Research Article

Bioactivity Study of Kombucha Black tea and Kombucha with Skim Milk on Some of Physiological and Biochemical Parameters in Male Albino Rats

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ABSTRACT

Kombucha is known as a probiotic, and used in several products to promotions of food. The present research was aimed to determine and investigate the beneficial and protective effects for the beverage of kombucha black tea alone and with skim milk fermented on concentrations of serum glucose, total lipids profile and body weights in male albino rats. The animals were distributed into four groups, the control group (I), group of skim milk (II), kombucha black tea group (III) and Kombucha in skim milk fermented group (IV), the dose of materials under study administered orally for experimental groups by gastric gavage method once daily for period (21) days. Blood samples were collected at the final of experiment and analyzed. The results revealed a significantly higher ($p<0.05$) in levels of serum glucose, total lipid profile, and significantly decrease ($P<0.05$) in HDL-C, AST and ALP in the skim milk group (II) in comparison with control group. While, animal groups that treated with Kombucha black tea (III) and Kombucha in skim milk fermentation (IV) lead to significantly decrease in concentrations of serum glucose, total lipid profile, ALT, AST, ALP and significantly higher in HDL-C and body weights in comparison with group (II) and normal control group consecutively. Moreover, administrated of Kombucha in skim milk fermentation showed significant decrease in above mentioned parameters compared with groups which treated with Kombucha black tea only. The obtained present results demonstrate that use Kombucha as a probiotic in skim milk fermented and with black tea lowers the harmful lipids, glucose and aminotransferases in blood, and it's maybe have a beneficial effects on the liver and the health of human body.

Keywords: Kombucha, Total lipid profile, Glucose, Probiotics, Skim milk.

INTRODUCTION

Kombucha is a sour beverage prepared from the fermentation of sugared black tea with a symbiotic culture of acetic acid bacteria and yeasts such as *Acetobacter xylinoides*, *Acetobacter xylinum*, *Bacterium xylinoides*, *Bacterium xylinum* and the novel species, *Acetobacter nitrogenifigens sp. nov.* and *Gluconacetobacter kombuchae sp. nov*., *Saccharomyces ludwigii*, *Saccharomyces cerevisiae*, *Saccharomyces apiculatus varieties*, *Schizosaccaromyces pombe*, *Zygosaccharomyces bailii* and *Pichia fermantans* (Jayabalan *et al*., 2014). In recent researches has been applied for milk fermentation with the aim to enlarge variety of the dairy products as a functional food. Fermentation of milk by kombucha is concerned, only a some of investigations have been reported; Lončar *et al*. (2001) they findings of metabolic-activity of kombucha on milk, also, Belloso and Hernández, (2003) refer to the production of beverage from cheese whey. During the fermentation process, bacteria and yeasts metabolize sucrose to produce a number of organic acids such as glucuronic acid, acetic acid, amino acids, vitamins, anti-infection agents and an assortment of micronutrients (Chu & Chen, 2006).

Figure (1): kombucha black tea.

Kombucha is rich in antioxidants and probiotic acids that can detoxify disease-causing toxins and hence alleviates a wide spectrum of pathological conditions. Therefore, the present study was aimed to determine a beneficial and protective effects of kombucha black tea with probiotic in

skim milk fermented on concentrations of lipids and glucose in male albino rats.

MATERIALS AND METHODS

Prepare of experimental samples:

The products of fermented milk were industrialized in laboratory conditions, include pasteurized skim milk (ALSAFI, Saudi) with 5% sucrose. The starter cultures were utilized for fermentation:

1- Probiotics starter culture-lacteol fort® contains LB, *Lactobacillus delbruekii, Lactobacillus fermentum*, French.

2- Kombucha-HiMedia company, India.

Kombucha black tea inoculum, kombucha cultured on 50 g/L of sucrose as substrate with addition of 1.5 g/L black tea at 35°C, and incubate for 12h, then, samples were prepared by addition 90 ml of inoculum of kombucha and 0.100g/L from cultures of probiotic starter in 900 ml skim milk at 37°C. Fermentation was continued until 4.5h then cooled and kept in refrigerator (4°C) (Abdul-Rahman *et al*., 2011; Spasenija *et al*., 2012).

Experimental animals:

This study was conducted on uses laboratory animals which are healthy adult a wistar strain male albino rats, weighing between 100-105 g, obtained from the animal house of faculty of veterinary medicine, university of Mosul, Iraq. They were placed under laboratory standard conditions of temperature and humidity, in addition to allowing for 12 hour light-dark cycle. A standard pellet diet and water were supplied in adequate amounts *ad libitum* and throughout the performance period of experiments between February-June, 2017.

Design of Experiments:

The laboratory rats which used in this study (20) animals. They were divided into four (4) groups, with five (5) rats in each group, as follows: The healthy control group (I): supplied only with food and drinking tap water daily for a period (21) days, skim milk control group (II): administered orally skim milk (3.6 ml/200g of body wt.) by using an gavage for a period (21) days. a Kombucha black tea group (III): this group had been given Kombucha black tea (3.6 ml/200g of body wt.) daily for a period (21) days, and group (IV): this group treated with milk fermented by kombucha inoculum and probiotic starter (3.6 ml/200g of body wt.) daily by using an oral gavage for a period (21) days.

Finally, after end of the experiment (21) days, the laboratory animals were fasted overnight, then anaesthetized with chloroform, and blood was obtained by jugular vein severance and collected approximately (4) ml from every animal, then put in test tubes devoid of anticoagulant and then it lets in water bath for period (15) minutes at 37°C, after that centrifuged and separate the serum for measuring concentrations of glucose, high density lipoprotein-cholesterol (HDL-C), triglycerides (TG), cholesterol, low density lipoprotein-cholesterol (LDL-C), very low density lipoprotein-cholesterol (VLDL-C), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP).

The biochemical tests:

The body weights of laboratory animals were determined by a digital balance. While analysis of serum glucose, cholesterol, TG and HDL-C by using a diagnostic kits which depending on clinical chemistry methods according to the manufacturer's recommended procedure from Biolabo, Rondex Laboratories Ltd (Tietz, 2005). In addition, assessment of serum ALT, AST and ALP enzymes by an enzymatic method (Deneke & Rittersdorf,1984; Deneke *et al*., 1985; Rosalki,1993) successively, by using kits supplied from (Roche Diagnostics GmbH, Germany).

STATISTICAL ANALYSIS

The data of results in the present study were analyzed by using the ANOVA analysis, utilized the general linear model of the Statistically Analysis System (SAS, 2001). Also, significant differences were evaluated by using Duncan's multiple-range test (Duncan, 1955), and significance level is based on level of probability ($P<0.05$).

THE RESULTS AND DISCUSSION

Results in table (1) showed that the laboratory animal groups which administrated of Kombucha black tea and Kombucha in skim milk fermented, respectively caused a significant increase ($P<0.05$) of body weight gain compared with normal control group. While observed no significant variations in body weight gain in animals group that treated only with skim milk in comparison with normal control group.

Table 1. Effect of Kombucha treatment on body weight in male albino rats

Groups	Initial body weight (g)	Final body weight (g)	Body weight gain (g)
Normal control (I)	100 ± 2.04 a	120 ± 2.00 b	20 ± 1.21 b
Skim milk Control (II)	102 ± 2.05 a	124 ± 2.05 b	22 ± 1.40 b
Kombucha black tea (III)	101 ± 2.07 a	130 ± 2.03 a	29 ± 1.34 a
Kombucha in Skim milk fermented (IV)	103 ± 2.10 a	133 ± 1.90 a	30 ± 1.30 a

- The values represent mean±S.E.
- Different of letters vertically means significant difference at significance level (P <0.05).

Whereas the tables (2,3) showed that the laboratory animal group which administrated of only skim milk caused significant increase (P<0.05) in concentrations of glucose, cholesterol, TG and LDL-C, and significant decrease (P<0.05) in concentration of HDL-C, whereas observed no significantly differences (p>0.05) of VLDL-C level in comparison with normal control group.

Table 2. Effect of Kombucha treatment on concentrations of triglycerides, cholesterol and glucose in serum of male albino rats

Groups	Glucose(mg\dl)	Cholesterol(mg\dl)	Triglycerides(mg\dl)
Normal control (I)	94.20 ± 0.37 b	91.35 ± 0.38 b	100.6 ± 0.40 b
Skim milk Control (II)	95.60 ± 0.50 a	94.30 ± 0.37 a	103.2 ± 0.37 a
Kombucha black tea (III)	86.00 ± 0.31 c	85.20 ± 0.39 c	95.20 ± 0.37 c
Kombucha in Skim milk fermented (IV)	81.30 ± 0.48 d	82.70 ± 0.30 d	91.60 ± 0.50 d

- The values represent mean±S.E.
- Different of letters vertically means significant difference at significance level (P <0.05).

While the animal groups that treated with Kombucha black tea and Kombucha in skim milk fermentation lead to significant decrease in concentrations of glucose, cholesterol, LDL-C, TG and VLDL-C, and significantly increase of HDL-C concentration in comparison with group which administrated skim milk and normal control group consecutively.

Table 3. Effect of Kombucha treatment on concentrations of serum HDL-C, LDL-C and VLDL-C in male albino rats

Groups	HDL-C(mg\dl)	LDL-C(mg\dl)	VLDL-C(mg\dl)
Normal control (I)	24.00 ± 0.31 c	47.23 ± 0.21 b	20.12 ± 0.08 a
Skim milk Control (II)	22.40 ± 0.40 d	51.38 ± 0.48 a	20.64 ± 0.07 a
Kombucha black tea (III)	26.20 ± 0.39 b	39.66 ± 0.34 c	19.04 ± 0.07 c
Kombucha in Skim milk fermented (IV)	28.20 ± 0.45 a	36.18 ± 0.60 c	18.32 ± 0.10 d

- The values represent mean±S.E.
- Different of letters vertically means significant difference at significance level (P <0.05).

Moreover, the groups that administrated of Kombucha in skim milk fermentation showed significant decrease in concentrations of glucose, cholesterol, TG and VLDL, and significant higher (P<0.05) of HDL-C in comparison with groups which treated by Kombucha black tea only.

Also, a results in table (4) showed that the laboratory animals group which administrated of only skim milk caused a significantly decrease (P<0.05) in concentrations of AST and ALP, whereas observed no significant difference (p>0.05) in concentration of ALT in compared with normal control group. in the same situation, the animal groups that treated with Kombucha black tea and Kombucha in skim milk fermentation lead to significant decrease in concentrations of ALT, AST and ALP in comparison with group which administrated skim milk and normal control group consecutively.

Table 4. Effect of Kombucha treatment on concentrations of serum ALT, AST and ALP enzymes in male albino rats

Groups	ALT (U\L)	AST (U\L)	ALP (U\L)
Normal control (I)	54.60±0.67 a	312.20±0.66 a	284.20±1.28 a
Skim milk Control (II)	55.60±0.25 a	310.00±0.63 b	280.60±0.40 b
Kombucha black tea (III)	52.00±0.40 b	305.0±0.31 c	265.800±1.01 c
Kombucha in Skim milk fermented (IV)	50.00±0.30 b	300.0±0.40 d	257.20±0.80 d

- The values represent mean±S.E.
- Different of letters vertically means significant difference at significance level (P <0.05).

On the other hand, the groups that administrated of Kombucha in skim milk fermentation revealed a significant decrease in concentrations of AST and ALP, whenas showed no significant variation in ALT concentration in compared with a group which treated only with Kombucha black tea respectively.

The results in present study may due to the following causes: There are suggestion that increased of animals body weight post-treatment by Kombucha black tea and Kombucha in skim milk fermented, respectively, that could be attributed to the fact that the fermentation of skimmed milk by kombucha inoculum maybe leads to a changes in the milk contents by synthesis and modification of some important nutrients such as B-complex vitamins and multi-minerals such as Ca, P, K, Mg, Cu, Fe, Zn, Mn and Cr, which has been studied extensively for its medicinal properties (Bekatorou *et al.*, 2006). Further, in additional to the nutrients and minerals; also, Kombucha beverage can be considered as a naturally source rich in vitamins and amino acids specially essential amino acids (Smith, 1996). On the other hand, may be explained the positive effect of kombucha at increase of body weight due to the containment cell wall of yeast on Mannanoligosaccharides that are important in the health status and increased of body weight, in addition to longer villi and improve mucosal architecture for intestines (Yang *et al.*, 2007). Also, Mannanoligosaccharides may lead to promoting growth of the body by reducing the depth of mucosal crypts in the small intestine (Yang *et al.*, 2009).

The results in present study may due to the following causes: The lowering concentration of serum glucose may be refer to insulino-mimetic action or by peripheral utilization of glucose stimulated by the metabolites produced during fermentation (Bhattacharya *et al.*, 2013). On the other side, the approximative decrease in level of glucose may be attributed to the utilization of glucan compounds which produced from probiotics microbes in this fermentation process, and action as conjugated with glucose molecules, causing a defect in a glucose absorbed from intestine and increased the raised rates with feces (Tietz, 2005; Karaca *et al.*, 2013).

In addition to, oral administration of kombucha beverage for animals showed lowering concentration in parameters of lipid profile, this may be due to the antioxidant activity of kombucha which contain a group of antioxidants mainly the presence of polyphenols such as flavonoids and catechins (Jayabalan *et al.*, 2007), thereby maybe eliminates the oxidative stress and free radicals that cause of lipid peroxidation, subsequently, lead to lowering the levels of cholesterol and harmful lipids (LDL-C) and increasing in concentration of beneficial lipid (HDL-C). On the other hand, the synergistic action of different compounds in Kombucha beverage such as organic acids that produced during the fermentation period (Veli´canski *et al.*, 2013), and improve energy and lipids metabolism may cause decrease in concentrations of serum cholesterol, LDL-C, TG and VLDL-C.

Also, results in the present study with treatment by Kombucha beverage reversed these parameters to nearby of normal levels which could be due to decreased metabolic disturbances of other pathway such as protein and nucleic acid metabolism and improved glycemic control. Or maybe reverted to be found of acetic acid and glucuronic acid that important in removal of free radicals and get rid of waste products from the blood and body (Flora & Chandrakala, 2016), therefore, Kombucha may lead to served in decrease concentrations of cholesterol, TG, LDL-C and VLDL-C and increase level of HDL-C.

In the same situation, decreased of lipids were perhaps attributed to the ability for production of glucan oligosaccharides from yeast and Lactic acid bacteria (LAB) species that found in Kombucha at skim milk fermentation, which the consumed from animals was action to increase the viscosity of the small intestine causing to increase the secretion of bile salts and decreased the shifting of these salts for formation of cholesterol and thus causing to decrease the cholesterol in plasma blood (Kumar *et al.*, 2012; Roselino *et al.*, 2012).

On the other hand, the decreased in the levels of cholesterol and TG of rats in present study, may be refer to multiple mechanisms which taken by such as, that the products of fermentation of Kombucha such as niacin

(Vitamin B3), that employed for reducing of high LDL, cholesterol and TG (fats) in blood and increasing of effectively HDL (Adriani *et al*., 2011), a stimulatory effect of Vitamin B3 in early formation for HDL within biogenesis process in liver, that suggests a mechanism engage in niacin role to increase early biogenesis of HDL by stimulating phospholipids/cholesterol effluence via a DR4-dependent transcription for gene of ABCA1, also niacin use as mediated to emergence of apoA-I biogenesis and lipidation of nascent HDL, and thus may be leading to increase formation and concentration of HDL-C (Zhang *et al*., 2012).

Moreover, fermentation of milk causing to be contains the different vitamins which produced by yeasts or LAB, especially folic acid (Rad *et al*., 2016), folate may be decrease of acetyl CoA concentration by reduction of ß-oxidation process for fatty acids, as acetyl CoA is the ingredient key in cholesterol synthesis, as a result, decrease concentrations of cholesterol and LDL-C (Owoyele *et al*., 2005).

Also, *Lactobacillus* bacteria can produce ferulic acid (Tomaro-Duchesneau *et al*., 2012), that can inhibit HMG-CoA reductase (3-hydroxy-3-methyl-glutaryl-coenzymeA reductase) which contribute in hepatic cholesterol synthesis, subsequently, decreased of cholesterol concentration in bloodstream. Moreover, the products of Probiotics such as short-chain fatty acids like butyric acid, which have inhibition effect for cholesterol synthesis in liver by blocking of HMG-CoA reductase activity (Kumar *et al*., 2012). As well as, a previous reports refer to that supplementing rations with probiotics reduced total lipid profile levels in the serum (Chiu-Hsia *et al*., 2006; Abd-alwahab & Al-dulaimi, 2018).

The lowering concentrations of serum ALT, AST and ALP enzymes, that refer to prevention the leakage of enzymes to interstitial or extracellular compartment as a result cell membrane stability or cellular regeneration (Daisy & Feril, 2013), this effect observed in animal groups which treated with Kombucha black tea and Kombucha in skim milk fermentation that may be due to produce several of antioxidants such as flavonoids, catechins and vitamin E which have a removable or neutralize effect for free radicals that can cause oxidative stress and peroxidation of phospholipids in the cell membranes.

In conclusion, this study suggest that from beneficial applications of Kombucha may due to its effects for reducing a harmful lipids and amelioration of liver functions.

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