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Comparative antidiabetic activity and hypolipidemic potential of cow urine and its preparations

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Abstract

Diabetes is a disease that occurs when your blood glucose, also called blood sugar, is too high. Insulin enables cells to absorb glucose in order to turn it into energy. In diabetes, the body can't use its own insulin as well as it should doesn't make enough insulin and sometimes both. This causes glucose to accumulate in the blood, often leading to various complications. All over the world the diabetes is a biggest problem because there are no effective and non expensive treatment are available. The cow urine therapy is capable of curing several curable and incurable diseases. The holy texts, like Atharva Veda, Charak Samhita, Rajni Ghuntu, Vridhabhagabhatt, Amritasagar, Bhavprakash, Sushrut Samhita contain beautiful description about these things. Cow Urine Treatment is capable of curing diabetes, blood pressure, asthma, psoriasis, eczema, heart attack, blockage in arteries. The objective of the present work was to investigate anti-diabetic activity of cow urine. To avoid the side effect and expensive associated with allopathic drugs there is need of research of drugs without side effects. The approval of experimental protocol was taken from IAEC of institute before starting the study. Albino rats of wistar strain (150-250g) were used for study. The chemical tests for various constituents and pH & specific gravity were determined. Treatment with cow urine preparations on blood glucose level, lipid profile and lever & kidney function tests was evaluated. Oral administration of cow urine preparations (5 ml/kg) for 21 days, resulted in significant decrease of blood glucose. Fresh cow urine produced highly significant antidiabetic activity i.e. 275±23.75 to 129.1±2.27. However in case of gawarc and ganwati also produced significant decrease in blood glucose but less than fresh cow urine. The study clearly shows that the cow urine preparations possess potent antidiabetic activity.

Keywords: Antidiabetic, Hypolipidemic, Cow urine

Introduction

Diabetes is a disease that occurs when your blood glucose, also called blood sugar, is too high. Blood glucose is your main source of energy and comes from the food you eat. Insulin, a hormone made by the pancreas, helps glucose from food get into your cells to be used for energy. Sometimes your body doesn't make

enough-or any-insulin or doesn't use insulin well.[1] Glucose then stays in your blood and doesn't reach your cells. Diabetes, often referred to by doctors as diabetes mellitus, describes a group of metabolic diseases in which the person has high blood glucose (blood sugar), either because insulin production is inadequate, or because the body's cells do not respond properly to insulin, or both. Patients with high blood sugar will typically experience polyuria (frequent urination), they will become increasingly thirsty (polydipsia) and hungry (polyphagia). Insulin enables cells to absorb glucose in order to turn it into energy. In diabetes, the body can't use its own insulin as well as it should doesn't make enough insulin, and sometimes both. This causes glucose to accumulate in the blood, often leading to various complications. The American Diabetes Association reported in 2009 that there are 23.6 million children and adults in the United States 7.8% of the population, who have diabetes. While an estimated 17.9 million in the US alone have been diagnosed with diabetes, nearly one in four (5.7 million) diabetics are unaware that they have the disease.[2]

Types of diabetes

Type 1: Insulin-dependent diabetes mellitus (IDDM), juvenile onset diabetes mellitus: There is β cell destruction in pancreatic islets majority of cases are autoimmune (type 1A). Antibodies that destroy β cells are detectable in blood, but some are idiopathic (type 1B). In all type 1 cases circulating insulin levels are low or very low, and patients are more prone to ketosis. This type is less common and has a low degree of genetic predisposition results from the body's failure to produce insulin. It is estimated that 5-10% of persons who are diagnosed with diabetes have type 1diabetes. Presently almost all persons with type 1 diabetes must take insulin injections.

Type 2 : Non insulin-dependent diabetes mellitus (NIDDM), maturity onset diabetes mellitus: There is no loss or moderate reduction in β cell mass; insulin in circulation is low, normal or even high, no anti β-cell antibody is demonstrable; has a high degree of genetic predisposition; generally has a late onset (past middle age). Over 90% cases are type 2 diabetes mellitus(DM). Causes may be: Type 2 Results from Insulin resistance, a condition in which cells fail to use insulin properly, sometimes combined with relative insulin deficiency. Many people designated to develop type 2 diabetes spend many years in a state of Pre-diabetes: termed as a condition that occurs when a person's blood glucose levels are higher than normal but not high enough for a diagnosis of type 2 diabetes. As of 2009 there are 57 million Americans who have pre-diabetes.

- Abnormality in gluco-receptor of β cells so that they respond at higher glucose concentration or relative β cell deficiency.
- Reduced sensitivity of peripheral tissues to insulin: reduction in number of insulin receptors, 'down regulation' of insulin receptors. Many hypertensives are hyperinsulinaemic, but normoglycaemic; exhibit insulin resistance associated with dyslipidaemia (metabolic syndrome). Hyperinsulinaemia *per se* has been implicated in causing angiopathy.
- Excess of hyperglycaemic hormones (glucagon, etc.)/obesity: cause relative insulin deficiency—the β cells lag behind.
- Gestational diabetes: Pregnant women who have never had diabetes before but who have high blood sugar (glucose) levels during pregnancy are said to have gestational diabetes. Gestational

diabetes affects about 4% of all pregnant women. It may precede development of type 2 (or rarely type 1).

- Many other forms of diabetes mellitus are categorized separately from these. Examples include congenital diabetes due to genetic defects of insulin secretion, cystic fibrosis-related diabetes, steroid diabetes induced by high doses of glucocorticoids, and several forms of monogenic diabetes.[1,2]

The classical symptoms of diabetes are polyuria, polydipsia, weight loss, fatigability and increased thirst and consequent increased fluid intake.

Insulin is the principal hormone that regulates uptake of glucose from the blood into most cells (primarily muscle and fat cells, but not central nervous system cells). Therefore deficiency of insulin or the insensitivity of its receptors plays a central role in all forms of diabetes mellitus. [4]

Most of the carbohydrates in food are converted within a few hours to the monosaccharide glucose, the principal carbohydrate found in blood and used by the body as fuel. The most significant exceptions are fructose, most disaccharides (except
sucrose and in some people lactose), and all more complex polysaccharides, with the outstanding exception of starch. [4]

Treatment of Diabetes

Insulin

Insulin was discovered in 1921 by Banting and Best who demonstrated the hypoglycaemic action of an extract of pancreas prepared after degeneration of the exocrine part due to ligation of pancreatic duct. It was first obtained in pure crystalline form in 1926 and the chemical structure was fully worked out in 1956 by Sanger. Insulin is a two chain polypeptide having 51 amino acids and MW about 6000. The A-chain has 21 while B-chain has 30 amino acids. Pork insulin is more homologous to human insulin than is beef insulin. The A and B chains are held together by two disulfide bonds. Insulin is synthesized in the β cells of pancreatic islets as a single chain peptide Preproinsulin (110 A A) from which 24 AAs are first removed to produce Proinsulin. The connecting or 'C' peptide (35 AA) is split off by proteolysis in Golgi apparatus; both insulin and C peptide are stored in granules within the cell. The C peptide is secreted in the blood along with insulin.

Oral hypoglycaemic drugs

These drugs lower blood glucose levels and are effective orally. The main drawback of insulin is-it must be given by injection. Orally active drugs have always been searched.

(A) Sulfonylureas

First generation

Tolbutamide
Chlorpropamide

Second generation

Glibenclamide
(Glyburide)
Glipizide
Gliclazide
Glimepiride

(B) Biguanides

Metformin

(C) *Meglitinide / Phenyl Alanine Analogues*

Repaglinide, Nateglinide

(D) *Thiazolidinediones*

Rosiglitazone, Pioglitazone

(E)α-*Glucosidase Inhibitors*

Acarbose, Miglitol

Herbal Treatment of Diabetes

According to ayurveda, diabetes is a metabolic kapha type of disorder in which diminished functioning of agni leads to a tendency toward high blood sugar.

(Ayurveda recognizes 24 forms of the disease commonly classified under Prameha - 4 are due to Vata dosha, 6 are due to Pitta dosha, and 10 are caused by Kapha dosha. The main causes of these diseases are fat, urine, and Kapha buildups due to foods, liquids, lifestyle and others.

Ayurvedic practitioners attack diabetes using a multiprong approach. First, they address diet modification, eliminating sugar and simple carbohydrates, and emphasizing complex carbohydrates. Protein is limited, since excessive intake can damage the kidneys. Fat is also limited because there is often a deficiency of pancreatic enzymes, making fat digestion difficult. Since many diabetics have autoantibodies, a cleansing program is instituted. Panchakarma is typically used for this purpose. This begins with herbal massages and an herbal steam sauna, followed by fasting to cleanse the body. This is followed by an herbal purge for the liver, pancreas and spleen. Colon therapy is next, first to cleanse the digestive tract and then to reconstitute the system. .[4,5]

Ayurvedic practitioners also use several herbal preparations for diabetics. Exercise is another cornerstone of ayurvedic treatment of diabetes. Yoga and breathing exercises are traditionally used.

The most important herbs for all doshas are shilajit, gudmar turmeric, neem, amalaki, guggul, and arjuna. Turmeric with aloe vera gel (1 to 3 gms./.035 to .1 oz) is best used during the early stages of diabetes for regulating pancreas and liver functions.

Cow Urine Therapy

Cow is a mobile dispensary. It is the treasure of medicines. The cow urine therapy is capable of curing several curable and incurable diseases. The holy texts, like Atharva Veda, Charak Samhita, Rajni Ghuntu, Vridhabhagabhatt, Amritasagar, Bhavprakash, Sushrut Samhita contain beautiful description about these things. Cow Urine Treatment and Research Center, Indore has conducted a lot of research in the past few years on patients directly and claimed that it is capable of curing diabetes, blood pressure, asthma, psoriasis, eczema, heart attack, blockage in arteries, fits, cancer, AIDS, piles, prostrate, arthritis, migraine, thyroid, ulcer, acidity, constipation, gynecological problems, ear and nose problems, abortion and several other diseases.[6,7]

Cow urine has a unique place in Ayurveda and has been described in "sushrita samhita" and a ashtanga sangraha to be the most effective substance/secrection of animal origin with innumerable therapeutic value. It has been recognized as water of life or amrita. This kind of alternative treatment as panchgavya therapy or cowpathy has been reported to be beneficial even for dreaded disease like cancer, AIDS, and diabetes. Improvement has been shown or reported with those suffering from flu, allergies, colds, rheumatoids arthritis, bacterial/viral infection, tuberculosis, chicken pox, hepatitis, leucorrhuea, leprosy, ulcer, heart disease, asthma, skin infection, aging, chemical intoxication. Through extensive research studies of cow urine distillated fraction popularly know as ark has been identified as bioenhancer of the activity of commonly used antibiotic, antifungal and anticancer drug. Cow urine enhances the immunocompetence and improve general health of an individual prevent the free radicals formation and act as anti-aging factor reduce apoptosis in lymphocytes and help them to survive and efficiently repair the damaged DNA and this is effective for cancer therapy. [8]

The analysis of cow urine has shown that it contains nitrogen, sulphur, phosphate, sodium, manganese, carbolic acid, iron, silicon, chlorine, magnesium, malic, citric,

tartric and succinic acid, calcium salts, Vitamin A, B, C, D, E, minerals, lactose, enzymes, creatinine, hormones and gold. A person falls ill when there is deficiency or excess of the substances inside the body. The cow urine contains those substances, which are present in the human body. Therefore consumption of cow urine maintains the balance of these substances and cures incurable diseases. [9]

Materials and methods

Collection of Cow urine and it's preperations.

The cow urine was collected from Kanhiya Gau shala, Pal Road, Jodhpur and cow urine preparations also collected from there.

Cow urine and it's preperations.

Fresh cow urine:

Fresh cow urine was collected in the morning, daily from kanhiya Gau shala, Pal Road, Jodhpur

Distillate cow urine (gau arc):

Gau arc was prepared by distillation process. Cow urine was boiled in an iron pot to which a vapour condensing device was attached. The vapour through tube was collected in a pot put over cold water.

Residue of cow urine (ganavati):

This was residue of cow urine after distillation process. Deep iron pan was used and boiled cow urine till it become concentrated and salts remained. When the cow urine was concentrated remove it from fire and let it cool.

Chemicals

Alloxan monohydrates a most widely used chemical diabetogen was procured from Chemdyes co. Ahemdabad, India. Glicalazide, a standard antidiabetic drug was purchased Nulife co. India. Other chemical which were used in the study were procured from Loba chem., Ahmadabad. Diagnostic kits (Logotech diagnostic kit) were used in the estimation of biochemical parameters.

Drugs:

(1) Diabetic induced by: Alloxan monohydrate

(2) Standard drug: Glicalazide

Experimental animal

The approval of experimental protocol was taken from IAEC of institute before starting the study. Albino rats of wistar strain (150-250g) were used for study. Animals were housed under standard condition of temperature (25̊c) for 12 hours in light/dark cycle and fed with standard pellet diet and water ad-libitum. Animals were acclimatized to laboratory condition at least 24 hours before conducting the experiment.

Chemical composition of cow urine and it's preparations:

Chemical tests for various constituents of cow urine and its preparations were carried out as per tests given below:

Table 1: Chemical tests for various constituents of cow urine and its preparations.

Component	TEST	OBSERVATION	GAU ARK	GHAN-WATI	FRESH COW URINE
Urea	TEST FOR UREA: UREASE TEST Sample+ Soya bean meal + Phenol red	Red color was obtained	+ve	+ve	+ve
Chloride	Sample+Conc.HNO_3 +$AgNO_3$	White Precipitate was obtained	+ve	+ve	+ve
Sulphate	Sample +$Bacl_2$	White Precipitate was obtained	+ve	+ve	+ve
Calcium	Sample + Amino oxalate	White Precipitate was obtained	+ve	+ve	+ve
Phosphorus	Sample + Conc. Nitric acid	White Precipitate was obtained	+ve		+ve
Carbohydrate	Sample + molish reagent + H_2SO_4	violet ring at the junction was obtained	-ve	-ve	-ve
Malic acid	2-3 ml sample +Added 2-3 drops 5% $fecl_3$ solution	Yellowish color appearance was obtained	+ve	+ve	+ve
Citric acid	2-3 ml sample + Added one drop dilute NH_4 OH & excess $Agno_3$ solution, boil for 15 min. on water bath	White gelatinous Precipitate was obtained	+ve	+ve	+ve
Bile pigment	(PETEN KOFERS TEST) Took 5 ml of cow urine +Dissolved crystal of sucrose +3 ml of conc. Sulphuric acid	Red or reddish purple ring was formed	-ve	+ve	+ve
Ketone bodies	(ROTHER's TEST) Took 5 ml of cow urine + Saturated with solid ammonium sulphate + 2-3 drop of 5% solution of sodium nitroprusside + 2 ml conc. ammonia	No permanent color formed	-ve	-ve	-ve
Creatinine	(JAFFE'S TEST) Took 5 ml of cow urine + 2 ml saturated picric acid + 10 % NAOH	Deep orange color was formed	-ve	-ve	+ve
Protien	(HELLER'S TEST) 3 ml of urine + 3 ml conc. Nitric acid	White Precipitate at the junction was	-ve	+ve	+ve

		obtained			
Ammonia	Took 5 ml of cow urine + red litmus paper	Litmus paper turns to blue	+ve	+ve	+ve
Uric acid	(SCHIFF'S TEST) Moistened a strip of filter paper with Silver nitrate solution & added to it a drop of urine	Black or yellow brown strain formed	+ve	+ve	+ve
Bi-carbonate	3 ml of urine + dilute HCL	Effervescence of co2	+ve	+ve	+ve
Iron	5 ml of test solution , added few drops of 2% potassium Ferro cyanide	Dark blue coloration was obtained	-ve	-ve	+ve
Salisylic acid	Sample +Bromine solution	Cream color Precipitate was obtained	+ve	+ve	+ve
Tartaric acid	2-3 ml 0f test solution ,added one drop dilute NH_4OH & excess 5% $Agno_3$ solution ,boiled for 15 min. on water bath	White gelatinous Precipitate observed	+ve	+ve	+ve
Magnesium	Sample +Ammonium carbonate	White Precipitate was obtained	+ve	+ve	+ve
Succinic acid	In a test tube, took the neutral solutions of the acid, added calcium chloride solutions, shaked & boiled for 2 min. on stretching the sides of the test tube	White Precipitate was obtained	+ve	+ve	+ve
Sulphur	Dilute odiumnitroprusside + sample	Purple color was obtained	+ve	+ve	+ve
Oxalic acid	2ml sample + added few drops 5% lead acetate	White Precipitate was obtained	+ve	+ve	+ve
Potassium	3 ml sample + added few drops of sodium cobalt nitrite solutions	Yellow Precipitate was obtained	+ve	-ve	+ve

Determination $_{P}$H and Specific gravity of Cow urine and it's preparations:

Procedure of pH determination:

1. At first pH meter was set with reference of stranded buffer of pH 4 and pH 7 and then we adjusted the pH of distilled water and then calculated the pH of samples of cow urine preparations.

2. Procedure of specific gravity determination:

The weighing bottle was weighed and then a fix amount of sample of cow urine preparations was filled in weighing bottle and weighed again after this the empty bottle again weighed and then calculated the specific gravity.

Table 2: pH and Specific gravity of Cow urine preparations:

S. No.	Cow urine preparations	pH of Cow urine preparations	Specific gravity of Cow urine preparations
1.	Fresh cow urine	9.0	1.027
2.	Distillate cow urine (Gau arc)	9.5	0.997
3.	Ganavati	10.0	1.035

Determination of Succinic acid (Quantitative)

Procedure - Succinic acid was estimated by the Naflo-turbidometer. In this standard solution of succinic acid was prepared by dissolving 5gm in 100ml distilled water. Dilutions were made by taking 1,2,3,4,5,6,7,8,9 & 10 of stock solution and volume made up to 10 ml with distilled water in test tubes. Saturated solution of $CaCl_2$ (1ml) was added in all dilutions and then heated gently and cooled. Precipitate was produced in all test tubes. Turbidity was measured in all standard samples by Naflo-turbidometer and reading was noted down. 10 ml quantity of each cow urine preparations (fresh cow urine, Gawarc) was taken and 1 ml of saturated solution of $CaCl_2$ added. For ganwati we prepared 1% w/v solution distilled water and then took 10ml of this solution for quantification. Turbidity in all test samples were analysed by Naflo-turbidometer. A standard plot was drawn between reading & quantity of samples. Quantity of succinic acid in test samples was determined from standard plot.

Diabetes induction

Male wistar rats weighing 150-250gm were taken for the study. Animals were allowed to fast for 24 hours and then they were injected with a freshly prepared aqueous solution of Alloxan monohydrate (120mg/kg, i.p). After 72 hrs rats with marked hyperglycemia (fasting blood glucose more than 200mg/dl) were used for the study and the animals which failed discarded from the study. [10,11]

Experimental design

Diabetic rats having sugar level more than 200mg/dl were used for the study of antidiabetic activity of the cow urine preparations. Animals were divided into different groups each containing of 6 rats.

Animals were divided into six groups (n = 6) and treated orally as follow:

Group 1. Normal control, received the normal saline (0.9% w/v NaCl, 5 ml/kg).

Group 2. Standard, received glyclazide (10mg/kg).

Group 3. Diabetic control, not received anything.

Group 4. Test, received fresh cow urine (5ml/kg).

Group 5. Test, received Gau ack (5ml/kg).

Group 6. Test, received Ganwati (500mg/kg/ 5ml).

Collection of Blood

Blood samples were collected in eppendroff bullet on study days 0,7,14,21 and 28 by retro - orbital plexus and serum was separated by centrifugation (for 10 min at 10000 rpm). Separated serum samples were analysed for glucose.

Estimation of Biochemical Parameters

Serum glucose, serum cholesterol, serum triglycerides, and serum HDL, were estimated by commercially available kits (Logitech diagnostic kit). All biochemical parameters were determined by using autoanalyser (Star – 21 model, adinose company)

Diabetic Profile test

Estimation of blood glucose levels:-
Serum was separated and the standard glucose reagent (1ml) was added in the samples and then they were allowed to stand for 15 minutes in Incubator and then reading were recorded for glucose level at 505 nm by auto analyser. Glucose was determined on study days 0,7,14,21 & 28.

Estimation of lipid profile:-
Estimation of Triglyceride level
Triglyceride level was checked in the different animal groups by using logotech diagnostic kit. Serum was separated and the standard triglyceride reagent (1ml) was added in the samples and then they were allowed to stand for 15 minutes in Incubator and then reading were noted for triglyceride level at 505 nm by auto analyser.

Estimation of HDL
HDL was analyzed by logotech diagnostic kit. Serum was separated and the standard HDL reagent (1ml) was added in the samples and then they were allowed to stand for 15 minutes in Incubator and then they were analysed for HDL level at 505 nm by auto analyser.

Estimation of Cholesterol
Cholesterol was analyzed by logotech diagnostic kit. By using cholesterol oxidase phenol 4-aminoantipyrine peroxidase method. Serum was separated and the standard Cholesterol reagent (1ml) was added in the samples and then they were allowed to stand for 15 minutes in Incubator and then reading were noted for Cholesterol level at 505 nm by auto analyser.

Estimation of Liver and kidney function test:-
Estimation of SGOT
SGOT was analyzed by logotech diagnostic kit Serum was separated and the standard SGOT reagent (1ml) was added in the samples and then they were allowed to stand for 15 minutes in Incubator and then they were analysed for SGOT level at 340s by auto analyser.

Estimation of SGPT
SGPT was analyzed by logotech diagnostic kit. Serum was separated and the standard SGPT reagent (1ml) was added in the samples and then they were allowed to stand for 15 minutes in Incubator and then they were analysed for SGPT level at 340 nm by auto analyser.

Estimation of Serum creatinine
Serum creatinine was analyzed by logotech diagnostic kit. Serum was separated and the standard Serum creatinine reagent (1ml) was added in the samples and then they were allowed to stand for 15 minutes in Incubator and then they were analysed for Serum creatinine level at 505 nm by auto analyser.

Recording of body weight
Body weight of each animal was recorded before alloxan injection, and on study days 0,7,14,21 & 28

Statistical Analysis
All results are expressed as the mean ± SEM. The results were analyzed for statistical significance using one-way analysis of variance (ANOVA); comparison was done by using Dunnett's test. P values <0.05 were considered as significant and P values <0.01 & 0.001 were considered as highly significant.

Results and discussion
Chemical constituents in cow urine and it's preparations
Fresh cow urine was collected from Kanihya guashala in the morning and gauarc and ganavati were also obtained from guashala. Chemical tests to find out

various constituents present in cow urine and its preparations were carried out in laboratory as per tests described. Components found in cow urine preparations are as given in table-3.

Determination $_{P}$H and Specific gravity of Cow urine and its preparations:

pH and specific gravity of cow urine and its preparations were determined and result are shown in table -4.

Determination of succinic acid (Quantitative)

Succinic acid was determined by the Naflo-turbidometer. Succinic acid in cow urine preparations was estimated quantitatively and results are given in table - 5.

Estimation of blood glucose

Blood samples were collected in eppendroff bullet on study days 0,7,14,21 and 28 by retro - orbital plexus and serum was separated by centrifugation (for 10 min at 10000 rpm). Serum was separated and glucose was determined on study days 0,7,14,21 & 28.

Table 3: Chemical constituents detected in cow urine and its preparations.

Component	GAU ARK	GHAN-VATI	FRESH COW URINE
Urea	+ve	+ve	+ve
Chloride	+ve	+ve	+ve
Sulphate	+ve	+ve	+ve
Calcium	+ve	+ve	+ve
Phosphorus	+ve	+ve	+ve
Carbohydrate	+ve	+ve	+ve
Malic acid	+ve	+ve	+ve
Citric acid	+ve	+ve	+ve
Bile pigment	-ve	+ve	+ve
Ketone bodies	-ve	-ve	-ve
Creatinine	-ve	-ve	+ve
Protien	-ve	+ve	+ve
Ammonia	+ve	+ve	+ve
Uric acid	+ve	+ve	+ve
Bicarbonate	+ve	+ve	+ve
Iron	+ve	-ve	+ve
Salisylic acid	+ve	+ve	+ve
Tartaric acid	+ve	+ve	+ve
Magnesium	+ve	+ve	+ve
Succinic acid	+ve	+ve	+ve
Sulphar	+ve	+ve	+ve
Oxalic acid	+ve	+ve	+ve
Potassium	+ve	-ve	+ve

Table 4: pH and specific gravity determined of cow urine preparations:

S.No.	Cow urine preparations	pH of Cow urine preparations	Specific gravity of Cow urine preparations
1	Fresh cow urine	9.0	1.027
2	Distillate cow urine (Gau arc)	9.5	0.997
3	Ganavati	10	1.035

Table 5: Determination of succinic acid of cow urine and its preparations.

S.No.	Cow urine preparations	Succinic acid (Quantitative) mg/ml
1	Fresh cow urine	0.39
2	Distillate cow urine (Gau arc)	0.19
3	Ganavati	0.28

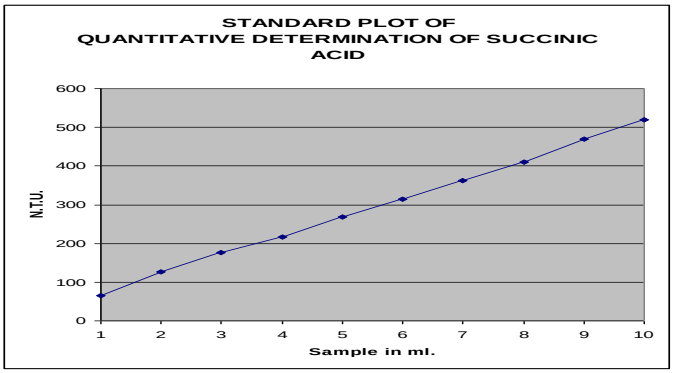

Figure 1: The Standard Plot of Quantitative determination of Succinic acid.

Table 6: Group I: Blood glucose levels (mg/dl) measured in alloxan induced diabetic rats treated with saline solution before treatment, during treatment period and after treatment. (Normal control group)

S.no.	Marking	Body wt.	Before treatment 0 th day	Treatment period			After treatment 28th
				7 th	14th	21st	
1	Head and back	178g	112.2	119.2	120	120	115
2	Tail	164g	96.4	106	102	102	108
3	Head and Tail	156g	120.17	116	118	118	119
4	Head	182g	106.5	113	114	114	118
5	Unmarked	194g	109.2	104	105	105	110
6	back	190g	115	117	116	120	112
		MEAN	109.91	112.53	112.5	113.16	113.66
		SD	8.13	6.19	7.31	7.85	4.41
		SEM	3.32	2.53	2.98	3.2	1.80

Table 8: Group II: Blood glucose levels (mg/dl) measured in alloxan induced diabetic rats treated with standard gliclazide before treatment, during treatment period and after treatment. (Standard group)

S.no.	Markings	Body wt.	Before treatment 0 th day	Treatment period			After treatment 28th
				7 th	14th day	21st day	
1	Head and back	172 g	499	419	242	152	190
2	Tail	189g	352	244	192	149	178
3	Head and Tail	193 g	300	195	187	132	134
4	Head	165g	279	207	198	126	147
5	Unmarked	158 g	288	219	195	131	152
6	back	200g	259	183	183	147	157
		MEAN	329.5	244.5	199.5	139.5	159.66
		SD	88.71	88.02	21.51	11.07	20.69
		SEM	36.21	35.93	8.78	4.52	8.44

Table 8: Group III: Blood glucose levels (mg/dl) measured in alloxan induced diabetic rats, without any treatment before treatment, during treatment period and after treatment. (Diabetic control group)

S.no.	Markings	Body wt.	Before treatment 0 th day	Treatment period			After treatment 28th
				7 th	14th day	21st day	
1	Head & Back	170	252	248	251	258	268
2	Tail	195	243	240	242	Extis letalis	Extis letalis
3	Head &Tail	184	248	246	245	Extis Letalis	Extis letalis
4	Head	160	270	260	280	Extis letalis	Extis letalis
5	Unmarked	159	278	268	278	280	278
6	back	159	278	268	278		
		MEAN	261.5	255	262.33	269	273
		SD	15.69	11.98	18.14	15.55	7.07
		SEM	6.4	4.89	7.4	11	5.0

Table 9: Group IV: Blood glucose levels (mg/dl) measured in alloxan induced diabetic rats, treated with fresh cow urine before treatment, during treatment period and after treatment.

S.no	Marking	Body Wt.	Before treatment 0 day	Treatment period			After treatment 28th day
				7th day	14th Day	21th day	
1	Head & Back	212	252	237	179	135	127
2	Tail	250	312	278	158	124	125
3	Head &Tail	185	219	185	147	134	131
4	Head	215	205	178	140	131	132
5	Unmarked	245	312	188	135	121	111
6	back	242	350	238	121	130	127
		MEAN	275	217.33	146.66	129.16	125.5
		SD	58.18	39.86	20.06	5.56	7.58
		SEM	23.75	16.27	8.19	2.27	3.09

Table 10: **Group V: Blood glucose levels (mg/dl) measured in alloxan induced diabetic rats, without any treatment before treatment, during treatment period and after treatment.**

S.no	Marking	Body Wt.	Before treatment 0 day	Treatment period			After treatment 28th day
				7th day	14th day	21thday	
1	Head & Back	210	315	298	278	159	134
2	Tail	185	243	181	149	99	110
3	Head &Tail	190	312	185	148	120	124
4	Head	220	273	195	140	125	119
5	Unmarked	212	215	180	146	109	107
6	back	225	219	195	147	103	104
		MEAN	262.83	205.66	168	119.16	116.33
		SD	44.37	45.71	53.98	21.87	11.46
		SEM	18.11	18.66	22.03	8.93	4.68

Table 11: Group VI: Blood glucose levels (mg/dl) measured in alloxan induced diabetic rats, without any treatment before treatment, during treatment period and after treatment.

S.NO	Marking	Body Wt.	Before treatment 0 day	Treatment period			After treatment 28th Day
				7th day	14th day	21th day	
1	Head & Back	245	287	227	168	121	123
2	Tail	179	267	207	195	126	125
3	Head &Tail	200	205	187	151	134	127
4	Head	165	212	193	178	119	110
5	Unmarked	172	235	201	189	122	123
6	back	200	218	169	139	127	114
		MEAN	237.33	197.33	170	124.83	120.33
		SD	13.43	19.57	21.79	5.41	6.74
		SEM	5.48	7.99	8.89	2.21	2.75

Table 12: Comparison in mean ± S.E.M. blood glucose level measured in different groups on study days 0,7,14,21 & 28.

Treatment group	Before treatment 0 day	Treatment period			After treatment 28th Day
		7th day	14th day	21th day	
Normal control	109.91±3.32	112.53±2.53	112.5±2.98	113.16±3.20	113.6±1.8
standard	329.5±38.21	244.5±35.93	**b** 199.5±8.78	**b** 139.5±4.52	**b** 159.6±8.
positive control	261.5±6.40	255±4.89	262.33±7.40	269±11	273±5
fresh cow urine	275±23.75	**a** 217.33±16.27	**b** 146.66±8.19	**c** 129.1±2.27	**c** 125.5±3
Gau Arc	262.83±18.11	**a** 205.66±18.66	**b** 168±22.03	**c** 119.16±8.93	**c** 116.3±4.6
Ganwati	237.33±5.48	**a** 197.33±7.99	**b** 170±8.89	**b** 124.83±2.21	**c** 120.3±2.7

Values shows the effect of treatments with various cow urine preparations on blood glucose level in diabetic and treated animals (n=6). Values are given as mean ± S.E.M. (a; b; c denotes statistically significantly mean) **a** $p<0.05$, **b** $p<0.01$, **c** $p<0.0001$, when compared 0 day with 7,14 & 21 days treatment with cow urine preparations and standard drug (Using ANOVA test).

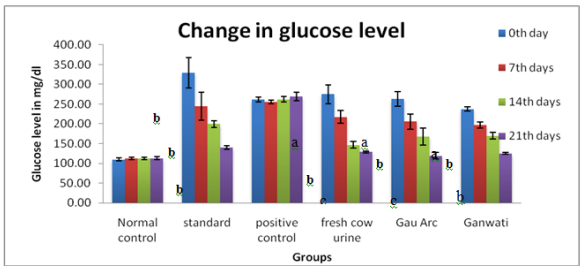

Figure 2: Comparison in mean ± S.E.M. value of blood glucose level measured in different groups on study days 0,7,14,21 & 28.

Vertical columns bars represents the effects of treatment with various cow urine preparations on blood glucose level in diabetic animals. Values are expressed as Mean ± S.E.M. of 6 animals in each group. Values shows the effect of treatments with various cow urine preparations on blood glucose level in diabetic and treated animals (n=6). Values are given as mean ± S.E.M. (a; b; c denotes statistically significantly mean) **a** $p<0.05$, **b** $p<0.01$, **c** $p<0.0001$, when compared 0 day with 7,14 & 21 days treatment with cow urine preparations and standard drug (Using ANOVA test).

Estimation of lipid profile

Blood samples were collected in eppendroff bullet on study day 21st by retro - orbital plexus and serum was separated by centrifugation (for 10 min at 10000 rpm). Serum was separated and lipid profile tests (cholesterol, HDL, Triglyceride) were determined on study day 21st in the different animal groups by using logotech diagnostic kit.

Mean ± S.E.M. lipid profile measured in different groups on study day 21. The results are shown in table 13.

Table 13: Comparison of different groups of lipid profile determined on study day 21. Values shown mean ± S.E.M, (n=6) Of lipid profile determined on study day 21.

Parameters	Diabetic Control	Normal Control	Standard	Fresh cow urine	Gau Arc	Ganwati
TC	111.15±10.15	**a** 72.23±5.4	**a** 78.55±2.27	**a** 80.03±1.05	**a** 76.9±1.33	**a** 76.1±1.2
TG	151.5±53.5	**c** 72.24±14	**a** 105.21±3.1	**b** 103.16±4.41	**a** 106.33±1.97	**b** 102±2.43
HDL	24.1±5.25	**a** 27.64±2.11	14.95±0.78	**b** 30.06±0.75	**a** 21.06±1.86	19.63±1.89
LDL	30.3±5.7	30.32±4.9	**a** 20.24±0.62	**a** 20.63±1.75	**a** 21.26±2.67	**a** 20.4±2.27
VLDL	45.25±10.7	**c** 14.28±2.92	38.35±0.62	**b** 29.33±0.88	34.9±0.39	36.08±0.48

Values shows the effect of treatments with various cow urine preparations on lipid profile in diabetic and treated animals (n=6). Values are given as mean ± S.E.M. (a; b; c denotes statistically significantly mean) **a** $p<0.05$, **b** $p<0.01$, **c** $p<0.0001$, when compared with diabetic control animals versus treatment with cow urine preparations and standard drug (Using ANOVA test).

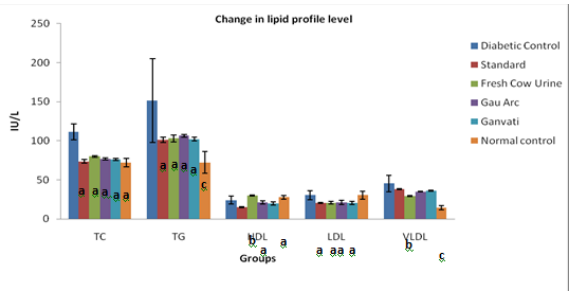

Figure 3: Comparison in mean ± S.E.M. value of lipid profile level measured in different groups on study days 21.

Vertical columns bars represents the effects of treatment with various cow urine preparations on lipid profile in diabetic animals. Values are expressed as Mean ± S.E.M. of 6 animals in each group. Values shows the effect of treatments with various cow urine preparations on lipid profile in diabetic and treated animals (n=6). Values are given as mean ± S.E.M. (a; b; c denotes statistically significantly mean) **a** p<0.05, **b** p<0.01, **c** p <0.0001, when compared with diabetic control animals versus treatment with cow urine preparations and standard drug (Using ANOVA test).

Estimation of liver and kidney function tests

Blood samples were collected in eppendroff bullet on study day 21st by retro - orbital plexus and serum was separated by centrifugation (for 10 min at 10000 rpm). Serum was separated and liver and kidney function tests (SGOT, SGPT, Serum creatinine) were determined on study days 21st in the different animal groups by using logotech diagnostic kit.

Table 14: Comparison in different groups of liver & kidney function tests determined on study days 21. Values shown mean ± S.E.M. n=6. of liver & kidney function tests determined on study day 21.

Parameters	Diabetic Control	Normal Control	Standard	Fresh cow urine	Gau Arc	Ganvati
SGPT	93.8±4.4	**b** 29.5±3.0	**b** 29.7±1.77	**b** 29.26±1.24	**b** 28.15±1.01	**b** 28.38±1.06
SGOT	80±1.4	**b** 29.4±1.7	**a** 34.08±1.15	**b** 32.26±1.28	**b** 31.86±1.35	**a** 34.15±1.37
Creatinine	1.61±0.08	**a** 0.86±0.05	**b** 0.78±0.05	**a** 0.85±0.04	**b** 0.75±0.02	**b** 0.71±0.03

Values shows the effect of treatments with various cow urine preparations on SGOT, SGPT & Serum creatinine in diabetic and treated animals (n=6). Values are given as mean ± S.E.M. (a; b; c denotes statistically significantly mean) **a** p<0.05, **b** p<0.01, **c** p <0.0001, when compared with diabetic control animals versus treatment with cow urine preparations and standard drug (Using ANOVA test).

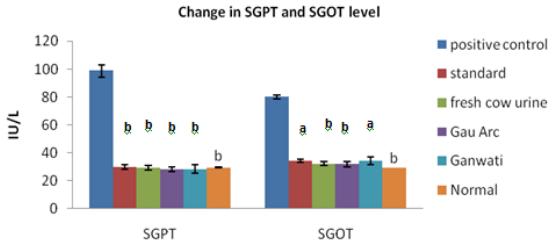

Figure 4 : Comparison in mean ± S.E.M. value of liver and kidney function tests measured in different groups on study days 21.

Vertical columns bars represents the effects of treatment with various cow urine preparations on liver and kidney function tests in diabetic animals. Values are expressed as Mean ± S.E.M. of 6 animals in each group.

Values shows the effect of treatments with various cow urine preparations on SGOT, SGPT & Serum creatinine in diabetic and treated animals (n=6). Values are given as mean ± S.E.M. (a; b; c denotes statistically significantly mean) **a** p<0.05, **b** p<0.01, **c** p <0.0001, when compared with diabetic control animals versus treatment with cow urine preparations and standard drug (Using ANOVA test).

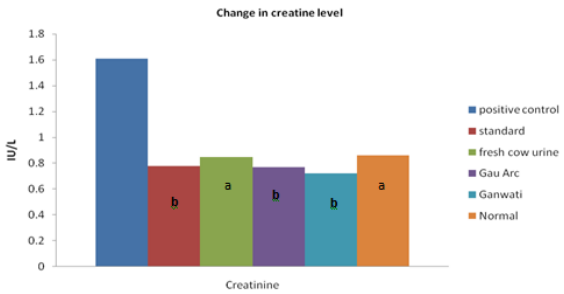

Figure 5: Comparison in mean ± S.E.M. value of liver and kidney function tests measured in different groups on study days 21.

Vertical columns bars represents the effects of treatment with various cow urine preparations on liver & kidney function tests in diabetic animals. Values are expressed as Mean ± S.E.M. of 6 animals in each group.

Values shows the effect of treatments with various cow urine preparations on Serum creatinine in diabetic and treated animals (n=6). Values are given as mean ± S.E.M. (a; b; c denotes statistically significantly mean) **a** p<0.05, **b** p<0.01, **c** p <0.0001, when compared with diabetic control animals versus treatment with cow urine preparations and standard drug (Using ANOVA test).

Recording of body weight

Body weight was taken before alloxan injection, on study days 0,7,14,21 and 28 by animal weighing balance.

Recordings of body weight are given in table no.15.

Table 15: Comparison in mean ± S.E.M. value of body weight recorded in different groups on study days 0,7,14,21 & 28.

Treatment group	Body weight Before Alloxan injection	Before treatment 0 day	Treatment period			After treatment 28th day
			7th day	14th day	21th day	
Normal control	177.33±6.03	178.16±5.97	179.66±5.91	181.16±6.31	182.33±6.1	184.16±6.09
standard	179.5±6.88	161.16±5.3	162.16±5.19	163.8±4.88	**a** 164.66±5.0	163.33±11.4
positive control	173±5.73	151.66±4.46	148.83±4.22	147±4.36	138±5	136±5
fresh cow urine	224.83±10.3	186.66±5.9	189±5.76	**a** 191.3±5.97	**a** 205.5±6.56	**c** 207.836.1
Gau Arc	207±6.58	182.5±3.9	185.6±3.95	189±3.9	**a** 195.6±4.8	**b** 198.5±4.99
Ganwati	176.7±11.86	145.7±9.23	148.3±9.0	150.8±9.3	**a** 158.2±9.7	**b** 160.8±10.0

Values shows the effect of treatments with various cow urine preparations on body weight in diabetic and treated animals (n=6). Values are given as mean ± S.E.M. (a; b; c denotes statistically significantly mean) **a** p<0.05, **b** p<0.01, **c** p <0.0001. When compared 0 day with 7,14,21 & 28 days treatment with cow urine preparations and standard drug (Using ANOVA test).

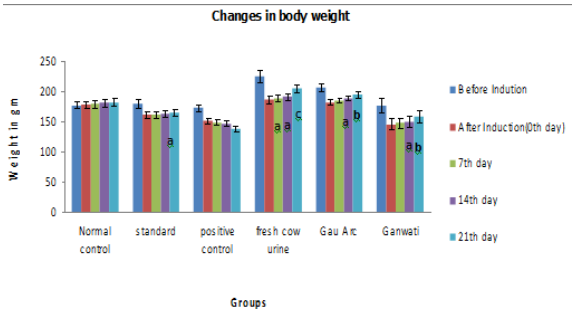

Figure 6: Comparison in mean ± S.E.M. value of body weight recorded in different groups on study days before, 0,7,14, 21 & 28.

Vertical columns bars represents the effects of treatment with various cow urine preparations on body weight in diabetic animals. Values are expressed as Mean ± S.E.M. of 6 animals in each group.

Values shows the effect of treatments with various cow urine preparations on body weight in diabetic and treated animals (n=6). Values are given as mean ± S.E.M. (a; b; c denotes statistically significantly mean) **a** p<0.05, **b** p<0.01, **c** p <0.0001, when compared 0 day with 7,14 & 21 days treatment with cow urine preparations and standard drug (Using ANOVA test).

Discussion

Management o diabetes with the agents devoid of any side effects is still a challenge to the medical system. This has led to an increase in the demand for natural products with antihyperglycemic activity and fewer side effects. The cow urine preparations exhibited dose-dependent antidiabetic property. The antidiabetic effect of these preparations at the dose of 5 ml/kg is even slightly higher then gliclazide 10 mg/kg. Our results are supporting its use as folklore medicine for the treatment of diabetes.

In present study blood glucose level was taken on study days 0,7,14,21 & 28 of

different groups. Mean ± S.E.M. blood glucose level values are shown in table-12. The anti-hyperglycemic effects of the cow urine preparations and gliclazide on the blood sugar levels of diabetic rats were shown in table-13. After daily treatment with cow urine preparations and gliclazide led to a dose dependent fall in blood sugar levels. The reduction of hyperglycemia were significant ($p<0.01$) on 7^{th},14^{th} & 21^{st} days after treatment with the cow urine preparations, as compared with the day 0 observations. The antihyperglycemic effects exhibited by 5 ml/kg of cow urine preparations was slightly higher than that of gliclazide 10 mg/kg. Boden G. Front. Biosci, demonstrated for the first time induce insulin resistance in human in a dose dependent fashion. Lowring of plasma free fatty acid levels is accordingly effective in the treatment of insulin resistance in a mammal. It has now been found that administration of and effective amount of succinic acid or salt thereof to insulin resistant mammals is effective therapy for treating of insulin resistance. Lowering of plasma free fatty acid levels accompanies a lowering of pathologically elevated insulin and glucose levels that reflects and improving in insulin sensitivity.[11-17] Cow urine preparations contain succinic acid as observed in the study. Cow urine preparations were given a dose 5 ml/kg. The cow urine preparations as (1 ml) contain 0.39 mg i.e. 1.95 mg/kg. This amount of succinic acid show the antidiabetic activity in the study. The dose used of succinic acid in previous study is 5 mg/kg of body weight. Lipids play an important role in the pathogenesis of diabetes mellitus. [18,19] Hyperlipidemia is a recognized consequence of diabetes mellitus demonstrated by the elevated levels of tissue cholesterol, phospholipids and free fatty acids. [24,25] Diabetes-induced hyprtlipidemia is attributable to excess mobilization of fat from the adipose tissuee due to the under utilizations of glucose. The abnormal high concentration of serum lipids in diabetes is mainly due to the increase in the mobilization of free fatty acids from the peripheral depots since insulin inhibits the hormone sensitive lipase. On the other hand, glucagons, catecholamine, and other hormones enhance lipolysis. The level of serum lipids is usually raised in diabetes and such an elevation represents a risk factor for coronary heart disease. The levels of serum cholesterol and triglycerides were raised in diabetic rats but which lowered significantly with the treatment of cow urine preparations. It indicates that the cow urine preparations are more useful in the treatment of diabetes as it has hypolipidemic effect. Moreover, its hypolipidemic effect could represent a protective mechanism against the development of atherosclerosis, which is usually associated with diabetes. The levels of HDL cholesterol were significantly increased in the groups treated with cow urine preparations.

The levels of SGOT, SGPT and Serum creatinine were increased very were increased in diabetic rats as compared with normal control rats. [20-23] Treatment with cow urine preparations reduced the SGOT, SGPT and Serum creatinine very significantly decreased ($P<O.05$), when compared with the diabetic control group. The findings indicate that these preparations may have hepatoprotective effects and could be effective therapy for both diabetes and hepatotoxicity. The effects of the cow urine preparations on body weight in the alloxan-induced diabetic rats are shown in [Table 14]. The results of the body weight analysis indicate that the body weight of the untreated diabetic rats was found to be significantly ($P<0.05$) decreased when compared with the normal control group. The body weight was slightly increased in the normal

control group compared to initial weight. Treatment with cow urine preparations and glyclazide prevented reduction in body weight and the weight was increased after the treatment. This shows that cow urine preparations increase body weight reduced due to diabetes and this may help to maintain normal body weight.

Conclusion

The cow urine preparations have significant hypoglycaemic effect in alloxan induced diabetic rats. Cow urine preparations also lowers hypertriglyceridemia and hyperlipidemia in alloxan induced diabetic rats. There were significant improvement in the animals in lowering blood glucose level. The reduction of hyperglycemia were significant (P<O.01) on 7^{th}, 14^{th}, and 21^{th} days after treatment with the cow urine preparations, as compared with the 0 day observations. The reduction of hyperglycemia was significant comparable with the standard gliclazide. The levels of serum cholesterol and triglycerides were increased very significantly and the levels of HDL were decreased in diabetic rats as compared with normal control rats. Cholesterol and triglycerides level were decreased in the cow urine preparations treatd groups at the dose of 5ml /kg as compared with diabetic control group. HDL levels were increased in diabetic rats treated with cow urine preparations as compared with diabetic control group. Effect of cow urine preparations specially fresh cow urine have more significant reduction in lipid profile (TG, TC, LDL) & increase in HDL-Cholestrol than standard gliclazide. The present work has detected that cow urine preparations were contained succnic acid that administration of and effective amount of succinic acid or salt thereof to insulin resistant mammals is effective therapy for treating of insulin resistance. The levels of SGOT, SGPT and Serum creatinine were increased in diabetic rats as compared with normal control rats. Treatment with cow urine preparations reduced the SGOT, SGPT and Serum creatinine very significantly decreased (P<O.01), when compared with the diabetic control group. From findings of present study we can conclude that cow urine and its preparations could be effective in treating diabetes, arthrosclorsis, hyperlipidaemia, hepatic & renal toxicity.

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