



PROTECTIVE ROLE OF PUNGANUR COW URINE ON STREPTOZOTOCIN INDUCED DIABETES IN RATS

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ABSTRACT

Punganur Cattle is the world's smallest *Bos indicus* cattle originated in Punganur town in Chittoor district of Andhra Pradesh, India. This breed is known for its short stature, high milk production efficiency and efficient reproductive characters. In 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 was aimed to evaluate 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). The antidiabetic effect of three different doses and a standard drug, Glibenclamide (0.25 mg/kg, p.o), was studied in diabetic rats. Fasting blood glucose levels, serum lipid profiles, liver glycogen levels and initial and final changes in body weight were assessed. 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 anti-diabetic effects. The present communication records the first ever report on protective role of cow urine on diabetic in a bizarre situation of tropical climate of Andhra Pradesh (AP), India.

Keywords: Punganur Cow, anti diabetes, cow urine distillate, streptozotocin, glibenclamide.

INTRODUCTION

Nature has provided us with all Natural Amenities like Air, Water, Sun light etc., which are essential for our Body. It has also provided us with "Divine Nectar" known as "Urine" which flows from our Body. Urine has a Natural Healing Power to Control and Cure all kinds of Diseases. Just like Nature has provided milk in the mother's breast for nourishment of the infant child, similarly Nature has also provided Urine in human body for preservations of their health and cure of various diseases. "Urine Therapy" is the Most Effective Natural Remedy and the safest method of treatment which does not have any side effects. It can Prevent, Control and Cure all kinds of chronic diseases such as Cancer, Diabetes, Blood Pressure HIV / AIDS, Kidney failure, Muscular Dystrophy, Arthritis, Psoriasis, Hair loss, Mental Retardation and Cerebral Palsy etc. It can boost the Immune System, Improve Nervous Disorder, Dissolves and Removes the Toxins Accumulated in our body. It can revive dead Tissues, Rebuilds Resistance Power of the Vital Organs like, Brain, Heart, Lungs, Pancreas, Liver and Intestine etc. It rejuvenates our body and safe guards the general Health of the people. The whole world at large can get rid of dreadful diseases and blessed with the Natural healing Power by "Urine Therapy." The very feeling that the "Devine Panacea" the solution for all Ailments is within us can fill your life with immense pleasure. Person's self confidence and positive attitude can solve all their problems and they will be able to maintain healthy and happy life. Lord Shiva has himself narrated the "Benefits of Urine Therapy" to Mother Parvati which has been referred in the ancient book "Damar Tantra" in Vedas. In Ancient Books and Vedas Urine is referred as "Shivambu" (Auto Urine) meaning Water of Shiva. Urine Therapy is the ancient method of treatment. The Powerful practice for healing "Self-Urine Therapy" has been referred in "Shivambu Kalpa Vidhi" part of 5000 years old document called Damar Tantra linking this practice to Vedas

the sacred Hindu texts. Reference of Urine Therapy is also found in almost all the volume of Ayurveda and in one of the volumes Bhavprakasha Urine is termed as "Vishaghna" killer of all poisons and "Rasayana" which can rejuvenate even old person and "Raktapamaharam" which purifies blood and cures all skin diseases. In Tantrik Yoga culture this practice is termed as "Amroli." Amroli comes from the root word "Amar." They termed "Shivambu" as Holy Liquid. According to them Urine is more nutritious than even milk as you are not only physically benefited by the practice, but you become spiritually advanced because it is an Elixir for body, mind and spirit¹. God has given us this precious Gift (Urine) right from our very birth. The proverb 5:15 have also been referred in the Holy Bible: "Drink water out of thine own cistern." In India, Cowpathy is known as an old system of traditional medicine mentioned in ancient Indian literature (Ayurveda) as Panchgavya Chikitsa. The Ayurvedic medicines of animal origin are mainly prepared from Panchgavya (five things from Indian cow viz., urine, dung, milk, butter oil and curd), which boost up the body immune system and makes body refractory to various diseases². The specificity of immune system depends upon the number and activity of lymphocytes.³ studied the immunomodulatory effect of cow urine in mice and found that cow urine enhances both T- and B-cell blastogenesis and also increases the level of IgG. Kumar and Chauhan *et al.*, reported increase in both cellular and humoral immune responses due to cow urine^{2,4}. The present study was planned to investigate the blastogenic activity of lymphocytes and effect of *in-vivo* cow urine treatment on it so as to find out their potential to mount protective immune response against diseases. 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, mostly spread over worldwide and the 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 suggested that free radical activity is increased in diabetes. Under physiological conditions, glucose produces oxidants that exhibit reactivity similar to that of hydroxyl free radicals⁵. 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 was aimed to study the protective role of cow urine as an anti-diabetic and potentiality. Punganur cow 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-Wagbhata, as being bitter, pungent, spicy and warm. Now a day's Cow urine is used as an insecticide and as a regulator for various disorders like intestinal gas, acidity and cough and is claimed to make humans wiser and can be used as a universal easily digestible medicine⁶. In classical texts Vedas like Ayurveda, like Charaka samhita and Shushruta samhita, several medicinal properties of cow urine are described very much with examples. 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 vitiligo and considered as an appetizer and is known to reverse inflammation, and acts as a diuretic as well as a nephro-protective 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 support⁷. Hence, the present study was undertaken.

MATERIALS AND METHODS

Cow Urine Distillate Preparation

The first early morning voided urine of punganur cow (*Bos indicus*) was collected from the cow sheds Livestock Research Station, Palamaner, under Sri Venkateswara Veterinary University, immediately distilled by using double distillation unit (at 1000°C using a temperature- controlled distillation apparatus and then stored below 100°C) used for further studies.

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 named Hetiro Pharmacy, Tirupati. Chittoor Dist, India.

Dose Selection

Evaluations 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)⁸. The second dose selected was twice that of the first dose, i.e. 10.8 ml / kg body weight and the third dose were selected as 50 % of the first dose i.e. 2.7 ml / kg body weight.

Animal Treatment

Healthy Wistar albino rats (150 to 180 g) Animals were housed in a room with temperature maintained at $22 \pm 2^{\circ}\text{C}$ and humidity 55 ± 4 %. They were fed with standard laboratory diet (SKS Feed, India). Rats were divided into 6 groups each containing 8 animals and allowed food and water *ad libitum* throughout the investigation. All the procedures are approved by the institutional animal ethics committee.

Preparation of Streptozotocin Solution

Preparation of 0.1 M citrate buffer solution of 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).

Experimental Induction of Diabetes

Diabetes was induced in rats by streptozotocin intraperitoneally injection at a dose level of 50 mg / kg b.wt. It is dissolved in citrate buffer (0.1M, pH 4.5) in the volume of 1 ml / kg. In order to prevent hypoglycemia during the first day after the streptozotocin 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 h prior to streptozotocin injection. Animals were divided into six groups of 8 rats per group. The test samples were administered orally for 2 weeks⁹.

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 to14.

Group IV: Diabetic animals received daily a single oral dose of Cow urine distillate 2.7 ml / kg body weight from day 1 to14.

Group V: Diabetic animals received daily a single oral dose of Cow urine distillate 5.4 ml / kg body weight from day 1 to 14.

Group VI: Diabetic animals received daily a single oral dose of Cow urine distillate 10.8 ml / kg body weight from day 1 to 14.

The effects 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 day 1, 5, 10 and 14 of the test sample administration period. Other parameters were determined on 15th day of experimentation, after the animals were sacrificed by decapitation.

Blood Sampling

Blood samples were collected retro-orbitally from the inner canthus of the eye under light ether anesthesia using capillary tubes. Blood was transferred into fresh vials and serum was

separated by centrifuging at 2000 rpm for 2 minutes. Blood glucose levels were measured using glucose kit.

Statistical Analysis

The data were expressed as Mean \pm SEM and analyzed using one way analysis of variance (Anova), followed by a post hoc Sheffe's multiple comparison tests using SPSS computer software version 10. The values were considered significant when $P < 0.05$.

Table 1: Effect of Cow Urine Distillate on Blood Glucose Levels in Streptozotocin-treated Diabetic Rats

Group	Dose	Glucose levels in blood (mg / dl)			
		Day 1	Day 5	Day 10	Day 14
Group I	--	85.89 \pm 0.96 ^{bc}	84.88 \pm 1.44 ^{bc}	84.89 \pm 0.89 ^{bc}	85.66 \pm 0.73 ^{bc}
Group II Diabetic control	--	336.10 \pm 4.88 ^a	359.54 \pm 2.78 ^{ac}	367.78 \pm 1.65 ^{ac}	374.84 \pm 8.11 ^{ac}
III- Standard glibenclamide	0.25	340.52 \pm 0.99 ^a	261.47 \pm 1.01 ^{ab}	194.88 \pm 1.00 ^{ab}	141.19 \pm 2.87 ^{ab}
Group IV	2.70	336.011 \pm 2.89 ^a	250.89 \pm 1.77 ^{ab}	231.11 \pm 1.76 ^{ab}	200.15 \pm 1.70 ^{ab}
Group V	5.40	341.44 \pm 1.88 ^a	250.11 \pm 0.89 ^{ab}	209.78 \pm 2.88 ^{ab}	173.96 \pm 1.83 ^{ab}
Group VI	10.80	339.91 \pm 2.81 ^a	230.96 \pm 2.11 ^{ab}	199.16 \pm 1.79 ^{ab}	145.59 \pm 1.78 ^{ab}

All the values are expressed as mean \pm SEM ($n = 8$), 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

Group	Glycogen content mg / g	Body weight % change	Cholesterol mg / dl	Triglycerides mg / dl	HDL mg / dl
Group I	35.26 \pm 88 ^{bc}	+8.99 \pm 1.89 ^{bc}	62.019 \pm 2.44 ^{bc}	79.09 \pm 1.16 ^{bc}	59.16 \pm 0.67 ^{bc}
Group II Diabetic control	16.57 \pm 1.01 ^{ac}	-13.87 \pm 1.08 ^{bc}	126.74 \pm 0.71 ^{ac}	189.24 \pm 0.69 ^{ac}	36.09 \pm 1.11 ^{ac}
III- Standard glibenclamide	30.89 \pm 2.09 ^{ab}	-3.88 \pm 0.79 ^{ab}	68.45 \pm 1.21 ^{ab}	89.99 \pm 1.10 ^{ab}	49.71 \pm 1.19 ^{ab}
Group IV	23.88 \pm 2.12 ^{ab}	-7.01 \pm 2.03 ^{ab}	89.03 \pm 1.08 ^{ab}	125.81 \pm 1.006 ^{ab}	38.21 \pm 1.10 ^{ab}
Group V	24.11 \pm 0.99 ^{ab}	-7.10 \pm 0.28 ^{ab}	87.88 \pm 0.10 ^{ab}	120.19 \pm 2.01 ^{ab}	40.65 \pm 1.11 ^{ab}
Group VI	25.72 \pm 2.71 ^{ab}	-5.12 \pm 0.19 ^{ab}	82.81 \pm 1.11 ^{ab}	134.11 \pm 1.91 ^{ab}	44.32 \pm 1.011 ^{ab}

All the values are expressed as mean \pm SEM ($n = 8$), 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

RESULTS AND DISCUSSION

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 and 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 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. 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 is 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 nitrosoureidogluco pyranose obtained from the fermentation of *Streptomyces achromogenes* 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 necrosis¹⁰. The fundamental mechanism underlying hyperglycemia in diabetes mellitus involves the over-production (excessive hepatic glycogenolysis and gluconeogenesis) and decreased use of glucose by the tissues¹¹. 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 a characteristic loss of body weight, which is due to increased muscle wasting in

diabetes¹². 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 and Kekki¹³. 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 are impaired during diabetes¹⁴. 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 diabetes¹⁵. Streptozotocin produces oxygen radicals in the body, which causes pancreatic injury and could be responsible for the increased blood glucose¹⁶. The compounds responsible for the anti-diabetic activity of cow urine are at presently not known. Studies have been carried out to examine the antioxidant potential of cow urine. For example¹⁷ 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.⁷ 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. Further research in this field can be carried out by assessing the anti diabetic protective role of Punganur cow urine. Presence of antioxidants, free radical scavengers in cow urine could be responsible for its anti- diabetic action. There are estimation that there are over 5.8 crore people who are diabetic in India. Diabetes is more common almost everywhere in the world. It is considered to be the root cause of many chronic diseases. Urine Therapy is the safest and easiest method of treatment to prevent, Control and cure Diabetes. It can safe guard all other complications arising from diabetes include heart disease, hypertension, and diabetic retinopathy.

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