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POTENTIAL *INVIVO* ANTIDIABETIC ACTIVITY OF "AAVARI KUDINEER FORMULATION" (AKF) IN NORMAL AND STREPTOZOTOCIN INDUCED TYPE-II DIABETIC RATS

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ABSTRACT

The present study demonstrates that "Aavarai Kudineer formulation" (AKF) exhibits promising antidiabetic activity and help to maintain good glycemic and metabolic control. The herbal formulation, AKF elicit hypo-glycaemic and antidiabetic effects in both normal and Streptozotocin (STZ) induced type- II hyperglycemic rats. The herbal formulation under acute toxicity studies shows, it is non toxic upto 2000mg/kg/BW. It is possible that the herbal formulation may act through both, pancreatic and extra-pancreatic mechanism(s). This Aavarai Kudineer formulation elicited a significant antidiabetic effect in Streptozotocin induced diabetic rats as reflected by its ability to inhibit lipid peroxidation and to elevate the enzymatic antioxidants in pancreatic tissue. This extract showed improvement in parameters like body weight, food consumption, organ weight and biochemical parameters and might be of great valuable in diabetic treatment. The histopathological studies during 28 days long term treatment have shown to ameliorate the Streptozotocin induced histological damage of Islets of Langerhans. Moreover the inhibitory effects on biochemical and histological parameters induced by herbal formulation at a dose of 500mg/kg were almost comparable to that of standard drug, Glibenclamide (5mg/kg).

KEY WORDS: **Aavarai Kudineer formulation, Antidiabetic, Streptozotocin, Biochemical parameters, Histopathological studies.**

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INTRODUCTION

Diabetes is a metabolic disorder in which a person has high blood sugar level because either the pancreas does not produce enough insulin or cells do not respond to it. Type 1 or Insulin Dependent Diabetes Mellitus known as Juvenile diabetes Mellitus. 5-10% cases are diagnosed as Type 1. It is an autoimmune disorder when the immune system goes against its own self causing destruction of the pancreatic cells that produce insulin. Type 2 or Non Insulin Dependent Diabetes Mellitus is the most common type. Slow onset with symptoms like increased thirst, increased urination and weight loss. Gestational Diabetes is seen during pregnancy. About 3-5% ladies have chances to develop Gestational diabetes. [1] In Siddha system of medicine, many single and polyherbal formulations and higher medicines like parpam, chendooram and chunnam have been practiced to cure or control diabetes mellitus from time immemorial. In modern medicine no satisfactory effective therapy is still available to cure diabetes mellitus. There is increasing demand by patients to use natural products with anti-diabetic activity due to side effects associated with the use of insulin and oral hypoglycemic agents. Recent overwhelming attention to plant products and alternative medicine has encouraged plant chemists, pharmacologists, biochemists and molecular biologists to combine their efforts in a search for natural agents that can limit diabetes mellitus and its complications. Siddha polyherbal formulations like Seenthil Kudineer, Seenthi Choornam, Vilva kudineer, Aavarai kudineer, Madhumegha choornam, Nyavalkottai Choornam, Silasathu parpam, Abraka chendooram, Triphala Choornam etc are used in treatment of Diabetes. [2] For the current research, Aavarai Kudineer formulation-AKF is selected based on survey among the Siddha physicians in and around Puducherry. It consists of equal parts of leaves of *Cassia auriculata* & *Cassia fistula*, seeds of *Syzygium cumini*, roots of *Salacia chinensis,* rhizomes of *Costus speciosus & Cyprus rotundus* and bark of *Terminalia arjuna*. The literature review revealed that there is no scientific work has been carried out on this formulation. Hence it was thought worth to carryout *invivo* antidiabetic studies on this traditional anti-diabetic herbal formulation.

MATERIALS AND METHODS

Experimental animals

Albino Wistar rats (180-220g) of either sex bred in the animal house were used in this study. The animals were fed on a standard pellet diet (Hindustan Unilever Ltd, Mumbai-400 099) and had free access to ozonised filter water *ad libitum*. The animals were maintained in their respective groups under controlled conditions of temperature and humidity. All the studies were conducted in accordance with CPCSEA guidelines and the experiments were carried out as per the approval of institutional ethics committee (IAEC-MTPG&RIHS/6040/JAN-16).[3-4]

Dose and drug solution

Traditionally 1 to 2g of the AKF is used in diabetes. Further for this study, in-house prepared AKF was extracted with distilled water, concentrated under vacuum, dried and dissolved in 1% CMC solution to have a desired dose of 125, 250 and 500 mg/kg BW in 1ml solution. Glibenclamide was obtained as a gift sample from USV Ltd, Mumbai, India. All other reagents and chemicals used were of analytical grade and procured locally.

Acute toxicity studies

The study was carried out according to the OECD guidelines 423. Female Wistar rats of weight 180-220g were taken for the study and kept for overnight fasting. Next day, body weight was taken and AKF was administered orally at a dose of 5, 50, 300 and 2000mg/kg in distilled water. Then the animals were observed for mortality and morbidity at 0, ½, 1, 2, 4, 6, 8, 12 and 24 hr. Feed was given to the animals after 4 hr of the dosing and the body weight was checked prior and at 6 hr after dosing. The animals were observed twice daily for 14 days and body weight was taken. The same experiment was repeated again on 3 rats as there was no observable clinical toxicity for the animals on the acute toxicity study.[5]

Antidiabetic screening of aavarai kudineer formulation (akf) in normal and streptozotocin induced diabetic rats.

Antidiabetic activity of Avarai Kudineer Formulation (AKF) in normal rats

Normal fasted rats: Normal albino rats (180-220 mg) were first used for the screening of the herbal formulation AKF for hypoglycemic activity. Overnight fasted normal rats were randomly divided into 5 groups, of 6 rats each. The group I served as control, which received vehicle i.e. 1%CMC (1ml/kg, orally). Group II, III and IV were treated orally with test AKF 125, 250 and 500 mg/kg, respectively. Group V received standard drug Glibenclamide 5 mg/kg orally.[6] Blood samples were collected from tail vein prior and 1, 2 and 4 hour after treatment using CONTOUR^{TM}TS blood glucose meter with same test strips. Fasting blood glucose was estimated by glucose oxidase and peroxidise (GOD/POD kit) method. Intensity of the red quinoneimine was measured at 540 nm in auto analyzer.[7-8]

Oral Glucose Tolerance Test

Overnight fasted rats were divided into 5 groups of six animals each as mentioned as above except the diabetic control and received the respective treatments. After 30 minutes of drug administration the rats of all the groups were orally administered with 2g/kg of glucose. Blood samples were collected from tail vein just prior to drug administration and at 30, 60, 120 and 240 minutes after glucose loading. Blood glucose levels were measured immediately using CONTOUR^{TM}TS blood glucose meter with same test strips.[9]

Antidiabetic activity of Aavari Kudineer Formulation (AKF) in Streptozotocin (STZ) induced diabetic rats

Adult albino Wistar rats (180-220g) of either sex were made diabetic with an intra-peritoneal injection of 65mg/kg body weight of Streptozotocin (Sigma Aldrich chemicals, USA) dissolved in 0.1 M cold citrate buffer, pH4.5, immediately before use. Streptozotocin injected animals exhibited massive glucosuria and hyperglycemia within 2-4 days. Diabetes was confirmed in STZ rats by measuring the fasting blood glucose concentration, on 4th day after the injection with STZ. Adult albino Wistar rats with blood glucose levels more than 200mg/dl were considered to be diabetic and were used in this experiment.

Single dose, short term study

After induction of diabetes, the rats were divided into 6 groups of six animals each and screened for anti-hyperglycemic activity of the various concentration of AKF in overnight fasted diabetic rats. The AKF at the dose of 125, 250 and 500mg/kg body weight were administered orally after suspending in 1% CMC solution. The blood samples were collected from tail vein and the blood glucose levels were determined using CONTOUR^{TM}TS blood glucose meter with same test strips.[10-11] The blood samples were collected from tail vein and the blood glucose levels were determined using CONTOUR^{TM}TS blood glucose meter with same test strips.

Multiple doses, long term study

In multiple dose studies the AKF at the dose of 125, 250 and 500mg/kg bodyweight once daily was given for 28 days and blood glucose levels were monitored only at seven days intervals. Blood sample were collected from tail veins of the animals. Blood glucose levels were determined using CONTOUR^{TM}TS blood glucose meter with same test strip at intervals of seven days. After 4 weeks of drug treatment, animals were sacrificed, blood withdrown for biochemical detection. The pancreas was removed and a portion of pancreatic tissue was homogenized and the extract was used for the estimation of activity of enzymes namely superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), lipid peroxidase (LPO), serum glutamate oxaloacetate transaminases (SGOT), serum glutamate pyruvate transaminases (SGPT), alkaline phosphosphatase (ALP) by colorimetric method. The body weights of all the animals of all the groups were recorded before starting the treatment and at end of the treatment period[12-15].

Haemoglobin and glycosylated Hb (HbA_1C)

Haemoglobin was estimated by the method of Drabkin's method. Intensity of the color formed by oxidized haemoglobin with potassium ferricyanide was measured at 530 nm in UV-Visble spectrophotometer (Shimadzu, Japan). Glycosylated Hb (HbA_1C) was estimated by following the method of Sudhakar Nayak and Pattabiraman, 1982. Briefly, saline washed red cells were treated with water/CCl_4 for lysis and incubated at 37^{O}C for 15minutes and oxalate or HCl solution was then added and mixed. The filtrate was heated in a boiling water bath for 4 hrs, cooled with ice-cold water, treated with 40% TCA and again centrifuged at 1000g for 10 minutes. The supernatant obtained was then heated with 80% phenol and sulphuric acid and the colour developed using thiobarbituric acid was read at 480nm after 30 minutes.[16-17].

Histopathological study of pancreas

Pancreas were isolated and preserved in 10% formalin. All paraformaldehyde fixed tissues were embedded in paraffin, sections 6µm thick cut with a cryostat microtome and then stained with haematoxylin and eosin. Histopathological observation of the tissues was carried out under a light microscope. Photomicrograph was taken to substantiate the findings under low power (10X). The alteration and changes in the histology of pancreas were shown in vide plate and the results with photomicrograph were given in the result section.[18]

Statistical analysis

The data obtained was analyzed using prism software and the results were expressed as mean ± SEM, n=6. Statistical significance was determined by using one way analysis of variance (ANOVA) followed by dunnett's multiple comparison tests. The AKF and Glibenclamide treated groups were compared with the corresponding normal or diabetic control. $P<0.01$ and $p<0.05$ were considered to be significant.

RESULTS AND DISCUSSION

Acute toxicity studies

In the acute toxicity study, Aavarai kudineer formulation up to the dose level of 2000mg/kg of body weight did not exhibit any lethality or toxic symptoms. Further dosing to estimate the LD_{50} of the formulation was not performed. According to Organization for Economic Cooperation and Development (OECD) guidelines for acute oral toxicity, an LD_{50} dose of 2000mg/kg and above is categorized as unclassified and hence the drug is safe. As 2000mg/kg of body weight was well tolerated by the animals without any behavioral changes further studies were carried out with in the 500mg/kg of body weight and the drug was found to be safe.

Antidiabetic activity in normal fasted rats

The onset of hypoglycemic activity of Aavarai kudineer formulation at 125 and 250 mg/kg was evident between 1-2 hrs, the peak was found to be at 4hs. The rats receiving 500mg/kg of Aavarai kudineer formulation showed the onset of effect at 1 hr with peak effect at 4hr. The hypoglycemic effect of Aavarai kudineer formulation at 500mg/kg was found to be nearly comparable to that of Glibenclamide 5mg/kg (Table 1).

Oral glucose tolerance test

Results of OGTT are presented in Table 2. An over dose of glucose (2g/kg BW) was fed to normal rats to evaluate the efficacy of various concentration of Aavarai kudineer formulation on anti-hyperglycemic properties. Results from this study showed that 250 mg and 500mg/kg were highly effective in bringing down the BGL from 170.3 to 127mg/dl and 109 to 85.10 mg/dl at the end of 240 minutes which was on par with the Glibenclamide that reduce BGL from 108 to 75.80 mg/dl. These results are in relationship with the earlier experiments suggesting that all concentrations are anti-hyperglycemic.

Antidiabetic activity in STZ induced diabetic rats (single dose, short term study)

Acute effect of various concentration of Aavarai kudineer formulation in overnight fasted diabetic rats presented in table 2. Blood glucose level (BGL) of rats of group-I was compared with BGL of group-II, diabetic control rats to confirm that the drug STZ has induced diabetes in experimental animals ($p<0.01$) at all interval of sampling. It was noticed that all the concentration of Aavarai kudineer formulation resulted in reduction of BGL significantly except 125mg/kg BW. 250mg and 500mg were significantly ($p<0.01$) effective in reducing initial BGL of 352.3 to 210 mg/dl and 360 to 170 mg/dl respectively which was on par with Glibenclamide that reduce BGL from 370 to 158 mg/dl at the end of 240 minutes (Table 3).

Antidiabetic activity in STZ induced diabetic rats (Multiple doses, long term studies)

The changes in BGL and body weight are reported in the Table 4 and 5. Changes in serum lipid profile are reported Table 6. There was significant ($p<0.01$) reduction in body weight in all diabetic rats within 28 days ranging from 13.78 to 27.78% (Table 5). Significant ($p<0.01$) decrease in BGL was observed in rats treated with 500mg/kg BW which was on par with Glibenclamide in reducing the BGL from 240 to 72.2 mg/dl and 258 to 64.5 mg/dl respectively. The 125mg and 250mg/kg BW also lowered BGL significantly ($p<0.05$) compared to diabetic control by bringing down from 241.2 to 162.1 and 260.2 to 102.2mg/dl respectively (Table 4). The triglycerides level of the animal treated with all the concentration have come down significantly compared to normal control group and Glibenclamide treated group which is a desired effects. Further the concentration of TC and TG decreased in 500mg/kg but in 250 and 125mg/kg it was less (table 7). This results suggested that 250 and 500mg/kg of Aavarai kudineer formulation are better than 125mg/kg BW and equivalent to standard drug Glibenclamide 5mg/kg BW.The anti-hyperglycemic activity of a drug is the ability of drug to lower very high blood sugar levels to acceptable lower levels. In literature very less work has been reported for this Aavarai kudineer formulation.[19]. In this study the report that resulted from three different independent experiments suggest that all the three concentration were anti-hyperglycemic. 250 and 500mg/kg BW were superior to 125mg/kg BW in bringing down the BGL from very high level to acceptable level within 240 minutes and the same was verified for it reproducibility of results in long duration multiple dose studies.It was confirmed that 250mg and 500mg/kg BW were on par with standard drug Glibenclamide, 5mg/kg BW in maintaining pancreatic enzyme profiles (Table 6). Further the Aavarai kudineer formulation in 250mg and 500mg/kg caused reduction in triglycerides (TG) and showed significant decreased in total cholesterol (TC) and raised insulin levels (Table 7). The Aavarai kudineer formulation in above mentioned effects was comparable with Glibenclamide 5mg/kg. Glycosylated haemoglobin (HbA_1C) level in STZ induced diabetic rat was decreased significantly after treatment with Aavarai kudineer formulation for 28 days (Table 7). In addition to decreased blood glucose level, the formulation has also effect on overall metabolic variables as evidenced by its property to lower lipid profile. Since type 2 diabetes mellitus is a metabolic disorder characterized by hyperglycemia, hyperlipidemia and insulin resistance drug therapy aiming at overall amelioration of the disorder is more desirable than a drug which decreases blood glucose alone. The results of our study shows that AKF not only normalizes blood glucose but also the hyperglycemia. It also showed in improvement in body weight and insulin levels and lipid profile of the animals. Thus the current study my pave a pathway to develop a novel formulation from a traditional dosage forms to combat diabetes mellitus and its associated complications.

Table 1

Effect of Aavarai kudineer formulation on blood glucose level in normal fasted rats

Group Treatment (dose, mg/kg, po)	Blood Glucose level (mg/dl)			
	0 hr	1 hr	2 hr	4 hr
Normal control	80.3±1.40	81.5±1.12	78.0±1.60	76.2±0.84
Aavarai kudineer formulation (125)	82.0±2.42	81.0±0.66	79.0±1.10	78.0±2.81
Aavarai kudineer formulation (250)	84.1±0.98	75.0±2.10	73.5±0.64**	70.3±1.18**
Aavarai kudineer formulation (500)	80.0±1.82	68.0±4.01	64.1±2.12**	59.0±2.26**
Glibenclamide (5)	81.1±2.18	71.0±2.14	61.3±1.20**	56.3±0.98**

Results are mean ±SEM of 6 rats in each group. One way ANOVA followed by Dunnett's multiple comparisons test was applied for comparing with the control group and treatment groups. **p value < 0.01 were considered as significant.

Table 2

Effect of Aavarai kudineer formulation on blood glucose level in glucose over loaded rats (Oral Glucose Tolerance Test)

Group & Treatment (dose mg/kg, po)	Blood Glucose Level (mg/dl)			
	30 minutes	60 minutes	120 minutes	240 minutes
Normal control	88.8±2.40	85.6±1.22	88.5±2.60	81.1±2.62
Aavarai kudineer formulation (125)	180.5±2.07	161.0±1.80	157.0±1.60	138.0±2.21
Aavarai kudineer formulation (250)	170.3±1.00	152.0±1.12*	147.3±1.06*	127.0±2.10*
Aavarai kudineer formulation (500)	109.0±1.46	99.1±2.00**	92.5±2.20**	85.1±2.31**
Glibenclamide (5)	108.0±2.20	87.0±1.20**	80.5±1.87**	75.8±2.60**

Results are mean ±SEM of 6 rats in each group. One way ANOVA followed by Dunnett's multiple comparisons test was applied for comparing with the control group and treatment groups. P value: <0.01(**), < 0.05(*) when compared to normal group.

Table 3

Effect of Aavarai kudineer formulation on blood glucoselevel in Streptozotocin-induced diabetic rats (single dose, short term study)

Group & Treatment (dose mg/kg, po)	Blood Glucose Level (mg/dl)			
	30 minutes	60 minutes	120 minutes	240 minutes
Normal control	80.8±2.40	81.6±1.22	82.5±2.60	80.1±2.62
Diabetic control	355.8±1.96 [a]	366.1±2.80 [a]	356.6±1.26 [a]	362.3±1.82 [a]
Aavarai kudineer formulation (125)	369.5±2.07	310.0±1.80 [b]	312.0±1.60 [b]	292.0±2.21 [b]
Aavarai kudineer formulation (250)	352.3±1.00	291.0±1.12 [b]	262.3±1.06 [b]	210.0±2.10 [b]
Aavarai kudineer formulation (500)	360.0±1.46	310.1±2.00 [b]	262.5±2.20 [b]	170.1±2.31 [b]
Glibenclamide (5)	370.0±2.20	298.0±1.20 [b]	200.5±1.87 [b]	158.8±2.60 [b]

Results were represented as mean ±SEM of n=6 rats in each group. One way ANOVA followed by Dunnett's multiple comparisons test was applied for comparing with the control group and treatment groups. P value: <0.01; compared to [a] normal group, [b] diabetic group.

Table 4

Effect of Aavarai kudineer formulation on blood glucose level in Streptozotocin-induced diabetic rats (multiple doses, long term study of 28 days)

Group & treatment (dose, mg/kg; p.o)	Blood glucose level (mg/dl)				
	Day 1	Day 7	Day 14	Day 21	Day 28
Normal control	76.1±1.02	81.0±1.20	81.0±0.82	82.8±2.10	78.9±4.40
Diabetic control	352.5±1.20 [a]	356.1±2.12 [a]	340.8±0.42 [a]	369.2±2.12 [a]	368.2±2.20 [a]
Aavarai kudineer formulation(125)	241.2±2.26 *	200.5±1.40 *	190.2±2.30 *	182.0±4.02 *	162.1±0.62 *
Aavarai kudineer formulation(250)	260.2±0.01*	240.2±4.20*	228.0±0.48 [b*]	174.0±2.70 [b*]	102.2±1.50 [b*]
Aavarai kudineer formulation(500)	240.0±2.21 **	122.2±2.12 **	110.0±2.12 [b**]	084.4±2.10 [b**]	072.2±2.10 [b**]
Glibenclamide(5)	258.5±2.30 **	100.2±2.12 **	090.6±1.40 [b**]	082.5±0.86 [b**]	64.5±2.12 [b**]

Results are mean ±SEM of 6 rats in each group. One way ANOVA followed by Dunnett's multiple comparisons test was applied for comparing with the control group and treatment groups. p values: <0.01** and <0.05* as compared to [a] normal group; [b] diabetic control.

Table 5

Effect of Aavarai kudineer formulation on body weight in Normal and Streptozotocin induced diabetic rats.

S. No	Groups	Initial Body weight (g)	Final Body weight (g)	%increased/ decreased of body weight
1	Normal control	182.22±2.70	210.00±8.22	+ 27.78
2	Diabetic control	189.60±0.20	160.12±2.90	- 29.48
3	Aavarai kudineer formulation (125)	182.60±2.34	161.82±8.92	- 20.78
4	Aavarai kudineer formulation (250)	184.22±1.22	168.44±2.10	- 15.78
5	Aavarai kudineer formulation (500)	188.12±8.44	174.10±4.22	- 14.02
6	Glibenclamide (5mg/kg)	190.00±1.02	176.22±8.52	- 13.78

Values are mean ± SD from 6 animals in each group. Where (+) indicates % increase of body weight. (-) indicates % decrease of body weight.

Table 6

Effect of Aavarai kudineer formulation on biochemical parameters in Streptozotocin induced diabetic rats.

Group & treatment (dose mg/kg, po)	SGOT (IU/L)	SGPT (IU/L)	Alkaline phosphatase (IU/L)	% Lipid Peroxi-dation	CAT (U/mg)	GP_X (U/mg)
Normal control	62.12±1.10	51.08±0.30	137.90±1.20	67.22±5.02	3.26±2.14	2.67±0.82
Diabetic control	142.70±1.12 [a]	104.10±0.44 [a]	260.20±1.12 [a]	112.0±0.50	1.600.16 [a]	1.66±0.14 [a]
AKF 125	115.8±1.06 [b]	85.16±0.64 [b]	194.30±1.17 [b]	80.56±0.24 [b]	2.20±0.17 [b]	2.10±1.09 [b]
AKF 250	95.23±5.02 [b]	74.66±1.58 [b]	164.20±0.42 [b]	74.36±0.50 [b]	2.46±0.69 [b]	2.26±0.04 [b]
AKF 500	80.36±2.11 [b]	65.34±0.24 [b]	149.20±0.49 [b]	65.32±0.85 [b]	2.96±0.13 [b]	2.68±1.17 [b]
Glibenclamide (5 mg/kg)	73.33±0.18 [b]	58.30±2.45 [b]	145.30±0.14 [b]	60.93±0.13 [b]	3.10±3.32 [b]	2.50±0.15 [b]

Results are mean ±SEM of 6 rats in each group. One way ANOVA followed by Dunnett's multiple comparisons test was applied for comparing with the control group and treatment groups. p value: <0.01; compared to [a] normal group [b] diabetic

Table 7

Effect of Aavarai kudineer formulation on Total Cholesterol (TC), Triglycerides (TG), Insulin (I), Hemoglobin (Hb) and Glycosylated hemoglobin (HbA_1C) levels in diabetic rats.

Group Treatment (dose, mg/kg, po)	TC (mg/dl)	TG (mg/dl)	I (µU/ml)	Hb (%g)	HbA_1C (%g)
Normal control	101.5±1.40	67.6±1.12	33.6±1.60	13.2±0.84	4.63±1.20
Diabetic control	307.3±0.46	179.6±2.02	25.9±1.30	9.7±0.61	7.14±0.07
AKF (125)	282.0±2.42	147.0±0.66	24.0±1.10	8.0±2.81	6.08±0.06
AKF (250)	174.1±0.98**	75.0±2.10**	33.5±0.64 **	10.3±1.18**	5.70±0.98 **
AKF (500)	169.0±1.82**	69.0±4.01**	32.1±2.12**	13.0±2.26**	5.29±4.10 **
Glibenclamide (5)	166.1±2.18**	71.0±2.14**	35.3±1.20 **	12.3±0.98 **	5.27±1.96**

Results are mean ±SEM of 6 rats in each group. One way ANOVA followed by Dunnett's multiple comparisons test was applied for comparing with the control group and treatment groups. P value < 0.01(**) were considered as significant.

HISTOPATHOLOGICAL STUDIES

Pancreas section of rat of normal group (Fig.1) revealing endocrine Islets of Langerhans and exocrine pancreatic acini of serous epithelial cells. No fibrosis or inflammation was found. Pancreas section of rat of diabetic control group (Fig.2 and 2A) Islets showing disrupted beta cells and reduction in beta cell mass. Degenerative changes and necrosis in islets of langerhans and acini were observed in this group. Pancreas section of rat treated with Aavarai kudineer formulation 125mg/kg (Fig.3) showed that minimal necrosis and mild to moderate atrophy of islets and fibrotic changes were found. Pancreas section of rat treated with Aavarai kudineer formulation 250mg/kg (Fig.4) showed moderate degenerative changes in endocrine and exocrine pancreas along with disruption of islets of Langerhans observed. Minimal necrosis and mild to moderate atrophy and fibrotic changes were found. Pancreas section of rat treated with Aavarai kudineer formulation 500mg/kg (Fig.5) clearly projects clear restoration of beta cells and exocrine glands. Mild degenerative changes in pancreatic parenchyma were observed. Acini and intercalated ducts are showing normal morphology. No necrosis and mild to moderate atrophy and fibrotic changes were found. Pancreas section of rat treated with 5 mg/kg Glibenclamide (Fig.6) showed that restoration of beta cells and exocrine glands. Acini and intercalated ducts are showing normal morphology. No necrosis and mild to moderate atrophy and fibrotic changes were found.

Histopathological observations of Aavarai kudineer formulationand Glibenclamide treated pancreas in streptozotocin-induced diabetic rats (under low power)

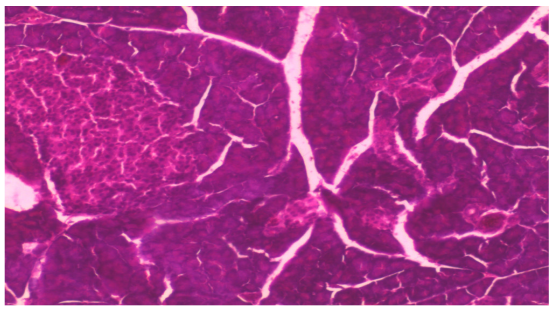

Figure 1
Group I – Normal Control showing presence of normal pancreatic islet cells

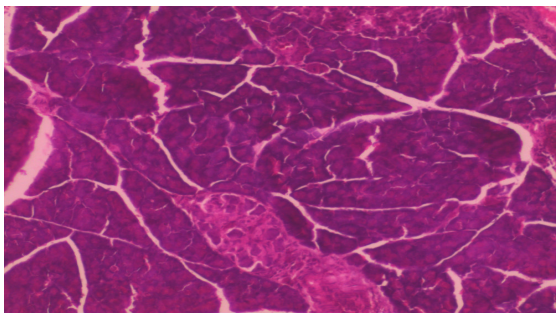

Figure 2
Group II- Diabetic control showing reduction in islet cell mass

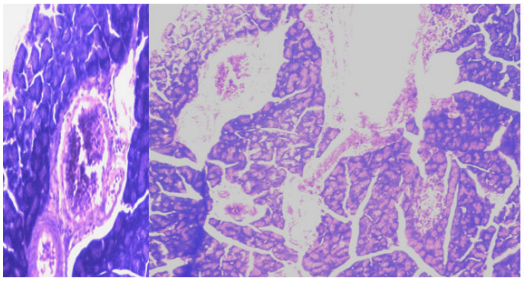

Figure 2A
Group II- Diabetic control showing necrosis of islets

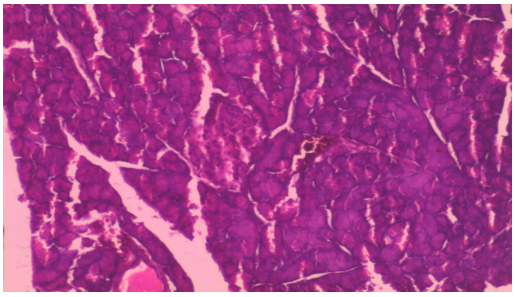

Figure 3
Group III- Test drug low dose treated group (125mg/kg) showing reduction in islets cell mass

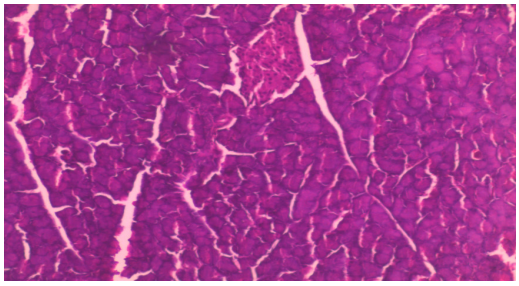

Figure 4
Group IV- Test drug mid dose treated group (250mg/kg) also showing reduction in islets cell mass

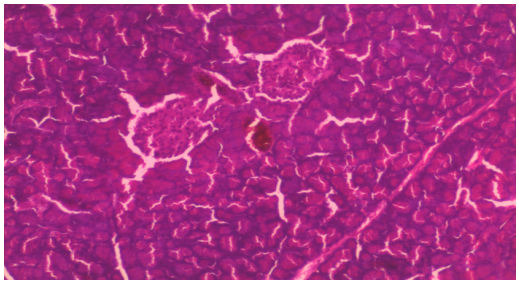

Figure 5
Group V- Test drug high dose treated group (500mg/kg) showing regeneration and restoration of islet cells

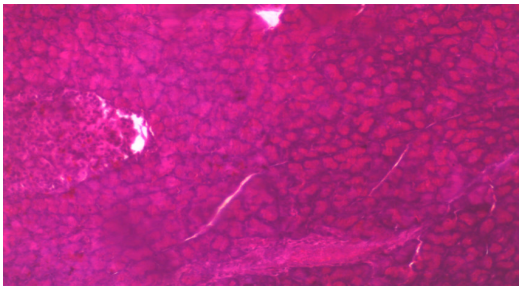

Figure 6
Group VI- Standard drug treated group showing mild reduction of islets cell mass

CONCLUSION

The extract of Aavarai Kudineer formulation exhibited significant anti-hyperglycemic activity in normal and Streptozotocin (STZ) induced diabetic rats. This extract showed improvement in parameters like body weight, biochemical parameters and histopathological changes which might be of great valuable in diabetic treatment. The results are encouraging and on par with currently used allopathic drug Glibenclamide. This study strongly proves the traditional use of this Siddha, polyherbal, antidiabetic formulation Aavarai kudineer scientifically.

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CONFLICT OF INTEREST

The authors are declare that there is no conflict of interest.

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