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EVALUATION OF THE ANTI-DIABETIC ACTIVITY OF THETRAN VIDHAI KUDINEER ON FRUCTOSE AND STREPTOZOTOCIN INDUCED DIABETIC RATS

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Keywords:

TVK, Siddha medicine, Wistar albino rats, Streptozotocin (STZ), Diabetic model

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ABSTRACT: Aim and Objectives: The present study was an attempt to investigate the effect of Thetran vidhai kudineer (TVK) on streptozotocin (STZ) induced diabetes in male Wistar rats. **Methods:** TVK, is a Siddha polyherbal formulation containing four ingredients includes *Strynchnos potatorum*, *Terminalia chebula*, *Cassia auriculata*, and *Limonia acidissima*. 6-8 week old 30 male Wistar albino rats fed with normal diet *ad libitum* and free access to water containing 10% fructose (w/v) for 5 weeks. After five weeks of dietary manipulation, all groups except the normal control group received a single injection intraperitoneal (ip) of STZ (40 mg/kg b.w.) dissolved in citrate buffer (pH 4.4). One week after the STZ injection, animals with non-fasting blood glucose levels > 250 mg/dl were considered as diabetic. Four groups of STZ-induced diabetic rats were orally treated with metformin (100 mg/kg) and TVK (300, 600, and 1200 mg/kg b.w), respectively. Bodyweight recording was done weekly; fasting blood glucose levels were measured at the end of 8th week, 10th week, 12th week. Lipid profile, SGOT, SGPT, Serum Insulin, Serum creatinine, TNF-alpha were measured at the end of the 12th week. **Results:** TVK and metformin were found to be reducing the blood glucose level, lipid profile, creatinine level, SGOT, and SGPT levels when compared to diabetic control, whereas the treatment with TVK increased body weight, HDL levels and decreased TNF-alpha levels, and insulin levels when compared to the diabetic control. Histopathological studies reinforce the healing of damaged organs by TVK and metformin as a possible mechanism of their anti-diabetic activity. **Conclusion:** Thus, it may be concluded that TVK, in addition to the anti-diabetic activity, also possess anti-hyperlipidemic activity in the fructose and STZ induced diabetic model.

INTRODUCTION: Traditional systems of medicines are playing a key role in meeting global health care needs.

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India has seven familiar systems of medicine; Ayurveda, Siddha, Unani, Yoga, Naturopathy, Homoeopathy, and Sowa-Rigpa [1]. Among all the alternative medicinal systems, Siddha system of medicine is unique and has originated from Tamil language and culture [2].

Literally, the word "Siddha" means "established truth" [3]. Fundamental principles of Siddha include theories of five elements (Aim pootham) and three forces/faults (Mukkuttram). The eight methods of

examination (Envakai Thervukal) are used to determine diagnosis, etiology, treatment, and prognosis. Siddha has safe herbal and herbo mineral treatment for Psoriasis, eczema, alopecia, asthma, premenstrual syndrome, arthritis, migraine, hypertension, diabetes, cancer, warts, vitiligo, pemphigus, pompholyx, leprosy and many more very common and rare diseases [4]. Siddha formulations are presented in the books of GUNAVAGADAM (Siddha pharmacology) quoted by Siddhars5. The World Health Organization (WHO) has documented that the vast majority of people (75-80%) mostly living in the "developing countries" and a significant number in the "developed industrialized nations prefer and request to alternate (traditional) medicine for treating common ailments and chronic diseases [6].

Diabetes mellitus (DM) is in the top 5 of the most significant diseases in the developed world, and is gaining in significance there and elsewhere. Present number of diabetics worldwide is 171 million, and this is likely to increase to 340 million or more by the year 20307 [8]. India currently represents 49% of the world's diabetes burden, and every year, nearly 1 million deaths were noted in Indians due to diabetes [9]. DM is a complex metabolic syndrome with an absolute or relative deficiency of insulin resulting in disturbed intermediary metabolism and manifestations. DM affect all body systems, and the main burnt is borne by eyes, kidneys, skin and nerves. The WHO estimates that by 2025, worldwide, there will be 300 million diabetes [10]. Several pathogenic processes are involved in the development of diabetes; these range from autoimmune destruction of the β-cells of the pancreas with consequent insulin deficiency to abnormalities that result in resistance to insulin action. Deficient action of insulin on target tissues and hyperglycemia are the basis of the abnormalities in carbohydrates, fat, and protein metabolism, causing diabetes characteristic clinical features, micro and macrovascular complications and increased risk of cardiovascular diseases [11].

Treatment targets for type-2 diabetes include restoring blood glucose to normal levels so as to abolish diabetic symptoms; and the risk of acute and chronic metabolic complications. There are different approaches to the treatment in type 2 diabetes, like Sulfonylurea's, which release insulin from the pancreas by blocking the ATP sensitive potassium channels. Sulfonylurea's can potentially produce many adverse effects, such as weight gain and increased cardiovascular risk [12]. Sulfonylurea's cause hypoglycemia, premature atherosclerosis, and hypersensitivity [13]. Biguanides, which decrease the insulin resistance, the most common side effects are mild diarrhea, abdominal pain, nausea, metallic taste, and Vitamin B_{12} deficiency, and the most important and serious side effect is lactic acidosis [13]. Thiazolidinediones, which increase insulin sensitivity. Thiazolidinediones can produce many adverse effects such as cardiac risk like myocardial infarction and congestive heart failure, plasma volume expansion, edema, weight gain, headache, myalgia, and mild anemia [13]. The Alpha-glucodase inhibitors like acarbose, which decrease glucose absorption from the intestine, thereby decreasing postprandial hyperglycemia, meti-glinides like repaglimide and nateglemide, which are insulin secretogogues [14]. Alpha-glucodase inhibitors have many adverse effects such as flatulence, abdominal discomfort, and loose stool are produced in 50% patients, and hepatic transaminases may raise and rarely causes liver damage [13]. A scientific investigation of traditional herbal remedies for diabetes may provide valuable leads for the development of alternative drugs and strategies.

In the Siddha system of medicine DM is called by different names such as neerizivunoi (neer means urine and izhivu means excessive discharge), madhumegam (madhu means sweet, and megam means venereal disease) and neerperukkal noi (polyuric condition) [15]. Siddha system of medicine is claimed to alleviate the root cause of the diseases by maintaining the ratio of tridoshas; Vatham, Pitham and Kapham [16]. In Siddha system of medicine, diabetes (Neerizhivu) is portrayed as Aiyyam (symbolizes earth and water) humour derangement disease that can be neutralized by vali (represents air and space) humor predominant drugs. To nullify the detrimental effects of diabetes, the proposal drug should possess either bitter or pungent or astringent taste, which will retract the deranged Aiyyam humor, because of its predominant vali humor [17].

Kudineer - These are decoctions prepared by adding water to dry herbs, or fresh ones and the

boiling them so that the water content is greatly reduced to 1/16th or 1/8 of the water added. Sometimes, some substances are not directly added to the water, but instead, they are kept in a clean white cloth, tied and immersed in the water. They also could be used for 3 h. Thus, the aim of the present study was to investigate the anti-diabetic potential of TVK on fructose and STZ induced diabetic rats. The objectives of the study were to study the effect of TVK on body weight, feed and water intake of experimental animals, biochemical changes *viz.*, changes in blood glucose levels, lipid profile, liver markers, renal markers, insulin and TNF-alpha levels in serum of experimental animals and to perform the histopathological examination of pancreas, aorta, fat tissue, heart, liver and kidneys of experimental animals at the end of study.

MATERIALS AND METHODS:

Drugs and Chemicals: TVK, a siddha formulation consists of Thetran vidhai seed (Strychnous potatorum), Kadukkai thol seed (*Terminalia chebula*), Aavarai vidhai flower (*Cassia auriculata*) and Vilam pisin fruit pulp (*Limonia acidisimma* Linn.). It is commonly indicated in the treatment of Neerizhivu [1, 2]. TVK was collected from Siddha Central Research Institute, Chennai - 600106. STZ (Sigma Aldrich), Fructose (SRL chemicals) was purchased from local scientific company, Chennai and all other chemicals and reagents used were of analytical grade.

Animal Maintenance and Induction of Diabetes: The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC approval number: 163/PHARMA/SCRI, 2017). Male albino Wistar rats age range 6-8 weeks were obtained from TANUVAS, Madhavaram, Chennai, maintained and treated in the animal house of Siddha Central Research Institute, and the procedures were performed according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA, India). The experimental animals provided food and drinking *water ad libitum*.

Induction of Diabetes Mellitus: The total duration of study period was 12 weeks. Induction of diabetic insulin-resistant rats was done according to the method described previously [20] by Rachel D Wilson & Md. Shahidul Islam with slight modifications. Briefly, 30 male rats were fed with normal diet *ad libitum* and free access to water containing 10% fructose (w/v) for 5 weeks. 6 animals were used as vehicle control throughout the study receiving only normal RO water and feed *ad libitum* without any treatment. At the beginning of the 6th week, rats received a single i.p. injection of a sub-diabetogenic dose of freshly prepared STZ (40 mg/kg) in citrate buffer (0.09 M, PH 4.4) after overnight fasting and were given 5% glucose solution to drink during the first 24 h after STZ administration to overcome hypoglycaemia. Then the animals were fed with normal diet & normal R.O. water for the rest of the study. One week after STZ injection, *i.e.* at the beginning of 7th week, animals that fulfilled the criteria of fasting blood glucose level exceeding 250 mg/dl, were used in the study.

Experimental Design: The total duration of study period was 12 weeks. One week after STZ injection, *i.e.* at the beginning of 7th week, animals that fulfil the criteria of diabetic were used in the study. The animals will be randomly allocated into 5 groups (n = 6): Group II: Diabetic rats: rats that fulfilled the previously mentioned criteria,: Group III: diabetic rats treated with metformin (100 mg/kg/ day), Group IV: diabetic rats treated with TVK (300 mg/kg), Group V: diabetic rats treated with TVK (600 mg/kg), Group VI: diabetic rats treated with TVK (1200 mg/kg) upto 12th week. Group I vehicle control (12 weeks). After the experimental period, all individuals were sacrificed for biochemical studies.

Drug Administration: All the animals were administered the respective doses on daily basis at the same time for the period of the study (6 weeks). RO water was used as vehicle. The volume given was not more than 2 ml / 100 gm body weight.

Biochemical Analysis: Blood glucose was estimated at 8, 10 and 12 weeks, lipid profile, serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), serum creatinine, Tumour necrosis factor-alpha (TNF-alpha) and serum insulin were estimated by the standard method of practical in biochemistry after 12th week.

Histopathological Examination: Pancreas, liver, aorta, heart, abdominal fat, and kidney were removed, washed with cold saline, and preserved in 10% formalin in buffered form.

Blocks from tissues were routinely processed and embedded in paraffin. Thin sections were cut using rotary microtome and stained with hematoxylin and eosin for histomorphology evaluation.

Statistical Analysis: All the data were expressed as mean ± SEM. Statistical significance between more than two groups was tested using one-way ANOVA followed by Tukey's post hoc using Graph pad prism version-5.

RESULTS:

Effect of TVK on Body Weight: There was gradual increase in body weight in normal control rat while the diabetic control rat continued to lose weight.

However, TVK and the metformin-treated diabetic group gained weight as compared to diabetic control and the bodyweight of diabetic treated towards normal range (P<0.01). The changes in body weight were tabulated in **Table 1**.

TABLE 1: EFFECT OF TVK ON BODY WEIGHT IN STZ INDUCED DIABETIC RATS

S. no.	Treatment	Body weights (gm)	
		Initial	Final
1	Vehicle control	398.67 ± 10.07	359.50 ± 13.05*
2	Diabetic control	341.25 ± 12.31	334.00 ± 8.95
3	Diabetic + Metformin (40 mg/kg)	365.20 ± 23.06	345.00 ± 13.08*
4	Diabetic + TVK (300 mg/kg)	324.60 ± 23.36	335.00 ± 27.24*
5	Diabetic + TVK (600 mg/kg)	291.00 ± 21.07	309.75 ± 10.16*
6	Diabetic + TVK (1200 mg/kg)	337.00 ± 11.14	335.40 ± 5.90*

All values are expressed as mean ± SEM (n = 6). One-way ANOVA followed by Tukey's post hoc test. *P<0.05, when compared with diabetic control

Effects of TVK on Serum Glucose Levels: Diabetic rats showed a significant increase in serum glucose level (P<0.5) with respect to normal control group rats. The oral administration of TVK at 300 mg/kg, 600 mg/kg, 1200 mg/kg, and metformin 100 mg/kg (P<0.5) significantly reversed the increase in serum glucose levels in diabetes-induced rats. TVK and metformin showed similar effect on serum glucose levels. The changes in serum glucose estimation in all groups of the animal were given in **Table 2**.

TABLE 2: EFFECT OF TVK ON SERUM GLUCOSE LEVEL IN STZ INDUCED DIABETIC RATS

S. no.	Treatment	Serum blood glucose (mg/dl)		
		8th week	10th week	12th week
1	Vehicle control	108.50 ± 2.20*	104.00 ± 1.79*	106.50 ± 1.38*
2	Diabetic control	195.00 ± 2.27	191.25 ± 4.27	189.50 ± 2.10
3	Diabetic + Metformin (40 mg/kg)	149.60 ± 2.50	114.00 ± 0.45*	108.80 ± 2.22*
4	Diabetic + TVK (300 mg/kg)	149.80 ± 2.48	122.25 ± 1.93*	117.75 ± 1.93*
5	Diabetic + TVK (600 mg/kg)	144.80 ± 2.40*	116.00 ± 2.02*	111.50 ± 1.19*
6	Diabetic + TVK (1200 mg/kg)	146.80 ± 3.43*	114.80 ± 1.24*	109.00 ± 3.76*

All values are expressed as mean ± SEM (n=6). One-way ANOVA followed by Tukey's post hoc test. *P<0.05, when compared with diabetic control

TABLE 3: EFFECT OF TVK ON SERUM LIPID AND LIPOPROTEIN PROFILE IN STZ INDUCED DIABETIC RATS

S. no.	Treatment	Serum lipid and lipoprotein profile (mg/dl)		
		Triglyceride	Total cholesterol	HDL
1	Vehicle control	54.17 ± 2.63*	60.67 ± 1.56*	21.17 ± 1.54*
2	Diabetic control	85.00 ± 5.34	83.50 ± 0.87	15.50 ± 0.65
3	Diabetic + Metformin (40 mg/kg)	60.20 ± 3.43	69.40 ± 0.60	21.60 ± 1.40*
4	Diabetic + TVK (300 mg/kg)	66.75 ± 1.65	62.00 ± 5.45*	22.00 ± 1.08*
5	Diabetic + TVK (600 mg/kg)	62.25 ± 0.63*	63.00 ± 1.22*	25.00 ± 0.41*
6	Diabetic + TVK (1200 mg/kg)	62.00 ± 1.73*	62.00 ± 1.78*	25.75 ± 1.38*

All values are expressed as mean ± SEM (n=6). One-way ANOVA followed by Tukey's post hoc test. *P<0.05, when compared with diabetic control

Effect of TVK on Serum Lipid and Lipoprotein Profile: Diabetic rats were found to have significantly increased triglyceride, total cholesterol levels, and markedly decreased HDL levels as compared to the normal control group (P < 0.05). Oral treatment with TVK 300 mg/kg, 600 mg/kg and 1200 mg/kg significantly decreased the triglyceride, total cholesterol levels and markedly

increased HDL levels in diabetes induced rats (P< 0.05). Metformin group was significantly prevented the increase in serum triglyceride, total cholesterol levels, and decrease in HDL levels in STZ induced diabetic rats. Thus, the TVK and metformin likely restored all these changes near to normal value. The changes in serum lipid and lipoprotein profiles were tabulated in **Table 3**.

Effect of TVK on Serum SGOT and SGPT Levels: Diabetic rats showed a significant increase in serum SGOT and SGPT levels (P<0.5) with respect to normal control group rats. The oral administration of TVK 300 mg/kg, 600 mg/kg, 1200 mg/kg, and metformin 100 mg/kg (P<0.5) significantly reversed the increase in serum SGOT and SGPT levels in diabetic rats. The changes in SGOT and SGPT levels in all groups of the animal were given in **Table 4**.

TABLE 4: EFFECT OF TVK ON SGOT AND SGPT LEVELS IN STZ INDUCED DIABETIC RATS

S. no.	Treatment	SGOT (U/L)	SGPT (U/L)
1	Vehicle control	163.50 ± 9.00*	45.33 ± 2.17*
2	Diabetic control	200.00 ± 22.11	68.00 ± 14.99
3	Diabetic + Metformin (40 mg/kg)	169.80 ± 3.25*	50.60 ± 1.36
4	Diabetic + TVK (300 mg/kg)	170.00 ± 8.09	52.25 ± 4.27
5	Diabetic + TVK (600 mg/kg)	166.75 ± 12.26*	48.75 ± 2.66*
6	Diabetic + TVK (1200 mg/kg)	169.25 ± 24.42*	49.00 ± 2.68*

All values are expressed as mean ± SEM (n=6). One-way ANOVA followed by Tukey's post hoc test. *P<0.05, when compared with diabetic control

TABLE 5: EFFECT OF TVK ON SERUM INSULIN LEVELS IN STZ INDUCED DIABETIC RATS

S. no.	Treatment	Insulin (U/L)
1	Vehicle control	10.07 ± 0.58*
2	Diabetic control	5.60 ± 0.76
3	Diabetic + Metformin (40 mg/kg)	9.43 ± 0.47
4	Diabetic + TVK (300 mg/kg)	8.93 ± 0.21
5	Diabetic + TVK (600 mg/kg)	9.50 ± 0.29
6	Diabetic + TVK (1200 mg/kg)	9.86 ± 0.14*

All values are expressed as mean ± SEM (n=6). One-way ANOVA followed by Tukey's post hoc test. *P<0.05, when compared with diabetic control

Effect of TVK on Serum Insulin Levels: STZ treatment produced significant decrease in serum Insulin levels (P<0.5) with respect to normal control group rats. The oral administration of TVK 300 mg/kg, 600 mg/kg, 1200 mg/kg and metformin 100 mg/kg (P<0.5) significantly reversed the serum insulin depletion in diabetic condition and brought back to normal level. The changes in Insulin levels in all groups of the animal were given in **Table 5**.

Effect of TVK on Serum Creatinine Levels: STZ treatment produced a significant increase in serum creatinine levels (P<0.5) with respect to normal control group rats. The oral administration of TVK 300 mg/kg, 600 mg/kg, 1200 mg/kg and metformin 100 mg/kg (P<0.5) significantly decreased the serum creatinine levels in STZ induced rats. Thus the TVK restored all these changes near to normal value. The changes in serum creatinine levels in all groups of animal were given in **Table 6**.

TABLE 6: EFFECT OF TVK ON SERUM CREATININE LEVELS IN STZ INDUCED DIABETIC RATS

S. no.	Treatment	Creatinine (mg/dl)
1	Vehicle control	0.48 ± 0.02*
2	Diabetic control	0.95 ± 0.04
3	Diabetic + Metformin (40 mg/kg)	0.48 ± 0.02*
4	Diabetic + TVK (300 mg/kg)	0.40 ± 0.00*
5	Diabetic + TVK (600 mg/kg)	0.43 ± 0.02*
6	Diabetic + TVK (1200 mg/kg)	0.45 ± 0.05*

All values are expressed as mean ± SEM (n=6). One-way ANOVA followed by Tukey's post hoc test. *P<0.05, when compared with diabetic control.

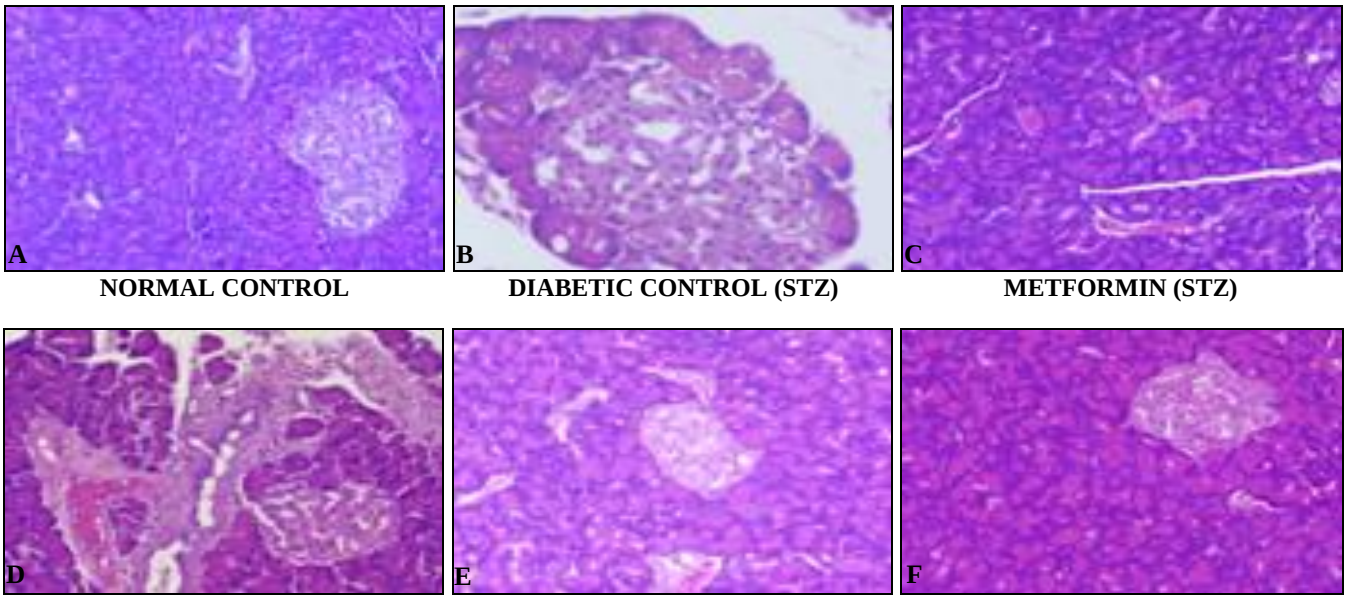
Effect of TVK on TNF-alpha Levels: STZ treatment produced significant increase in TNF-alpha levels (P<0.5) with respect to normal control group rats. The oral administration of TVK 300 mg/kg, 600 mg/kg, 1200 mg/kg and metformin 100 mg/kg (P<0.5) significantly decreased the TNF-alpha levels in STZ induced rats. Thus, the TVK restored all these changes near to normal value. The changes in TNF-alpha levels in all groups of animal were given in **Table 7**.

TABLE 7: EFFECT OF TVK ON TNF-ALPHA LEVELS IN STZ INDUCED DIABETIC RATS

S. no.	Treatment	TNF-alpha
1	Vehicle control	156.50 ± 1.48
2	Diabetic control	222.25 ± 25.61
3	Diabetic + Metformin (40 mg/kg)	155.80 ± 3.65
4	Diabetic + TVK (300 mg/kg)	151.50 ± 11.73
5	Diabetic + TVK (600 mg/kg)	155.75 ± 6.55
6	Diabetic + TVK (1200 mg/kg)	154.00 ± 2.35

All values are expressed as mean ± SEM (n=6). One-way ANOVA followed by Tukey's post hoc test. *P<0.05, when compared with diabetic control

Histopathology: Histopathological studies **Fig. 1** showed normal acini and normal cellular population in the Islets of Langerhans in the pancreas of control rats (Group I). Extensive damage to the islets of Langerhans and reduced dimensions of islets diabetic control (Group II), restoration of a normal cellular population size of islets with hyperplasia by metformin (Group III) was also shown. The partial restoration of normal cellular population and enlarged size of cells with hyperplasia were shown by TVK (300 mg/kg, 600 mg/kg, and 1200 mg/kg) (**Fig. 1**. Group IV to Group VI).

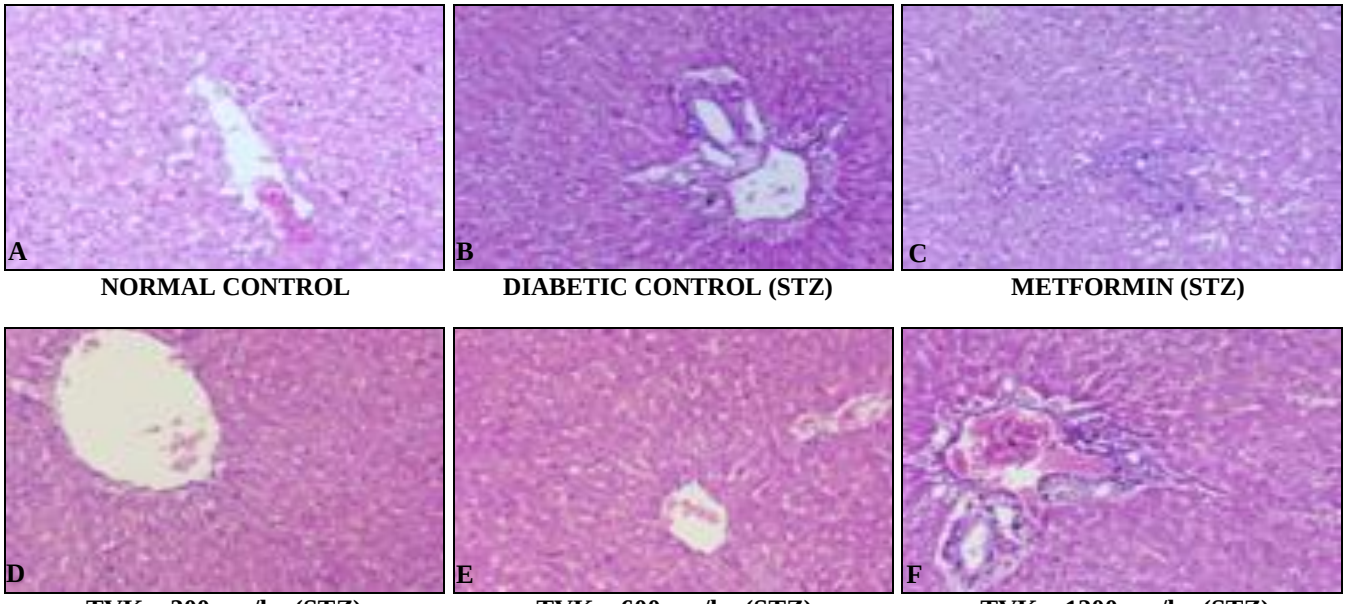


NORMAL CONTROL | **DIABETIC CONTROL (STZ)** | **METFORMIN (STZ)**

TVK – 300 mg/kg (STZ) | **TVK – 600 mg/kg (STZ)** | **TVK – 1200 mg/kg (STZ)**

FIG. 1(A-F): DEPICTS THE ISLETS OF THE PANCREAS OF RATS IN DIFFERENT GROUPS

Histopathological studies **Fig. 2** showed normal hepatic cells in the liver of control rats (Group I). Congestion, diffuse hydropic degeneration of hepatocytes, and multifocal mild mononuclear cell infiltration were observed in diabetic control rats (Group II), restoration of infiltration of hepatic cells by metformin (Group III) was also shown. The partial restoration of degenerated hepatocytes and mononuclear cell infiltration was shown by TVK (300 mg/kg, 600 mg/kg, and 1200 mg/kg) (**Fig. 2**. Group IV to Group VI).



NORMAL CONTROL | **DIABETIC CONTROL (STZ)** | **METFORMIN (STZ)**

TVK – 300 mg/kg (STZ) | **TVK – 600 mg/kg (STZ)** | **TVK – 1200 mg/kg (STZ)**

FIG. 2(A-F): DEPICTS THE HEPATIC CELLS OF THE LIVER OF RATS IN DIFFERENT GROUPS

Histopathological studies **Fig. 3** showed normal renal cells in kidney of control rats (Group I). Tubular epithelial cell degeneration, atrophy of glomerulus and focal mild nodular glomerulo sclerosis was observed in diabetic control rats (Group II), restoration of epithelial cell degeneration, and nodular glomerulosclerosis by metformin (Group III) was shown. The kidney damage was partially healed by TVK (300 mg/kg, 600 mg/kg, and 1200 mg/kg) (**Fig. 3**. Group IV to Group VI).

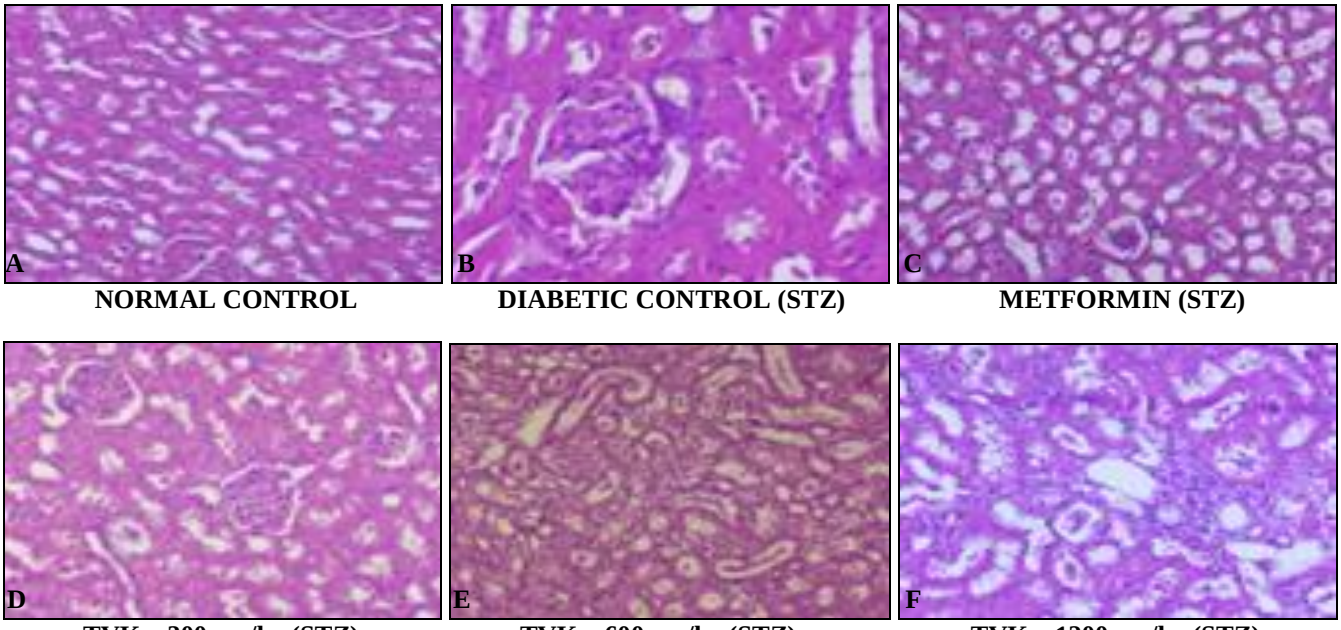


FIG. 3(A-F): DEPICTS THE RENAL CELLS OF THE KIDNEY OF RATS IN DIFFERENT GROUPS

Histopathological studies **Fig. 4** showed no damage to aorta in control rats (Group I). Few vacuolations in the tunica media and tunica intima were observed in the aorta of diabetic control rats (Group II), restoration of aortic damage was observed in metformin (Group III) and TVK (300 mg/kg, 600 mg/kg and 1200 mg/kg) treated groups (**Fig. 4**. Group IV to Group VI).

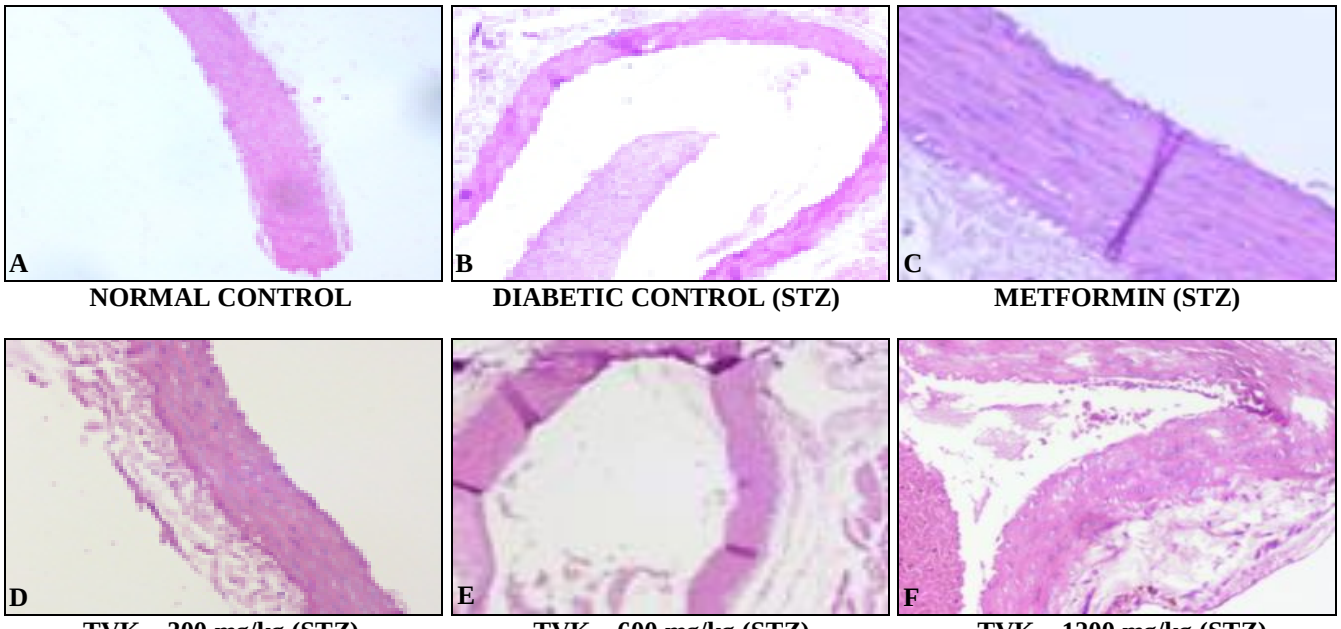
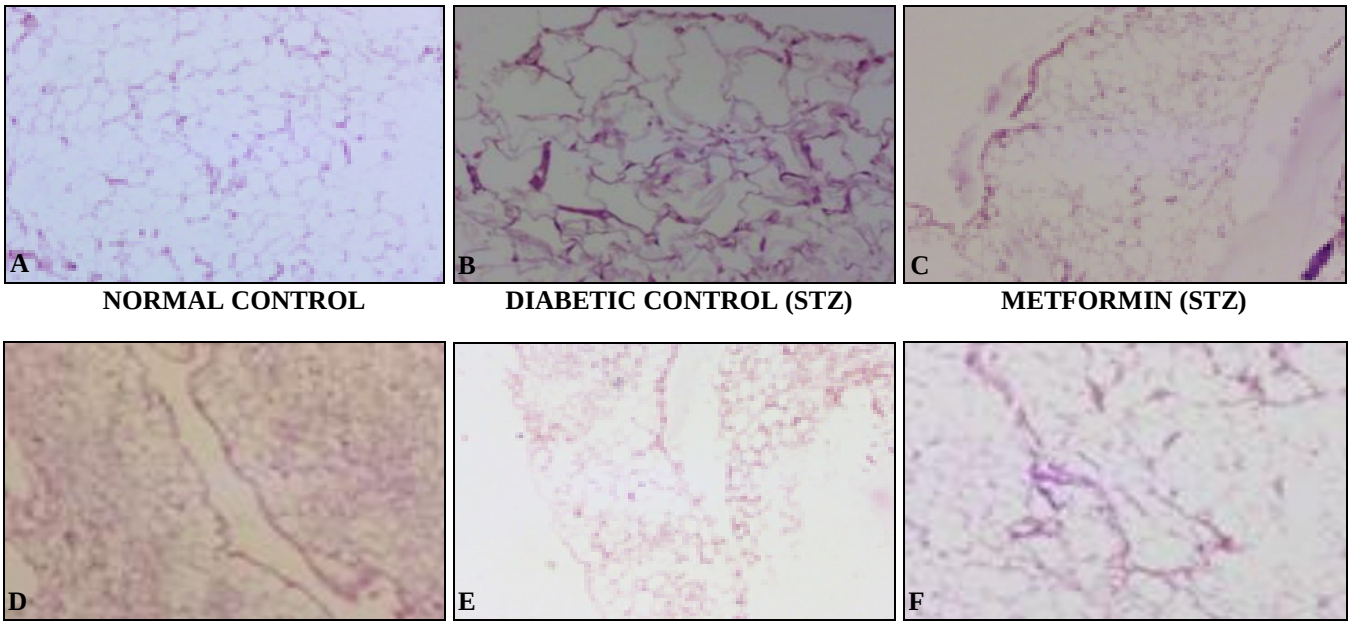


FIG. 4(A-F): DEPICTS THE TUNICA MEDIA AND TUNICA INTIMA OF THE AORTA OF RATS IN DIFFERENT GROUPS

Histopathological studies **Fig. 5** showed no abnormality in abdominal fat of control rats (Group I). Increased size of fat vacuoles was observed in abdominal fat of diabetic control rats (Group II), the size of fat vacuoles restored to a normal level by metformin (Group III) and TVK (300 mg/kg, 600 mg/kg and 1200 mg/kg) treated groups was observed. **Fig. 5** Group IV to Group VI.

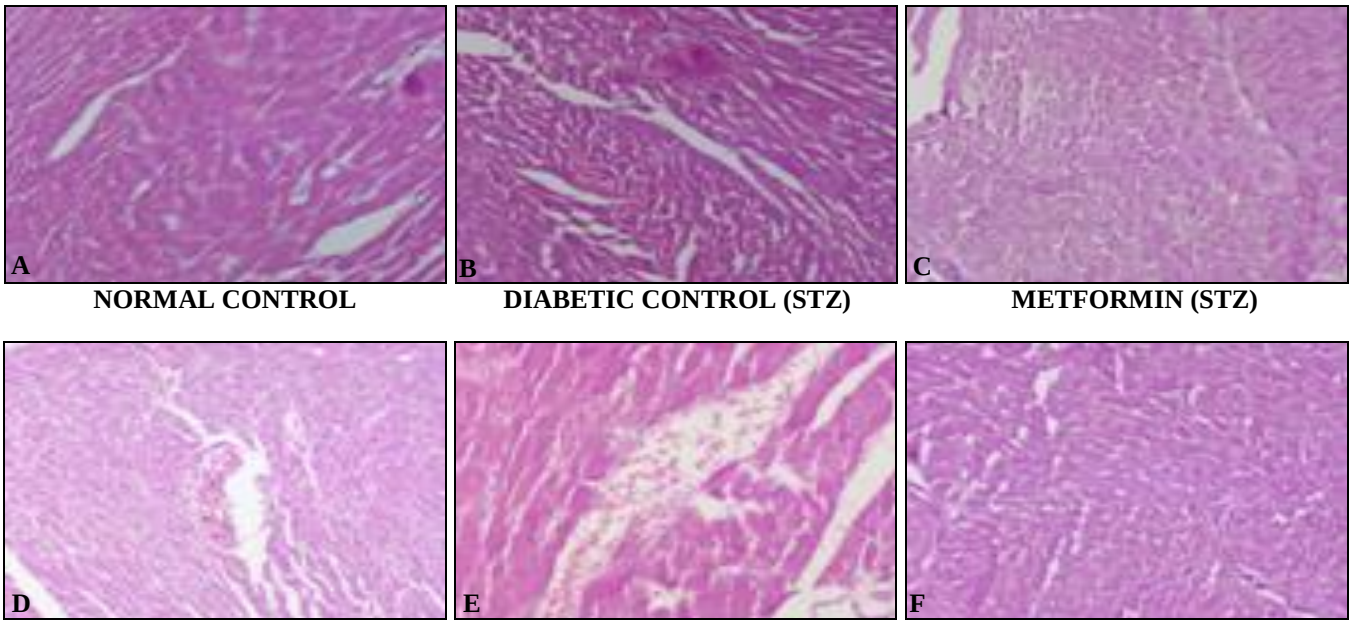


NORMAL CONTROL | **DIABETIC CONTROL (STZ)** | **METFORMIN (STZ)**

TVK – 300 mg/kg (STZ) | **TVK – 600 mg/kg (STZ)** | **TVK – 1200 mg/kg (STZ)**

FIG. 5(A-F): DEPICTS THE FAT VACUOLES OF THE ABDOMINAL FAT OF RATS IN DIFFERENT GROUPS

Histopathological studies **Fig. 6** showed no damage to the heart in control rats (Group I). Multifocal myocardial degeneration was observed in the heart of diabetic control rats (Group II), restoration of heart damage was observed in metformin (Group III) and TVK (300 mg/kg, 600 mg/kg and 1200 mg/kg) treated groups (**Fig. 6**. Group IV to Group VI).



NORMAL CONTROL | **DIABETIC CONTROL (STZ)** | **METFORMIN (STZ)**

TVK – 300 mg/kg (STZ) | **TVK – 600 mg/kg (STZ)** | **TVK – 1200 mg/kg (STZ)**

FIG. 6(A-F): DEPICTS THE MYOCARDIAL CELLS OF THE HEART OF RATS IN DIFFERENT GROUPS

DISCUSSION: In the modern era, herbal formulations have gained greater importance than ever before, mainly due to their efficacy and easy availability [21], as well as less side effects as compared to the synthetic drugs [22]. These advantages have led the people move toward herbal

preparations for disease treatment and prevention, as they are claimed to display synergistic, potentiation and agonistic/antagonistic actions, and the mixture of species in them shows better therapeutic effect than either species on its own [23]. TVK, is a Siddha polyherbal formulation containing four ingredients, namely *Strynchnos potatorum*, *Terminalia chebula*, *Cassia au*riculata and *Limonia acidissima*. It corrected the function of the pancreas, stimulated it to produce insulin in the natural way, which in turn maintains the blood sugar level. TVK revitalized and rejuvenated the organs, the dysfunction of which is caused by the disease. TVK brought back the normal functioning of the organs. Since no artificial chemicals are involved, TVK doesn't cause any side effects.

Diabetes mellitus (DM) is an endocrine disorder in which glucose metabolism is impaired because of a total loss of insulin after the destruction of pancreatic beta cells or because of the inadequate release of insulin from the pancreatic beta-cell or insensitivity of target tissue to insulin. The fundamental mechanism underlying hyperglycemia involved overproduction (excessive hepatic glyconeogenesis and gluconeogenesis) and decrease utilization of glucose by the tissue. In the present study, TVK was evaluated for its antihyperglycemic action in Fructose and STZ induced diabetic rats. STZ, a beta cytotoxin induces chemical diabetes in a wide variety of animal species including rats by selectively damaging the insulin-secreting beta cells of the pancreas - I.P injection of STZ produces fragmentation of DNA of beta cells of the pancreas which stimulates poly (ADP-ribose and deflects NAD ultimately leading to destructions of beta cells and it is evidenced by clinical symptoms of hyperglycemia [24].

However, the induction of Insulin resistance (IR) through fructose-feeding in animals has been employed previously. Fructose has been supplied *ad libitum* either in drinking water or with diets with a concentration of 10-15% for a short or longer period to induce IR or T2D, respectively, in experimental animals [25]. Unfortunately, the induction of IR, as well as T2D only by fructose-feeding, requires several weeks, which increases the cost of study. Additionally, it has been reported that only fructose feeding for a long period of time can lead to nutritional tolerance without developing classical signs and symptoms of IR and impaired glucose tolerance [26].

Hence, we employed the combination of fructose-feeding for a shorter period of time and a lower dose of STZ injection for inducing all major pathogeneses of T2D in rats using the method described by Rachel D. Wilson & Md. Shahidul Islam with slight modifications.

In the present study, there was a significant weight gain in TVK treated diabetic rats compared with vehicle control rats, and this observation shows the anabolic effect of TVK on body weight on the diabetic rats. Hyperglycemia and insulin resistance both seem to have important roles in the pathogenesis of macrovascular complications. DM causes a disturbance in the uptake of glucose as well as glucose metabolism. The hyperglycemia in diabetes might inhibit tissue repair in macrovascular beds. In the present study, TVK treated groups show hypoglycemia, and it confirms the presence of anti-diabetic activity. Biguanides such as metformin are often used as a standard anti-diabetic drug in STZ-induced diabetes to compare the efficacy of a variety of anti-hyperglycemic compounds. In the present study, there was a significant elevation in blood glucose levels in the diabetic control group as compared with normal animals. The TVK treated group exhibited a significant reduction of fasting plasma glucose levels as compared to the diabetic control group. Overproduction of glucose by means of excessive hepatic glycogenolysis and gluconeogenesis is one of the fundamental bases of hyperglycemia in diabetes mellitus.

The most commonly observed lipid abnormalities in diabetes are hypertriglyceridemia and hypercholesterolemia. A marked increase in total cholesterol and a decrease in HDL cholesterol have been observed in diabetic control rats compared to normal rats. Insulin deficiency results in failure to activate lipoprotein lipase, thereby causing hypertriglyceridemia. There was a significant control of the levels of serum lipids in TVK treated diabetic rats. In diabetes, LDL carries cholesterol to the peripheral tissues where it is deposited, whereas HDL transports cholesterol from peripheral tissues to the liver and thus aids its excretion. In our present study, TVK treated groups showed a

significant decrease in TG and total cholesterol levels, whereas there was a significant increase in the HDL level.

In the present study, there was a significant elevation in SGOT, SGPT, and serum creatinine levels in the diabetic control group as compared with normal animals. The TVK treated group exhibited a significant reduction of SGOT, SGPT, and serum creatinine levels as compared to the diabetic control group. Elevated levels of SGOT and SGPT usually indicate the presence of liver disease, although they can also indicate muscle damage. Serum creatinine (a blood measurement) is an important indicator of renal health because it is easily measured by the product of muscle metabolism that is excreted unchanged by the kidneys.

In the present study, there was a significant decrease in Insulin and TNF-alpha levels in the diabetic control group as compared with normal animals. The TVK treated group exhibited a significant increase in insulin and TNF-alpha levels as compared to the diabetic control group. Type 2 diabetes is characterized by hyperglycemia caused by defects in insulin secretion (impaired β-cell function) and insulin action (insulin resistance by the liver and muscle tissue) [26-27]. These defects occur early in the course of the disease and are often present before diagnosis. Insulin is a hormone made by the pancreas that allows your body to use sugar (glucose) from carbohydrates in the food that you eat for energy or to store glucose for future use. Insulin helps your blood sugar level from getting too high (hyperglycemia) or too low (hypoglycemia). TNF-α is an adipocytokine involved in systemic inflammation and stimulates the acute phase reaction [28]. TNF-α is primarily secreted by macrophages and also by a broad variety of other cells, including adipocytes. TNF-α inhibits insulin transduction and has an effect on glucose metabolism [29]. Disturbances in the TNF-α metabolism have been implicated in metabolic disorders, such as obesity and insulin resistance34, indicating that perturbations of TNF-α metabolism may affect the onset of type 2 DM and the progression of the disease.

Histopathological studies of pancreas, liver, kidney, aorta, heart, and abdominal fat also supported our findings. Histopathological examination of pancreas, liver, kidney, aorta, heart, and abdominal fat showed the recovery of damaged tissues when the section of metformin and TVK treated groups compared with diabetic control.

CONCLUSION: The present study is an attempt to investigate the effect of TVK on STZ induced diabetes in Wistar albino rats. The serum glucose, lipid profile, SGOT, SGPT, and TNF-alpha levels shown to be decreased in TVK treated diabetic animals. Bodyweight, HDL, Insulin levels shown to be increased in TVK treated diabetic animals. The findings of the present investigation suggest that TVK has the potential for its evaluation as an anti-diabetic agent against Fructose and STZ induced diabetes. Assessment of TVK for its underlying mechanism(s) will be an interesting topic and requires further study.

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