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Anti-diabetic activity of *Kataka Khadirādi Kasāyam* in Wistar albino rats

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Katakakhadirādi Kaṣāyaṃ is an Ayurvedic herbal preparation used for treating diseases such as diabetes, skin, and urinary tract ailments. The objective of present study was to assess antidiabetic potential of *Kataka Khadirādi Kaṣhāyaṃ*, in Streptozotocin (STZ) induced diabetic Wistar rats for a period of 90 days. Treatment with *Katakakhadirādi Kaṣhāyaṃ* demonstrated significant efficacy, reducing blood glucose levels to near normal by the 5th week in diabetic rats, showing a 58% reduction compared to day 1 and a 47% reduction in comparison to the diabetic control group's blood glucose levels. However from 7th week onwards, a marked elevation was observed in blood sugar levels of all diabetic rats. *Katakakhadirādi Kaṣāyaṃ* treatment resulted in 35% reduction in blood level glucose values when compared to that of diabetic control group in 13th week. Hence it can be inferred that, *Kaṣāyaṃ* had maintained notable hypoglycemic activity despite insulin resistance. *Kaṣāyaṃ* treatment shows protective nature on liver tissues by reducing the increased levels of serum ALT, AST and ALP. Also it significantly reduced TG levels with a marked reduction in TC, HDL and LDL values. Histological examination also revealed mild to moderate reduction in degenerative changes of liver and kidney tissues and marked regeneration of islet architecture in pancreatic tissues. *Katakakhadirādi Kaṣāyaṃ* might be able to control the pancreatic beta-cell damage and has hypoglycemic, hypolipidemic and organ protective potential in STZ -induced diabetic rats. Mechanisms of action during insulin resistance is yet to be explored.

Keywords: Anti-Diabetic, *Katakakhadirādi Kaṣhāyaṃ,* Streptozotocin, Wistar rats

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Diabetes mellitus (DM) is a metabolic disorder characterized by increase in blood glucose due to relative deficiency of insulin secretion from pancreatic cells[1]. There are two main types of diabetes mellitus (DM): 1) Type 1 DM, also known as 'insulin-dependent diabetes mellitus' (IDDM) which occur due to the pancreas's inability to generate enough insulin due to the loss of beta cells; and 2) Type 2 DM, also known as 'non-insulin-dependent diabetes mellitus' (NIDDM) which begins with insulin resistance, a condition where cells do not respond properly to insulin. As the disease progresses, lack of insulin may also develop. Despite significant progress in managing diabetes through conventional strategies and drugs, the disease and its complications remain a key concern. Nearly all synthetic oral hypoglycemic agents used in treatment come with serious side effects and are also costly[2]. As a result, traditional herbal medicines are gaining popularity among masses for prevention and treatment of diabetes across the world[3]. There is therefore an increasing interest in traditional medicinal plants.

Polyherbal Ayurvedic formulations are a cornerstone of Ayurvedic medicine due to their enhanced efficacy and least side effects as compared to single herbs. However, scientific validation of these polyherbal formulations is limited in comparison to that of individual herbs. *Katakakhadirādi Kaṣāyaṃ* is such a herbal preparation, which is mostly used for the treatment of diabetes, skin, and urinary tract ailments and also manages both Vata and Kapha associated ailments[4,5]. But this formulation does not have any scientific evidence on the efficacy aspects. To develop *Katakakhadirādi Kaṣāyaṃ* as a product that meets both domestic and international regulatory standards, *in vivo* safety and efficacy data are indispensable. Therefore, the present study aimed to evaluate the antidiabetic potential of *Katakakhadirādi Kaṣāyaṃ* in experimentally induced diabetic rats.

Materials and Methods

Drugs and chemicals

Katakakhadirādi Kaṣāya, a dark brown suspension liquid, was manufactured by CARe Keralam Ltd. The raw materials, all of pharmacopeial quality, included

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Kataka (*Strychnos potatorum*) seeds, Khadira (*Acacia catechu*) heartwood, Dhatri (*Emblica officinalis*) pericarp, Vairi (*Salacia reticulata*) root, Darvi (*Berberis aristata*) stem, Samanga (*Biophytum sensitivum*) whole plant, Rajani (*Curcuma longa*) rhizome, Pata (*Cyclea peltata*) rhizome, Chootabija (*Mangifera indica*) seed, Abhaya (*Terminalia chebula*) pericarp, Abda (*Cyperus rotundus*) rhizome, and Kola (*Ziziphus jujube*) seed, all in equal quantities. These were boiled in 16 parts of fresh water and reduced to 1/4th of the original volume. The mixture was then filtered, concentrated further to 1/4th of its volume, and preserved with a 0.1% solution of sodium benzoate. Streptozotocin (Sigma, USA) was used to induce diabetes in experimental animals.

Experimental animals

In this study, male Wistar albino rats (8 to 12 weeks old) were used. The animals were housed under standard laboratory conditions (temperature 22±3ºC, humidity 50-60%), with air-conditioning and adequate fresh air supply, using an Individually Ventilated Caging (IVC) system (15 air changes per hour), and a 12 h light/dark cycle. The rats were acclimatized to the laboratory conditions for seven days and were monitored daily for clinical signs. They were provided with a pelleted laboratory rat diet and purified water ad libitum. The protocol was approved by Institutional Animal Ethics Committee (IAEC), protocol no. CKL/TOX/IAEC/026-2014.

Study design

Induction of diabetes

A total of 30 rats (6 normal and 24 STZ-induced diabetic surviving rats) were used in the study. Twenty-four rats, fasted for 16 hours, were administered a single in traperitoneal injection of 35 mg/kg body weight of streptozotocin dissolved in freshly prepared citrate buffer (0.1 M, pH 4.5). Rats with fasting blood glucose levels above 200 mg/dL, measured 3 days after streptozotocin administration, were considered diabetic. The rats were then divided into five groups (n=6) and treated orally for 90 days. The Vehicle Control (I) and Diabetic Control (II) groups received 1 mL/100 g body weight of distilled water (vehicle). Group-III received Glibenclamide (standard drug) at 600 µg/kg body weight, while drug-treated Groups IV and V received *Katakakhadirādi Kaṣāyaṃ* at 4.32 and 8.64 mL/kg body weight, respectively.

Upon completion of the treatment period, all animals were fasted overnight. The following day, blood samples were collected from the retro-orbital plexus under anesthesia, allowed to clot for 30 minutes at room temperature, and then serum was separated by centrifugation for various biochemical estimations. The animals were subsequently euthanized by an overdose of thiopental sodium (intraperitoneal injection).

Changes in body weight

The body weight of each rat was recorded preceding to treatment, weekly afterward and at terminal sacrifice of the study. Calculations were done for group mean body weights and body weight gains.

Blood glucose estimation

Fasting blood glucose levels were measured on Day 1, before starting the treatment, and then every two weeks to evaluate the effect of the treatment on the diabetic animals. Blood samples were collected using the tail-vein method, and glucose levels were immediately measured with glucose oxidase-peroxidase reactive strips using a glucometer (OneTouch SelectSimple®).

Serum biochemistry

Serum was used for determining various clinical chemistry parameters using automatic clinical chemistry analyzer (Vitalab Selectra Junior). Various parameters like Glucose, Lipid profile, Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Alkalinephosphatase (ALP), Total Protein (TP), Albumin, Bilitubin (Total & Direct), Urea and Creatinine were analyzed.

Histopathology

Representative tissue samples from the pancreas, liver, and kidney were collected from the euthanized animals and preserved in 10% neutral buffered formalin. The tissues were then embedded in paraffin wax, sectioned at 5 micrometers, and stained with hematoxylin and eosin for histopathological assessment.

Statistical analysis

The data on body weight, blood glucose and clinical chemistry parameters, generated from the present study were statistically analysed using GraphPad Prismsoftware, Version 5.00, USA. 2007. One way ANOVA with Dunnets post-test was carried out for diverse treatment groups comparing with the diabetic control group data. All analysis and comparisons was evaluated at 5% significance level.

Results

Effect of treatment on body weight

During the study period, diabetes induced rats showed significant reduction in body weight compared to normal control animals. Even though a gradual elevation was observed in body weight of rats which received either glibenclamide or *Katakakhadirādi Kaṣāyaṃ*, it was not found to be statistically significant when compared to the diabetic control group (Table 1).

Effect of treatment on blood glucose levels

A significant change in the blood glucose levels of diabetic control group was recorded as compared to normal control animals throughout the treatment period. An effective reduction in blood glucose levels of treatment group animals, were observed from 3rd week onwards up to 5th week. Blood glucose levels of rats treated with *Katakakhadirādi Kaṣāyaṃ* (both low and high doses) and standard drug, during the 5th week of treatment, were significantly lower ($p<0.001$) as compared to diabetic control group. *Katakakhadirādi Kaṣāyaṃ* (at 4.32 mL/kg bwt.) showed 58% reduction, when compared to day 1, and 47% reduction compared to the blood glucose values of the diabetic control group on 5th week of treatment. Similarly, glucose levels of diabetic rats treated with *Katakakhadirādi Kaṣāyaṃ* (at 8.64 mL/kg bwt.) and glibenclamide, was reduced by 43% and 37%, respectively, compared to day 1 and 39% and 44% respectively, compared to the values of the diabetic control group (Table 2).

However from 7th week onwards, a marked elevation in blood glucose levels of diabetic rats was observed which was consistently on the higher side till the end of experiment period. On the last (13th) week of treatment, blood glucose levels of rats treated with *Katakakhadirādi Kaṣāyaṃ* and standard drug were significantly higher ($p<0.001$) as compared to normal control group and comparable to that of diabetic control group. During the last week of treatment, *Katakakhadirādi Kaṣāyaṃ* (8.64 mL/kg bwt.) and glibenclamide treated groups showed 35% and 19% reduction respectively, in blood glucose values, compared to the values of diabetic control group; while on comparing with the respective Day 1 blood glucose levels, there was 7% and 62% increase in glucose levels in *Katakakhadirādi Kaṣāyaṃ* (8.64 mL/kg bwt.) and glibenclamide treatment groups, respectively. However, *Katakakhadirādi Kaṣāyaṃ* (4.32 mL/kg bwt.) showed 48% increase, compared to day 1, and 5% increase, when compared to the blood glucose values of the diabetic control group, on 13th week of treatment.

Effect of treatment on serum biochemistry

Significant increase in serum glucose was noted in the diabetic control group compared to the normal animals ($p<0.001$). But, any significant reduction in glucose levels were not detected in any of the treatment groups at the end of experiment. Serum glucose levels of *Katakakhadirādi Kaṣāyaṃ* (both low and high doses) and glibenclamide treated

Table 1 — Effect of *Katakakhadirādi Kaṣāyaṃ* treatment on bodyweight (g) of diabetic Wistar rats

Groups	Day	Week												
	1	1	2	3	4	5	6	7	8	9	10	11	12	13
I	222.50	255.83	259.17	275.83	282.50	290.83	288.333	295.00	305.00	293.33	302.50	300.83	305.83	303.33
Normal	±	±	±	±	±	±	±	±	±	±	±	±	±	±
Control	7.16	9.70***	8.51***	12.41**	12.50**	13.69***	13.82**	13.60**	14.72**	12.76**	15.64*	14.63*	15.68*	15.79*
II	218.33	216.67	225.00	238.33	244.17	235.00	239.17	250.83	251.67	245.83	263.33	259.17	262.50	262.50
Diabetic	±	±	±	±	±	±	±	±	±	±	±	±	±	±
Control	4.22	4.01$^{\#\#\#}$	3.65$^{\#\#\#}$	5.43$^{\#\#}$	5.39$^{\#\#}$	4.66$^{\#\#\#}$	4.55$^{\#\#}$	3.96$^{\#\#}$	5.27$^{\#\#}$	5.69$^{\#\#}$	7.60$^{\#}$	9.08$^{\#}$	7.93$^{\#}$	8.73$^{\#}$
III	222.50	215.83	223.33	234.17	244.17	235.00	235.00	260.00	254.17	241.67	267.50	260.83	267.50	268.33
Standard	±	±	±	±	±	±	±	±	±	±	±	±	±	±
	4.43	4.73$^{\#\#\#}$	5.43$^{\#\#\#}$	8.11$^{\#\#}$	7.12$^{\#\#}$	7.42$^{\#\#\#}$	7.42$^{\#\#\#}$	10.57$^{\#}$	10.91$^{\#\#}$	10.70$^{\#\#}$	9.38	10.44	11.96	9.97
IV	215.83	215.83	220.83	230.00	235.83	223.33	235.00	223.33	230.83	229.17	220.00	222.50	237.50	237.50
Low dose	±	±	±	±	±	±	±	±	±	±	±	±	±	±
	1.54	3.52$^{\#\#\#}$	3.96$^{\#\#\#}$	5.16$^{\#\#\#}$	7.90$^{\#\#}$	6.91$^{\#\#\#}$	8.94$^{\#\#\#}$	6.91$^{\#\#\#}$	10.12$^{\#\#\#}$	6.64$^{\#\#\#}$	7.53$^{\#\#\#}$	9.20$^{\#\#}$	9.38$^{\#\#\#}$	9.90$^{\#\#\#}$
V	212.50	217.50	225.00	233.33	238.33	230.00	237.50	230.00	245.00	243.33	238.33	240.83	248.33	254.17
High dose	±	±	±	±	±	±	±	±	±	±	±	±	±	±
	3.35	3.35$^{\#\#\#}$	1.83$^{\#\#\#}$	3.80$^{\#\#}$	5.87$^{\#\#}$	5.32$^{\#\#\#}$	6.92$^{\#\#}$	5.32$^{\#\#\#}$	7.53$^{\#\#}$	10.54$^{\#\#}$	9.01$^{\#\#\#}$	12.00$^{\#\#}$	9.46$^{\#\#}$	7.90$^{\#}$

Values as mean ± SEM
* $p<0.05$, ** $p<0.01$, *** $p<0.001$ vs Diabetic Control
# $p<0.05$, ## $p<0.01$, ### $p<0.001$ vs Normal Control

rats were comparable to that of diabetic control group (Table 3).

Serum liver enzyme levels (AST, ALT, and ALP) in the diabetic rats were significantly elevated when compared to the normal rats. Serum ALT values in diabetic control, low dose of *Katakakhadirādi Kaṣāyaṃ* and glibenclamide groups were significantly elevated than that of normal control group at p<0.001, p<0.001andp<0.05, respectively. However treatment of *Katakakhadirādi Kaṣāyaṃ* at high dose resulted in significant reduction (p<0.01) in ALT levels compared to that of diabetic control rats and it was also comparable to that of normal control rats.

Serum AST values in diabetic control and glibenclamide groups were significantly higher than that of normal control group. However, there observed treatment related significant normalization of AST levels in *Katakakhadirādi Kaṣāyaṃ* treated rats. AST levels of *Kaṣāyaṃ* treated rats at 8.64 mL/kg bwt. was lower as compared to the diabetic

Table 2 — Effect of *Katakakhadirādi Kaṣāyaṃ* treatment on blood glucose levels of diabetic Wistar rats

Groups	Blood Glucose (mg/dL)							
	1st day	1st week	3rd week	5th week	7th week	9th week	11th week	13th week
I Normal Control	100.33 ± 4.34**	104.67 ± 6.86**	101.67 ± 4.83***	97.17 ± 2.52***	95.33 ± 2.47**	86.17 ± 2.98*	101.67 ± 3.12**	113.00 ± 3.01***
II Diabetic Control	282.17 ± 35.14##	274.00 ± 34.86##	253.17 ± 27.68###	264.33 ± 28.80###	232.00 ± 17.95##	292.17 ± 16.49#	309.17 ± 21.95##	470.17 ± 32.94###
III Standard	236.17 ± 18.77#	234.00 ± 17.22#	148.67 ± 14.82*	148.33 ± 16.84***	142.83 ± 13.90*	172.17 ± 40.69	215.33 ± 18.53	382.83 ± 68.57##
IV Low dose	334.33 ± 56.41###	307.33 ± 44.36###	179.67 ± 39.02	139.00 ± 16.06***	208.00 ± 42.92##	311.33 ± 64.56##	233.50 ± 34.44	494.33 ± 27.62###
V High dose	285.33 ± 43.59##	281.17 ± 43.41##	180.67 ± 3.41	161.50 ± 2.93***	220.50 ± 17.87##	253.33 ± 72.54	243.83 ± 69.12#	306.33 ± 64.35#

Values as mean ± SEM
* p<0.05, ** p<0.01, *** p<0.001 vs Diabetic Control
# p<0.05, ## p<0.01, ### p<0.001 vs Normal Control

Table 3 — Effect of *Katakakhadirādi Kaṣāyaṃ* treatment on serum biochemistry of diabetic Wistar rats

Groups	Glucose (mg/dL)	AST (U/L)	ALT (U/L)	ALP (U/L)	Total Protein (g/dL)	Albumin (g/dL)	Bilirubin Total (mg/dL)	Bilirubin Direct (mg/dL)	Urea (mg/dL)	Creatinine (mg/dL)
I Normal Control	84.70 ± 12.39***	129.17 ± 19.73**	47.95 ± 4.44***	269.63 ± 29.92*	7.90 ± 0. 91	1.70 ± 0.11	0.37 ± 0.07**	0.15 ± 0.03	35.85 ± 1.89	0.23 ± 0.03***
II Diabetic Control	517.67 ± 54.74###	250.48 ± 16.24##	108.83 ± 9.91###	753.25 ± 107.62#	5.80 ± 0.57	1.48 ± 0.09	1.07 ± 0.07##	0.37 ± 0.09	43.33 ± 2.87	0.47 ± 0.03###
III Standard	311.02 ± 70.11#	231.78 ± 22.09##	80.60 ± 9.22#	677.80 ± 135.95#	7.87 ± 0.65	1.77 ± 0.10	1.02 ± 0.13#	0.367 ± 0.09	43.65 ± 1.09	0.45 ± 0.03###
IV Low dose	528.22 ± 46.34###	199.28 ± 31.08	103.52 ± 10.45###	733.15 ± 122.90#	6.40 ± 0.55	1.48 ± 0.11	0.70 ± 0.27	0.28 ± 0.07	43.85 ± 2.08	0.47 ± 0.04###
V High dose	327.90 ± 84.93#	158.87 ± 6.11*	66.07 ± 7.77**	524.28 ± 99.67	6.37 ± 0.60	1.50 ± 0.12	0.33 ± 0.09**	0.22 ± 0.07	39.80 ± 3.56	0.45 ± 0.04###

Values as mean ± SEM
* p<0.05, ** p<0.01 *** p<0.001 vs Diabetic Control
# p<0.05, ## p<0.01, ### p<0.001 vs Normal Control

control rats ($p<0.05$). *Katakakhadirādi Kaṣāyaṃ* (at both low and high doses) resulted in AST values comparable to that of the normal control group.

ALP levels of diabetic rats also were significantly ($p<0.05$) elevated compared to normal control animals. Serum ALP values of *Kaṣāyaṃ* at dose 4.32 mL/kg bwt. and glibenclamide (677.80±135.95) treated rats were comparable to that of diabetic control rats. However, ALP levels of *Kaṣāyaṃ* treated rats at 8.64 mL/kg bwt. was comparable to that of normal control group (Table 3).

Serum total protein and albumin levels were notably reduced in the diabetic control group compared to the normal animals, although this difference was not statistically significant. No significant differences in serum protein levels were observed between the treatment groups and the diabetic control group (Table 3). Serum urea levels were slightly elevated in the diabetic control animals compared to the normal control group, but this difference was not statistically significant. Additionally, treatment did not lead to a significant reduction in the elevated urea levels in the diabetic rats. The serum lipid profile was significantly altered in the diabetic control group compared to the normal animals, with marked increases in total cholesterol (TC) and triglyceride (TG) levels in the diabetic control group ($p<0.05$ and $p<0.01$, respectively). Animals treated with high-dose *Katakakhadirādi Kaṣāyaṃ* and glibenclamide showed a significant reduction ($p<0.05$) in TG levels compared to the diabetic control group, with TG levels comparable to those of the normal control group. However, only a mild reduction in TC, HDL, and LDL levels was observed in the treatment groups, which was not statistically significant when compared to the diabetic control group (Table 4).

Table 4 — Effect of *Katakakhadirādi Kaṣāyaṃ* treatment on serum lipid profile of diabetic Wistar rats

Groups	TC (mg/dL)	TG (mg/dL)	HDL (mg/dL)	LDL (mg/dL)
I Normal Control	60.32 ± 10.33*	50.12 ± 6.40**	23.17 ± 4.65	27.13 ± 5.86
II Diabetic Control	97.90 ± 9.68#	113.97 ± 14.08##	43.43 ± 6.24	33.34 ± 5.14
III Standard	75.17 ± 4.72	72.53 ± 11.36*	30.80 ± 3.46	29.86 ± 4.50
IV Low dose	74.50 ± 6.37	94.15 ± 8.65#	43.77 ± 9.27	11.90 ± 11.22
V High dose	68.72 ± 11.52	66.02 ± 11.41*	32.02 ± 3.43	23.50 ± 7.05

Values as mean ± SEM
* $p<0.05$, ** $p<0.01$, *** $p<0.001$ vs Diabetic Control
# $p<0.05$, ## $p<0.01$, ### $p<0.001$when vs Normal Control

Histopathological evaluation

Microscopic examination of rat tissue sections from the pancreas, liver, and kidney, stained with Hematoxylin and Eosin (H&E) was conducted. In normal control rats, the exocrine component of the pancreas consisted of tightly packed acinar cells organized into small lobules. These pancreatic lobules were separated by intact in tralobular and interlobular connective tissue septa. The islet cells were interspersed among the acinar cells and appeared lightly stained in comparison to the surrounding acinar cells. In diabetic control rats, the islets were damaged, shrunken, and showed lymphocytic infiltration. In contrast, rats treated with *Katakakhadirādi Kaṣāyaṃ* (8.64 mL/kg body weight) or Glibenclamide displayed islets that were almost similar to those of normal rats, with only a mild decrease in islet shrinkage, though slight damage was still noticed (Fig. 1).

Histopathological examination of the liver in normal control animals showed normal hepatic cells with well-preserved sinusoids, nuclei, portal tracts, and central veins. While diabetic control animals exhibited maintained lobular architecture but showed vacuolation of hepatocytes, mild to moderate degeneration and necrosis, sinusoidal dilation and congestion, and mild portal inflammation. However, diabetic rats treated with *Katakakhadirādi Kaṣāyaṃ* (8.64 mL/kg body weight) or glibenclamide exhibited hepatocytes with minimal degenerative changes (Fig. 2).

Histopathological analysis of renal tissues in control rats showed normal renal tubules, glomeruli, and corpuscles. In contrast, the kidneys of diabetic rats exhibited acute cellular swelling, tubular degeneration, and necrosis. Treatment with (8.64 ml/kg body weight) or glibenclamide improved the kidney architecture (Fig. 3).

Discussion

The STZ induced diabetic rats are one of the animal models of DM. It is well known for its selective pancreatic islet beta cell cytotoxicity and has been extensively used to induce diabetes in

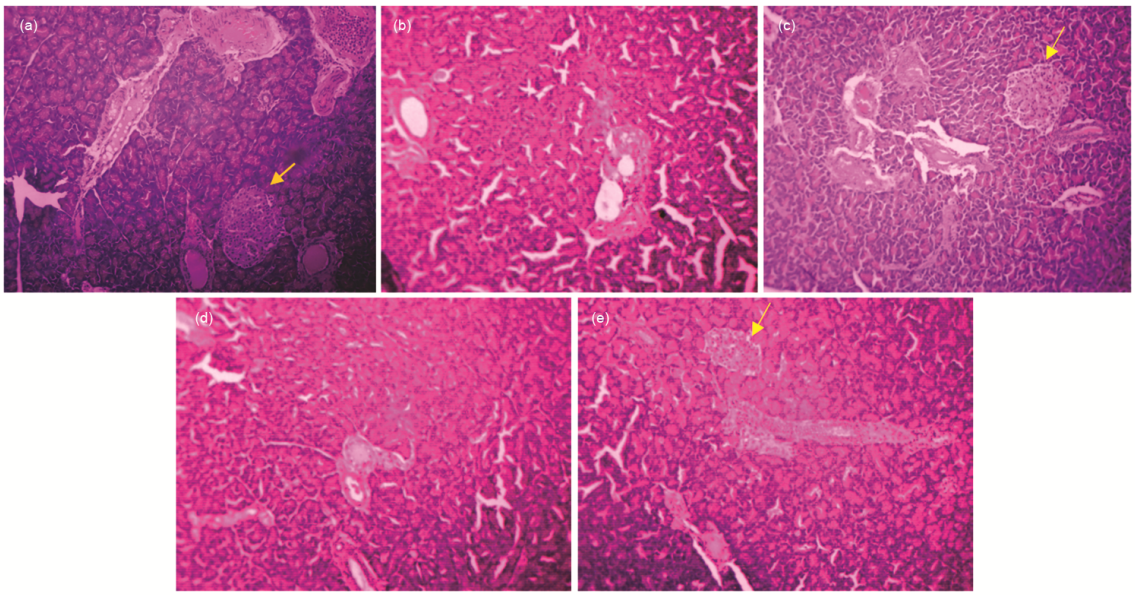

Fig. 1 — Effect of *Katakakhadirādi Kaṣāyaṃ* treatment on pancreas of diabetic Wistar rats
Photomicrograph of pancreatic tissue (H&E, 100X) – (a) Normal control group-Normal architecture with normal appearance of Islets of Langerhans (arrow), (b) Diabetic control group-degenerative changes in acinar cells, marked atrophy of Islets of Langerhans and dilatation of lobular ducts, (c) Glibenclamide group-restoration of normal architecture with increase in size of Islets (arrow), (d) *Katakakhadirādi Kaṣāyaṃ* (4.32 mL/kg bwt) group- degeneration of acinar cells with Islet atrophy, (e) *Katakakhadirādi Kaṣāyaṃ* (8.64 mL/kg bwt) group- marked normalization of acinar cells and increase in size of Islets(arrow)

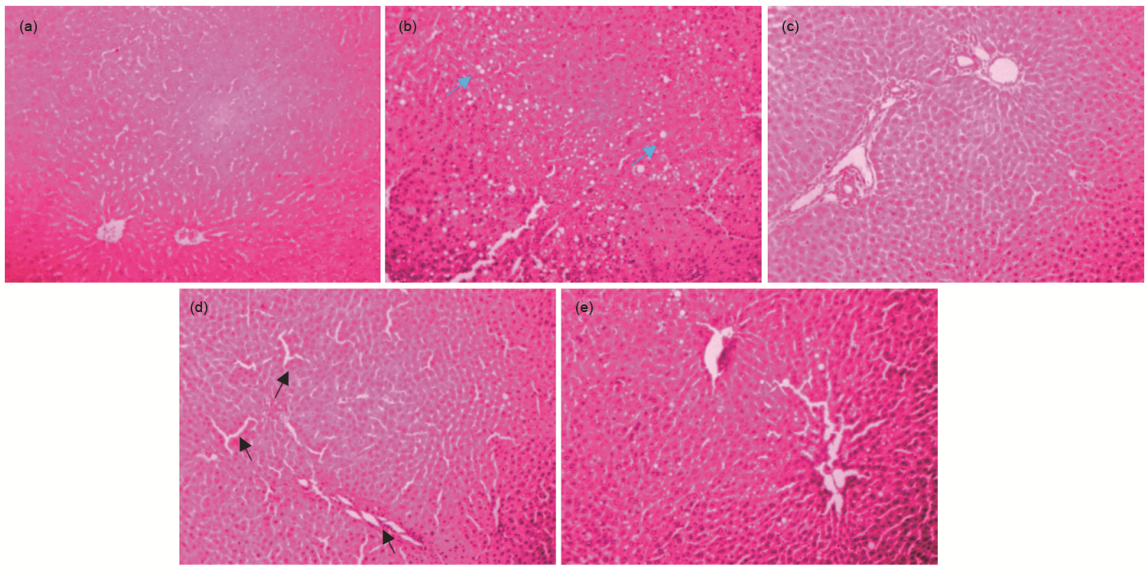

Fig. 2 — Effect of *Katakakhadirādi Kaṣāyaṃ* treatment on Liver of diabetic Wistar rats
Photomicrograph of Liver tissue (H&E, 100X) – (a) Normal control group-Normal histological structure of hepatocytes, (b) Diabetic control group-fatty degeneration and vacuolations (blue arrow) in the hepatocytes, (c) Glibenclamide group- normal hepatic histostructure with reduced degenerative changes, (d) *Katakakhadirādi Kaṣāyaṃ* (4.32 mL/kg bwt) group-hepatocyte degeneration with sinusoidal dilatation (black arrow), (e) *Katakakhadirādi Kaṣāyaṃ* (8.64 mL/kg bwt) group- almost normal hepatic histostructure with reduced degenerative changes

experimental rat model. Glibenclamide was often used as a standard antidiabetic drug in STZ induced diabetes to compare the efficacy of variety of hypoglycemic drugs[6]. STZ-induced diabetes mellitus is associated with the generation of reactive oxygen species causing oxidative damage. Diabetes and

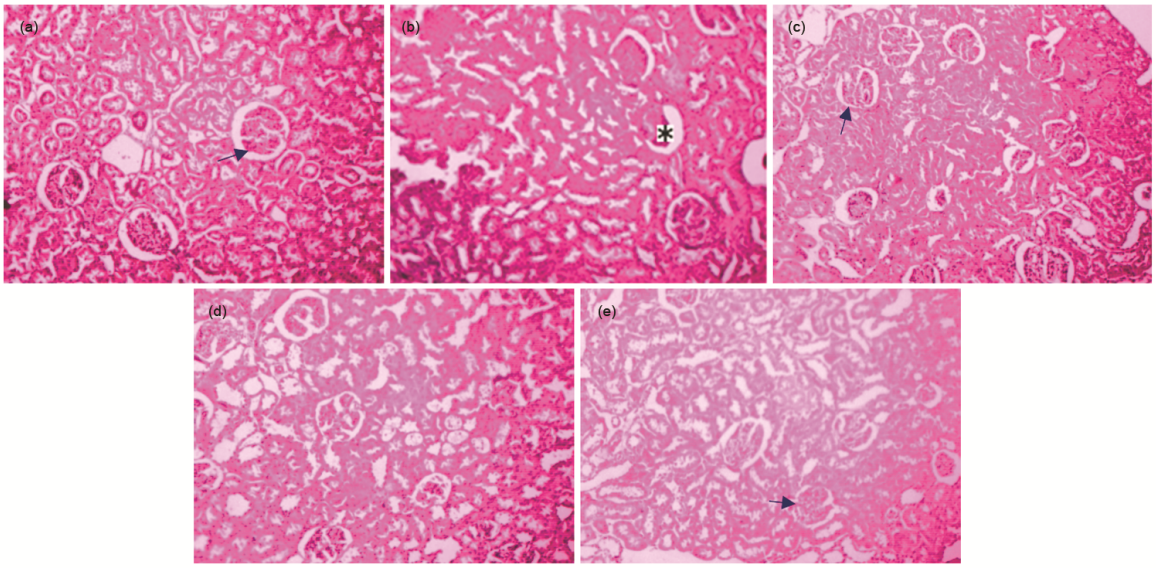

Fig. 3 — Effect of *Katakakhadirādi Kaṣāyaṃ* treatment on Kidneyof diabetic Wistar rats
Photomicrograph of kidney tissue (H&E, 100X) – (a) Normal control group-normal architecture of renal tubules and glomeruli (arrow), (b) Diabetic control group- marked tubular degeneration and swelling with glomerular atrophy and widened Bowman's space (asterisk), (c) Glibenclamide group-almost normal appearance of tubules and mild glomerular atrophy, (d) *Katakakhadirādi Kaṣāyaṃ* (4.32 ml/kg bwt) group- marked tubular degeneration and widened Bowman's space, (e) *Katakakhadirādi Kaṣāyaṃ* (8.64 mL/kg bwt) group- reduced tubular degeneration and normal appearance of glomeruli

animal experimental models exhibit oxidative stress due to persistent and chronic hyperglycemia which thereby depletes the activity of antioxidant defense system, and thus promotes denovo generation of free radicals[7]. Natural antioxidants present in the plants scavenge harmful free radicals from the body[8]. Several studies revealed that phenols mainly a type of flavonoids, from medicinal plants are safe and bioactive and possess antioxidant properties[9]. *Katakakhadirādi Kaṣāyaṃ* is a polyherbal preparation in Ayurvedic medicine consisting of 12 different types of plants; main ingredients being *Strychnos potatorum, Acacia catechu, Emblica officinalis, Berberis aristata,* etc. The phytochemical analysis of these ingredients had revealed the presence of secondary metabolites such as Alkaloids, Phenols, Flavonoids, tannins, and saponins[10-15].

In the present study, DM was induced in rats through a single intraperitoneal injection of STZ and the effect of destruction of beta-cells of islets of Langerhans was demonstrated through the elevation of blood glucose levels in these rats. Throughout the experiment, all the rats were monitored daily and/or weekly for the symptoms of DM, including polydipsia, polyuria, polyphagia, hyperglycemia and muscle wasting leading to weight loss and insulin deficiency. The body weight of normal control rats increased gradually with time whereas in diabetes induced groups body weight added on much slowly. The STZ induced diabetes is characterized by a severe loss in body weight. Due to absolute or relative deficiency of insulin and decreased production of ATP and protein synthesis decreases in all tissues. This insulin deficiency cause hyperglycemia and when blood glucose level exceeds the renal threshold, glucose excretes in urine. The loss and ineffective utilization of glucose leads to breakdown of fat and protein. Structural proteins are known to contribute to body weight, the loss or degradation of these structural proteins reflects the reduction in body weight[16].

Diabetes induced by STZ was characterized by apoptosis of cells of pancreas, attenuation of gene expression of insulin and reduced synthesis of insulin. Apoptosis of pancreatic cells is believed to be the primary factor which ultimately results in hyperglycemia[17]. In the present study, there was significant elevation in the blood glucose levels of diabetic control group compared to normal control animals. A significant reduction in blood glucose levels, in treatment group animals, were observed from 3rd week onwards up to 5th week, compared to diabetic control group which was in accordance with that reported by Kim *et al.*[18] *Katakakhadirādi Kaṣāyaṃ* treatment demonstrated significant anti-

hyperglycemic activity by bringing down the blood glucose level to near normal by 5th week in diabetic rats similar to standard drug glibenclamide. However from 7th week onwards, there observed a marked elevation in blood glucose levels of diabetes induced rats of all groups, which was consistently on the higher side till end of experiment period. It has been reported that in STZ –induced diabetes, hyperglycemia leads to progressive insulin resistance of the peripheral tissues. Most previous studies have shown that, in rodents, STZ-induced type 1 DM results in a reduced response to insulin, despite increased numbers of insulin receptors (IR), owing to hyperglycemia[19]. High glucose concentrations cause the development of insulin resistance in peripheral tissues, including skeletal muscle, owing to impairment of both insulin secretion and insulin sensitivity[20-22]. Several researchers claimed that hyperglycemia not only represents the manifestation of DM owing to the development of insulin resistance in peripheral tissues, but that it is also a self-perpetuating factor in the diabetic state, known as 'glucose toxicity'[23,24]. When exposed to chronic hyperglycemia, body tissues (especially skeletal muscle) seem to protect themselves against excessive glucose utilization, at least in part. These protective mechanisms include a reduction in insulin-stimulated glucose disposal[20]. However, chronic hyperglycemia is detrimental to both insulin sensitivity and beta-cell function[25]. Previous works demonstrated that insulin resistance in peripheral tissues and beta-cell failure may result from a defect in insulin signaling. Bevilacqua *et al.*[26] tested whether insulin resistance can evolve from a primary lesion of the beta-cell secretory function using STZ induced diabetic dogs and reported that experimentally induced insulin deficiency can lead to the development of insulin resistance; both the liver and the peripheral tissues contribute to the insulin resistance and the insulin resistance is directly related to the impairment in insulin secretion and to the degree of fasting hyperglycemia. Interestingly, another hypothesis proposed was that mitochondrial dysfunction may cause both insulin resistance in peripheral tissues and impairment of glucose-induced insulin secretion in beta cells[27]. Hence in the present study, marked elevation in blood glucose levels after a certain period of STZ injection, could be attributed to development of STZ-induced chronic hyperglycemia mediated insulin resistance in peripheral tissues since the increase in glucose level was observed in all STZ-induced diabetic rats, including Diabetic control group. Also an important observation made in this study was *Katakakhadirādi Kaṣāyaṃ* (high dose) treated rats demonstrated only mild elevation in blood glucose values when compared to the values of diabetic control and standard groups on 13th week. Hence it can be inferred that, *Katakakhadirādi Kaṣāyaṃ*, have maintained notable hypoglycemic activity, despite insulin resistance. However, its mechanism of action is yet to be studied further.

In the present study, serum lipid profile was found to be markedly increased in diabetic control group compared to the normal animals and statistically significant elevation was noted in TC and TG levels of diabetic control group animals. Animals which received *Katakakhadirādi Kaṣāyaṃ* (at high dose) and glibenclamide showed significant reduction in TG levels when compared to diabetic control group, while a reduction in TC, HDL and LDL values noted in treatment groups was not found to be statistically significant. The hyperglycemia in DM mechanism involves overproduction (excessive hepatic glycogenolysis and gluconeogenesis) and decrease in glucose utilization by the tissues and the diabetes pathogenesis associated with disturbances in carbohydrate, fat and protein metabolism. These complex multifactorial changes of metabolism often lead to functional impairment of various organs in both types of diabetes and the associated disturbances are usually characterized by hyperglycemia, hypertriglyceridemia combined with low level of insulin[28]. The serum levels of TC, LDL, and TG increases, contributing to secondary complications of diabetes[29,30]. Acute insulin deficiency initially causes an increase in free fatty acid mobilization from adipose tissue which results in an increased production of LDL[31]. Thus insulin deficiency and increased blood glucose levels lead to hyperglycaemia and hypercholesterolaemia, as observed in this study. However, the positive change in lipid levels observed in *Katakakhadirādi Kaṣāyaṃ* administered diabetic rats suggests its potential to improve serum lipid profiles.

Diabetic control rats in the present study, exhibited reduced concentrations of total protein and albumin. Serum creatinine, urea, and bilirubin were elevated, while significant increases in the activities of serum ALT, AST and ALP were observed. The negative alterations in these toxicity markers reflect the

significant impact on liver and kidney functions caused by STZ-induced diabetes. ALT and AST are common intracellular enzymes that increase the liver damage induced by diabetes[32]. In the present study, the significant increase in serum AST and ALT levels that was observed in STZ-induced diabetic rats represents liver damage compared to control rats. Most probably the liver necrosis in STZ-induced diabetic rats increased the activities of AST and ALT in serum by leakage of these enzymes from liver cytosol into the blood stream. The increase in ALP activity observed may also be a result of hepatic damage, which led to the leakage of the enzymes from the tissues to the serum[33]. However, despite hyperglycemia associated with peripheral insulin resistance, oral administration of *Katakakhadirādi Kaṣāyaṃ* showed its protective nature on liver tissue by reducing the elevated levels of AST, ALT and ALP. The increased levels of serum urea and creatinine in diabetic rats observed could be due to insulin deficiency and the consequent inability of glucose to reach extrahepatic tissues which activate gluconeogenesis as an alternative source of glucose, as reported by Gastaldelli *et al*.[34]. Also, because of the increased proteolysis needed to sustain this route, deamination of glucogenic amino acids released into the plasma consequently leads to increased urea in the blood. Creatinine is a metabolite of creatine and its concentration in serum is proportional to the body muscle mass. Elevated levels of urea and creatinine in the serum could therefore signify mild renal impairment.

Histological examinations of the rat pancreatic tissue sections with (H&E) stain revealed structural changes reflecting alterations in metabolic processes of secretion; sensitivity and regulation of insulin. Atrophy of islets, decrease in the beta cells, cellular degeneration, vacuolation and the decrease in the number of pancreatic islets are indicating features of pancreatic destruction[35]. In the present study, diabetic control group pancreatic tissues showed occasional islets with severe destruction. However in most of the diabetic rats administered with *Katakakhadirādi Kaṣāyaṃ* (high dose) resulted in normalization of the pancreatic histoarchitecture quite appreciably. The reduction in the extent of lesions in the diabetic rats treated with *Katakakhadirādi Kaṣāyaṃ* could be due to suppression of further damage to the pancreas, prevention of beta-cell death and/or recovery of partly injured beta-cells.

Histopathology of the liver revealed degenerative and necrotic changes in diabetic control animals. The observed degeneration of the hepatocytes in untreated diabetic rats could be attributed to insulin deficiency and suppression of mitochondrial β-oxidation of fatty acids, leading to deposition of triglycerides in the hepatocytes[36]. However, diabetic rats treated with *Katakakhadirādi Kaṣāyaṃ* (high dose) or glibenclamide showed hepatocytes with nearly normal appearance and minimal necrosis, supporting the results of serum liver function analysis. The hepatoprotective activity of *Katakakhadirādi Kaṣāyaṃ* may be attributed to the combined free-radical scavenging activities of polyphenols, especially flavonoids.

The histopathology of renal tissues in diabetic rats exhibited acute cellular swelling, glomerular atrophy, tubular degeneration and necrosis. Long-term damage, dysfunction and failure of the kidneys are major complications of diabetes mellitus. Hyperglycemia combined with insulin resistance have been shown to lead to oxidative stress, which is considered as one of the causative factors of diabetes-associated kidney disorders such as apoptosis, tubular atrophy and necrosis[37,38]. However, administration of *Katakakhadirādi Kaṣāyaṃ* (high dose) or glibenclamide resulted mild improvement in the architecture of the renal tissues.

Hence the results of this study reveal that *Katakakhadirādi Kaṣāyaṃ,* at high dose, has hypoglycemic, hypolipidemic and organ protective potential in Streptozotocin -induced diabetic rats. *Katakakhadirādi Kaṣāyaṃ* is rich in antioxidants, since most of the ingredients of it have been reported to have antioxidant activities[39,40]. Previous *in vivo* studies on *Strychnos potatorum* (Kataka) have established its antidiabetic activity, which may be attributed to the presence of antioxidants such as flavonoids and phenols[41]. As the development of diabetes by STZ is related to increased generation of free radicals, it may be extrapolated that the antidiabetic role of *Katakakhadirādi Kaṣāyaṃ* could be due to its antioxidant properties. However, its mechanisms of action during insulin resistance is yet to be explored.

Conclusions

From the findings of the present study with reference to bloodglucose levels, serum biochemical parameters and Histopathological analysis,

*Katakakhadirādi Kaṣāyaṃ*was found to have antidiabetic effect on Streptozotocin induced diabetic Wistar rats and it could justify the traditional use of this formulation in the management of Diabetes mellitus. However, the present study also highlights the need for further research to explore its mechanisms of action in insulin resistance. Additionally, clinical trials should be conducted in the future to validate these findings, which could potentially contribute to the development of a more effective antidiabetic formulation.

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None

Conflict of Interest

Authors declare no competing interest.

Author Contributions

VTJ: Conceptualization and guidance; SMS: Experimental study conduction and original draft preparation; HP: Methodology, Drug preparation and guidance; SP: Experimental study conduction; JM: Drug preparation; SS: Writing support. All authors reviewed the manuscript and approved the final version of the same.

Ethics Approval

The protocol was approved by Institutional Animal Ethics Committee (IAEC), protocol no. CKL/TOX/IAEC/026-2014.

Data Availability

Data will be made available by the corresponding author upon reasonable request.

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