



Research Paper

Antidiabetic effect of Vidangadi Kvatha in STZ-NAD induced diabetic rats

Authors

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Abstract

The largest population in the world is suffered from diabetes mellitus type 2 which are characterized by hyperglycemia associated with minor and major complications and it is caused by acquired deficiency in production of insulin by the pancreas, or by the resistance of the insulin. The experimental study includes the acute oral toxicity study, OGTT and antidiabetic effect of Vidangadi Kvatha in STZ-Nicotinamide induced diabetes in rats. Oral Glucose Tolerance Test (OGTT) was performed in normal rats and results shows that 200 mg/kg reduces the blood glucose level 89.45 ± 1.24 mg/dl in 2 hrs. The 100 mg/kg of Vidangadi Kvatha reduces the blood glucose level 93.34 ± 2.25 mg/dl. Oral Glucose Tolerance Test (OGTT) in diabetic rat shows that 200 mg/kg dose of Vidangadi Kvatha lowers the blood glucose level 123.46 ± 2.74 mg/dl in 2 hrs. Diabetic control rats shows blood glucose level 405.98 ± 2.33 mg/dl and Glibenclamide 4 mg/kg lowers blood glucose level up to 98.42 ± 3.14 mg/dl. In STZ-NAD induced diabetic rats Vidangadi Kvatha lowers the post prandial blood glucose level, SGOT, SGPT, Lipid profile and C-Peptide level. The study found that Vidangadi Kvatha have antidiabetic property.

Keywords: Diabetes Mellitus, Streptozotocin, Nicotinamide, OGTT, Vidangadi Kvatha, Glibenclamide, Gliclazide.

Introduction

Diabetes mellitus is a disorder characterized by hyperglycemia with minor and major complications and it is caused by inherited and/or acquired deficiency in production of insulin by the pancreas, or by the ineffectiveness of the insulin produced, i.e. insulin resistance¹. This leads into increased concentrations of glucose in the blood, which in turn damage many of the body's systems, in particular the blood vessels and nerves². The number of people with diabetes has been raised from 108 million in 1980 to 422 million in 2014. The global prevalence of diabetes among adults

over 18 years of age has been raised from 4.7% in 1980 to 15.5% in 2020. Diabetes prevalence has been rising more rapidly in middle- and low-income countries³. Diabetes is a major cause of blindness, kidney failure, heart attacks, stroke and lower limb amputation⁴. In 2015, an estimated 1.6 million deaths were directly caused by diabetes. Almost half of all deaths attributable to high blood glucose occur before the age of 70 years. WHO projects that diabetes will be the seventh leading cause of death in 2030⁶. Diabetes might be one of the most talked about diseases across the world and especially in India. In India, today have more

people with type-2 diabetes (more than 50 million) than any other Nation⁷. In India, this increase is estimated to be 58 %, from 51 million people in 2010 to 87 million in 2030⁸. Diabetes mellitus is the leading cause of end stage renal disease (ESRD)⁸. In comparative to synthetic medicines, Ayurvedic formulations are effective, less side effects, broad range of action and relatively low cost, makes polyherbal formulation as a good choice in management of diabetes type 2. So many polyherbal formulations are reported in ancient Ayurvedic literatures but due to lack of scientific evidences such formulations are still hidden. Vidangadi Kvatha is a polyherbal formulation of total five ingredients. The aim of this study is to scientifically prove the Vidangadi Kvatha as antidiabetic polyherbal formulation for public welfare.

Material and Methodology

Experimental Animal Requirements: Albino rats (Charles foster strain of weight 150-200 g male and female) purchased from registered animal house of the IMS BHU. The proposal was approved from Central Animal Ethical Committee, Reg. No. 542/GO/ReBi/S/02/CPCSEA. Approval letter no is DEAN/2019/IAEC/1242 dated 26.05.2019. The rats were kept in polypropylene cages containing paddy husk bedding. The cages were stored inside a ventilated room of animal house in Department of Dravyaguna, IMS, BHU Varanasi. Each cage contains 6 rats and standard condition was maintained (temperature 20-30°C, humidity 65-70% and 12 hrs light & dark cycle. Rats were feed with standard pellet diet (Ashirwad trade,) and water.

Chemicals Requirements: Streptozotocin (STZ), Nicotinamide (NAD) and saline, SGPT kit, SGOT kit, HDL kit, Total cholesterol kit, C-Peptidase kit, LDL kit, Vidangadi Kvatha, Glibenclamide, Gliclazide.

Preparation of decoction of Vidangadi Kvatha:

The ingredients of Vidangadi Kvatha was Vidanga (*Embelia ribes Burm F.*)⁹, Haridra (*curcuma*

longa)¹⁰, Shunthi (*zingiber officinale Rose*)¹¹, Yashtimadhu (*Glycyrrhiza glabra Linn.*)¹², Gokshura (*Tribulus terrestris Linn*)¹³. All ingredients were subjected for size reduction using the pulverizer. Equal amount of all crude drugs was soaked in 4 times water in vessel and kept overnight for 12 hrs. After 12 hrs contents were boiled at 90⁰ C – 95⁰ C with stirring. Water was evaporated till 1/4th amount was remains and galenicals was filtered through cotton cloth. Filtrate was dried with rotatory evaporator and dried powder was used for quality control standard test and in vitro antidiabetic activity¹⁴.

Physicochemical evaluation of Vidangadi Kvatha (Decoction):

Prepared Decoction of Vidangadi Kvatha and dried powder of decoction was evaluated for their organoleptic properties, microbial contaminations, presence of heavy metals and presence of pesticide residues.

Phytochemical analysis of Vidangadi Kvatha (Decoction):

The Phytochemical screening of different extracts of crude drugs and Kvatha (decoction) were performed according to the procedure mentioned in Ayurvedic Pharmacopoeia of India (API)¹⁵.

Acute Oral Toxicity Study:

Acute oral toxicity was performed as per OECD guidelines 423 for toxicity study and estimation of LD₅₀ dose after treatment with Vidangadi Kvatha at different doses within 24 hours for dose optimization. Rats were distributed into total four groups and each groups contains 5 rats. Group 1 treated with Vidangadi Kwatha 5 mg/kg po, Group 2 treated with Vidangadi Kwatha 50 mg/kg po, Group 3 treated with Vidangadi Kwatha 300 mg/kg po, Group 4 treated with Vidangadi Kwatha 2000 mg/kg po. The rate of mortality and following parameters (Tremor, clonic convulsion, tonic convulsion, straub tail erection, catatonia, ataxia, loss of lighting reflex, sedation, hypnosis, lacrimation, diarrhea, writhing and rate of respiration) were observed for 24 hours¹⁶.

Oral Glucose Tolerance Test (OGTT) in normal and diabetic Rats

The oral glucose tolerance test was performed in overnight (18-h) fasted normal rats. Rats divided into groups were administered either drinking water or Vidangadi Kvatha and standard drug (Glibenclamide). Glucose (2 g/kg) was fed 30 min

after the administration of the Kvatha. Blood sample was withdrawn at each 30, 60, and 120 min after glucose administration and blood glucose was estimated¹⁷. The normal and diabetic rats were divided into different groups for the treatment, shown in table 1 & 2.

Table 1: The rats were divided into 4 groups of 6 rats each group (n=6)

S.N.	GROUPS	TREATMENT
1	Group 1: Normal rats	Receive 0.9 % saline
2	Group 2: Normal rats	Vidangadi Kvatha 100 mg/kg po
3	Group 3: Normal rats	Vidangadi Kvatha 200 mg/kg po
4	Group 4: Normal rats	Glibenclamide 600 µg/kg po

Table 2: The rats were divided into 5 groups of 6 rats in each group (n=6)

S.N.	GROUPS	TREATMENT
1	Group 1: Normal control	Receive 0.9 % saline
2	Group 2: Diabetic control	Receive 0.9 % saline
3	Group 3: Diabetic rats	Vidangadi Kvatha 100 mg/kg po
4	Group 4: Diabetic rats	Vidangadi Kvatha 200 mg/kg po
5	Group 5: Diabetic rats	Glibenclamide 600 µg/kg po

Assessment: After 60 minute of Vidangadi Kvatha administration, the rats were orally fed with 2g/kg of glucose. The blood samples were collected at 0, 30, 60, 90, 120 minutes and blood glucose level was estimated.

Effects of Vidangadi Kvatha in STZ-NAD induced Diabetic Rats:

Induction of diabetes and treatment: Streptozotocin (STZ) was freshly prepared in citrate buffer (pH 4.5) and Nicotinamide was prepared in normal saline. Hyperglycemia was

confirmed by the elevated random blood glucose levels (>200 mg/dl). Diabetes mellitus type 2 in rats was induced administration of Nicotinamide 120 mg/kg ip followed by administration of 60 mg/kg Streptozotocin (Sigma, Germany) after 15 minute. The rats with fasting plasma glucose >126 mg/dl & post prandial blood glucose level > 200 mg/dl were used in the study¹⁸. Grouping of diabetic rats in different groups described in table 3.

Table 3: The rats were divided into 6 groups and 6 rats in each group (n=6)

GROUPS	TREATMENT
Group 1- Normal control	Normal diet and water
Group 2 – Per-see study	Vidangadi Kvatha 100 mg/kg po
Group 3- Diabetic control	Normal diet and water
Group 4- Diabetic rats	Vidangadi Kvatha 100 mg/kg po
Group 5- Diabetic rats	Vidangadi Kvatha 200 mg/kg po
Group 6- Diabetic rats	Gliclazide 4 mg/kg po

Vidangadi Kvatha and Gliclazide were treated for 15 days and blood fasting glucose was measured on day 1, 5, 10 and day 15. After completion of treatment for 15 days body weight, Lipid profiles, liver profile, c-peptide level was measured.

Results

Physicochemical and Phytochemical evaluation of Vidangadi Kvatha: Dried Vidangadi Kvatha powder is dark brown in color, astringent-sweet taste and pungent smell. Heavy metals (Arsenic,

Lead, cadmium and mercury), microbial contamination and pesticide residue was absent in Vidangadi Kvatha. Phytochemical screening of Vidangadi Kvatha was performed and found that carbohydrate, flavonoids, alkaloids, proteins, tannins saponins and amino acids was present in Kvatha. The volatile oils and steroids are absent in Vidangadi Kvatha.

Acute oral toxicity study:

The Acute oral toxicity shows that there was no mortality found at dose of 5, 50, 300, and 2000 mg/kg. So The LD₅₀ dose was greater than 2000 mg /kg. Therefore the dose i.e. 100 mg/kg and 200 mg /kg were safe for treatment.

Oral Glucose Tolerance Test (OGTT) in normal and diabetic rats:

Oral Glucose Tolerance Test (OGTT) was performed in normal rats and results shows that 200 mg/kg reduces the blood glucose level 89.45±1.24 mg/dl in 2 hrs. The 100 mg/kg of Vidangadi Kvatha reduces the blood glucose level 93.34±2.25 mg/dl. Oral Glucose Tolerance Test (OGTT) in diabetic rat shows that 200 mg/kg dose of Vidangadi Kvatha lowers the blood glucose level 123.46±2.74 mg/dl in 2 hrs. Diabetic control rats shows blood glucose level 405.98±2.33 mg/dl and Glibenclamide 4 mg/kg lowers blood glucose level up to 98.42±3.14 mg/dl. The results of OGTT in normal and diabetic rats given in table 4,5 fig 1,2.

Table 4: Oral Glucose Tolerance Test (OGTT) in normal rats

GROUPS	TREATMENT	OBSERVATIONS FOR 2 HOURS				
		0 Hr	30 Min	60 Min	90 Min	120 Min
GROUP 1	Normal Saline	85.62 ± 2.05	170.32 ± 2.14	142.28 ± 9.34	113.24 ± 3.13	110.23 ± 7.23
GROUP 2	Vidangadi Kvatha 100 mg/kg	84.5 ± 2.14	134.32 ± 1.84	153.12 ± 3.21	132.13 ± 3.21	93.34 ± 2.25
GROUP 3	Vidangadi Kvatha 200 mg/kg	80.56 ± 1.09	194.77 ± 1.19	131.86 ± 3.23	121.55 ± 4.91	89.45 ± 1.24
GROUP 4	Glibenclamide	81.34 ± 1.30	185.63 ± 3.41	143.54 ± 3.33	109.83 ± 3.36	80.13 ± 2.14

The values represent the mean ± SEM with in the column and analyzed by one way ANOVA followed by Dunnett's test are significantly different at the P < 0.05

Table 5: Oral Glucose Tolerance Test (OGTT) in diabetic rats

Groups	Treatment	Blood Glucose Level (Mg/Dl)				
		0 Hr	30 Min	60 Min	90 Min	120 Min
GROUP 1 NC	Normal Saline	76.22 ± 1.05	151.52± 1.32	162.63±1.34	133.54±3.23	112.63 ± 5.64
GROUP 2 DC	Normal Saline	190.13 ± 2.38	298.14± 2.87	342.24±2.12	398.78±3.21	405.98 ± 2.33
GROUP 3 D	Vidangadi Kvatha 100 Mg/Kg	185.26± 3.14	298.34±2.64	313.42± 4.16	232.43± 3.61	181.24± 3.42
GROUP 4 D	Vidangadi Kvatha 200 Mg/Kg	180.58 ± 1.59	264.72± 1.63	252.36± 5.26	200.67±4.11	123.46 ± 2.74
GROUP 5 D	GLIBENCLAMIDE 600 µg/Kg	179.44 ± 1.63	265.23± 3.21	155.54± 3.23	110.24±2.27	98.42± 3.14

The values represent the mean ± SEM with in the column and analyzed by one way ANOVA followed by Dunnett's test are significantly

different at the P < 0.05, NC= Normal control, DC = Diabetic control, D = Diabetic Group

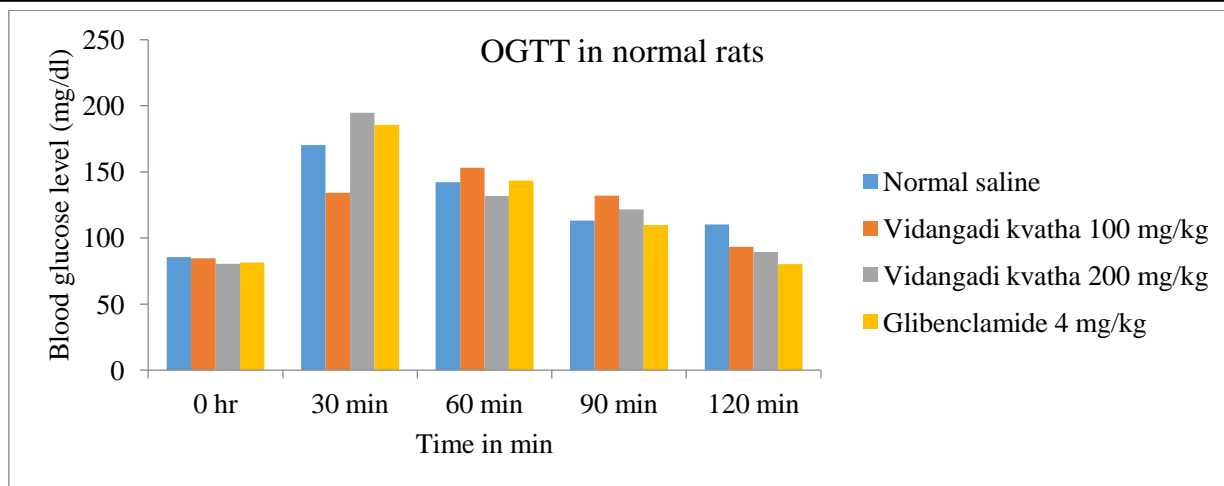


Figure 1: OGTT in normal rats

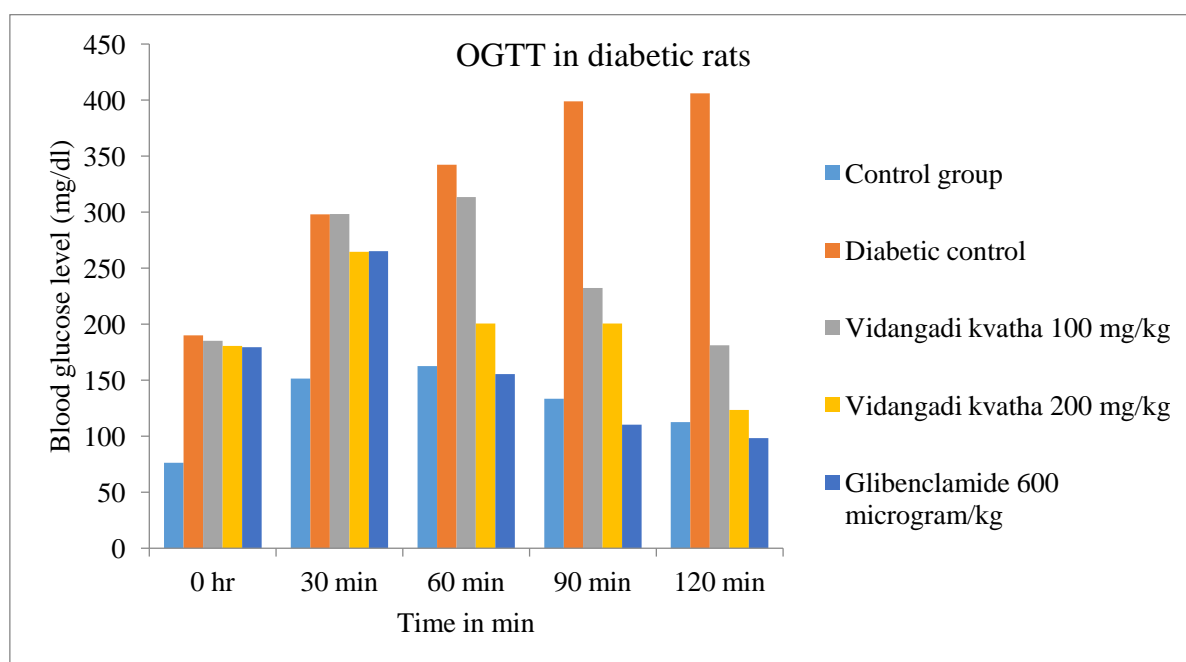


Figure 2: OGTT in diabetic rats

Effect of Vidangadi Kvatha on blood glucose level, lipid profile and liver profile in STZ-NAD induced diabetic rats: After treatment of diabetic rat with Vidangadi Kvatha for 15 days post prandial blood glucose level was found 129.35 ± 6.29 mg/dl at dose 200 mg/kg of Vidangadi Kvatha. 100 mg/kg of Vidangadi Kvatha lowers blood glucose level 140.34 ± 2.45 mg/dl, diabetic control rat shows 392.54 ± 2.13 mg/dl blood glucose level and Gliclazide 4 mg/kg lowers blood glucose level up to 96.32 ± 2.90 mg/dl. After 15 days treatment of diabetic rats with Vidangadi Kvatha at 200 mg/kg and 100 mg/kg increases the HDL level and decreases the

triglyceride level along with decrease in LDL, VLDL and total cholesterol. In liver profile the Vidangadi Kvatha also lowers the SGOT and SGPT level in both 100 mg/kg and 200 mg/kg dose in diabetic rats in comparison to diabetic control rats. The values are highly significant in comparison to normal control group rats, diabetic control group rats and standard (Gliclazide 4 mg/kg po treated rats). When a comparison is made between different doses of Vidangadi Kvatha (100 mg/kg and 200 mg/kg) the 200 mg/kg dose of Vidangadi Kvatha shows better result (93.34 ± 2.25 IU/L) in comparison to 100 mg/kg (120.16 ± 7.71 IU/L). Vidangadi Kvatha

increases the C-Peptide level 0.50 ± 0.015 ng/dl at dose 200 mg/kg of Vidangadi Kvatha in comparison to diabetic control group rats $0.32 \pm$

0.034 ng/dl. The study shows that Vidangadi Kvatha at dose 200 mg/kg gives better response in comparison to 100 mg/kg.

Table 6: Post Prandial blood glucose level (mg/dl)

S.N.	Groups	Post Prandial (pp) Blood Glucose Level (mg/dl) at 0-15 Day			
		0 Day	5 th Day	10 th Day	15 th Day
1	Normal control	$89.12 \pm 3.31^{**}$	$85.23 \pm 1.34^{**}$	$90.37 \pm 4.56^{**}$	$82.23 \pm 2.55^{**}$
2	Diabetic control	$229.44 \pm 3.23^*$	$224.11 \pm 4.28^*$	$318.12 \pm 3.72^*$	$392.54 \pm 2.13^*$
3	Per-see (study Vidangadi Kvatha 100 mg/kg)	$85.38 \pm 3.69^{***}$	$86.67 \pm 1.73^{***}$	$82.44 \pm 2.35^{***}$	$86.89 \pm 3.34^{**}$
4	Vidangadi Kvatha 100 mg/kg	$228.17 \pm 1.34^*$	$233.23 \pm 2.54^*$	$191.22 \pm 3.13^*$	$140.34 \pm 2.45^*$
5	Vidangadi Kvatha 200 mg/kg	$240.42 \pm 4.26^*$	$166.46 \pm 2.44^{**}$	$154.78 \pm 6.12^{**}$	$129.35 \pm 6.29^{***}$
6	Gliclazide 4 mg/kg	$211.56 \pm 7.34^*$	$208.32 \pm 4.78^{**}$	$115.21 \pm 7.12^*$	$96.32 \pm 2.90^{**}$

Values represents in mean \pm SEM (n = 6), analyzed by one way ANOVA, * P<0.05, ** P<0.001, ***P<0.0001 compared with diabetic control.

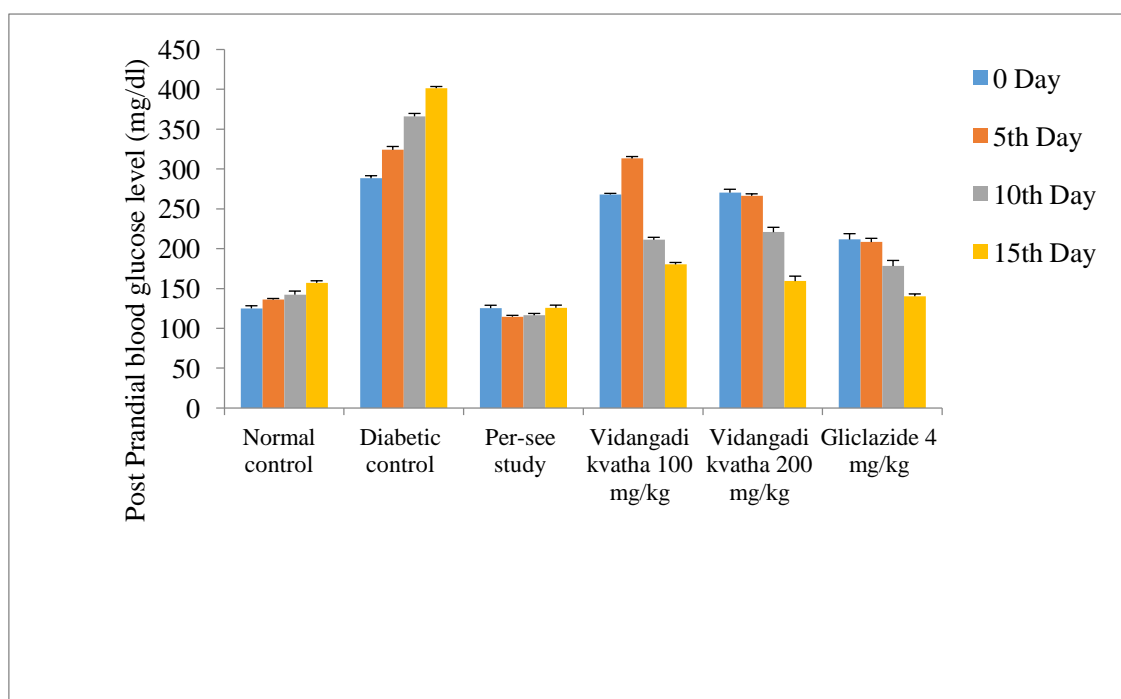


Figure 3: Graph representing post prandial blood glucose level

Table 7: Liver Profile in Diabetic Rats

Group	Treatment	SGPT (IU/L)	SGOT (IU/L)
I	Normal control	89.58 ± 6.66	76.30 ± 1.72
II	Diabetic control	123.60 ± 3.94	232.02 ± 4.39
III	Per-see study (Vidangadi Kvatha 100 mg/kg)	$115.06 \pm 6.1^{***}$	$125.78 \pm 2.6^{***}$
IV	Vidangadi Kvatha 100 mg/kg	$120.16 \pm 7.71^{***}$	$185.69 \pm 3.3^{***}$
V	Vidangadi Kvatha 200 mg/kg	$93.34 \pm 2.25^{**}$	$80.89 \pm 3.76^{**}$
VI	Gliclazide 4 mg/kg	80.12 ± 3.65	815.23 ± 2.36

Values are Expressed as Mean \pm S.E.M.,(n=6) analyzed by one way ANOVA followed by Dunnett test *P value<0.05, **P value <0.01,***<P value<0.001 compared with control group. DF=5,30.

Table 8: Lipid Profile of rats after treatment

GROUP	TREATMENT	TOTAL CHOLESTEROL (mg/dl)	TRIGLYCERIDES (mg/dl)	HDL CHOLESTEROL (mg/dl)	VLDL CHOLESTEROL (mg/dl)	LDL CHOLESTEROL (mg/dl)
I	Normal control	85.6 ± 3.33	75.6 ± 2.81	35.63 ± 2.06	14.32 ± 0.39	38.68 ± 1.52
II	Diabetic control	170.23 ± 3.56	165.47 ± 2.3	25.32 ± 0.95	36.85 ± 0.65	185.56 ± 2.32
III	Per-see study Vidangadi Kvatha 100 mg/kg	86.74 ± 2.39	78.14 ± 1.14	27.36 ± 0.12	26.12 ± 3.12	58.45 ± 1.45
IV	Vidangadi Kvatha 100 mg/kg	113.56 ± 2.56	77.36 ± 2.13	34.32 ± 2.13	20.41 ± 2.53	57.58 ± 2.33
V	Vidangadi Kvatha 200 mg/kg	92.56 ± 3.24	71.65 ± 5.26	36.56 ± 5.23**	21.05 ± 4.62	52.41 ± 2.11**
VI	Gliclazide 4 mg/kg	88.89 ± 6.25	70.45 ± 3.56**	36.58 ± 4.15	28.14 ± 5.12***	42.35 ± 1.62***

Values are Expressed as Mean ± S.E.M.,(n=6) analyzed by one way ANOVA followed by Dunnett test

* P value<0.05, **P value <0.01,***<P value<0.001 compared with control group. DF=5,30.

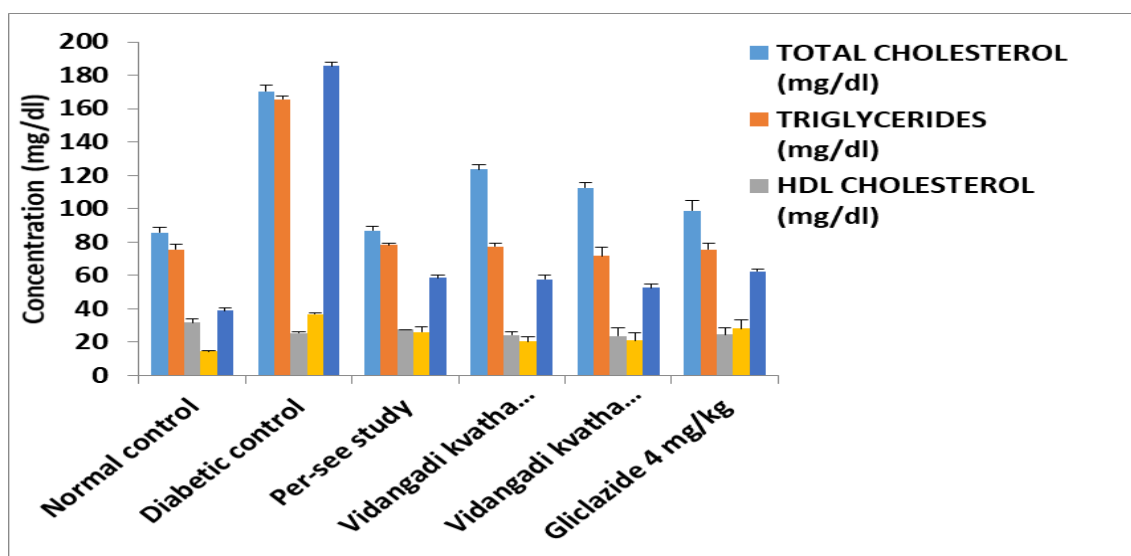


Figure 4: Lipid profile of treated diabetic rats

Table 9: C-Peptide level in rats after treatment with Vidangadi Kvatha

GROUP	TREATMENT	C-Peptide (ng/dl)
I	Normal control	0.58 ± 0.025
II	Diabetic control	0.32 ± 0.034***
III	Per-see study Vidangadi Kvatha 100 mg/kg	0.55 ± 0.063*
IV	Vidangadi Kvatha 100 mg/kg	0.45 ± 0.031**
V	Vidangadi Kvatha 200 mg/kg	0.50 ± 0.015**
VI	Gliclazide 4 mg/kg	0.59 ± 0.025***

Values are expressed in mean ± SEM, *P<0.05 significant value when compared with control, **P<0.001 highly significant compared with diabetic control, ***P<0.0001 highly significant. DF=5, P value = 0.007

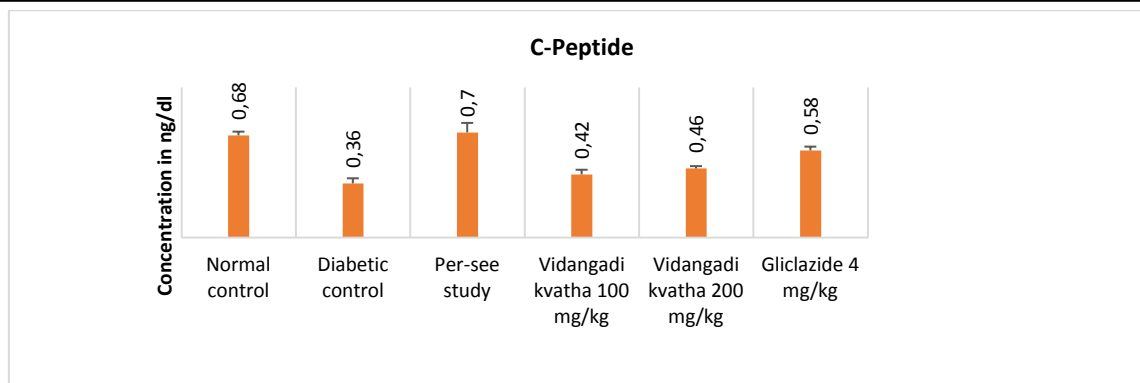


Figure 5: Graph representing C-Peptide level in diabetic rats after treatment

Discussion

In-vivo antidiabetic study of Vidangadi Kvatha in rats was performed for estimation of acute oral toxicity¹⁹, Oral glucose tolerance test (OGTT)²⁰ and antidiabetic activity in STZ-NAD induced diabetic rat models²¹. Acute oral toxicity study shows greater than 2000 mg/kg was safe and effective in rats. The OGTT was performed in normal and diabetic rats and the results indicates that 200 mg/kg dose of Vidangadi Kvatha is more effective in comparison to 100 mg/kg of Vidangadi Kwatha in reducing blood glucose level. Vidangadi Kwatha lowers the elevated SGOT and SGPT level, increases the HDL level and reduces LDL, VLDL, total cholesterol and triglyceride level. Vidangadi Kwatha increases the C-Peptide level indicated that it mimic the action of Gliclazide.

Conclusion

The Vidangadi Kvatha a polyherbal formulation reduces the blood glucose level significantly in diabetic rats. Kvatha reduces the enzymatic activity of alpha amylase, alpha glucosidase which are responsible for hyperglycemia. Kvatha also inhibit the glucose absorption from dialysis membrane, hence we can says that Vidangadi Kvatha can be used as a potent antidiabetic formulation for the management of hyperglycemia.

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Conflict of Interest: No conflict of interest

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