Protective Effect of Kombucha on Diabetic Nephropathy in Streptozotocin-Induced Diabetic Rats

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Abstract: Kombucha is a fermented black tea, claimed to have wide range of beneficial effects on human health functions. The present study was aimed to investigate the effect of kombucha on diabetes induced alterations in the kidney. The effect of kombucha on blood glucose, plasma protein, albumin, urea and creatinine was examined in the control and experimental groups along with histopathological studies. The results of biochemical analysis showed a remarkable improvement in metabolic profile after kombucha administration. The histological studies showed glomerular and tubular damage in diabetic control rats. The kombucha treated group showed restoration in renal architecture. Thus kombucha may be of supportive treatment to combat diabetes complications, diabetic nephropathy.

Keywords: Diabetes, Kombucha, Diabetic Nephropathy, Streptozotocin

1. Introduction

Diabetes mellitus (DM) is a metabolic disease associated with secondary complications such as cardiovascular and renal disease. Among the complications, nephropathy seems to be highly prevalent (Selby et al., 1990). Diabetic nephropathy refers to a spectrum of renal diseases from microalbuminuria to the progressive decline in glomerular filtration rate which may lead to end-stage renal failure (Ritz et al., 2011). At present, diabetic kidney disease affects about 15%–25% of diabetes patients (Hovind et al., 2003). Reactive oxygen species play an important role in high glucose-induced renal injury (Ha and Lee, 2000).

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 xylinum, A. xylinoides, Bacterium xylinum, Bacterium xylinoides and the novel species, Acetobacter nitrogenifigens sp. nov. and Gluconacetobacter kombuchae sp. nov., Saccharomyces ludwigii, Saccharomyces apiculatus varieties, Schizosaccharomyces pombe, Saccharomyces cerevisiae, Zygosaccharomyces bailii and Pichia fermentans (Jayabalan et al., 2014). During the fermentation process, bacteria and yeasts metabolize sucrose into a number of organic acids such as acetic acid, gluconic acid, amino acids, antibiotics and a variety of micronutrients (Chu and Chen, 2006). 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 the protective effect of kombucha on diabetic nephropathy in streptozotocin (STZ) induced DM.

2. Materials and Methods

Chemicals

STZ was purchased from Sigma Chemical Company (St Louis, MO, USA). All the other chemicals used were of analytical grade and were purchased from commercial sources.

Preparation of Kombucha

The tea decoction was prepared by adding 10% of sucrose to tap water and 0.75% of black tea leaves, boiled for 3 minutes and then cooled to room temperature. The tea decoction was filtered and poured into clean glass bottles and kombucha pellicle from previous culture was added to it. Under aerobic condition, the sugared tea was allowed to ferment for 7 days at room temperature. The kombucha obtained was filtered, sterilized and refrigerated.

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. They were maintained under standard laboratory conditions of temperature and humidity, 12 hour light dark cycle. A standard pellet diet and water were supplied ad libitum. 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

Diabetes was induced in overnight fasted rats by a single intraperitoneal injection of freshly prepared Streptozotocin (50mg/kg body weight) in 0.1 M chilled citrate buffer (pH 4.5) (Parthasarathy et al., 2007). 72 hours after diabetes induction, fasting blood glucose was determined and the rats with blood glucose above 250 mg/dl were used for the study. Rats were maintained in the diabetic state for 14 days and the treatment was started on the 15th day after STZ injection and this was considered as the 1st day of treatment. The treatment was continued 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

Kombucha (1.71 ml/kg) and Glibenclamide (5 mg/kg) was given once daily using an oral gavage for 14 days. At the end of the 2 weeks of treatment period, the overnight fasted rats were anaesthetized using ketamine 22-24 mg/kg i.m., and blood was collected by heart puncture. The organs were excised after sacrifice for histological studies.

Biochemical Analysis
The body weight in experimental animals was determined by a digital balance. The fasting blood glucose concentration was determined by means of one touch ultra glucometer. Total protein, albumin, were analyzed using commercial kits (Agappe Diagnostics Ltd., Kerala). Biuret method was employed for the determination of protein (Gornall et al., 1949). Albumin was estimated spectrophotometrically by reaction with the dye bromocresol green (Doumas et al., 1977). Urea, creatinine and uric acid were assayed in blood plasma using standard kits supplied from Agappe Diagnostics Ltd., Kerala, India.

Histological Study
Renal tissues were collected, washed in ice cold saline, fixed in 10% formalin solution and embedded in paraffin. Sections were obtained by a microtome and stained with hematoxylin and eosin. The sections were then examined under a light microscope.

3. Result and Discussion

DM causes serious injury to the kidney tissue that leads to renal dysfunction (Eid et al., 2013). STZ-induced diabetic rats result in development of nephropathy similar to the early stage of human diabetic nephropathy (Rasch and Mogensen, 1980). STZ-induced diabetes is characterized by severe loss in body weight which was observed in the present study. The decrease in body weight observed in diabetic rats might be the result of protein wasting due to unavailability of carbohydrate as an energy source (Musabayane et al., 2005). Kombucha administration controlled this loss in body weight and also reduced symptomatic conditions such as polydipsia and polyuria along with diarrhoea.

Kidney enlargement is an early feature in both experimental and human diabetes due to an increase in the capillary length and diameter and was correlated with the degree of glycaemic control (Jefferson et al., 1983). Administration of kombucha reduced and prevented diabetes induced kidney enlargement. Oral administration of kombucha to the diabetic rats showed a marked hypoglycaemic effect by restoring the blood glucose levels to near normal. The hypoglycaemic action may be due insulinomimetic action or by stimulation of glucose uptake by peripheral tissues (Burcelain et al., 1995).

The decrease in protein and albumin may be due to microproteinuria and albuminuria, and/or may be due to increased protein catabolism, which are important clinical markers of diabetic nephropathy (Mauer, 1981). The treatment of diabetic rats with kombucha caused a noticeable elevation in the plasma total protein and albumin levels as compared with their normal levels. Urea, uric acid and creatinine concentrations are also considered as a significant marker of renal dysfunction (Almdal and Vilstrup, 1988). Protein glycation in diabetes may lead to muscle wasting and increased release of purine, the main source of uric acid, as well as increased activity of xanthine oxidase and lipid peroxidation. Kombucha reversed these parameters to near normal which could be due to decreased metabolic disturbances of other pathway such as protein and nucleic acid metabolism and improved glycaemic control. This may also be due to the presence of acetic acid and glucuronic acid which helps in detoxification and removal of waste products from the blood.

Histopathology of kidney of diabetic rats showed marked degeneration of the Bowman’s capsule, tubular damage, haemorrhage in the Bowman’s space due to glomerular damage and dilatation of renal tubules. However, the administration of kombucha to diabetic rats exhibited an improvement in these pathological changes in the kidney. This suggests that kombucha may restore antioxidant activity and thereby reduces renal damage. The nephroprotective effect may be also be attributed to the synergistic action of the bioactive compounds such as flavonoids present in kombucha.

| Group (n = 6)                  | Parameters | Body Weight (g) | Kidney Weight (g) | Fasting Blood Glucose (mg/dl) |
|--------------------------------|------------|-----------------|-------------------|------------------------------|
|                                |            | Initial         | Final             |                              |
| Normal Control rats            |            | 204.6 ± 2.19    | 232.9 ± 3.66      | 1.04 ± 0.10                  | 81.32 ± 3.63                 |
| Diabetic Control rats          |            | 209.76 ± 2.38   | 148.5 ± 4.42***   | 1.61 ± 0.18***               | 324 ± 6.66***                |
| Diabetic + Kombucha treated rats |          | 206.75 ± 2.99   | 198.1 ± 2.69***   | 0.95 ± 0.11***               | 114 ± 5.76***                |
| Diabetic + Glibenclamide treated rats |    | 207.6 ± 2.43    | 201.8 ± 2.38***   | 0.9 ± 0.13***                | 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 KT treated (P<0.001) Diabetic versus Glibenclamide treated (P<0.001)
Table 2: Effect of kombucha on urea, creatinine and uric acid in control and experimental rats

| Groups (n=6)                      | Urea (mg/dl) | Creatinine (mg/dl) | Uric Acid (mg/dl) |
|----------------------------------|--------------|--------------------|-------------------|
| Normal Control rats              | 31±3.74      | 0.4±0.23           | 0.97±0.1          |
| Diabetic Control rats            | 52.03±2.94***| 1.03±0.33***       | 2.6±0.35***       |
| Diabetic + Kombucha treated rats | 39.7±4.19*** | 0.5±0.2*           | 1.2±0.38***       |
| Diabetic + Glibenclamide treated rats | 42.5±2.37*** | 0.5±0.2*           | 0.91±0.06***      |

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 KT treated (P<0.05) (P<0.001), Diabetic versus Glibenclamide treated (P<0.05) (P<0.001)

Table 3: Effect of kombucha on protein, albumin and globulin in control and experimental rats

| Group (n=6)                      | Protein (g/dl) | Albumin (g/dl) | Globulin (g/dl) | A/G       |
|----------------------------------|----------------|---------------|-----------------|-----------|
| Normal Control rats              | 6.53±0.95      | 3.76±0.27     | 2.77±1.12       | 1.53±0.54 |
| Diabetic Control rats            | 4.12±0.42***   | 1.75±0.29***  | 2.37±0.63***    | 0.8±0.30* |
| Diabetic + Kombucha treated rats | 5.5±0.58**     | 2.46±0.34**   | 3.04±0.72***    | 0.87±0.35 NS |
| Diabetic + Glibenclamide treated rats | 6.1±0.28**     | 3.13±0.09***  | 2.97±0.22***    | 1.05±0.06 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<0.001), (P<0.05), Diabetic versus KT treated (P<0.01), NS, Diabetic versus Glibenclamide treated (P<0.001), NS

4. Histopathological Studies

![Figure (a): Normal control](image1)
![Figure (b): Diabetic Control](image2)
![Figure (c): Kombucha treated](image3)
![Figure (d): Glibenclamide treated](image4)

Figure: a – d: Histopathological changes of kidney in control and experimental rats

5. Conclusion

Based on the findings, it can be concluded that kombucha acts effectively against diabetes. These results support its implication of controlling blood glucose levels and may be slowing or even reverse some of the pathological conditions of diabetic nephropathy.

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