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Antidiabetic potential of cow urine in streptozotocin-induced diabetic rats

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Abstract

The sacred Indian cow, *Bos indicus* known as "Kamadhenu" in Indian scripts, is believed to be a "mobile hospital" for the treatment of many diseases. Ancient Ayurvedic scriptures such as Charaka samhita, Shushruta samhita and Brahad-Wagbhatt mention various medicinal properties of cow urine. It is used as an insecticide and in disorders like intestinal gas, acidity and cough. Although Indian Ayurvedic literature cites many medicinal properties of cow urine, there is no scientific evidence to support this. Hence, the present study of the antidiabetic activity of cow urine was undertaken. The effect of a distillate of cow urine was studied in vivo in rats given streptozotocin. Diabetes was induced by administration of streptozotocin (50 mg/kg body wt., i.p) dissolved in citrate buffer (0.1 M, pH 4.5). The antidiabetic effect of the (three different doses) and a standard drug, Glibenclamide (0.25 mg/kg, p.o) ,was studied in these diabetic rats. The parameters used in the study included assessment of fasting blood glucose levels, serum lipid profiles, liver glycogen levels and initial and final changes in body weight. The cow urine distillate produced a significant (*P*<0.05) reduction of the elevated blood glucose, serum cholesterol and serum triglycerides levels when compared with the diabetic control. The diabetic animals treated with cow urine distillate also showed a significant increase in HDL levels and a gain in body weight when compared with the diabetic control. Earlier studies have revealed the presence of antioxidants and free radical scavengers in cow urine which might be responsible for the observed anti- diabetic effects.

Key words: antidiabetes, cow urine distillate, streptozotocin, glibenclamide.

1. Introduction

Oxidative stress, defined as an imbalance between oxidants and anti-oxidants leads to many biochemical changes and is an important causative factor in several human chronic diseases, such as atherosclerosis and cardiovascular diseases, mutagenesis and cancer, several neurodegenerative disorders and the aging process. Diabetes mellitus is one such disease and it is estimated that the number of diabetic patients will continue to increase worldwide in the future. It has been postulated that the etiology of the complications of diabetes involves oxidative stress perhaps as a result of hyperglycemia. The elevated level of blood glucose in diabetes produces oxygen free radicals which cause membrane damage due to peroxidation of membrane lipids and protein glycation. It has been

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suggested that free radical activity is increased in diabetes. Under physiological conditions, glucose produces oxidants that exhibit reactivity similar to that of hydroxyl free radicals [1].

In recent years, there has been a renewed interest in variety of natural products with antioxidant potential which can play a major role in protecting against the molecular damage induced by reactive oxygen species. The present study of cow urine is aimed at its anti-diabetic potential. The sacred Indian cow, *Bos indicus,* is believed to be a "mobile hospital" for the treatment of many diseases. A number of diseases can be cured by the use of medicines derived from the cow. Cow urine is described in detail in ancient Ayurvedic scriptures, such as Charaka samhita, Shushruta samhita and Brahad-Wagbhatt, as being bitter, pungent, spicy and warm. It is used as an insecticide and as a regulator for various disorders like intestinal gas, acidity and cough. It is claimed to make humans wiser and can be used as a universal easily digestible medicine [2].

In classical texts of Ayurveda, like Charaka samhita and Shushruta samhita, several medicinal properties of cow urine are described. Cow urine is known to cause weight loss, and reverse certain cardiac and kidney problems, as well as indigestion, stomach ache and edema. Cow urine is considered useful in treating renal colic, jaundice, anemia, diarrhea, gastric infection, piles and skin diseases including vitilago. It is also considered as an appetizer and is known to reverse inflammation, and acts as a diuretic as well as a nephroprotective agent. However the anti-diabetic properties of cow urine have not been described in the literature. Further, although Indian Ayurvedic literature cites various medicinal properties of cow urine, there is very little scientific evidence to support this. Hence, the present study was undertaken to provide this [3].

2. Materials and methods

2.1. Cow urine distillate

The first early morning voided urine of *Bos indicus* was collected from the local cow sheds belonging to Sri Ramachandrapura math, Hosanagara, and immediately distilled at 100^0C using a temperature- controlled distillation apparatus and then stored below 10^0C for further use.

2.2. Chemicals

All chemicals and reagents used were of analytical grade and obtained from Sigma Chemical Company (St. Louis, MO, USA). The kits for the estimation of blood glucose levels and serum lipid profiles were obtained from Ranbaxy Diagnostics and Reckon Diagnostics Pvt. Ltd., India. The standard drug glibenclamide was purchased from a local pharmacy in Mangalore, India.

2.3 Selection of dose

For the evaluation of the anti-diabetic activity of the cow urine distillate, three dose levels were selected. The rat dose was calculated from the human dose (60 ml per day), multiplied by a factor of 0.018 × 5 which is equal to 5.4 ml/kg body weight (first dose) [4]. The second dose selected was twice that of the first dose. i.e. 10.8 ml / kg body weight and the third dose selected was 50% of the first dose i.e. 2.7 ml/ kg body weight.

2.4. Animal treatment

Male albino Wistar rats of both sex (180-260 g) were obtained from the laboratory of K.S Hegde Medical Academy (KSHEMA), Deralakatte, Mangalore ,India and were maintained under a 12 h light/dark cycle and allowed food and water *ad libitum*. The institutional animal ethics committee of K.S Hegde Medical Academy (KSHEMA), Deralakatte, Mangalore, India, approved the

experimental protocol in accordance with the guidelines provided by the committee for the control and supervision of experiments on animals (CPCSEA) with registration number KSHEMA/AEC/049/2007.

2.5. Preparation of streptozotocin solution:

Preparation of 0.1 M citrate buffer solution pH 4.5: An accurately weighed quantity of Trisodium citrate (14.9 g) was dissolved in sufficient distilled water to produce 1000 ml and the pH was adjusted to 4.5 using conc. HCl.

The solution of streptozotocin was prepared by dissolving the weighed quantity of streptozotocin in 0.1 M freshly prepared ice -cold citrate buffer (pH 4.5).

2.6. Experimental induction of diabetes [5]

Diabetes was induced in rats by the intraperitoneal injection of streptozotocin (Sigma –Aldrich, Germany) at a dose of 50 mg/kg b.w. dissolved in citrate buffer (0.1 M, pH 4.5) in the volume of 1ml/kg. In order to prevent hypoglycemia during the first day after the STZ administration, the diabetic rats were given 5 % *w*/*v* glucose solution orally. Three days after the injection, the blood glucose levels were measured and the animals with blood glucose levels above 300 mg/dl were considered to be diabetic and were used in the subsequent experiments. In all the experiments, rats were fasted for 16 hr prior to STZ injection.

Animals were divided into six groups of six rats per group. The test samples were administered orally for 15 days.

Group I–Normal control group-Animals received only vehicle

Group II–Diabetic control group (streptozotocin-treated)-Animals received only vehicle.

Group III–Standard drug group-Diabetic animals received daily a single oral dose of the reference drug glibenclamide (0.25 mg/kg) from day 1 to15.

Group IV–Diabetic animals received daily a single oral dose of Cow urine distillate 2.7 ml/kg body weight from day 1 to15

Group V–Diabetic animals received daily a single oral dose of Cow urine distillate 5.4 ml/kg body weight from day 1 to 15.

Group VI–Diabetic animals received daily a single oral dose of Cow urine distillate 10.8 ml/kg body weight from day 1 to 15.

The effect of administration of cow urine distillate to diabetic rats were determined by measuring the fasting blood glucose levels , serum lipid profiles, liver glycogen levels and initial and final changes in body weight.

Day 3 of induction was designated as day 1 for administration of the test sample to diabetic rats. Fasting blood glucose levels were measured on days 1, 5, 10 and 15 of the test sample administration period. Other parameters were determined on day 15 after the animals were sacrificed by decapitation.

2.7. Blood sampling

Blood samples were collected retro-orbitally from the inner canthus of the eye under light ether anesthesia using capillary tubes (Micro Hemocrit Capillariea,Mucaps). Blood was transferred into fresh vials and serum was separated by centrifuging at 2000 rpm for 2 min. Blood glucose levels were measured using GOD/POD glucose kit.

3. Statistical analysis

The data were expressed as Mean ± SEM and analyzed using one way analysis of variance

(ANOVA), followed by a post hoc Sheffe's multiple comparison test using SPSS computer software version 10. The values were considered significant when $P<0.05$.

4. Results

Streptozotocin administration to experimental animals resulted in a significant ($P<0.05$) rise in blood glucose levels. The changes in body weights and fasting blood glucose levels, before and after treatment with the test drug in streptozotocin -induced diabetic animals are shown in Table 1 & 2. Fasting blood glucose levels of untreated diabetic rats were significantly higher and the body weights were lower than those in normal rats. Diabetic animals treated with cow urine distillate showed significant lowering of blood glucose levels and

Table 1. Effect of cow urine distillate on blood glucose levels in streptozotocin-treated diabetic rats

Groups	Dose (mg/kg)/(ml/kg)	Blood glucose level (mg/dl)			
		1st day	5th day	10th day	15st day
I) Normal control	----	85.50±0.95 [b,c]	84.20±1.41 [b,c]	84.88±0.71 [b,c]	85.66 ±0.91 [b,c]
II) Diabetic control	----	336.33±4.80 [a]	356.83±2.49 [a,c]	364.66±2.31 [a,c]	372.83±2.58 [a,c]
III) Std glibenclamide	0.25	343.33±3.49 [a]	263.66±3.46 [a,b]	193.50±4.36 [a,b]	140.16±2.71 [a,b]
IV)	2.70	334.20±2.88 [a]	252.83±2.68 [a,b]	232.21±1.78 [a,b]	206.43±2.34 [a,b]
V)	5.40	340.33±3.48 [a]	249.50±3.28 [a,b]	208.33±3.75 [a,b]	172.65±3.26 [a,b]
VI)	10.80	339.50±2.95 [a]	235.10±3.51 [a,b]	198.33±2.82 [a,b]	150.80±1.98 [a,b]

All the values are expressed as mean ± SEM ($n = 6$), values are statistically significant at $P<0.05$

[a] $P<0.05$ when compared with the normal control group

[b] $P<0.05$ when compared with the diabetic control group

[c] $P<0.05$ when compared with the standard group

Table 2. Effect of cow urine distillate on the glycogen content, body weight and lipid profiles in streptozotocin-treated diabetic rats

Groups	Glycogen content (mg/g)	change in body wt.(%)	Cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL (mg/dl)
I) Normal control	39.80±2.17 [b,c]	+9.93±1.29 [b,c]	61.40±1.46 [b,c]	78.10±1.26 [b,c]	58.70±1.41 [b,c]
II) Diabetic control	16.47±1.18 [a,c]	-12.06±0.55 [a,c]	125.14±2.71 [a,c]	190.14±1.76 [a,c]	35.30±1.14 [a,c]
III) Std glibenclamide	30.71±2.34 [a,b]	-3.66±0.63 [a,b]	69.81±3.19 [a,b]	90.15±3.10 [a,b]	49.44±1.37 [a,b]
IV)	22.41±2.72 [a,b]	-7.39±1.74 [a,b]	87.47±33 [a,b]	123.11±1.48 [a,b]	38.71±1.43 [a,b]
V)	23.71±1.61 [a,b]	-7.10±0.22 [a,b]	85.32±1.77 [a,b]	118.70±2.16 [a,b]	40.64±2.01 [a ,b]
VI)	24.25±2.26 [a,b]	-5.18±0.25 [a,b]	80.71±1.69 [a,b]	112.10 ±2.11 [a,b]	43.49±1.61 [a,b]

All the values are expressed as mean ± SEM ($n = 6$), values are statistically significant at $P<0.05$

[a] $P<0.05$ when compared with the normal control group

[b] $P<0.05$ when compared with the diabetic control group

[c] $P<0.05$ when compared with the standard group

a significant increase in body weights (P<0.05). Serum cholesterol, triglycerides and HDL levels in all the groups of streptozotocin-treated diabetic animals are given in Table 2. The cholesterol and triglyceride levels were significantly higher and the HDL levels were significantly lower in the untreated diabetic rats compared with the values in normal rats. The treated diabetic rats had lower levels of cholesterol, and triglycerides and a higher level of HDL compared with those in the untreated diabetic group. The treatment with cow urine distillate produced almost normal levels of cholesterol, triglyceride and HDL. Table 2 shows the hepatic glycogen levels in all the animal groups. The liver glycogen levels in streptozotocin- treated diabetic rats were significantly lower than those in normal rats. Treatment with cow urine distillate improved the liver glycogen significantly, as indicated by the higher levels of hepatic glycogen in the treated diabetic group compared with those in the untreated diabetic group.

5. Discussion

Cow urine is one of a number of traditional remedies that have several pharmacological actions. The use of cow urine as an anti-diabetic agent has been described in various Ayurvedic texts. However, there are no information about its activity in experimental diabetes. The present study indicates that cow urine was able to provide significant protection against diabetes in streptozotocin-treated diabetic rats. Streptozotocin is widely used to induce experimental diabetes in animals. Streptozocin or Streptozotocin is a cytotoxic nitrosoureido-gluco pyranose obtained from the fermentation of *Streptomyces achcromogenesis* and it produces diabetes in a number of animals such as rats, rabbits, and mice. The mechanism of their action on pancreatic β cells has been intensively investigated. The cytotoxic action of this diabetogenic agent is mediated by reactive oxygen species. Streptozotocin enters β cells via a glucose transporter (GLUT2) and causes alkylation of DNA. DNA damage induces activation of poly ADP-ribosylation, a process that is more important for the diabetogenic effect of streptozotocin than DNA damage itself. Poly ADP-ribosylation leads to depletion of cellular NAD+ and ATP. Enhanced ATP dephosphorylation after streptozotocin treatment supplies a substrate for xanthine oxidase resulting in the formation of superoxide radicals. Consequently, hydrogen peroxide and hydroxyl radicals are also generated. Furthermore, streptozotocin liberates toxic amounts of nitric oxide that inhibits aconitase activity and participates in DNA damage. As a result of the streptozotocin action, β cells are destroyed by necrosis [6]. The fundamental mechanism underlying hyperglycemia in diabetes mellitus involves the over-production (excessive hepatic glycogenolysis and gluconeogenesis) and decreased use of glucose by the tissues [7]. Studies in animals with streptozotocin-induced diabetes treated with cow urine distillate revealed a significant reduction in the blood glucose level when compared with diabetic control groups at the end of the experimental period. Induction of diabetes with streptozotocin is associated with the a characteristic loss of body weight, which is due to increased muscle wasting in diabetes [8]. Diabetic rats treated with the cow urine showed an improvement in weight gain compared with the diabetic control. The marked increase observed in serum triglycerides and cholesterol and the decrease in HDL in untreated diabetic rats is in agreement with the findings of Nikkila&Kekki [9]. Diabetic rats treated with the cow urine exhibited a significant decrease in cholesterol and triglycerides and an increase in HDL compared with the diabetic control. Glycogen syntheses in the rat liver and skeletal muscles is impaired during diabetes [10].

The decreased glycogen levels may probably be due to the lack of insulin in the diabetic state, which results in the inactivation of the glycogen synthase systems. In the present investigation, a significant increase in glycogen levels was observed in the treated groups, which might be due to the reactivation of the glycogen synthase system. Oxidative stress has been shown to play an important role in the etiology of diabetes [11]. Streptozotocin produces oxygen radicals in the body, which causes pancreatic injury and could be responsible for the increased blood glucose [12]. The compounds responsible for the anti-diabetic activity of cow urine are at presently not known. Studies have been carried out to examine the anti-oxidant potential of cow urine. For example, Jarald *et al*. [13] described the anti oxidant properties of cow urine using two *in vitro* models, DPPH radical scavenging activity and superoxide scavenging activity using ascorbic acid as a reference standard. Krishnamurthi *et al*. have described the antioxidant action of cow urine using an ABTS assay model and the antioxidant effect of cow urine was b due to the presence of volatile fatty acids [14]. Hence the presence of antioxidants, free radical scavengers in cow urine could be responsible for its anti- diabetic action.

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