The Indian Journal of Veterinary Sciences & Biotechnology ***(2017) Volume 12, Issue 3, 127-131***

ISSN (Print) : 2394-0247 : ISSN (Print and online) : 2395-1176, abbreviated as IJVSBT

http://dx.doi.org/10.21887/ijvsbt.v12i3.7111

Submitted : 02-01-2017 ***Accepted : 12-01-2017*** ***Published : 15-02-2017***

Protective Effect of Cow Urine Distillate in Streptozotocin Induced Type I Diabetes in 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. Although Indian Ayurvedic literature cites many medicinal properties of cow urine, there is no scientific evidence to support this. Hence, this study was undertaken to validate antidiabetic property of cow urine. The effect of cow urine distillate was studied *in vivo* in induced diabetic rats. Diabetes was induced by administration of single injection of streptozotocin (60 mg/kg b.wt.) intraperitoneally. The antidiabetic effect of cow urine distillate in two different doses (5.0 ml and 10.0 ml/kg b.wt.) was studied in these adult diabetic rats. The cow urine distillate was administered orally to the experimental rats from 8th day and continued for 42 days thereafter. The assessment included fasting blood glucose levels, serum lipid profiles and body weight changes. The cow urine distillate produced a significant ($P<0.01$) reduction in the elevated blood glucose, serum cholesterol, serum triglycerides and serum creatinine levels when compared with the diabetic control. The diabetic animals treated with cow urine distillate also showed a significant gain in body weight. The presence of antioxidants and free radical scavengers in cow urine might be responsible for the observed anti-diabetic effects. The study concluded that cow urine distillate has a protective effect in diabetic rats.

Keywords: Diabetes mellitus, Cow urine distillate, Streptozotocin, Blood glucose, Lipid profile.

Introduction

Diabetes is a disease known to exist since ancient times. In modern medicine no satisfactory effective therapy is still available to cure diabetes mellitus (DM) which is a metabolic disorder of the endocrine system affecting carbohydrate, fat and protein metabolism (FDA, 2008). Diabetes mellitus is an oxidative stress induced disease 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. Under physiological conditions, glucose produces oxidants that exhibit reactivity similar to that of hydroxyl free radicals (Vijaykumar *et al.,* 2006).

In recent years, there has been a renewed interest in the variety of natural products with antioxidant potential which can play a major role in protecting against the molecular damage induced by reactive

oxygen species. 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, and it is used as an insecticide and as a regulator for various intestinal disorders like gas, acidity and cough. It is claimed to make humans wiser and can be used as a universal easily digestible medicine (Chauhan, 2005). In classical texts of Ayurveda, several medicinal properties of cow urine are described. However the anti-diabetic properties of cow urine have not been described. Further, although Indian Ayurvedic literature cites various medicinal properties of cow urine, there is very little scientific evidence to support this (Krishnamurthi *et al.*, 2004). Hence, the present study was aimed to know the protective effect of cow urine distillate on body weight gain and biochemical parameters in streptozotocin induced type I diabetes in rats.

Materials and Methods

The study was conducted on 36 adult male albino Wistar rats (Age: 6-8 weeks, weight: 150-200 g). Prior to study, the approval of institutional animal ethics committee was taken vide letter No AAU/GVC/CPCSEA-IAEC/129/2015. Rats procured from Animal Research Facility, Zydus Research Center, Moraiya, Ahemdabad, Gujarat, were kept under standard management conditions at temperature 22±3°C, humidity 70±5%, and 12:12 hrs light-dark cycles. Animals were maintained in polypropylene cages on rat pellets (Keval Sales Corporation, Laboratory animal feed suppliers, Baroda) and water was given *ad-libitum*. Streptozotocin (STZ) injection was obtained from Himedia chemical Ltd Mumbai. The distilled cow urine was procured from BAPS Swaminarayan herbal care in pure form.

Experimental Groups and Induction of insulin-dependent diabetes mellitus (IDDM)

Study was conducted for a period of 42 days. 36 rats were divided into 6 groups (n=6). Group I was kept as normal control. Group II was kept as diabetic control. Groups III and IV received 5.0 ml/kg and 10.0 ml/kg b. wt. cow urine distillate orally, while groups V and VI were kept as their respective controls. At the commencement of experiment, all rats except normal control and CUD (cow urine distillate) control I and II were injected with streptozotocin (@ 60 mg/kg b.wt.) intraperitoneally for induction of diabetes (Pellegrino *et al.,* 1998). Hyperglycemia was confirmed by the elevated glucose levels in blood, which was determined by glucometer (Easytouch G, code No. 7128) weekly after injection of STZ till 7th week. The threshold value of fasting glucose to diagnose diabetes was taken as >200 mg/dl (Pari and Suman, 2010). Rats found with permanent IDDM (insulin-dependent diabetes mellitus) were used for the anti-diabetic study. At the end of the study period, blood was collected by retro-orbital sinus puncture for estimation of biochemical parameters.

Serum Biochemical Estimation

Blood samples were centrifuged at 2000 RPM and serum was separated. Serum was used for various biochemical investigations. Cholesterol level was measured by Oxidase-Peroxidase (CHOD-PAP) method. Triglycerides were estimated by Glycerol Phosphate oxidase peroxidase (GPO-POD) method while creatinine was estimated by modified Jaffe kinetic method using Coral Kits (Coral Clinical Systems, Goa, India) on Chemistry Analyzer (BS 120, Mindray). The data generated on various parameters was subjected to statistical analysis using completely randomized design (CRD) on IBM SPSS 20.00 Statistics (Snedecor and Cochran, 1986). One-way-analysis of variance (ANOVA) was used and statistical significance was assumed, if $P<0.01$.

Results and Discussion

Body weight: Induction of diabetes with streptozotocin (60 mg/kg, i.p.) significantly reduced the body weight of rats as compared to normal and control groups. While treatment of rats in groups III, IV with cow urine distillate @ 5.0 ml and 10.0 ml per kg b.wt., respectively, significantly increased body weight gain in comparison with streptozotocin control group (Table 1).

Table 1: Comparison of weekly body weight of different groups of Wistar rats

No	Description of group	Body weight, Mean ± SE (g)							
		First wk (d7)	Second wk (d14)	Third wk (d21)	Forth wk (d28)	Fifth wk (d35)	Sixth wk (d42)	Seventh wk (d49)	Overall
I	Normal control	266.67 ±10.54^{a}	295.00 ±13.35^{ab}	325.00 ±10.88^{b}	360.00 ±11.55^{bc}	388.33 ±10.14^{c}	415.00 ±10.37^{cd}	435.67 ±12.58^{cd}	355.09 ±9.37^{t}
II	Diabetic control	225.00 ±17.08^{a}	211.67 ±15.36^{ab}	190.00 ±12.38^{abc}	171.67 ±13.76^{bc}	150.00 ±13.90^{c}	135.33 ±15.22^{cd}	121.00 ±12.36^{cd}	172.10 ±7.86^{p}
III	CUD-I 5.00 ml/kg	235.00 ±12.84^{a}	251.67 ±13.52^{b}	271.67 ±15.58^{ab}	295.00 ±14.55^{bc}	315.00 ±13.35^{c}	330.00 ±14.21^{cd}	349.33 ±15.77^{d}	292.52 ±7.90^{r}
IV	CUD-II 10.00 ml/kg	240.00 ±13.42^{a}	251.67 ±15.36^{ab}	270.00 ±13.90^{abc}	291.67 ±14.00^{bc}	311.67 ±13.02^{c}	326.33 ±15.87^{cd}	342.00 ±14.08^{d}	290.48 ±7.55^{r}
V	CUD control-I	251.67 ±9.46^{a}	288.33 ±6.54^{ab}	320.00 ±7.30^{b}	358.33 ±7.49^{bc}	395.00 ±6.19^{c}	413.33 ±7.22^{cd}	438.67 ±6.39^{d}	311.00 ±9.87st
VI	CUD control-II	221.67 ±10.77^{a}	265.00 ±8.46^{ab}	297.00 ±9.15^{b}	331.67 ±10.77^{bc}	370.00 ±11.55^{c}	394.00 ±10.39^{cd}	426.33 ±9.13^{d}	329.38 ±10.45^{rs}

Overall mean values with superscript p,q,r,s,t vary highly significantly between groups (P<0.01). Means with superscript a,b,c,d vary highly significantly between weeks within the group (P<0.01).

Biochemical parameters: Induction with Streptozotocin (60 mg/kg b.wt., i.p.) significantly increased the blood glucose, total cholesterol, creatinine and triglyceride levels in comparison to control groups. The blood glucose level on day of initiation of treatment (day 0) was identical in all groups, which rose almost 3 times in a week following STZ injection. Treatment of rats in groups III, IV with cow urine distillate @ 5.0 ml and 10.0 ml per kg b.wt., respectively, significantly reduced the elevated blood glucose, total cholesterol, creatinine and triglyceride levels in comparison to STZ control group (Table 2, 3). The period effect was significant only in group IV for blood glucose.

Table 2: Comparison of weekly blood glucose of different groups of Wistar rats

No	Description of group	Blood glucose, Mean ± SE (mg/dl)							
		First wk (d7)	Second wk (d14)	Third wk(d21)	Forth wk (d28)	Fifth wk (d35)	Sixth wk (d42)	Seventh wk (d46)	Overall
I	Normal control	143.67 ±4.21	139.67 ±10.55	151.00 ±5.29	139.67 ±5.62	136.17 ±8.36	149.33 ±4.73	150.67 ±5.68	147.34 ±3.13^{p}
II	Diabetic control	535.67 ±18.92	533.33 ±19.64	517.67 ±22.98	512.83 ±19.17	530.83 ±18.13	525.50 ±19.62	531.00 ±18.44	527.52 ±8.41^{r}
III	CUD-I 5.00 ml/kg	573.17 ±24.89	531.83 ±28.85	456.50 ±31.25	412.00 ±38. 80	381.00 ±37.02	335.67 ±27.69	276.50 ±24.73	380.37 ±14.91^{r}
IV	CUD-II 10.00 ml/kg	539.17 ±27.68^{d}	490.83 ±25.59^{cd}	410.50 ±22.73^{c}	305.67 ±23.73^{bc}	272.17 ±19.72^{b}	231.33 ±17.35^{ab}	180.00 ±16.24^{a}	307.75 ±10.95^{qr}
V	CUD control-I	146.67 ±10.49	153.83 ±9.46	151.17 ±8.75	153.17 ±4.35	145.00 ±7.34	143.00 ±7.64	146.50 ±5.36	146.49 ±3.53^{p}
VI	CUD control-II	149.17 ±8.17	156.17 ±8.00	149.50 ±7.02	156.00 ±3.80	149.83 ±3.07	145.67 ±4.89	153.00 ±4.30	150.27 ±2.72^{p}

Overall mean values with superscript p,q,r,s vary highly significantly between groups (P<0.01). Means with superscript a,b,c,d vary highly significantly between weeks within the group (P<0.01).

Table 3: Effect of various treatments on biochemical profile of Wistar rats at the end of study.

Group No.	Description of group	biochemical parameters, Mean ± SE (mg/dl)		
		Cholesterol	Creatinine	Triglycerides
I	Normal control	65.70 ± 3.09^{q}	0.39 ± 0.01^{q}	180.93 ± 15.57^{pq}
II	Diabetic control	214.54 ± 11.71^{p}	1.50 ± 0.12^{p}	391.08 ± 23.49^{r}
III	CUD-I 5.00 ml/kg	78.79 ± 4.43^{q}	0.36 ± 0.03^{q}	141.42 ± 8.11^{p}
IV	CUD-II 10.00 ml/kg	79.40 ± 4.31^{q}	0.42 ± 0.06^{q}	202.38 ± 20.44^{q}
V	CUD control-I	79.38 ± 7.61^{q}	0.32 ± 0.04^{q}	160.26 ± 15.21^{pq}
VI	CUD control-II	77.75 ± 7.68^{q}	0.24 ± 0.04^{q}	183.77 ± 15.82905^{pq}

Mean values with different superscript vary highly significantly between the groups ($p<0.01$).

Diabetes mellitus is one of the common metabolic disorders with micro and macro vascular complications that results in significant morbidity and mortality. Streptozotocin is a β-cytotoxin, induces “chemical diabetes” in a wide variety of animal species including rat by selectively damaging the insulin-secreting β-cells of the pancreas. The purpose of choosing streptozotocin as diabetes-inducing agent was its known property to produce diabetes mellitus irreversibly with a single dose of intraperitoneal administration by relative necrotic action on the β-cells of pancreas leading to insulin deficiency (Kokte *et al*., 2002). Generation of free radicals, DNA strand breaks, activation of the PARP and depletion of intracellular NAD appear to be common factors in β-cell death, whether mediated by oxygen radicals, nitric oxide, or STZ (Heller *et al.,* 1995).

Oxidative stress has been shown to play an important role in the etiology of diabetes (John, 1991). Streptozotocin produces oxygen radicals in the body, which causes pancreatic injury (Donald *et al.,* 1984). The compounds responsible for the anti-diabetic activity of cow urine are at present not known. Studies have been carried out to examine the anti-oxidant potential of cow urine. For example, Jarald *et al*. (2008) described the antioxidant 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.* (2004) have described the antioxidant action of cow urine using an ABTS assay model and the antioxidant effect of cow urine was due to the presence of volatile fatty acids. Hence the presence of antioxidants which account for the scavenging of free radical in cow urine could be responsible for its anti-diabetic action and further might have lead to its ameliorative effect.

In diabetes, fatty acids are increasingly taken up by the liver and, after esterification with glycerol phosphate, are deposited as triglycerides. In the current study, treatment with cow urine distillate significantly ($P<0.01$) improved the altered lipid profile. It significantly decreased circulating cholesterol and triglycerides levels. It promotes the fecal excretion of cholesterol and cholic acid derived bile acids. It also decreases serum triglycerides and cholesterol and improves hyper-triglyceridemia and hypercholesterolemia.

In conclusion, cow urine distillate represents a novel candidate for alternative medicine in the management of diabetes mellitus in view of its effects on the blood glucose level and associated biochemical parameters and also improvement in body weight gain in diabetic rats.

Acknowledgement

Authors are grateful to Dr. D.S. Nauriyal, Dr. K.N. Wadhwani and Dr. A.M. Pande, Professors and Head, Department of Medicine, LPM and Biochemistry, respectively, College of Veterinary Science & Animal Husbandry, Anand for the facilities and cooperation provided. Thanks are also due to Dr. Satish Patel, Animal house incharge, Animal Research Facility, Zydus Research Center,

Ahemdabad, for their support in animal experimentation.

Conflict of Interest: All authors declare no conflict of interest.

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