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Antihyperlipidemic And Antiatherogenic Effect of Kombucha in Streptozotocin induced Diabetic Rats

KEYWORDS: Diabetes, Kombucha, Streptozotocin, Atherogenic

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ABSTRACT *Oxidative stress has a major role in the pathogenesis of diabetic complications. The purpose of the present study was to investigate the possible protective effect of traditional fermented beverage kombucha on lipid profile and liver functions in streptozotocin (STZ) induced diabetic rats. The effect of kombucha on fasting blood glucose, lipid profile, atherogenic index (AI), cardiovascular indices, and the hepatic enzyme markers was examined in all the groups. Oral administration of kombucha significantly reduced fasting blood glucose, lipid parameters, AI, atherogenic coefficient, cardiac risk ratio and increased the levels of high density lipoproteins in diabetic rats. Thus, it can be concluded that kombucha treatment significantly alleviated the altered glycemic and lipidemic distress and protects against the risk of developing atherosclerosis in STZ - induced diabetic rats.*

INTRODUCTION

Diabetes mellitus (DM) is associated with long term complications and the incidence of diabetes is increasing worldwide. Lack of insulin during diabetes is associated with disturbances in carbohydrate, protein and fat metabolism [1]. In addition, DM is characterized by abnormalities in lipid profile [2] and an increase in atherogenic index [3]. DM has been recognised as a major risk factor for such as atherosclerosis, heartattacks, stroke etc. About 75% of deaths among men and 57% of death among women with diabetes are attributed to Cardiovascular disease [4].

Kombucha as an alternative therapy has been traditionally used for a wide range of health problems. Kombucha pellicle or tea fungus is a symbiotic association of acetic acid bacteria and yeasts such as *Bacterium xylinum, Bacterium xylinoides, Bacterium gluconicum Acetobacter ketogenum,* and *Saccharomyces ludwigii, Schizosaccaromyces pombe, , Torula varieties, Pichia fermantans* [5]. Kombucha is a fermented tea prepared by using a sugared black tea and kombucha pellicle. Fermentation with tea fungus converts the added sugar into various organic acids and other products. The components of kombucha include several organic acids, active enzymes, amino acids, vitamins and polyphenols which may support health benefits.

Hence, the objective of the study was to investigate the effect of Kombucha on lipid profile and antiatherogenic activity in STZ- induced diabetic rats.

MATERIALS AND METHODS

Preparation of Kombucha Tea

Kombucha tea was prepared by adding 10% sugar and 0.75% of tea leaves to tap water and allowed to boil for 3 minutes. Once the tea decoction attained room temperature, it was filtered into clean glass bottles and the kombucha pellicle was added to it. Under aerobic condition, the tea decoction was allowed to ferment for 7 days. The fermented tea thus obtained was filtered and sterilized before use.

Experimental Animals

The experiments were carried out in healthy adult Wistar strain Albino rats of either sex, weighing between 200-240g obtained from the Animal House, Department of Biosciences, Mangalore University, Mangalore. A standard pellet diet and water were supplied ad libitum. They were maintained under standard laboratory conditions of temperature and humidity, 12 hour light dark cycle. The experiments were conducted according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India, after the approval of the research proposal by the Animal Ethical Committee of Mangalore University (CPCSEA-Registration No. 232).

Induction of Diabetes

A freshly prepared streptozotocin (50mg/kg body weight) in 0.1 M chilled citrate buffer (pH 4.5) was injected intraperitoneally to induce diabetes to overnight fasted rats [6]. After 72 hours of diabetes induction, the rats with fasting blood glucose exceeding 250 mg/dl were considered diabetic and used for the study. After STZ- injection, the fifteenth day was considered as the 1st day of treatment. Kombucha (1.71ml/kg) and Glibenclamide (5mg/kg) was fed once daily for 14 days.

Experimental Protocol

The rats were divided into four groups with six rats in each group.

Group I: Normal control rats
Group II: Diabetic control rats
Group III: Diabetic rats treated with Kombucha
Group IV: Diabetic rats treated with Glibenclamide

Blood Sample Collection:

At the end of the fourteen days of kombucha treatment, the overnight fasted rats were anesthetized (ketamine 22-24mg/kg i.m.,) and blood was collected by heart puncture.

Biochemical Analysis:

The fasting blood glucose was determined by using one touch ultra glucometer. Total cholesterol (TC), triglycerides (TG), high density lipoprotein (HDL) Aspartate Transaminase (AST), Alanine Transaminase (ALT) and alkaline phosphates (ALP) were analyzed using commercial kits (Agappe Diagnostics Ltd., Kerala.). Estimation of triglycerides [7],

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HDL-C estimation [8] and estimation of total cholesterol using enzymatic method by Flegg [9]. Serum LDL and VLDL were calculated with the Fridewald formula [10].

LDL = Total cholesterol – (HDL cholesterol +Triglycerides / 5)
VLDL = Triglycerides /5
Atherogenic index of plasma (AIP) was calculated as log (TG / HDL-C) [11].

Coronary Risk Index (CRI) = Total cholesterol / HDL- C [12].
Atherogenic Coefficient (AC) = (TC - HDL-C) / HDL-C) [13]

Statistical Analysis
Data were statistically analyzed by one-way analysis of variance followed by Tukey HSD test. Results were considered statistically significant when ($P < 0.05$). The values of the experimental results were expressed as mean ± SD

RESULTS

Table 1. Effect of Kombucha on Body Weight, Liver Weight and Fasting Blood Glucose

Parameters		Group (n = 6)			
Normal Control rats		Diabetic Control rats	Diabetic + Kombucha treated rats	Diabetic+ Glibenclamide treated rats	
Body weight (g)	0 day	204.6 ± 2.19	209.76 ± 2.38	206.75 ± 2.99	207.6 ± 2.43
	14th day	218.4±2.71	173.3±5.22	179.6±2.44	172±2.78
	28th Day	232.9±3.66	148.5±4.42***	198.1±2.69***	201.8±2.38***
Liver weight (g)		6.7±0.46	4.89±0.30***	6.1±0.33***	5.83±0.43***
Blood Glucose (mg/dl)	0 day	79.5±2.09	81.1±2.08	79.6±1.44	80.4±1.49
	14th day	80.9±1.90	285.1±5.72	291.6±2.43	289.4±4.17
	28th Day	81.32 ± 3.63	324 ± 6.66***	114 ± 5.76***	109 ± 6.06***

Values are given as Mean ± SD. values are statistically significant at *P<0.05, **P<0.01, ***P<0.001.Diabetic versus normal (P<0.001)
Diabetic versus Kombucha treated (P<0.001)
Diabetic versus Glibenclamide treated (P<0.001)

Table 2: Effect of Kombucha on Lipid Parameters, Atherogenic index and Cardiovascular indices

Parameters		Group (n=6)			
Normal Control rats		Diabetic Control rats	Diabetic+ Kombucha treated rats	Diabetic + Glibenclamide treated rats	
Lipid Profile	Total Cholesterol	65 ± 5.4	149±4.69***	85±4.05***	101±4.42***
	Triglycerides	67 ± 4.19	103±5.32***	80±3.63***	73±2.89***
	HDL	22 ± 2.76	13 ± 2.37***	19 ± 2.1**	18 ± 2.53*
	LDL	29.6 ±0 .22	115.4 ± 0.24***	50 ± 3.84***	68.4 ± 3.24***
	VLDL	13.4 ±0 .84	20.6 ± 1.07***	16 ± 0.73***	14.6± 0.58***
Atherogenic index and Cardiovascular indices	Atherogenic Index	0.49 ± 0.06	0.9 ± 0.08*	0.63 ± 0.04*	0.61 ± 0.06*
	Artherogenic Coefficient	2.00±0.55	10.82±2.40***	3.51±0.46***	4.69±0.72***
	Cardiac risk ratio (LDL/ HDL-C)	1.39±0.49	9.19±2.13***	2.66±0.39***	3.87±0.60***
	Cardiac risk ratio (TC/ HDL-C)	3.00±0.55	11.82±2.40***	4.51±0.46***	5.69±0.72***

Values are given as Mean ± SD. Values are statistically significant at *P<0.05, **P<0.01, ***P<0.001
Diabetic versus normal (P<0.05), (P<0.001)
Diabetic versus Kombucha treated (P<0.05), (P<0.01), (P<0.001)
Diabetic versus Glibenclamide treated (P<0.05) (P<0.001)

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Table 3: Effect of Kombucha on Hepatic Enzymes

Group (n=6)	AST (UL)	ALT (UL)	ALP (UL)
Normal rats	62.75 ± 5.03	56.01 ± 5.87	611.68 ± 42.67
Diabetic rats	208.3 ± 6.95***	118.95 ± 12.42***	1147.5± 182.30***
Diabetic+Kombucha treated rats	91.73 ± 3.72***	63.5 ± 13.51***	629.83± 219.96***
Diabetic+ Glibenclamide treated rats	86.9 ± 20.11***	61.01 ± 9.15***	829.5 ± 221.49^{NS}

Values are given as Mean ± SD. Values are statistically significant at. *P<0.05, **P<0.01, ***P<0.001, NS: Non Significant
Diabetic versus normal (P<0.05) (P<0.001)
Diabetic versus Kombucha treated (P<0.05) (P<0.001)
Diabetic versus Glibenclamide treated (P<0.05) (P<0.001), NS

DISCUSSION

In the present work we have studied the effect of kombucha on lipid profile, hepatic enzymes and cardiovascular risk indices in STZ - induced diabetic rats through 2 weeks of treatment. STZ is cytotoxic and is taken up by the β cells via the glucose transporter GLUT2 and causes alkylation of DNA [14] inducing severe hyperglycemia in experimental animals. The results obtained from the present study are very much promising and comparable with Glibenclamide, a standard drug used to treat DM.

Decrease in bodyweight is a common feature in diabetes and is mainly due to derangement of metabolic pathways [15]. Kombucha administration controlled this loss in body weight. Diabetic animals showed a decrease in the liver weight and this might be due to an increased breakdown of glycogen and/or pronounced gluconeogenesis. Increase in liver weight after kombucha administration may be due to increase in the accumulation of glycogen in diabetic liver [16].

It was observed that oral administration of kombucha to the diabetic rats showed a marked hypoglycaemic effect by restoring the blood glucose. The lowering of blood glucose levels was in accordance with the previous studies [17]. The hypoglycemic action of kombucha in diabetic rats may be due to insulinomimictic action or by peripheral utilization of glucose stimulated by the metabolites produced during fermentation.

Diabetes is associated with profound alterations in lipid profile and with increased risk of coronary heart disease [18]. In the present study the oral administration of kombucha to STZ induced diabetic rats showed lipid lowering properties in addition to the hypoglycaemic activity. These reductions could be beneficial in preventing diabetic complications as well as improving lipid metabolism in diabetics [19]. This might be due to the antioxidant activity of kombucha, mainly due to the presence of polyphenols such as flavanoids and catechins and also due to the synergistic action of different compounds such as organic acids produced during the fermentation period.

STZ increased the plasma levels of hepatic enzymes AST, ALT and ALP. The elevation of plasma concentrations of ALT, AST and ALP enzymes indicates liver damage as in the case of complications in diabetes. Increased levels of these enzymes are an indicator of cellular infiltration and functional disturbance of liver cell membranes [20]. The return of the above enzymes to near normal after kombucha treatment may be due to prevention of intracellular enzyme leakage resulting from cell membrane stability or cellular regeneration [21]. This suggests that decrease in these enzyme activities may be due to the hepatic and cardiac protection offered by kombucha. The protective effect of kombucha might be due to the antioxidants such as flavanoids present in kombucha. There is a strong relationship between high level of total cholesterol concentration in the blood and cardiovascular disorder [22].The diabetic rats administered with kombucha showed relatively low cholesterol and low density lipoprotein. Thus, these results indicate the ability of kombucha to reduce atherosclerosis, a complication of diabetes.

CONCLUSION

Based on the results, it can be concluded that kombucha confirms its antihyperglycemic, antihyperlipidemic and antiatherogenic potential in diabetic rats. Therefore, kombucha could be clinically applicable for reducing complications of diabetes together with the ideal antidiabetic drugs.

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