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Therapeutic Efficacy of Some Commercially Sold Antidiabetic Herbal Teas in Streptozotocin-Induced Diabetes in Wistar Rats

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Abstract

The hypoglycaemic efficacy and the effect on haematology of some commercially available antidiabetic herbal teas were evaluated in Streptozotocin-induced diabetes in Wistar rats. Twenty-five (25) male Wistar rats were divided into 5 groups of five rats each. Diabetes was induced in 20 rats by a single intraperitoneal injection of Streptozotocin (STZ) at 50 mg/kg body weight. Group 1 received only distilled water. Group 2 was an untreated control, and Group 3 was treated with Metformin (METF) at 500 mg/kg body weight. Groups 4 and 5 were Tea A and Tea B at 200 mg/kg body weight, respectively, for 14 days. The Fasting blood glucose and body weights of rats were taken at day 0, 1, 3, 7, and 14, blood sample for haematology was taken after 14 days of treatment. The result shows a ($P<0.05$) increases in fasting blood glucose in STZ-treated rats. The groups treated with metformin and the antidiabetic teas A and B for 14 days in STZ-diabetic rats showed significantly reduced fasting blood glucose levels ($P < 0.05$). The haemogram after the treatment period revealed a significant ($P<0.05$) reduction in PCV, RBC, and Hb of non-treated and Tea A-treated diabetic rats, as well as a decrease in WBC of Tea A-treated group, compared to the METF-treated and normal control. Therefore, the two herbal anti-diabetic teas tested exerted an anti-diabetic effect by promoting hypoglycaemic action. Still, tea A showed a tendency to fail to ameliorate the haematological effects of Streptozotocin in Streptozotocin-induced diabetic rats.

Keywords: Herbal Teas, Diabetes, Streptozotocin, hypoglycaemia

Introduction

Type 1 diabetes mellitus results from the inability of the pancreas to produce enough insulin due to the loss of beta cells, manifesting as hyperglycaemia. The loss of beta cells is caused by an autoimmune reaction [1]. The cause of this autoimmune reaction is unknown [2]. Frequent urination, increased thirst, and increased hunger are common symptoms of this disease [2]. Recent reports showed that 537 million individuals worldwide had diabetes in 2021 and by 2030, this figure is expected to rise to 643 million, and by 2045, to 783 million [3]. More than 1.2 million children and teenagers had type 1 diabetes in 2021 [3]. Among other issues, retinopathy, nephropathy, and neuropathy are among the particular long-term effects of diabetes. Hyperglycemia causes almost 4 million deaths each year [4]. Beyond the person, diabetes' consequences also impact families and the entire society. Particularly in low and middle-income nations where diabetes is frequently associated with other diseases, it has significant socioeconomic effects and endangers national productivity and economies [5]. Diabetes-related direct health costs are now close to $1 trillion USD, and they will surpass this amount by 2030 [3].

The pancreatic beta cells are destroyed by streptozotocin injection in rats, which results in insulin shortage and may cause symptoms similar to type 1 diabetes in people, such as frequent urination, increased thirst, and high blood sugar levels [6]. As a result, it serves as an animal model for study to better understand the pathological process behind diabetes, which will help researchers create new therapeutic treatments for the disease [7].

Concerning treatments, people have also developed an interest in alternative (complementary) medicine that consists of herbal remedies and dietary supplements due to the various side effects and adverse effects associated with chemical drugs [8]. According to studies, 80% of people in developing countries rely on traditional medicines as their primary treatment for a variety of ailments because they are cheaper, have fewer side effects, and are readily available without a prescription [9]. Therefore, various products abound in the market with claims of anti-diabetic properties, hence the need to verify their therapeutic efficacies and safety.

Materials and Methods

Materials

The equipment's, reagents and drugs used in this study include; cages, feeding containers, water containers, fresh drinking water, distilled water, Whitman filter paper, beakers, spatula, analytical weighing balance, hot air oven, freezer, hand gloves, EDTA vacutainers, non EDTA

vacutainers, ONETOUCH® UltraMini glucometer, ONETOUCH® Ultra test strips, tissue paper, capillary tubes, sample bottles, centrifuge, microscope, tally counter, micropipette, micropipette tips, timer, 1 ml needle and syringe, test tubes, test tube rack, spectrophotometer, oral cannula, Sodium Citrate buffer, Streptozotocin (STZ), herbal anti-diabetic teas with tea A [containing *Radix ophiopogonis* - 6.2 %, *Polygonatum biflorum* (also called Fragrant Solomon Seal Rhizome - 6.2 %), *Discorea polystachya* (Chinese yam - 6.2 %), *Crataegus monogyna* (Hawthorn Berry – 10 %), *Radix pueraeiae* – 8 % and *Camellia sinensis* (White tea - 63.4 %)] and herbal anti-diabetic tea B [containing *Viscum album* – 20 %, Bitter Herbs – 20 %, *Taraxacum mongolicum* (Mongolicum – 10 %), *Origanum vulgare* (Oregano – 10 %), *Mimosa pudica* (Mimosa – 20 %), *Ocimum basilicum* – 20 %], Metformin and 1% buffered Sodium Chloride.

Extraction of Anti-diabetic herbal Teas

Extraction was done using warm maceration for each anti-diabetic herbal tea. A total of 18g of Tea A (T_A) and 24g of antidiabetic Tea B (T_B) was used. Both were extracted with 500 mL of warm distilled water, and kept at room temperature for 48 hours with frequent agitation every 3 hours of the day. Filtration was done with Whitman filter paper No. 1 after 48 hours, and the filtrate was concentrated in a hot air oven at 60 °C. The concentrated aqueous extracts were weighed and placed in universal bottles and were stored in a refrigerator until they were needed. Percentage yield for each tea was calculated using the following formulas:

$$\% \text{ Yield} = \frac{\text{Weight of Tea Used (a)} - \text{Weight of Extract (b)}}{\text{Weight of Tea Used (a)}} \times 100$$

Experimental Animals

A total of thirty-five (35) Wistar rats, weighing between 120 and 190 g and 10 weeks old, were obtained from the National Veterinary Research Institute in Vom, Plateau State and transported to Makurdi Benue State by road. The rats were kept in laboratory cages and housed at the Veterinary Physiology and Biochemistry Laboratory, Joseph Sarwuan Tarka University, Makurdi, and acclimatised for 2 weeks. They were fed standard pelleted commercial poultry feeds and given clean drinking water *ad libitum*. All animals were handled in accordance with the International Standards for Biomedical Research involving animals. Good laboratory animal practice regulations of TDR [10] and as well as the guidelines of laboratory animal use and care as documented by Natural research council, [11] were religiously followed. Ethical approval was obtained from the animal ethics and welfare committee (AEWC) in the College of veterinary medicine, Joseph Sarwuan Tarka University Makurdi, with the approval number; JOSTUM/CVM/ETHICS/2023/18.

Determination of the Median Lethal Dose (LD_{50})

Acute toxicity testing was carried out using the Modified up and down Method [12]. Ten (10) rats of both sexes were randomly assigned into two groups (A & B) of five (5) rats each. Thereafter, a test limit dose of 2000 mg/kg body weight of each tea extract was administered orally to the rats in groups A and B, respectively and observed for 14 days for mortality and signs of toxicity.

Experimental Protocol

Thirty (25) male Wistar rats, 10 weeks old, were randomly assigned into 5 groups, with 5 rats each. The rats were fasted overnight and fasting blood glucose (FBG) was measured. Blood samples were also collected from each rat via micro-capillary tube puncture of the retro-bulbar plexus of the medial canthus of the eye into EDTA vacutainers for pre-experimental haematological evaluation before induction of diabetes. Rats from groups 2 to 5 were administered freshly prepared Streptozotocin (STZ) solution in 0.1 M citrate buffer at 50 mg/kg body weight intraperitoneally to induce diabetes. Following the STZ injection, all rats were given free access to food and water. The diabetic status of the rats was confirmed by measuring the FBG after 72 hours using a glucometer and test strips. Rats with FBG values above 120 mg/dL were considered diabetic and used in the study. Group 1 served as the normal control and were administered distilled water ($_dH2O$) only for 14 days; group 2 were given distilled water for 14 days after streptozotocin injection; group 3 was treated with Metformin (METF) as the reference drug control at 500 mg/kg body weight for 14 days; group 4 and 5 were treated with T_A and T_B respectively at 200 mg/kg body weight for 14 days. The FBG was evaluated for all groups on day 0, 1, 3, 7, and 14 post-inductions of diabetes. The body weights of rats were also taken on day 0, 1, 3, 7, and 14. At day 14 blood samples were collected as previously described into EDTA vacutainers for haematological evaluation.

Determination of Haematological Parameters

Haematological parameters (Red blood cell (RBC) and white blood cell (WBC) counts, packed cell volume (PCV), haemoglobin concentration (Hb) and differential WBC counts) were determined using standard laboratory procedures as described by Cheesbrough, [13]. Haematological indices including; mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated from the values of RBC count, PCV and [13].

Statistical Analysis

The data collected were statistically analysed and expressed descriptively as mean ± standard error of mean (SEM). The means were compared using a One-Way Analysis of Variance (ANOVA) at 5 % ($P < 0.05$) significance level. Significant means were separated using the Least Significant Difference (LSD) Post Hoc Test. All data were analysed using SPSS version 21 and Microsoft Excel.

Results

Percentage yield and Median Lethal Dose (LD_{50}) of ADTa and ADTb

The percentage yield of ADTa and ADTb was calculated to be 66.67% and 81.40%, respectively.

Acute toxicity test (LD_{50}) of the Herbal Teas

No death was recorded, and no toxicity signs were observed after the 14-day post-administration of the two anti-diabetic teas at a test limit dose of 2000 mg/kg body weight. Therefore, the LD50 of ADTa and ADTb was estimated to be above 2000 mg/kg body weight in rats.

Effect of Antidiabetic Herbal Teas on Body Weights of Streptozotocin-Induced Diabetic Rats

The body weights of normal control rats and diabetic rats before and after diabetes induction and treatment with teas were monitored. Figure 1 shows that the body weights were generally decreased in streptozotocin-induced diabetic rats as compared to the normal control rats. At the end of the 14-day treatment period, there were no significant improvements in the body weights of the rats in groups 2, 3, and 5, as seen in Figure 1. However, for group 4, the body weights were maintained throughout the experimental period

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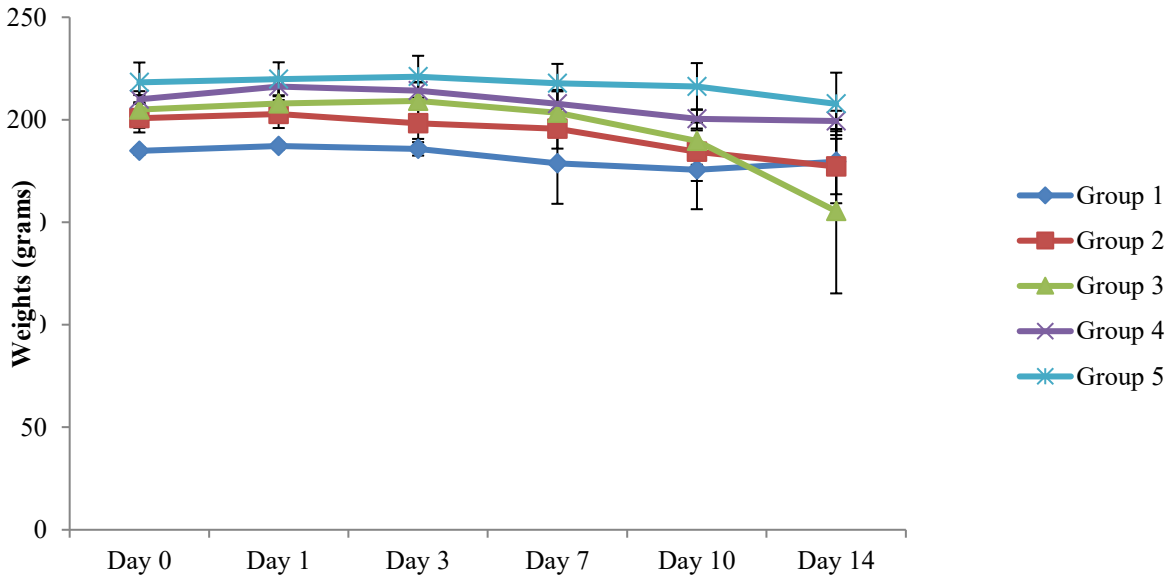

Figure 1: Mean body weight of diabetic rats treated with anti-diabetic teas (Values are expressed as mean ± SEM of five rats in each group; significance accepted at P < 0.05). Group 1 = Normal Control, Group 2 = STZ + dH_2O, Group 3 = STZ + MEFT, Group 4 = STZ + ADTa, Group 5 = STZ + ADTb.

Effect Antidiabetic Herbal Teas on Fasting Blood Glucose of Streptozotocin-Induced Diabetic Rats

Figure 2 showed the fasting blood glucose of streptozotocin-induced diabetic rats. Group 2 (untreated) showed significantly (P<0.05) higher FBG throughout the treatment period when compared with other normal control (Group 1) and other groups. The group (group 3) treated with metformin had FBG levels significantly (P<0.05) higher when compared with normal control and the groups treated with the teas (Groups 3 and 5) throughout the period but was significantly (P<0.05) lowered when compared with the untreated group (Group 2) on day 1. There was a steady and significant (P<0.05) reduction in FBG of untreated rats on day 3, 7 and 14 when compared with the metformin treated group. Groups 4 and 5 had significantly (P<0.05) higher FBG levels on day 1, 3 and 7 when compared with normal control but their FBG levels were significantly (P<0.05) lowered when compared with groups 2 and 3. Group 5 showed significantly (P<0.05) lowered FBG levels throughout the period when compared to groups 2, 3 and 4.

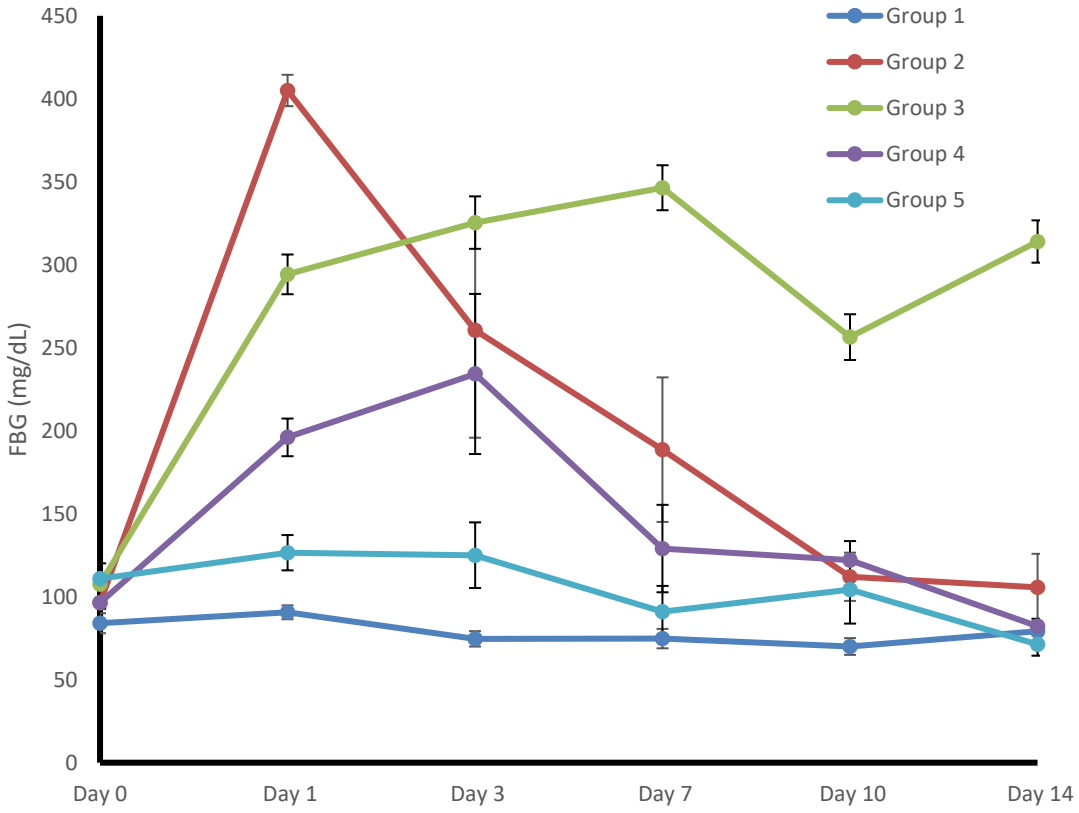

Figure 2: Mean fasting blood glucose level of diabetic rats treated with anti-diabetic teas (Values are expressed as mean ± SEM of five rats in each group; significance accepted at $p < 0.05$). Group 1 = Normal Control, Group 2 = STZ + dH_2O, Group 3 = STZ + MEFT, Group 4 = STZ + ADTa, Group 5 = STZ + ADTb.

Effect of Antidiabetic Herbal Teas on the Haematology of Streptozotocin-Induced Diabetic Rats

Table 2 shows the haematological values (mean ± SEM) of rats after induction of diabetes with streptozotocin and subsequent treatment with the anti-diabetic teas. Significant ($p<0.05$) increases in values of PCV (48.00±1.30), Hb (16.00 ± 0.43), MCV (59.32±4.20), MCH (19.11±1.40) were observed in group 4, while PCV (29.20±0.86), Hb (9.73±0.29), RBC (6.93±0.48), MCV (37.20±1.75) and MCH (12.40±0.58) values were significantly ($p < 0.05$) Low, when compared with the normal control and the other Groups. In groups 3, 4, and 5, WBC count (5.30±0.29, 5.30±0.35, 5.04±0.48 respectively), Neutrophils (39.00±1.70, 33.80±4.73, 32.00±1.97 respectively) and lymphocytes (55.40±1.60, 61.66±4.01, 62.40±1.83 respectively) were significantly ($p < 0.05$) lower than values of normal control. Eosinophils (1.80±0.49) of group 2 and monocytes (3.00±0.32, 3.60±0.40) of groups 2 and 3 respectively were significantly ($P<0.05$) higher when compared with normal control.

Table 2: Mean Haematology Values of Rats after STZ-Induced Diabetes

GROUP	PCV (%)	Hb (g/L)	RBC (×10⁹/L)	MCV (f/L)	MCH (g/L)	MCHC (g/L)	WBC (×10⁹/L)	Neut. (×10⁹/L)	Baso. (×10⁹/L)	Eosin. (×10⁹/L)	Mono. (×10⁹/L)	Lymp. (×10⁹/L)
1	42.80±1.50^{a}	14.27±0.50^{a}	9.52±0.69^{a}	45.46±1.81^{a}	15.16±0.60^{a}	33.33±0.00	8.00±0.27^{a}	25.60±1.12^{a}	1.80±0.49	0.80±0.20^{a}	1.80±0.37^{a}	70.00±0.89^{a}
2	40.40±1.17^{a}	13.46±0.39^{a}	6.88±0.32^{b}	59.34±3.72^{b}	19.78±1.24^{b}	33.33±0.00	9.68±0.71^{b}	31.80±1.77^{a}	1.80±0.49	1.80±0.20^{b}	3.00±0.32^{b}	61.60±1.34^{b}
3	35.40±1.69^{b}	11.80±0.57^{b}	7.16±0.61^{b}	51.06±5.11^{ab}	17.02±1.71^{ab}	33.33±0.00	5.30±0.29^{c}	39.00±1.70^{b}	1.40±0.60	0.60±0.40^{a}	3.60±0.40^{b}	55.40±1.60^{b}
4	48.00±1.30^{c}	16.00±0.43^{c}	8.51±0.49^{a}	59.32±4.20^{b}	19.11±1.40^{b}	33.33±0.00	5.30±0.35^{c}	33.80±4.73^{b}	1.00±0.55	1.00±0.32^{a}	2.60±0.40^{a}	61.60±4.01^{b}
5	29.20±0.86^{d}	9.73±0.29^{d}	6.93±0.48^{b}	37.20±1.75^{c}	12.40±0.58^{c}	33.33±0.00	5.04±0.48^{c}	32.00±1.97^{b}	1.60±0.24	1.40±0.40^{a}	2.60±0.24^{a}	62.40±1.83^{bc}

Values are mean ± SEM, n = 5. Values with alphabet superscripts on the same column are statistically significant from the rest of the group at $p < 0.05$. Key: Group 1 = Normal Control, Group 2 = STZ + dH_2O, Group 3 = STZ + MEFT, Group 4 = STZ + ADTa, Group 5 = STZ + ADTb, PCV = Packed Cell Volume, Hb = Haemoglobin concentration, RBC = Red Blood Cells, MCV = Mean Corpuscular Volume, MCH = Mean Corpuscular Haemoglobin, MCHC = Mean Corpuscular Haemoglobin Concentration, WBC = White Blood Cells, Neut. = Neutrophils, Baso. = Basophils, Eosin. = Eosinophils, Mono. = Monocytes, Lymp. = Lymphocytes.

Discussion

As seen in the course of this study, weight loss and persistent hyperglycaemia are two important symptoms that characterize type I diabetes. Streptozotocin-induced hyperglycaemia in experimental animals has been widely used as a valuable experimental model to study the effect of different hypoglycaemic agents. Over 150 medicinal plants are known to be used for the treatment of diabetes mellitus [14]. The purpose of this study was to assess the anti-diabetic effects of two herbal tea extracts on haematology, body weight, and blood glucose levels in streptozotocin-induced diabetic rats. The herbal teas comprise several medicinal plants.

A diabetic condition can affect red blood cells [15]. Treatment is often aimed at not just reducing blood glucose but also improving the blood picture. The use of herbal preparations for therapeutic or prophylactic purposes has been known to have diverse effects on haematology. These effects may be positive or negative, and they may also depend on the duration of use as well as the quantity consumed. The effect on haematology often depends on the phytochemical composition of the plant(s) used in the herbal preparation. The haematological parameters of streptozotocin-induced diabetic rats treated with anti-diabetic teas were evaluated in this study to determine their possible haematological effect. The administration of the herbal anti-diabetic teas to diabetic rats for 14 days revealed some effects on haematology. The increase in PCV, haemoglobin, MCV, and MCH in the group treated with the foreign tea (Tea A) indicates that the tea has components that support erythropoiesis. This same effect, however, can't be said with regard to its effect on the white blood cells (WBC) and lymphocytes, in which there was a decrease in the number of cells, indicating that the tea has no beneficial effect on the white blood cells. These immune cells are known to be depressed in diabetic conditions [16], as seen in the marked and sustained decrease in the total white blood cell count and lymphocytes in the diabetic rats not treated with the extract or metformin. The increase in neutrophils consistent in the treated and untreated rats may be attributed to an inflammatory response due to the effect of streptozotocin on pancreatic beta cells. Tea B had little or no erythropoietic effect, as revealed by the marked decrease in PCV, Hb, MCV, and MCH values. Total WBC and lymphocytes also were low compared with the control groups. The haematological findings in this study reveal that the use of these anti-diabetic herbal teas in the treatment or management of type I diabetes may not be helpful in improving the haematological parameters in diabetic conditions, despite the good hypoglycaemic effects observed.

Loss of body weight has been reported to be associated with type I diabetes [17]. Loss of body weight in diabetes is due to dehydration, loss of carbohydrates, and the excessive breakdown of tissue proteins and fat [18-20]. In agreement with the above reports, the body weights were significantly decreased in streptozotocin-induced diabetic rats. Treatment with the two anti-diabetic teas showed no significant improvement in the body weights of the diabetic rats after the 14-day administration, even though this could be attributed to the short period of the experimental design.

Daily administration of the anti-diabetic teas showed that both teas exhibited a significant blood glucose level reducing effect. Tea B, which is a local tea, showed a better hypoglycaemic effect compared to Tea A; the glucose reducing effect of B was similar to that of the standard drug metformin. Insulin regulates blood glucose homeostasis by stimulating the utilization of glucose by the liver, muscle, and adipose tissue. In the liver, insulin stimulates glycogen synthesis, glycolysis, and fatty acid synthesis, but unlike in muscle and adipose tissue, it does not stimulate glucose transport. The conversion of glucose to glycogen in liver cells is dependent on the extracellular glucose concentration and on the presence of insulin, which stimulates glycogen synthesis over a wide range of glucose concentrations [21, 22]. Type I diabetes mellitus is characterized by a progressive dysfunction of pancreatic beta cells and a decline in insulin secretion, leading to persistent hyperglycaemia. Streptozotocin moderately destroyed pancreatic beta cells in rats at lower doses (50 mg/kg body weight), resulting in inadequate insulin secretion [8]. Rats treated with the anti-diabetic teas and metformin showed a significant decrease in the level of blood glucose, which may be due to increased release of insulin from the existing and/or regenerated pancreatic beta cells [23, 24].

Conclusion

Our findings show that anti-diabetic teas have a hypoglycaemic effect on streptozotocin-induced type I diabetes mellitus in rats. This might be due to the presence of some active pharmacological principles in the herbal teas. The protective effect of the two anti-diabetic teas against hyperglycaemia may be due to the improvement in peripheral glucose utilization and glycolysis, similar to known diabetic drugs like meglitinides and metformin, etc. The beneficial anti-diabetic effect of the anti-diabetic teas was comparable with the known standard, Metformin. Thus, the dietary supplementation of the two anti-diabetic teas may be helpful for the management of type I diabetes mellitus and the prevention of diabetic complications.

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