Research Paper

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Evaluation of Kombucha Consumption Against Myocardial Infarction

Bioscience

KEYWORDS : Kombucha, Myocardial Infarction, cardiac troponin T, serum uric acid, serum iron

Reema Orison Lobo — Department of Biosciences, Mangalore University, Mangalagangothri-574199, Karnataka, India.

Chandrakala Shenoy K. — Department of Biosciences, Mangalore University, Mangalagangothri-574199, Karnataka, India

ABSTRACT

Kombucha is a fermented beverage produced from sugared black tea which is said to possess many health benefits. The present study was designed to know whether Kombucha is able to exert protective benefits against myocardial infarction. The animals were divided into negative control, positive control, black tea pre-treated and Kombucha pre-treated animals. The levels of cardiac marker (cardiac troponin T), antioxidant (ceruloplasmin) and the prognostic markers for the degree of cardiac injury (serum uric acid, serum iron, plasma iron binding capacity) were analyzed in the serum of rats belonging to all groups. An increase in the levels of cardiac troponin T, ceruloplasmin, serum iron, and serum uric acid along with a decrease in the levels of plasma iron binding capacity was observed in myocardial infarcted *rats. Kombucha pre-treatment was able to protect the myocytes from adverse effects during myocardial infarction by* decreasing *the degree of tissue injury and by stabilizing the cellular and sub-cellular organelles.*

INTRODUCTION

The diseases of the heart and blood vessels including angina, heart attack and diseases of the heart valves or muscles are together known as cardiovascular diseases (CVDs). Myocardial infarction (MI) is the principle cause of death in developed as well as developing countries (Yasin et al., 2009) and arises due to diminished blood supply to the heart known as ischemia (Bolooki and Askari, 2010). Ischemia and the resulting oxygen shortage for an extended period results in irreversible myocardial damage and necrosis. The prevalence and incidence of silent MI was found to increase with increasing age, in patients with diabetes mellitus, hypertension, and underlying CVDs (Valenci et al., 2011)

Kombucha is a beverage which is produced by the fermentation of tea and sugar by the symbiotic association of bacteria and yeasts which forms a "tea fungus". It is a refreshing beverage tasting somewhat like sparkling apple cider and is often produced at home by fermentation using a tea fungus passed from home to home. Bacteria and fungus present in Kombucha form a powerful symbiosis which is able to inhibit the growth of potential contaminating bacteria (Balentine, 1997). Based on the various cultures tested the main acetic acid bacteria found in the tea fungus are: *Acetobacter xylinum, A. xylinoides, Bacterium gluconicum, A. aceti, A. pasteurianus, Gluconacetobacter* sp. A4, and the novel species, *Acetobacter nitrogenifigens* sp. nov. and *Gluconacetobacter kombuchae* sp. nov.. The various yeast species identified are *Schizosaccharomyces pombe, Saccharomycodes ludwigii, Kloeckera apiculata, Saccharomyces cerevisiae, Zygosaccharomyces bailii, Brettanomyces bruxellensis, B. lambicus, B. custersii*, Candida and Pichia species (Jayabalan et al., 2014).

Even though the composition and properties of tea are well documented, scarce scientific information is available concerning the composition and the effects of Kombucha on health. In this context, the present study is aimed to explore the potential cardioprotective role of Kombucha on isoproterenol (ISO) induced MI in male Wistar rats.

MATERIALS AND METHODS:

Preparation of Tea and Kombucha:

The tea decoction was prepared by adding 10% of commercial sucrose and 7.5g/L of tea leaves (Brooke Bond Red Label) to boiling water and allowed to simmer for 3 minutes. After cooling to 30 ºC, the tea decoction was filtered into two clean glass bottles. One was considered as black tea and to the other a Kombucha pellicle from previous culture was added. 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 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*) 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).

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: Kombucha pre-treated + ISO-induced rats

Tea and Kombucha were administered (*per os*) daily at a concentration of 1.7ml/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., 2010).

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

Biochemical analysis:

Cardiac Troponin T (cTnT) was assayed in plasma using a chemiluminescence immunoassay kit (Roche Diagnostics, Basel, Switzerland). Plasma ceruloplasmin levels were estimated by the method of Ravin (1961). Plasma uric acid was analyzed using kit by Agappe Diagnostics Ltd., Kerala, India. Serum iron and plasma iron binding capacity were assayed by the methods of Ramsay (1969a, 1969b).

Figure 1: Black tea and 7th day Kombucha

RESULT AND DISCUSSION:

Black tea had a pH range between 5.2-5.0 which increased to 3.4-3.0 on fermentation using Kombucha pellicle for 7 days. The alcohol content of Kombucha was around 0.5, which is the permissible limit for non-alcoholic beverages. The resulting Kombucha has the color of honey with a mild flavor.

ISO induced MI is a well developed method for the study of the impact and mechanism of action of various natural products in the prevention of MI. This is because the effects of ISO administration closely resemble the biochemical and histological changes that occur during human MI. cTnT is a well known cardiac marker which is regularly used to predict the extent of cardiac injury. Thus the serum of ISO induced MI rats' shows significantly higher levels of cTnT when compared to that of normal control rats. This is because the damage that occurs to the myocytes during MI leads to the destabilization of the cellular membrane, leading to leakage of the cytoplasmic content (Acikel et al., 2005). On pre-treatment with Kombucha the levels of cardiac markers such as cTnT in the serum decreased significantly, which indicates the membrane stabilizing property of Kombucha.

Table 1: Effect of Kombucha on cardiac marker and antioxidant

Variable	Control	ISO Control	Tea + ISO	KT + ISO
Cardiac Troponin T	0.719±0.15	1.390±0.17	0.999±0.13*	0.796±0.15***
Ceruloplasmin	25.71±3.65	43.39±2.06	38.33±2.08**	34.13±1.97****

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 < 0.005, *****P* < 0.001. Activity is expressed as ng/ml for cTnT and mg/dl for ceruloplasmin

Ceruloplasmin acts as a chain breaking antioxidant independent of its catalytic ferroxidase activity (Atanasiu et al., 1998). It acts as an antioxidant by inhibiting the ferritin dependent lipid peroxidation by promoting the oxidative re-incorporation of released iron (Samokyszyn et al., 1989). Ceruloplasmin is an acute phase protein, synthesized by the liver in response to any tissue damage or inflammation. Pre-treatment with Kombucha protects the myocardium against ISO induced damage and resulting inflammation, thus preventing the over-synthesis of ceruloplasmin.

Contrary to earlier beliefs, an increase in serum uric acid is now considered to be a risk factor for MI and is being used as prognostic indicator in cases of mild to severe heart failure (Nadkar and Jain, 2008). Our study reveals a significant increase in the serum uric acid levels in ISO induced MI rats when compared to normal rats, while, pre-treatment with Kombucha successfully reduced the serum uric acid levels. Elevation in the levels of serum iron and a simultaneous decrease in the plasma iron binding capacity were also observed in rats administered with ISO. Free iron, a catalyst for the production of free radicals, is released from heme dependent proteins during MI along with a decrease in the plasma iron binding capacity which results in an increase in the *in vivo* lipid peroxidation (Halliwell and Gutteridge, 1989). Increase in free iron levels causes a proportionate increase in the concentration of the antioxidant ceruloplasmin, which scavenges free iron. Kombucha pre-treatment increases the plasma iron binding capacity, thereby keeping the serum free iron concentration in check.

Table 2: Effect of Kombucha on serum uric acid, serum iron and plasma iron binding capacity

Variable	Control	ISO Control	Tea + ISO	KT + ISO
Serum uric acid	2.550±0.34	4.331±0.19	3.846±0.18**	3.407±0.20****
Serum iron	47.38±4.60	74.55±3.25	61.68±5.02*	47.79±5.24****
Plasma Iron binding capacity	49.21±3.26	25.55±2.94	34.96±1.88*	38.47±2.22**

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. Activity is expressed as mg/dl for serum uric acid and µg/dl for serum iron and plasma iron binding capacity.

CONCLUSIONS:

The results of the present study indicate that Kombucha exerts a protective effect on the myocytes by decreasing or preventing the adverse effects of ISO in rats, mainly due to stabilization of the cellular and sub-cellular membranes.

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