Myocardial potency of Bio-tea against Isoproterenol induced myocardial damage in rats

Reema Orison Lobo · Chandrakala K. Shenoy

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Abstract Kombucha (Bio-tea) is a beverage produced by the fermentation of sugared black tea using a symbiotic association of bacteria and yeasts. Traditional claims about Kombucha report beneficial effects such as antibiotic properties, gastric regulation, relief from joint rheumatism and positive influence on the cholesterol level, arteriosclerosis, diabetes, and aging problems. The present investigation was carried out to understand the preventive effect of Kombucha on heart weight, blood glucose, total protein, lipid profile and cardiac markers in rats with myocardial damage induced using Isoproterenol. As Bio-tea is produced by fermenting tea, the parameters were compared in rats pre-treated with normal black tea and Bio-tea for 30 days followed by subcutaneous injection of Isoproterenol (85 mg/kg body weight). Normal rats as well as Isoproterenol induced myocardial infarcted rats were also used, which served as controls. Isoproterenol induced myocardial infarcted control rats showed a significant increase in heart weight, blood glucose and cardiac markers and a decrease in plasma protein. Increased levels of cholesterol, triglycerides, low density lipids (LDL) and very low density lipids (VLDL) were also observed, while the high density lipid (HDL) content decreased. Bio-tea showed a higher preventive effect against myocardial infarction when compared to tea, as was observed by the significant reduction in heart weight, and blood glucose and increase in plasma albumin levels. Bio-tea significantly decreased cholesterol, triglycerides, LDL and VLDL while simultaneously increasing the levels of HDL. Similarly a decrease in leakage of cardiac markers from the myocardium was also observed.

Keywords Kombucha · Isoproterenol · Myocardial infarction · Lipid profile · Cardiac markers

Introduction

Cardiovascular diseases comprise a group of diseases of the heart and the vascular system. At the beginning of the 20th Century, cardiovascular disease accounted for <10 % of all deaths worldwide. At the beginning of the 21st Century, cardiovascular disease accounted for nearly half of all deaths in the developed world and 25 % in the developing world (Murray and Lopez 1996; WHO 2002). In India about 1.59 million deaths were reported as a result of cardiovascular diseases in 2000, which rose to 2.03 million in 2010. Cardiovascular diseases are set to dominate as the major cause of death by 2020, accounting for at least one in every three deaths (WHO 2002). Myocardial infarction (MI) is a condition which arises due to imbalance between myocardial demand and coronary blood supply which in turn causes necrosis of the myocardium (Boudina et al. 2002; De Bono and Boon 1992) and is the principle cause of death in developed as well as developing countries (Agarwal et al. 2006). Despite the advances in the treatment of coronary artery disease, acute myocardial infarction is one of the leading causes of morbidity and mortality worldwide in both men and women (Adams 2002; Whellan 2005).

Bio-tea, also named as Kombucha, is a popular beverage among many traditional fermented foods, across the world. Bio-tea is a sugared black tea, fermented with a symbiotic association of acetic acid bacteria and yeasts. This beverage reportedly exerts a number of medicinal effects against metabolic disease, arthritis, psoriasis, constipation, indigestion and hypertension. Although there are numerous claims that drinking Bio-tea is beneficial to health, these individual testimonies have not been validated scientifically (Allen 1998). Many of

R. O. Lobo (✉) · C. K. Shenoy
Department of Biosciences, Mangalore University,
Mangalagangothri, Mangalore 574199, Karnataka, India
e-mail: reema.orison@gmail.com

C. K. Shenoy
e-mail: shenoychandrakala@gmail.com

the claimed beneficial effects of Bio-tea such as alleviation of inflammation and arthritis, cancer prevention and enhancement of immunity may be attributed to its antioxidant activities (Chu and Chen 2006). Wang et al. (2014) have demonstrated that the hepatoprotective effects of Kombucha are due to the presence of D-saccharic acid-1,4-lactone (DSL) produced by a *Gluconacetobacter* sp. present in Kombucha pellicle.

The present study has been carried out as there is paucity of information regarding the role of Kombucha in prevention of MI, thereby, enabling Kombucha to be used for drug development against cardiovascular diseases.

Materials and methods

Experimental animals and diet

All the experiments were carried out as per the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India and approved by the Institutional Animal Ethics Committee (IAEC) of Mangalore University (CPCSEA registration No.-232). The study was conducted on twenty four male albino Wistar rats (*Rattus norvegicus*) weighing 210–240 g, maintained in the Animal House, Department of Biosciences, Mangalore University, Karnataka, India. The rats were housed in polypropylene cages, lined with husk, renewed every 24 h under a 12:12 h light dark cycle at around 22 °C and food and water was supplied ad libitum. The rats were fed on a standard pellet diet (Pranav Agro Industries Limited, Maharashtra, India).

Drugs and chemicals

Isoproterenol hydrochloride was purchased from Sigma Chemical Company, St. Louis, MO, USA while all the other chemicals used were of analytical grade.

Preparation of ISO

ISO was prepared at a concentration of 85 mg/kg body weight in cold saline.

Preparation of Kombucha

Kombucha was prepared by adding 10 % commercial sucrose to boiling water, followed by 7.5 g/L tea leaves (Brooke Bond Red Label) and allowed to simmer for 3 min. After cooling to 30 °C, the tea decoction was filtered into clean glass bottles and Kombucha pellicle from previous culture was added to it. Incubation was carried out at room temperature under aerobic

conditions for 7 days. After 7 days, the Kombucha obtained was filtered and sterilized before refrigeration.

Experimental protocol

The animals were divided into 4 groups containing 6 rats each and the following treatment groups were used for the study:

- Group 1: Normal control rats
- Group 2: ISO-induced control rats
- Group 3: Tea pre-treated+ISO-induced rats
- Group 4: Bio-tea pre-treated+ISO-induced rats

Tea and Kombucha were administered (per os) daily at a concentration of 1.71 ml/kg body weight for a period of 30 days. Cardiotoxicity was induced by subcutaneous administration of ISO (85 mg/kg) to rats on the 29th and 30th days at a 24 h interval (Goyal et al. 2009).

At the end of the study period the animals were anesthetized using ketamine 22–24 mg/kg i.m., and blood was collected by heart puncture for biochemical estimations. The animals were sacrificed by cervical dislocation and the heart was excised.

Estimation of biochemical parameters

Assay of glucose, protein and lipid profile

Serum glucose, total protein, albumin, total cholesterol, triglycerides and high density lipoprotein (HDL) were analyzed using commercial kits (Agappe Diagnostics Ltd., Kerala).

Serum glucose was determined by using glucose oxidase, as per Trinder (1969). Biuret method was employed for the determination of proteins (Gornall et al. 1949). Albumin was estimated spectrophotometrically by reaction with the dye bromocresol green (Doumas et al. 1971). Estimation of total cholesterol was carried out using the protocol of Flegg (1973). Estimation of triglycerides involved the reactions by lipases, glycerol kinase, glycerol—phosphate oxidase and peroxidase, which produced a red quinone dye, read at 630 nm (Annoni et al. 1982). HDL cholesterol was estimated by precipitating out low density lipoproteins (LDL) and very low density lipoproteins (VLDL) from the serum as per Gordon et al. 1977.

Serum LDL and VLDL were calculated with the Friedewald formula (Friedewald et al. 1972):

$$\text{LDL} = \text{Total cholesterol} - (\text{HDL cholesterol} + \text{Triglycerides}/5)$$

$$\text{VLDL} = \text{Triglycerides}/5$$

The results were expressed in g/dl for total protein and albumin, and as mg/dl for the rest.

Assay of marker enzymes

The marker enzymes Creatine Kinase (CK), Creatine Kinase-MB (CK-MB), Lactate Dehydrogenase (LDH), Aspartate Transaminase (AST) and Alanine Transaminase (ALT) were assayed in serum and heart homogenate using standard kits supplied from Agappe Diagnostics Ltd., Kerala, India. The results were expressed as IU/L in serum, while CK in heart homogenate was expressed as μ moles of phosphorous liberated/min/mg protein and as nmoles of pyruvate liberated/min/mg protein for LDH, AST and ALT. The levels of total proteins were determined in heart homogenates of experimental animals by using the Lowry's method (Lowry et al. 1951).

Statistical analysis

The data was statistically analyzed using One-way ANOVA (SigmaPlot 11; Systat Software Inc., USA) followed by Duncan's multiple range test (DMRT). $P < 0.05$ was considered statistically significant.

Results

Heart weight

The heart weight of the ISO induced MI rats was significantly higher when compared to normal control rats as indicated by the significant elevation of the heart weight-body weight ratio. Significant ($P < 0.01$) reduction in heart weight was observed in MI rats which were pre-treated with Kombucha as compared to ISO induced control rats (Table 1). The reduction in heart weight was not significant in the MI rats pre-treated with tea.

Glucose levels

The serum glucose levels of the ISO induced MI rats were significantly higher compared to the normal control rats. Significant ($P < 0.01$) reduction in serum glucose levels were observed in MI rats pre-treated with Kombucha as compared to the ISO induced MI rats. Significant ($P < 0.05$) reduction in serum glucose levels were also observed in MI rats pre-treated with tea. However the degree of reduction by Kombucha is significantly higher when compared to tea (Table 2).

Serum protein

The total serum protein levels of the ISO induced MI rats were significantly lower compared to the normal control rats. However, even though increase in serum protein levels were

observed in MI rats pre-treated with tea and Kombucha, the increased levels were not quite significant. The albumin levels were significantly lowered in ISO induced MI rats in comparison to the normal control rats. Significant ($P < 0.01$) increase in serum albumin levels were observed in MI rats pre-treated with Kombucha, which was higher than that of MI rats pre-treated with tea ($P < 0.05$) (Table 2).

Lipid profile

Alteration in the lipid profile in MI rats and Kombucha pre-treated MI rats are shown in Fig. 1. The total cholesterol levels in the serum of ISO induced MI were significantly higher in comparison to the normal rats. Significant ($P < 0.01$) reduction in total cholesterol levels were observed in MI rats that were pre-treated with Kombucha. Significant ($P < 0.01$) reduction in total cholesterol levels were also observed in MI rats pre-treated with tea. However the reduction was more significant in rats pre-treated with Kombucha than with tea.

Similarly, the LDL and VLDL levels also showed a significant increase in the serum in ISO induced MI rats compared to normal rats. Kombucha pre-treatment was able to lower these lipoprotein levels significantly ($P < 0.05$ and $P < 0.01$) when compared to the ISO induced MI control rats. Tea pre-treated MI rats also showed significant reduction ($P < 0.05$) in LDL and VLDL levels but these were not as significant as those of Kombucha pre-treated rats.

Inversely, a significant decrease in HDL levels was observed in ISO induced MI in rats when compared to the normal control rats. Kombucha pre-treatment demonstrated an increase in the levels of HDL significantly ($P < 0.05$) in MI rats. However the increase in HDL levels in the MI rats on pre-treatment with tea was not significant.

Triglyceride levels in the serum showed a significant increase in ISO induced MI rats when compared against the normal control rats. Significant ($P < 0.05$) decrease was observed in triglyceride levels in MI rats pre-treated with Kombucha, but tea pre-treatment did not show any significant decrease.

Cardiac markers in serum

The variation in the cardiac markers, CK, CK-MB, LDH, AST and ALT in the serum of MI rats and Kombucha pre-treated MI rats are shown in Fig. 2.

The cardiac enzymes in the serum of ISO induced MI rats were significantly higher in comparison to the normal rats. Highly significant ($P < 0.001$) reduction in CK levels were observed in MI rats that were pre-treated with Kombucha. Significant ($P < 0.05$) reduction in CK levels were also observed in MI rats pre-treated with tea. The reduction in CK-MB levels in MI rats pre-treated with tea was not significant, but MI rats pre-treated with Kombucha showed significant

Table 1 Effect of Kombucha on heart weight-body weight ratio in MI rats

Variable	Body weight (g)	Heart weight (g)	Heart weight-body weight ratio
Control	226.5±10.35	0.68±0.09	3.49±0.37
ISO Control	239.18±9.95	1.03±0.08	4.65±0.56
Tea + ISO	233.68±10.17	0.92±0.07	4.22±0.13NS
KT + ISO	229.71±11.12	0.87±0.06	3.92±0.27**

All the groups contained 6 rats. Values are mean±S.D

ISO: Isoproterenol induced. KT: Kombucha

Treated rats *Vs* ISO Control: ** $P<0.01$, NS: Not Significant

decrease ($P<0.05$). Significant ($P<0.05$) decrease was observed in case of LDH, AST and ALT in rats pre-treated with tea. Prior treatment with Kombucha resulted in highly significant decrease ($P<0.01$) in case of LDH and AST whereas the decrease in ALT was found to be same as in MI rats pre-treated with tea.

Cardiac markers in heart tissue

The levels of CK, LDH, AST and ALT were significantly lowered in heart tissues of ISO induced MI rats in comparison to the normal control rats. Significant ($P<0.05$) increase in levels of CK, LDH, AST and ALT were observed in MI rats pre-treated with tea. Similar ($P<0.05$) increase was observed in the concentration of tissue ALT in case of prior treatment with Kombucha, while increase of higher significance ($P<0.01$) was observed in the levels of AST and LDH and highly significant ($P<0.001$) increase was observed in the levels of CK in MI rats pre-treated with Kombucha (Table 3).

Discussion

ISO-induced MI can be used as a well standardised model because the pathophysiological changes following isoproterenol administration occurring in rats are comparable to those

taking place in human MI (Goyal et al. 2009). Hence it is used widely as an experimental model for the evaluation of cardio protective effects of various drugs (Nandave et al. 2009).

The increase in heart weight-body weight ratio indicates cardiac hypertrophy (Vlasov and Volkov 2004), which can be explained by the increase in overall protein biosynthesis during development of hypertrophy accompanied by oedema or over expression of the genes encoding proteins involved in the contractile unit (Heather et al. 2009). The elevation of cytoplasmic calcium during MI could also be a factor responsible for the activation of intracellular signals governing the hypertrophic response of cardiac cells (Choukroun et al. 2002). The reduction in heart weight-body weight ratio indicates the antihypertrophic activity of Kombucha. Therefore Kombucha is able to protect heart cells against stress caused by ISO.

Elevated glucose level at admission has been noted to be a common finding in acute MI (Lavi et al. 2008; Marfella et al. 2005; Nordin et al. 2005). Experimental and clinical studies suggest that rapid increases in serum glucose increases infarct size (Esposito et al. 2002; Williams et al. 1998), impairs left ventricular function, and microvascular obstruction (Jensen et al. 2011). This could be because hyperglycemia increases interstitial fibrosis and myocyte apoptosis that exaggerate left ventricular remodelling (Shiomi et al. 2003). It could also be because hyperglycemia abolishes the cardioprotective effect of ischemic preconditioning by closing K^+ ATP channels (Ishihara et al. 2003; Kersten et al. 2001). However, the

Table 2 Effect of Kombucha on serum glucose, total serum protein and albumin in MI rats

Variable	Serum glucose (mg/dl)	Total serum protein (g/dl)	Albumin (g/dl)
Control	87±4.74	5.93±0.3	3.58±0.15
ISO Control	153±4.4	4.5±0.3	1.95±0.1
Tea + ISO	135±5.8*	4.55±0.34NS	2.1±0.12*
KT + ISO	109±5.9**	5.0±0.3NS	2.8±0.19**

All the groups contained 6 rats. Values are mean±S.D

ISO: Isoproterenol induced. KT: Kombucha

Treated rats *Vs* ISO Control: * $P<0.05$, ** $P<0.01$, NS: Not Significant

Fig. 1 Effect of Kombucha on lipid profile of MI rats. *ISO*: Isoproterenol induced. *KT*: Kombucha. Treated rats *Vs* ISO: * $P<0.05$, ** $P<0.01$

underlying mechanism by which hyperglycemia influences prognosis is still not completely understood. Pre-treatment with Kombucha protects the myocardium by lowering the glucose levels.

Although total protein measurement is a non-specific measurement, as it can be influenced by many variables, it provides general information reflecting disease states in many organ systems (Bishop and Duben-Engelkirk 2000; Luxton 1999). Among several important functions of albumin, one is the scavenging of reactive oxygen radicals, thereby acting as an important antioxidant in the plasma (Burtis and Ashwood 1999). Thus hypoalbuminemia leads to a reduction in the antioxidant scavenging capacity of plasma proteins and this in turn induces myocardial muscle damage by free oxygen radicals (Gosling and Beevers 1989). Decrease in serum total protein in MI, may be due to, the increased degradation of plasma proteins by free oxygen radical, as protein sulfhydryls serve as sacrificial antioxidant and prevent plasma lipid peroxidation as well as being targets for oxidative damage (Lopez et al. 2004). The results of the present study suggest that the increase in total serum protein may be due to the involvement of Kombucha in the defence against oxidative stress associated with acute MI.

Cardiovascular risk factors such as hypercholesterolemia and hypertriglyceridemia have long been studied in order to gain insight into pathophysiology, epidemiology and therapy of MI (Katz and Messineo 1981). Excess lipids such as circulating cholesterol and its accumulation in the myocardium are associated with cardiovascular damage. Altered lipid metabolism can alter the cardiac function by changing the properties of cardiac cell membrane, and these changes may contribute to the cell death that follows coronary artery occlusion (Yeagle 1986). Cholesterol is also a major component of the atherosclerotic plaque that is associated with MI. The increased levels of cholesterol lead to increase in the membrane fluidity, regulate membrane permeability, alter internal viscosity, and also the internal chemical composition (Yeagle 1986).

An increase in the VLDL, LDL and decrease in HDL are associated with raised risk for MI (Buring et al. 1992; Mediene-Benchekor et al. 2001) which has been confirmed in this study. The reason for the increase in LDL in blood is due to the hyperlipidemia caused by ISO, which in turn leads to the buildup of harmful deposits in the arteries thus favoring coronary heart diseases (CHD) (Rajadurai and Prince Rajadurai and Stanely Mainzen Prince 2006). ISO

Fig. 2 Effect of Kombucha on serum cardiac markers in MI rats. *ISO*: Isoproterenol induced. *KT*: Kombucha. Treated rats *Vs* ISO: * $P<0.05$, ** $P<0.01$, *** $P<0.001$, NS: Not Significant

Table 3 Effect of Kombucha on cardiac markers in heart tissue of MI rats

Variable	CK [†]	LDH [‡]	ALT [‡]	AST [‡]
Control	18.42±0.68	121.12±6.80	48.96±2.31	25.16±2.42
ISO Control	8.36±0.73	83.63±4.58	25.05±2.93	15.77±2.87
Tea + ISO	14.64±0.57*	99.03±5.84*	31.64±4.91*	18.96±3.04*
KT + ISO	17.49±0.47***	115.94±2.09**	39.36±2.19*	23.26±3.71**

All the groups contained 6 rats. Values are mean±S.D

ISO: Isoproterenol induced. KT: Kombucha

Treated rats *Vs* ISO Control: **P*<0.05, ***P*<0.01, ****P*<0.001, NS: Not Significant

[†] μmoles of phosphorous liberated/min/mg protein

[‡] nmoles of pyruvate liberated/min/mg protein

administration also increases the uptake of LDL from the blood by myocardial membranes thus increasing the myocardial cholesterol levels (Anandan et al. 2007).

An increase in levels of LDL positively correlates with MI, whereas HDL levels reveal a negative correlation. HDL is known to inhibit the uptake of LDL from arterial wall and also facilitates the transport of cholesterol from peripheral tissues to the liver where it is catabolised and excreted from the body (Prince and Kumaran 2012). Pre-treatment with Kombucha considerably minimized the alterations in the levels of serum lipoproteins by increasing HDL and decreasing LDL and VLDL levels in ISO induced MI.

Increased levels of triglycerides are associated with cardiovascular disturbances and ISO promotes lipolysis in the myocardium (Sushmakumari et al. 1990). This observed increase maybe be due to the impaired removal of VLDL from the serum (Sharmila and Rajadurai 2012; Sparagna and Hickson Bick 1999). An increase in the synthesis of TGs in the heart tissue could be due to accumulation of acyl CoA and an augmented production of glycerol by increased glycolytic flux as indicated by previous study (Subramanian et al. 2003). Pre-treatment with Kombucha decreased the levels of triglycerides in MI rats by inhibiting lipolysis in the myocardium by exhibiting membrane stabilizing property of Kombucha.

When myocardial cells are injured or destroyed due to ischemia (deficient oxygen supply or glucose), the cardiac membrane becomes permeable or may rupture which results in leakage of macromolecules from damaged tissue (Sasikumar and Shyamala Devi 2000). Among these macromolecules, enzymes are the best markers of tissue damage because of their tissue specificity and catalytic activity. These cellular enzymes are released as a response to β-adrenergic stimulation (Ebenezar et al. 2003) or due to the alterations in the integrity and permeability plasma membrane. These alterations may be due to the damage caused by the β-agonist (ISO) to the sarcolemma. Prior treatment with Kombucha

helps to effectively minimize the damage to the cell membrane.

The protective effect of Kombucha observed is attributed to increase in concentrations of Epigallocatechin gallate and Gallic acid as well as the formation of Gallic acid due to the metabolic activity of the Kombucha pellicle (unpublished data).

Conclusion

The results of the study, reveal that, Kombucha possesses significant antihypertrophic, antihyperglycemic, antihyperlipidemic and membrane stabilising properties. Therefore Kombucha can be recommended for consumption by people suffering from cardiovascular diseases as well as by healthy individuals, on a daily basis.

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