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ANTI-DIABETIC ACTIVITY OF NEERIZHIVU CHOORNAM IN NORMAL AND STREPTOZOTOCIN (STZ) INDUCED TYPE-II DIABETIC RATS

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ABSTRACT

There are several reputed anti-diabetic polyherbal drug are available in Siddha medical practice, all are time tested, proven and synergic herbal combined together for the management of Diabetes mellitus. One among them is Neerizhivu choornam consisting seven herbal ingredient of different families of diverse chemical nature but able to control blood sugar when ingested orally. Particularly this kind of medicine has high attention in Siddha and Ayurveda medicine when compared with other remedies prescribed for Type-II diabetes mellitus. But lack of scientific evidence worsens the use of valuable medicine and it is still under used. Hence the present attempt highlighted the pharmacological of effects of Neerizhivu choornam in normal and experimentally induced type-2 diabetes mellitus. The outcome of results might encourages the physician to clinically expedite the use of Neerizhivu choornam into a greater extent for the management of diabetes.

Key words: Neerizhivu Choornam, Siddha medicine, Type-II Diabetes mellitus, Pharmacological activity.

INTRODUCTION

Diabetes mellitus, a metabolic disorder, is becoming a serious threat to health of people. The prevalence of diabetes mellitus is expected to reach up to 4.4% in the world by 2030 (Patel *et al*., 2010). The people with diabetes in the world are expected to approximately double between 2000 and 2025. India leads the world with largest number of diabetic subjects being termed as ‘diabetes capital of the world (Sarah Wild *et al*., 2010; Mohan *et al*., 2010). Plants have been used since time immemorial for medicinal purposes and form the origin of much of modern Pharmacotherapy. Many plants are reported to be useful in the treatment of Diabetes mellitus. Neerizhivu Choornam is a polyherbal Siddha formulation useful in the treatment of Diabetes. Literature survey on Neerizhivu choornam was carried out for its pharmacological aspects. The literature review revealed that the Neerizhivu choornam is scientifically under explored. Further the ingredients of Neerizhivu choornam have been proved to be Hypoglycemic, Anti-hyperglycemic, Anti-diabetic and Hypocholestrolemic. Phyto-formulation of Neerizhivu choornam is not studied till now.

Neerizhivu Chooranam consists of fine powders of Kadukkai – *Terminalia chebula* (Pericarp), Karuveppilei – *Murraya konigii* (Whole plant), Nellivatral – *Embilica officianalis* (Pericarp), Naval Kottai – *Syzygium cumini* (Seed), Seenthil – *Tinospora cardifolia* (Stem), Kizanelli – *Phyllanthus amarus* (Whole plant) and Koraikizhangu – *Cyperus cariosus* (Rhizome). Polyherbal formulation in powdered form where the botanical ingredients are not more than ten can be identified microscopically (Anonymous, 2010). The

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hypoglycemic activity of alcoholic extract of individual ingredients of *Cyperus scariosus* (Gandhipuram Periasamy *et al*., 2006), *Terminalia chebula* (Nagaraja Pranik *et al*., 2010), *Tinospora cardifolia* (Shetti *et al*., 2010), *Phyllanthus amarus* (Mastan *et al*., 2009), *Syzygium cumini* (Vinuthan *et al*., 2004), *Embilica officianalis* and *Murraya konigii* (Baynes JW and THrope SR, 1999) is already reported. In the present investigation Pharmacological screening of the various concentration of this formulation was carried out.

MATERIALS AND METHODS

Experimental animals

Albino Wistar rats (180-220g) of either sex bred in the animal house of drug testing laboratory, Bangalore were procured and 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 (Kaneto *et al*., 2005). 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-XII/SRU/78/2008).

Dose and drug solution

Traditionally 1 to 2g of the Neerizhivu choornam is used in diabetes. Further for this study, in-house prepared Neerizhivu choornam was suspended in 1% gum acacia 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 Neerizhivu choornam was administered orally at a dose of 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 will be repeated once again on 3 rats (preferably female) if there is no observable clinical toxicity for the animals on the acute toxicity study (Arora *et al*., 1999; Mallurwar and Pathak, 2008).

Hypoglycemic activity of Neerizhivu choornam in normal rats

Normal fasted rats: Normal albino rats (180-220 mg) were first used for the screening of the herbal formulation Neerizhivu choornam 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% W/V Gum acacia solution (1ml/kg, orally). Group II, III and IV were treated orally with test Neerizhivu choornam 125, 250 and 500 mg/kg, respectively. Group V received standard drug Glibenclamide 5 mg/kg orally.

Blood samples were collected from tail vein prior and 1, 2, 4 and 6 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. The percentage (%) fall in blood glucose level was also calculated at peak hour of effect (Prince *et al*., 2004; Georg and Ludvik, 2000; Pesmi *et al*., 2001).

Antidiabetic activity of Neerizhivu choornam in Streptozotocin (STZ) induced diabetic rats

Induction of experimental diabetes

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 chemical company, Mumbai) dissolved in 0.1 M cold citrate buffer, pH4.5, immediately before use. Streptozotocin injected animals exhibited massive glucosuria and hyperglycemia within few 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. The Neerizhivu choornam at the dose of 125, 250 and 500mg/kg body weight were administered orally after suspending in 1% gum acacia 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 (Mukherjee *et al*., 2006; Grover *et al*., 2002).

Experiment no 1.

Evaluation of Neerizhivu choornam for anti-hyperglycemic properties in STZ induced diabetic rats (single dose, short term study)

After induction of diabetes, the rats were divided into 6 groups of six animal each and screened for anti-hyperglycemic activity of the various concentration of Neerizhivu choornam in overnight fasted diabetic rats. 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.

Experiment no 2.

Evaluation of Neerizhivu choornam for anti-hyperglycemic properties in STZ induced diabetic rats in presence of glucose load (oral glucose tolerance test).

Overnight fasted rats were divided into 6 groups of six animal each as mentioned as above and received the respective treatments. After 30 minutes of drug administration the rat 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.

Experiment no3.

Evaluation of Neerizhivu choornam for anti-hyperglycemic properties in STZ induced diabetic rats (multiple dose, long term study)

In multiple dose studies the Neerizhivu choornam 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 on every seven days. After 4 weeks of drug treatment, parameters such as fasting blood glucose, 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 (Sabu and Kuttan, 2000; Pandey *et al*., 2002).

Estimation of blood parameters

Blood samples were collected from the retro-orbital plexus of the rats and the blood glucose level was estimated by GOD-POD method, total cholesterol were estimated by CHOD-PAP method and triglycerides level was estimated by GPO-ESPAS method using Ranbaxy diagnostic kits, New Delhi following the kit's procedure. Serum insulin levels were estimated by Radio-immuno assay method by using R.I.A kit. (Baba atomic research centre, Mumbai, India). the results are expressed as µU of insulin ml[22].

Haemoglobin and glycosilated 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). Glycosilated 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^OC 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 (Chandra Shekhar Joshi *et al*., 2007).

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 were taken to substantiate the findings. 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 (Edwin *et al*., 2009; Rajesh *et al*., 2009).

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 test. The herbal Neerizhivu choornam 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, Neerizhivu choornam upto the dose level of 2000mg/kg of body weight did not exhibit any lethality or toxic symtoms. Further dosing to estimate the LD50 of the formulation was not performed. According to Organisation for economic cooperation and development (OECD) guidelines for acute oral toxicity, an LD50 dose of 2000mg/kg and above is categorized as unclassified and hence the drug is found to be 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 (results not shown).

Hypoglycemic activity in normal fasted rats

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

Hypoglycemic effects in STZ induced diabetic rats

Acute effect of various concentration of Neerizhivu choornam in overnight fasted diabetic rats presented table. Blood glucose level (BGL) of rats of group-I was compared with BGL of other 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 Neerizhivu choornam resulted in reduction of BGL significantly except 125mg/kg BW. 250mg and 500mg were significantly ($p<0.01$) effective in reducing initial BGL of 252 to 150 mg/dl and 260 to 100 mg/dl respectively which was on par with glibenclamide that reduce BGL from 270 to 98 mg/dl at the end of 240 minutes (table 5).

Oral glucose tolerance test

Results of OGTT are presented in table 6 . An over dose of glucose was fed to diabetic and normal rats to evaluate the efficacy of various concentration of Neerizhivu choornam on anti-hyperglycemic properties. Results from this study showed that 250 mg and 500mg/kg were highly effective in bringing down the BGL from 570 to 127mg/dl and 589 to 95 mg/dl at the end of 240 minutes which was on par with the Glibenclamide that reduce BGL from 588 to 75 mg/dl. These results are in consonance with the earlier experiments suggesting that all concentrations are anti-hyperglycemic.

Multiple dose studies

The changes in BGL and body weight are reported in the table 7 and 8. and changes in serum lipid profile are reported table 9. There was significant ($p<0.01$) reduction in body weight in all diabetic rats within 28 days ranging from 14.7 to 27.4%. 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 mg/dl and 258 to 64 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 to 162 and 260 to 102mg/dl respectively (table 7). 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 10). This results suggested that 250 and 500mg/kg of Neerizhivu choornam 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 Neerizhivu choornam. In this study we report that result from three different independent experiments suggested 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 serum lipid profiles (table 9). Further the Neerizhivu choornam in 250mg and 500mg/kg caused reduction in triglycerides (TG) and showed significant decreased in total cholesterol (TC) and raised insulin levels. The Neerizhivu choornam in above mentioned effects were comparable with Glibenclamide 5mg/kg. Glycosylated haemoglobin (HbA_1C) level in STZ induced diabetic rat was decreased significantly after treatment with Neerizhivu choornam for 28 days (table 10).

Table 1. Experimental protocol for hypoglycemic activity of Neerizhivu choornam in normal rats

Group I	Treated with 1% Gum acacia solution, 1ml/kg
Group II	Treated orally with Neerizhivu choornam,125mg/kg
Group III	Treated orally with Neerizhivu choornam,250mg/kg
Group IV	Treated orally with Neerizhivu choornam,500mg/kg
Group V	Treated orally with Glibenclamide,5mg/kg

Table 2. Experimental protocol for anti-hyperglycemic properties in STZ induced diabetic rats (Single dose, short term study)

Group	Treatment
Group-I	Healthy rats, treated with 1%w/v gum acacia
Group-II	Diabetic rats, 1%w/v gum acacia
Group-III	Treated with Neerizhivu choornam,125mg/kg
Group-IV	Treated with Neerizhivu choornam,250mg/kg
Group -V	Treated with Neerizhivu choornam,500mg/kg
Group -VI	Treated with Glibenclamide,5mg/kg

Table 3. Experimental protocol for anti-hyperglycemic properties in STZ induced diabetic rats (Multiple dose, long term study)

Group 1	Normal control and received vehicle i.e. 1% Gum acacia Solution,
Group 2	Diabetic control and received (STZ) + 1% Gum acacia solution,
Group 3	Treated orally with Neerizhivu choornam,125mg/kg
Group 4	Treated orally with Neerizhivu choornam,250mg/kg
Group 5	Treated orally with Neerizhivu choornam,500mg/kg
Group 6	Received Glibenclamide,5mg/kg

Table 4. Effect of Neerizhivu choornam 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	6 hr
Normal control	70.3±1.40	72.5±1.12	71.0±1.60	70.2±0.84	78.2±1.60
Neerizhivu choornam (125)	72.0±2.42	72.0±0.66	71.0±1.10	69.0±2.81	70.3±1.56
Neerizhivu choornam (250)	74.1±0.98	75.0±2.10	73.5±0.64	70.3±1.18**	72.0±0.98 **
Neerizhivu choornam (500)	80.0±1.82	78.0±4.01	74.1±2.12	69.0±2.26**	73.0±4.10 **
Glibenclamide (5)	71.1±2.18	77.0±2.14	71.3±1.20	66.3±0.98 **	70.3±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.

Table 5. Effect of Neerizhivu choornam on blood glucose level 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	70.8±2.40	71.6±1.22	72.5±2.60	70.1±2.62
Diabetic control	255.8±1.96 [a]	266.1±2.80 [a]	256.6±1.26 [a]	262.3±1.82 [a]
Neerizhivu choornam (125)	269.5±2.07	210.0±1.80 [b]	212.0±1.60 [b]	192.0±2.21 [b]
Neerizhivu choornam (250)	252.3±1.00	191.0±1.12 [b]	162.3±1.06 [b]	150.0±2.10 [b]
Neerizhivu choornam (500)	260.0±1.46	110.1±2.00 [b]	102.5±2.20 [b]	100.1±2.31 [b]
Glibenclamide (5)	270.0±2.20	118.0±1.20 [b]	100.5±1.87 [b]	98.8±2.60 [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 group

Table 6. Effect of Neerizhivu choornam on blood glucose level in Streptozotocin-induced diabetic 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	58.8±2.40	65.6±1.22	58.5±2.60	61.1±2.62
Diabetic control	574.8±1.96 [a]	534.1±2.80 [a]	379.6±1.26 [a]	333.34±1.82 [a]
Neerizhivu choornam (125)	580.5±2.07	431.0±1.80 [b]	317.0±1.60 [b]	288.0±2.21 [b]
Neerizhivu choornam (250)	570.3±1.00	262.0±1.12 [b]	217.3±1.06 [b]	127.0±2.10 [b]
Neerizhivu choornam (500)	589.0±1.46	269.1±2.00 [b]	192.5±2.20 [b]	95.1±2.31 [b]
Glibenclamide (5)	588.0±2.20	247.0±1.20 [b]	180.5±1.87 [b]	75.8±2.60 [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 group

Table 7. Effect of Neerizhivu choornam on blood glucose level in Streptozotocin-induced diabetic rats (long term study of 28 days at daily once)

Group & treatment (dose, mg/kg; p.o)	Blood glucose level (mg/dl)				
	Day 1	Day 7	Day 14	Day 21	Day 28
Normal control	66.1±1.02	71.0±1.20	71.0±0.82	72.8±2.10	68.9±4.40
Diabetic control	252.5±1.20 [a]	256.1±2.12[a]	240.8±0.42[a]	269.2±2.12[a]	268.2±2.20 [a]
Neerizhivu choornam (125)	241.2±2.26 [b]	240.5±1.40[c]	200.2±2.30[c]	190.0±4.02[c]	162.1±0.62 [c]
Neerizhivu choornam (250)	260.2±0.01 [c]	170.2±4.20[c]	128.0±0.48[c]	114.0±2.70[c]	102.2±1.50 [c]
Neerizhivu choornam (500)	240.0±2.21 [c]	102.2±2.12[c]	090.0±2.12[c]	082.4±2.10[c]	072.2±2.10 [c]
Glibenclamide (5)	258.5±2.30 [c]	100.2±2.12[c]	090.6±1.40[c]	082.5±0.86[c]	064.5±2.12 [c]

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, as compared to [a] normal group; [c] diabetic control group [b]<0.05 compared to diabetic group.

Table 8. Effect of Neerizhivu choornam 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	180.22±2.70	212.00±8.22	+ 31.78
2	Diabetic control	199.60±10.20	160.12±2.90	- 39.48
3	Neerizhivu choornam (125)	182.60±12.34	150.82±8.92	- 31.78
4	Neerizhivu choornam (250)	184.22±10.22	163.44±12.12	- 20.78
5	Neerizhivu choornam (500)	188.12±8.44	174.10±4.22	- 14.02
6	Glibenclamide (5mg/kg)	190.00±11.02	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 9. Effect of Neerizhivu choornam Neerizhivu choornam on biochemical parameters in Streptozotocin induced diabetic rats

Group & treatment (dose mg/kg, po)	SGOT (IU/L)	SGPT (IU/L)	Alkaline phosphatise (IU/L)	% Lipid Peroxi-dation	CAT (U/mg)	GP_X (U/mg)
Normal control	72.12± 0.12	41.08± 0.36	132.90± 0.25	60.22±1.02	3.16± 0.14	2.56± 0.10
Diabetic control	138.20±0.18 [a]	98.16± .44 [a]	250.23± 0.22 [a]	102.0± 1.59	1.88± 0.169 [a]	1.83± 0.175 [a]
Neerizhivu choornam 125	115.8± 0.16[b]	75.16± 0.58 [b]	216.30± 0.42 [b]	80.56± 0.04 [b]	2.20± 0.817 [b]	2.10± 0.09 [b]
Neerizhivu choornam 250	105.23±0.06 [b]	64.66± 0.60 [b]	194.20± 0.14 [b]	74.36± 0.15 [b]	2.46± 0.069 [b]	2.26± 0.04 [b]
Neerizhivu choornam 500	90.36± 0.06 [b]	55.34±0.45 [b]	169.20± 0.17 [b]	65.32± 0.58 [b]	2.76± 0.31 [b]	2.68± 0.17 [b]
Glibenclamide (5 mg/kg)	83.33± 0.18 [b]	48.30± 0.24 [b]	155.30± 0.40 [b]	60.93± 0.36 [b]	2.80± 0.23 [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 10. Effect of Neerizhivu choornam 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	71.5±1.40	67.6±1.12	33.6±1.60	13.2±0.84	0.232±1.60
Diabetic control	87.3±0.46	79.6±2.02	25.9±1.30	9.7±0.61	0.405±0.20
Neerizhivu choornam (125)	82.0±2.42	77.0±0.66	24.0±1.10	8.0±2.81	0.389±1.56
Neerizhivu choornam (250)	74.1±0.98**	75.0±2.10**	33.5±0.64 **	10.3±1.18**	0.300±0.98 **
Neerizhivu choornam (500)	69.0±1.82**	69.0±4.01**	32.1±2.12**	13.0±2.26**	0.290±4.10 **
Glibenclamide (5)	66.1±2.18**	71.0±2.14**	35.3±1.20 **	12.3±0.98 **	0.270±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

Microscopically examine pancreas section show the following features:

Pancreas section of rat of normal group (fig.1) showed that normal architecture of pancreas with acini of serous epithelial cells along with nest of endocrine cells separated by fibro-collagenous, stroma into lobules. No fibrosis or inflammation was found. Pancreas section of rat of diabetic control group (fig.2) showed that abnormal architecture of pancreas with acini of serous epithelial cells along with nest of endocrine cells separated by fibrocollagenous, stroma into lobules. Necrosis, atrophy and fibrotic changes were found. Pancreas section of rat treated with Neerizhivu choornam 125mg/kg (fig.3) showed that abnormal architecture of pancreas with acini of serous epithelial cells along with nest of endocrine cells separated by fibrocollagenous, stroma into lobules. Minimal necrosis and mild to moderate atrophy and fibrotic changes were found. Pancreas section of rat treated with Neerizhivu choornam 250mg/kg (fig.4) showed that slight normal architecture of pancreas with acini of serous epithelial cells along with nest of endocrine cells separated by fibrocollagenous, stroma into lobules. Minimal necrosis and mild to moderate atrophy and fibrotic changes were found. Pancreas section of rat treated with Neerizhivu choornam 500mg/kg (fig.5) showed that normal architecture of pancreas with acini of serous epithelial cells along with nest of endocrine cells separated by fibrocollagenous, stroma into lobules. No necrosis and mild to moderate atrophy and fibrotic mg/kg changes were found. Pancreas section of rat treated with 5 Glibenclamide (fig.6) showed that normal architecture of pancreas with acini of serous epithelial cells along with nest of endocrine cells separated by fibrocollagenous, stroma into lobules. no necrosis and mild to moderate atrophy and fibrotic changes were found.

Fig. 1. Normal Control

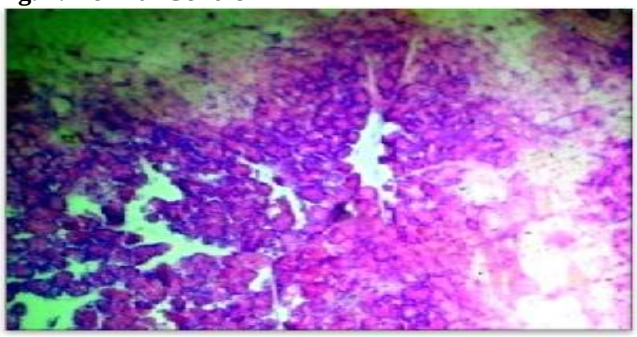


Fig. 2. Diabetic Control

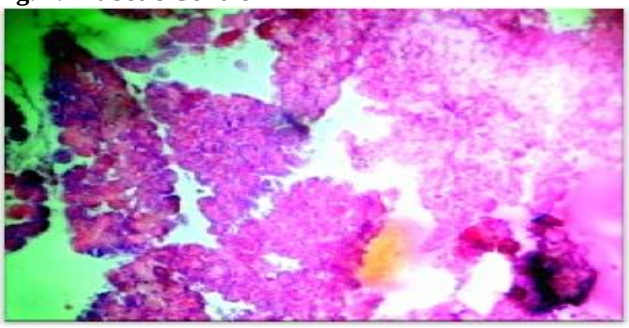


Fig. 3. Neerizhivu choornam (125mg/kg)

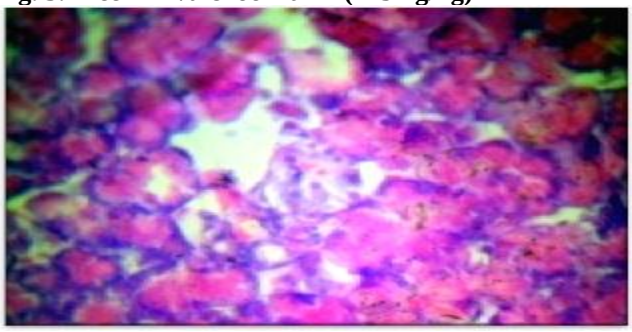


Fig. 4. Neerizhivu choornam (250mg/kg)

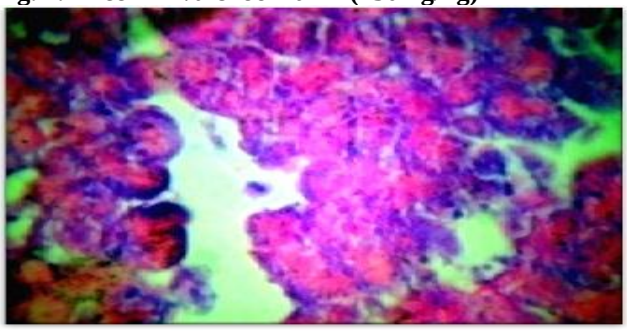


Fig. 5. Neerizhivu choornam (500mg/kg)

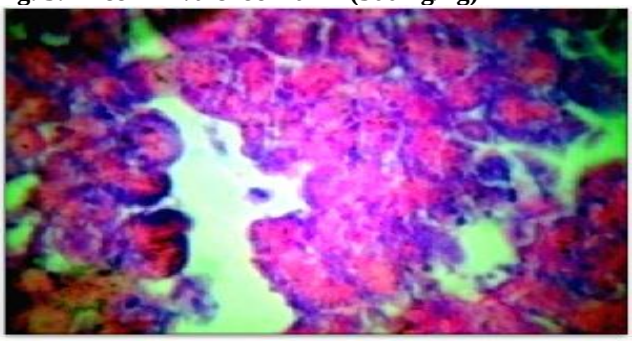
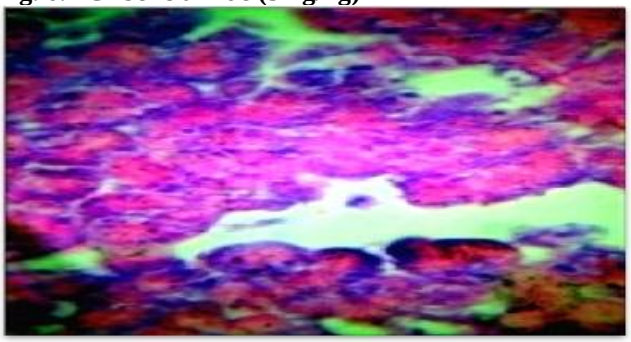


Fig. 6. Glibenclamide (5mg/kg)



CONCLUSION

In conclusion, it can be said that, Neerizhivu choornam (250-500mg/kg/bw) is a potential anti-diabetic herbal formulation and hence it is interesting to investigate its underlying molecular mechanisms of action. The 250 & 500mg/kg of Neerizhivu choornam exhibited significant anti-hyperglycemic activity in Streptozotocin induced type-II diabetic rats. This formulation showed improvement in parameters like blood glucose, pancreatic anti-oxidant enzymes, body weight, lipid profiles, insulin, hemoglobin and glycosylated hemoglobin levels and re-alteration in the pancreatic histopathological changes which might be of valuable in diabetic treatment.

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CONFLICT OF INTEREST

The authors do not have any conflict of interest for this review work on Neerizhivu choornam.

REFERENCES

Anonymous, Laboratory guide for the analysis of Ayurveda and Siddha Formulations, Central Council for Research in Ayurveda and Siddha. Dept of AYUSH, Ministry of Health and Family Welfare, Govt. of India, New Delhi, 2010.

Arora D, Dubey SD and Ojha JK. Role of Rasayana in preventing the complication of diabetes mellitus, *Journal Diab Assoc India*, 39, 1999, 4.

Baynes JW, THrope SR. Role of oxidative stress in diabetic complications. *Diabetes*, 48, 1999, 1-4.

Chandra Shekhar Joshi, Ekambaram Sanmuga Priya, and Subramanian Venkatraman. Acute and subacute Toxicity Studies on the polyherbal Antidiabetic Neerizhivu choornam Diakyur in Experimental Animal Models. *Journal of Health Science*, 53(2), 2007, 245-249.

Edwin J, Joshi SB and Jain DC. Biochemical study on the hypoglycemic effects of extract and fraction of barks of *Cassia fistula* Linn.in Streptozotocin-induced diabetic rats. *Indian drugs*, 46(7), 2009, 572.

Gandhipuram Periasamy Senthil Kumar, Palanisamy Arulselvan, Durairaj Sathish Kumar and Sorimuthu Pillai Subramanian. Anti-Diabetic activity of fruits of *Terminalia chebula* on streptozotocin induced diabetic rats. *Journal of Health Science*, 52(3), 2006, 283-291.

Georg P, Ludvik B. Lipids and Diabetes. *Journal of Clinical and Basic Cardiology*, 3, 2000, 159-162.

Grover JK, Yadav S, Vats V. Medicinal plants of India with anti-diabetic potential, *Journal of Ethnopharmacol*, 81, 2002, 81.

Kaneto H, Matsuoka T, Nakatani Y, Kamamori D, Matsunisa M and Yamasaki Y. Oxidative stress and the JNK pathway in diabetes. *Curr Diab Rev*, 1, 2005, 65.

Mallurwar V R and Pathak AK. Studies on Immuno modulatory activity of *Ficus religiosa*. *Indian Journal of Pharm Educ Res*, 2008, 42, 341.

Mastan SK, BhavyaLatha T, Sri Latha T, Srikanth A, Chaitanya G and Eswar Kumar K. Influence of Methanolic Extract of *Syzygium cumini* seeds on the activity of Gliclazide in normal and Alloxan-induced diabetic rats. *Pharmacologyonline*, 3, 2009, 845-850.

Mohammad AsrafulAlam, RownakJahan, Shahnaz Rahman, Asish Kumar Das and Mohammed Rahmatullah. Antinociceptive and Anti-hyperglycemic activities of methanol leaf extract of *Cyperus cariosus*. *Pak J Pharm Sci*, 24(1), 2011, 53-56.

Mohan V, Sandeep S, Deepa B, Shah and Varghese C. Epidemiology of type 2 diabetes, Indian scenario. *Indian J Med Res*, 125, 2007, 217-230.

Mukherjee PK, Maiti K, Mukherjee K, Houghton PJ. Leads From Indian medicinal plants with hypoglycemic potentials. *Journal of Ethnopharmacol*, 1, 2006,106.

Nagaraja Pranik, Kararashah Fakruddin kammar, Sheela devi. Anti-diabetic activity of *Tinospora cordifolia* in streptozocin diabetic rats, does it act like sulfonylureas. *Turk J Med Sci*, 40(2), 2010, 265-270.

Pandey VN, Rajagopalan SS, Chowdhorry DP. An effective Ayurvedic hypoglycemic Neerizhivu choornam. *Journal of Ethnopharmacol*, 81, 2002,155.

Patel DK, Kumar R, Laloo D and Hemalatha S. Natural medicines from plant source used for therapy of diabetes mellitus, An overview of its pharmacological aspects. *Asian Pacific Journal of Tropical Disease*. 2012, 239-250.

Pesmi CR, Fathima A, Silnilal B and Latha MS. Antidiabetic effect of a herbal drug in streptozotocin –diabetic rats. *Indian Drugs*, 38(6), 2001, 319-322.

Prince PS, Padmanabhan M and Menon VP. Restoration of antioxidant defense by ethanolic extract of *Tinospora cardifolia* root extract in streptozotocin –induced diabetic liver and Kindney, *Phytoher Res*, 18, 2004, 785.

Rajesh MS, Harish MS, Sathyaprakash RJ, Raghuram Shetty, Shivananda TN. Anti hyperglycemic activity of the various extracts of *Costus speciosus* rhizomes. *Journal of natural remedies*, 9(2), 2009, 235-241.

Sabu MC, Kuttan R. Antidiabetic activity of medicinal plants and its relationship with their antioxidant property. *Journal of Ethnopharmacol*, 81, 2000, 155.

Sarah Wild, GojkaRoglic, Anders Green. Richard Sicree and Hilary King, Global Prevalence of Diabetes Estimates for the year 2000 and projections for 2030. *Diabetes Care*, 27(5), 2004.

Shetti AA, Sanakal RD and Kaliwal BB. Antidiabetic effect of ethanolic leaf extract of *Phyllanthus amarus* in alloxan induced diabetic mice. *Asian Journal of Plant Science and Research*, 2012, 2(1), 11-15.

Vinuthan MK, Girish Kumar V, Ravindra JP, Jayaprakash and Narayana K. Effect of Extracts of *Murraya koenigii* leaves on the leaves of blood glucose and plasma insulin in Alloxan-induced diabetic rats. *Indian J Physiol Pharmacol*, 48(3), 2004, 348-352.