±0.08
Fat diet	1.79±0.08	0.50±0.02	0.18±0.01	4.06±0.21
Fat diet+fermented beverage (2.5 mL kg^{-1} b.wt.)	1.79±0.08	0.55±0.03	0.13±0.01	2.44±0.13

Table 2: Homocysteine status in rats after 60 days intake of fermented beverage

Group	Homocysteine (μmol L^{-1})
Control	5.57±0.28
Fat diet	7.54±0.34
Fat diet+fermented beverage (2.5 mL kg^{-1} b.wt.)	6.98±0.33

Table 3: Liver marker enzyme status in rats after 60 days of preparation intake

Group	ASAT (U L^{-1})	ALAT (U L^{-1})
Control	117±7	81±4
Fermented beverage (2.5 mL kg^{-1} b.wt.)	113±5	69±3
Fat diet	95±5	58±4
Fat diet+fermented beverage (2.5 mL kg^{-1} b.wt.)	89±4	59±4

could even more increase the risk of cardiovascular diseases. It was shown that high intakes of saturated fatty acids were associated with high plasma concentrations of homocysteine (Berstad *et al*., 2007). The possible relation between fat intake and plasma homocysteine may be explained by a biochemical link between homocysteine and lipid metabolism (Berstad *et al*., 2007).

In this study it was shown that fermented beverage intake decreased homocysteine concentration in high fat diet animals (Table 2). It could be suggested that the decrease in homocysteine concentration during fermented beverage intake is indirectly linked with lowered cholesterol levels but further research is needed to elucidate possible mechanisms of action of fermented beverage on homocysteine levels.

Table 3 shows that activities of liver marker enzymes ASAT and ALAT decreases due to fermented beverage consumption in both-normal and high fat diet group. Thus it was shown that the effect reported for native Kombucha consumption could be observed when defined starter cultures are used. It is known that increased levels of ALAT and ASAT indicate the liver cell damage. It has been reported that Kombucha acts as hepatoprotective agent when liver cell damage was induced by artificial hepatotoxins like acetominophen or carbon tetrachloride (Murugesan *et al*., 2009; Abshenas *et al*., 2012). In our study no exogenous hepatotoxins were applied however liver marker enzymes levels can increase also due to high fat intake and subsequent increase in cholesterol because liver is main organ for cholesterol metabolism (Dietschy *et al*., 1993). Thus Kombucha or yeast and acetic acid bacteria fermented beverage under study could be recommended to virtually healthy subjects as refreshing beverage or prophylactic nutraceutic for improvement of liver function especially taking into account the increased consumption of xenobiotics with food or due to environmental pollution.

This study on the physiological effects of fermented beverage obtained by defined starter suggests that it could be suitable for prevention of some metabolic disorders, like cardiovascular diseases or liver disorders, attributed to unhealthy lifestyle like high-fat diet. Obtained results showed similar health benefits as for native Kombucha symbiotic association. It should be noted that only products with constant composition can be used for health prophylaxis or treatment of illnesses. No significant influence were observed on complete blood count values after 60 days of intake of fermented beverage (data not shown) thus proving that the beverage is safe for consumption in concentration 2.5-7.5 mL kg^{-1} b.wt. Zero rat mortality was observed. However further research on physiological effects and possible mechanisms of action is needed and human studies would be welcomed.

ACKNOWLEDGMENT

This research was supported by ERDF project No. 2010/0322/2DP/2.1.1.1.0/10/APIA/VIAA/108.

REFERENCES

Abshenas, J., A. Derakhshanfar, M.H. Ferdosi and S. Hasanzadeh, 2012. Protective effect of kombucha tea against acetaminophen-induced hepatotoxicity in mice: A biochemical and histopathological study. Comp. Clin. Pathol., 21: 1243-1248.

Adriani, L., N. Mayasari, Angga and R. Kartasudjana, 2011. The effect of feeding fermented kombucha tea on HLD, LDL and total cholesterol levels in the duck bloods. Biotechnol. Anim. Husb., 27: 1749-1755.

Aloulou, A., K. Hamden, D. Elloumi, M.B. Ali and K. Hargafi *et al*., 2012. Hypoglycemic and antilipidemic properties of kombucha tea in alloxan-induced diabetic rats. BMC Complementary Altern. Med., Vol. 12. 10.1186/1472-6882-12-63.

Banerjee, D., S.A. Hassarajani, B. Maity, G. Narayan, S.K. Bandyopadhyay and S. Chattopadhyay, 2010. Comparative healing property of kombucha tea and black tea against indomethacin-induced gastric ulceration in mice: Possible mechanism of action. Food Funct., 1: 284-293.

Battikh, H., A. Bakhroufa and E. Ammar, 2012. Antimicrobial effect of Kombucha analogues. LWT-Food Sci. Technol., 47: 71-77.

Berstad, P., S.V. Konstantinova, H. Refsum, E. Nurk and S.E. Vollset *et al*., 2007. Dietary fat and plasma total homocysteine concentrations in 2 adult age groups: The hordaland homocysteine study. Am. J. Clin. Nutr., 85: 1598-1605.

Blanc, P.J., 1996. Characterization of the tea fungus metabolites. Biotechnol. Lett., 18: 139-142.

Chu, S.C. and C. Chen, 2006. Effects of origins and fermentation time on the antioxidant activities of kombucha. Food Chem., 98: 502-507.

Dayal, M.S., N. Goswami, A. Sahai, V. Jain, G. Mathur and A. Mathur, 2013. Effect of media components on cell growth and bacterial cellulose production from *Acetobacter aceti* MTCC 2623. Carbohydr. Polym., 94: 12-16.

Dietschy, J.M., S.D. Turley and D.K. Spady, 1993. Role of liver in the maintenance of cholesterol and low density lipoprotein homeostasis in different animal species, including humans. J. Lipid Res., 34: 1637-1659.

Dipti, P., B. Yogesh, A.K. Kain, T. Pauline and B. Anju *et al*., 2003. Lead induced oxidative stress: Beneficial effects of Kombucha tea. Biomed. Environ. Sci., 16: 276-282.

Dufresne, C. and E. Farnworth, 2000. Tea, Kombucha and health: A review. Food Res. Int., 33: 409-421.

Finkelstein, J.D. and J.J. Martin, 2000. Homocysteine. Int. J. Biochem. Cell Biol., 32: 385-389.

Gandotra, P. and M. Miller, 2008. The role of triglycerides in cardiovascular risk. Curr. Cardiol. Rep., 10: 505-511.

Gharib, O.A., 2009. Effects of Kombucha on oxidative stress induced nephrotoxicity in rats. Chin. Med., Vol. 4 10.1186/1749-8546-4-23

Greenwalt, C.J., K.H. Steinkraus and R.A. Ledford, 2000. Kombucha, the fermented tea: Microbiology, composition and claimed health effects. J. Food Prot., 63: 976-981.

Grundy, S.M., G.J. Balady, M.H. Criqui, G. Fletcher and P. Greenland *et al*., 1998. Primary prevention of coronary heart disease: Guidance from Framingham. A statement for healthcare professionals from the AHA task force on risk reduction. American Heart Association. Circulation, 97: 1876-1887.

Gursu, M.F., G. Baydas, G. Cikim and H. Canatan, 2002. Insulin increases homocysteine levels in a dose-dependent manner in diabetic rats. Arch. Med. Res., 33: 305-307.

Holven, K.B., K. Retterstol, T. Ueland, S.M. Ulven and M.S. Nenseter *et al*., 2013. Subjects with low plasma HDL cholesterol levels are characterized by an inflammatory and oxidative phenotype. PLoS One, Vol. 8. 10.1371/journal.pone.0078241

Jayabalan, R., P. Subathradevi, S. Marimuthu, M. Sathishkumar and K. Swaminathan, 2008. Changes in free-radical scavenging ability of kombucha tea during fermentation. Food Chem., 109: 227-234.

Kallel, L., V. Desseaux, M. Hamdi, P. Stocker and E.H. Ajandouz, 2012. Insights into the fermentation biochemistry of Kombucha teas and potential impacts of Kombucha drinking on starch digestion. Food Res. Int., 49: 226-232.

Kurtzman, C.P., C.J. Robnett and E. Basehoar-Powers, 2001. *Zygosaccharomyces kombuchaensis*, a new ascosporogenous yeast from Kombucha tea. FEMS Yeast Res., 1: 133-138.

Liu, C.H., W.H. Hsu, F.L. Lee and C.C. Liao, 1996. The isolation and identification of microbes from a fermented tea beverage, Haipao and their interactions during Haipao fermentation. Food Microbiol., 13: 407-415.

Markov, S.L., R.V. Malbasa, M.J. Hauk and D.D. Cvetkovic, 2001. Investigation of tea fungus microbe associations, 1: The yeasts. Acta Periodica Technol., 32: 133-138.

Marsh, A.J., O. O'Sullivan, C. Hill, R.P. Ross and P.D. Cotter, 2014. Sequence-based analysis of the bacterial and fungal compositions of multiple kombucha (tea fungus) samples. Food Microbiol., 38: 171-178.

Mayser, P., S. Fromme, C. Leitzmann and K. Grunder, 1995. The yeast spectrum of the tea fungus Kombucha. Mycoses, 38: 289-295.

McBride, P.E., 2007. Triglycerides and risk for coronary heart disease. JAMA., 298: 336-338.

Miller, M., N.J. Stone, C. Ballantyne, V. Bittner and M.H. Criqui *et al*., 2011. Triglycerides and cardiovascular disease: A scientific statement from the American heart association. Circulation, 123: 2292-2333.

Murugesan, G.S., M. Sathishkumar, R. Jayabalan, A.R. Binupriya, K. Swaminathan and S.E. Yun, 2009. Hepatoprotective and curative properties of kombucha tea against carbon tetrachloride-induced toxicity. J. Microbiol. Biotechnol., 19: 397-402.

Nygard, O., S.E. Vollset, H. Refsum, L. Brattstrom and P.M Ueland, 1999. Total homocysteine and cardiovascular disease. J. Internal Med., 246: 425-454.

Parks, E.J. and M.K. Hellerstein, 2000. Carbohydrate-induced hypertriacylglycerolemia: Historical perspective and review of biological mechanisms. Am. J. Clin. Nutr., 71: 412-433.

Sai Ram, M., B. Anju, T. Pauline, D. Prasad and A.K. Kain *et al*., 2010. Effect of kombucha tea on chromate (VI)-induced oxidative stress in albino rats. J. Ethnopharmacol., 71: 235-240.

Sreeramulu, G., Y. Zhu and W. Knol, 2001. Characterization of antimicrobial activity in Kombucha fermentation. Acta Biotechnol., 21: 49-56.

Steinkraus, K.H., K.B. Shapiro, J.H. Hotchkiss and R.P. Mortlock, 1996. Investigations into the antibiotic activity of tea fungus/Kombucha beverage. Acta Biotechnol., 16: 199-205.

Vina I., P. Semjonovs, R. Linde and I. Denina, 2013. Current evidence on physiological activity of kombucha fermented beverage and expected health effects. J. Med. Food, 10.1089/jmf.2013.0031

Yang, Z.W., B.P. Ji, F. Zhou, B. Li, Y. Luo, L. Yang and T. Li, 2009. Hypocholesterolaemic and antioxidant effects of kombucha tea in high-cholesterol fed mice. J. Sci. Food Agric., 89: 150-156.