Original Article

A creeper, Coccinia indica, has anti-hyperglycaemic and anti-ureogenic effects in diabetic rats

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Abstract

Objective: To explore how ethanolic extract of Coccinia indica affects normal and diabetic rats.

Methods: The case-controlled animal study was conducted in June 2008 at the Department of Biochemistry and Molecular Biology, University of Dhaka, Bangladesh. Two groups of 10 male rats each - one streptozotocin-induced diabetics and the other normal - were fed orally aqueous suspension of residue extracted from C. indica leaves with 60% ethanol after 18 hours of fasting. After 90 minutes of oral administration, the rats were sacrificed, and blood level of glucose and free fatty acids and hepatic arginase activity were analysed.

Results: The blood sugar level had significantly decreased by 23% ($p < 0.01$) and 28% ($p < 0.001$) in the normal and diabetic rats. Level of blood-free fatty acid was depressed by 15% ($p < 0.01$) and 25% ($p < 0.001$) in the two groups respectively. Moreover, the activity of hepatic arginase, a key urea cycle enzyme, was significantly depressed by 14% ($p < 0.05$) and 22% ($p < 0.02$) in the normal and diabetic groups.

Conclusion: Results suggested that C. indica extract had anti-hyperglycaemic and anti-ureogenic effects on the diabetic rats as judged by the decreased level of blood glucose and fatty acid and hepatic arginase activity.

Keywords: Coccinia indica, Non-diabetic and diabetic rats, Streptozotocin, Anti-hyperglycaemic, Anti-ureogenic.
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Introduction

Diabetes mellitus is a very common medical problem, caused by a deficiency of the secretion or action of pancreatic hormone insulin. Metabolic abnormalities of carbohydrate, protein and fats occur in this condition. Characteristic symptoms of diabetes are excessive thirst and frequent urination (polyuria) leading to increased water intake (polydipsia), weight-loss in spite of polyphagia (increased appetite).[1] In diabetic condition, blood sugar level rises as it cannot enter into the cells, resulting in hyperglycaemia, which is accelerated by gluconeogenesis in liver and kidney as it becomes the only one source of glucose for the survival of humans and animals in this condition. Under diabetic condition, fatty acids are excessively and incompletely oxidised in the liver which leads to over-production of kentone bodies in the liver.[2] In diabetic condition, body proteins are broken down into amino acids, later gluconeogenic amino acids contribute to hyperglycaemia by increasing gluconeogenesis, which, in turn, results in increased urea cycle in the liver. Under this condition, key enzyme of urea cycle arginase is also increased.[3]

Herbal medicine is well-known in the world for the treatment of many complicated diseases as a parallel to modern medical care. Diabetes mellitus is a very familiar metabolic complication from ancient times. Many medicinal plants are usually being used for its treatment from the very beginning when people came to know about this disease in the Indian sub-continent, especially in rural areas. Some plants have already been scientifically studied and some others are under study for the treatment of diabetes.[4-9]

There are several types of orally administered chemical drugs to reduce hypoglycaemia in diabetes. However, these are not sufficient. So it is necessary to design a novel oral agent which is more effective and safe to treat hypoglycaemia.[10] Previously, many studies showed anti-diabetic properties of Artemisia pallens,[11] Gophila reniformis,[12] Cassia klenii,[13,14] Azadirachta indica, Aloe vera, Trigonilla foenum graecum[15] and Coccinia indica.[4-7]

C. indica (family: Cucurbitaceae) is a creeper. It is locally known as Telakucha. It grows abundantly in Bangladesh and most people use it for the treatment of diabetes mellitus in Bangladesh and India.[16] This plant has also been extensively used in Ayurvedic and Unani practice (Herbal medicine) in the Indian sub-continent.[7] Leaves of this plant are used by the rural people of Bangladesh as vegetable. To the best of our knowledge, there is no report of toxicity of this plant in the human body. However, the physiological effect of this plant on animal is still unknown. Thus the objective of this study was to observe the effect of ethanolic extract of C. indicia leaves on the normal and diabetic rats.

Material and Methods

The study was conducted at the Department of Biochemistry and Molecular Biology, University of Dhaka, Bangladesh, in June 2008. The study was approved by the Academic Committee of the University. Male albino rats of 116-151kg were purchased from the International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDR,B) and were kept at a constant temperature of 25oC with a 14 hour of light and 10 hour of dark cycles. Approximately 5-6g of balanced pelleted rat food was supplied thrice a day with free access to drinking water. These conditions were maintained throughout the experimental period until the animals were sacrificed.

Diabetes was induced in the rats by an intraperitoneal injection of streptozotocin (STZ) dissolved in saline of pH 4.3 (65 mg/kg body weight) after 18 hours of fasting. Normal rats injected with citrate buffer were used as control.

C. indica plant was collected from the jungle area of Narayanganj, Bangladesh, and identified with the taxonomy. For the purpose, 1kg of fresh C. indica leaves was crushed with a blending machine. These crushed leaves were then soaked in 64% ethanol (v/v) at room temperature in a glass jar for 4 days with an occasional shaking. The suspension was filtered and the filtrate was then freeze dried in a Virtis Bench Freeze dryer (Model no 10-030, USA). The dried green oily semi-solid material was stored in a freezer until further use. The semi-solid material was suspended into water which was fed orally to the experimental rats at a dose of 200mg/kg of body weight after 18 hours of fasting.

The rats were sacrificed after 90min of oral administration and about 0.50ml of blood was collected from each rat into a vial containing heparin (35 units/ml of blood) solution. Blood glucose and fatty acid levels were estimated by method of Nelson and Somogyi[17-19] and Trout et al[20] respectively. Liver was also collected to determine the activity of arginase by the method of Roman et al.[21]

Rat liver (0.5 g) was chopped into small pieces on a pre-cooled water glass and homogenised for 2 min with ice-cold carbonate buffer at 0-4°C in a potter-Elvehjem type of homogeniser placed in ice bath. The homogenate was centrifuged for 30 min at 10,000 rpm at 2°C. The supernatant was diluted 10X with carbonated buffer and stored at -20°C for detection of arginase activity. The total protein was estimated by the method of Lowry et al.[1]

Rats were divided into two groups with 10 rats in each group. One group was normal, while the other was diabetic. One rat died during the induction of diabetes.

Out of 10 normal rats, 5 were administered with C. indica ethanolic extract, and the other 5 were treated with citrate saline as controls.

On the other hand, out of 9 diabetic rats, 5 were administered with C. indica ethanolic extract, and the other 4 were treated with citrate saline.

A comparison between the control and treated groups was performed by student's t-test. All statistical tests were considered significant at a level of $p < 0.05$. All data was analysed by using SPSS version 10.0. The quantitative data were presented as mean ± SD.

Results

The average body weight and blood sugar of the 20 rats were 135.95g and 74.30±3.49 mg% respectively. Half of these rats were treated with STZ and the remaining rats were treated with citrate saline as control after 18 hrs of fasting. Body weight and blood sugar level were again measured in these rats after first, second and third weeks of the injection (Figure-1). Although control rats gained weight by 20.5% at third week post-injection (164g) compared to first week (143g), but average blood glucose level was nearly constant. On the other hand, STZ-treated rats lost weight by 9% at third week post-injection (125g) compared to first week (131g), but average blood glucose level increased by 40%.

To study the effect of C. indica plant on one group of rats (five normal and five diabetics) they were orally given aqueous extract of this creeper at dose of 200mg per kg of body weight after 18 hrs of fasting. As control, another group of rats (five normal and four diabetics) were given only distilled water orally after 18 hrs of fasting. Rats were sacrifised after 90 min of the oral administration. Normal and diabetic rats showed no significant difference in their blood glucose levels before and after the oral administrations (Figure-2). On the other hand, the blood glucose levels significantly decreased in both normal and diabetic rats after the oral administration of C. indica extract. The decrease in blood glucose level was much more significant in the diabetic rats

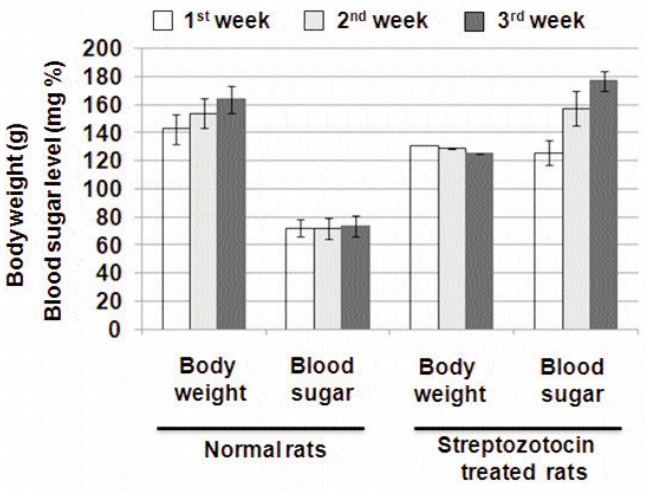

Figure-1: Generation of diabetic rats by Streptozotocin (STZ) treatment (65mg/kg body weight). Measurement of body weight (g) and blood sugar level (mg %) in normal and STZ-treated rats after 18 hrs of fasting at first, second and third weeks of injection.

(28%, $p < 0.001$) compared to normal one (23%, $p < 0.01$).

To exclude the possibility of the effect of C. indica on fatty acid production, we determined the blood-free fatty acid level in both control and experimental rats. The levels had not significantly changed in both normal and diabetic rats before and after the oral administration of distilled water (Figure-3A). On the other hand oral administration of C. indica extract caused depression of free fatty acid levels in blood in both normal and diabetic rats. The depression of free fatty acid levels was more significant in the blood of the diabetic rats (25%, $p < 0.001$) compared to that of normal rats (15%, $p < 0.01$) after oral administration of the plant extract.

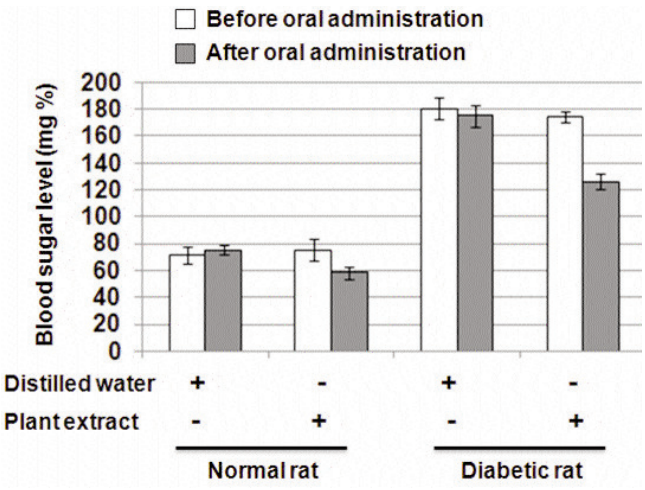

Figure-2: Effect of ethanolic extract of Coccinia indica leaves (200 mg/kg body weight) on blood sugar level in control and Streptozotocin-induced diabetic rats. Values are the mean ± S.D. of five control rats and four STZ-treated rats. The decrease of sugar level was significant in both control ($p < 0.01$) and diabetic ($p < 0.001$) rats by the oral treatment of plant extract.

The oral administration of ethanolic extract of C. indica led to depression of the activity of hepatic arginase (14% $p < 0.05$) compared to the oral administration of distilled water in normal rats (Figure-3B). The depression of the activity of hepatic arginase was much more significant (22%, $p < 0.02$) in the case of diabetic rats.

Discussion

Diabetic rats were created by a single intraperitoneal injection of STZ (65 mg/kg body weight). The diabetic condition was confirmed by the increased level of blood sugar level after 18 hrs of fasting condition compared to control rats. Mortality rate was found to be 10% in STZ-induced rats. The blood glucose level was found reduced in both control and diabetic rats after the oral administration of C. indica extract. The percentage of reduction of blood glucose level by C. indica extract was much more significant in the case of diabetic rats (28%) compared to the control rats (23%) under fasting condition. Under fasting condition in diabetic rats blood glucose level increases over the normal one causing a metabolic condition called hyperglycaemia. But our study showed that C. indica extract lowered the level of blood glucose in diabetic rats under fasting condition. It can be concluded that C. indica

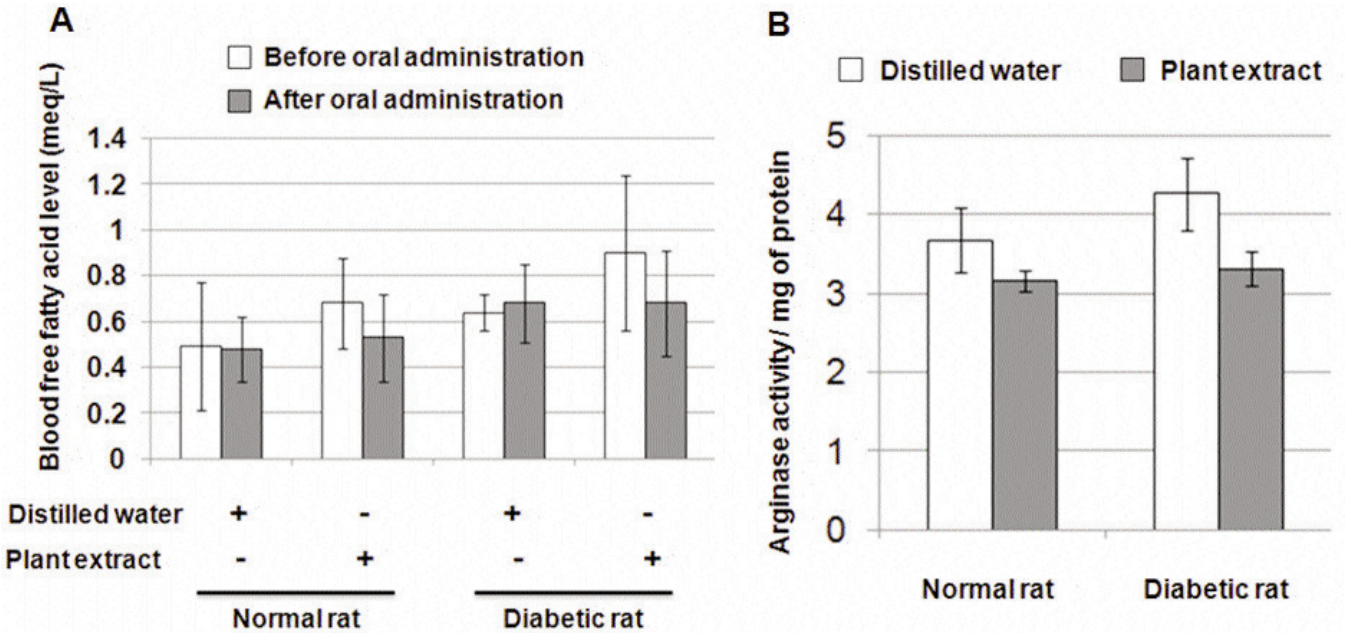

Figure-3: (A) Effect of ethanolic extract of Coccinia indica leaves (200 mg/kg body weight) on blood-free fatty acid level in control and Streptozotocin-induced diabetic rats. Values are the mean ± S.D. of 5 control rats and 4 STZ-treated rats. The decrease of blood-free fatty acid level was significant in both control ($p < 0.01$) and diabetic ($p < 0.001$) rats by the oral treatment of plant extract. (B) Effect of ethanolic extract of Coccinia indica leaves on hepatic arginase activity in control and STZ-induced diabetic rats. The decrease was significant in both control ($p < 0.05$) and diabetic ($p < 0.02$) rats.

extract appascutly has an anti-effect on hyperglycaemia. There should be some components in C. indica extract which might help in increased uptake of glucose by cells and/or tissues as the activity of glucose-6-phosphate dehydrogenase is enhanced.[23] Under fasting condition in diabetic rats, gluconeogenesis from amino acids in liver was the main source of blood glucose. Key enzymes of gluconeogenesis, glucose-6-phosphatese and fructose-1,6-bisphosphatase are inhibited by C. indica extract.[24,25] Thus the inhibition of gluconeogenesis should also contribute to lower the blood glucose level in diabetic rats under fasting condition after the oral administration of C. indica extract. Moreover, blood-free fatty acid levels were also found decreased in both normal and diabetic rats under fasting condition after the oral treatment of C. indica extract. This indicated that this creeper extract seems to have some chemicals which might enhance oxidation of blood-free oxidation.

Arginase is a key enzyme of urea cycle in the liver. The enzymatic activity of hepatic arginase was significantly reduced in diabetic rats (24%) compared to normal rats (14%) after the oral administration of C. indica extract under fasting condition, indicating the reduction of urea formation. In diabetic condition urea formation is abnormally high, but after oral administration of C. indica extract, arginase activity was seen to have significantly reduced in diabetic rats under fasting condition. Moreover, gluconeogenesis directly linked to urea cycle in liver was reduced by extract treatment. Thus it can be concluded that the oral administration of C. indica extract seems to have anti-ureogenic effect in diabetic rats under fasting condition together with anti-hyperglycaemic effect which are brought about by the depression of hepatic gluconeogenesis as the depression of hepatic glocuneogenesis is related to the depression of the activity of hepatic arginase in the urea cycle.

Conclusion

Creeper, C. indica has a highly significant effect on the control of blood glucose level compared to other herbs reported, and urea level in diabetic rats under fasting condition. The plant might be used in the future as herbal medicine for the treatment of diabetic patients as it reduces the metabolic complications under diabetic condition. Moreover, it would be much more interesting to detect metabolic elements which contribute to reduced metabolic complications in the diabetic rats.

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