EVALUATION OF ANTI-DIABETIC ACTIVITY OF HYDRO ALCOHOLIC EXTRACT OF SPROUTS OF *VIGNA RADIATA* ON STREPTOZOTOCIN INDUCED DIABETES IN WISTAR RATS

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ABSTRACT

Diabetes mellitus is a metabolic disorder of multiple etiology characterized by chronic hyper glycaemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both[1].In 2000, an estimated 171 million people in the world had diabetes and this is projected to increase to 366 million by 2030. The World Health Organization (WHO) has estimated that for some 3.4 billion people in the developing world. Plants represent the primary source of medicines. The mung bean *Vigna radiata* (L.) is a legume cultivated for its edible seeds and sprouts across Asia, belongs to Fabaceae family.*Vigna radiate*used for paralysis, rheumatism, coughs, fevers and liver ailments. The acute oral toxicity was performed as per OECD 423 guidelines. Wistar albino rats were divided into five groups of six animals each. Group I were normal, Group II were STZ (55 mg/kg b.w., i.p) induced diabetic animals. Group III STZ (55 mg/kg b.w., i.p) induced diabetic animals were treated with Glibenclamide 5mg/kg b.w/p.o, Group IV STZ (55 mg/kg b.w., i.p) induced diabetic animals were treated with HAEVR (Hydro alcoholic extract of *Vigna radiata* (L.)) 200mg/kg b.w/ p.o Group V STZ (55 mg/kg b.w., i.p) induced diabetic animals were treated with HAEVR 400mg/kg b w/p.o for 28 days. On 28th day all animals were sacrificed and HDL, LDL, SGOT, SGPT, triglycerides, urea, creatinine and total protein were estimated.

Keywords: Diabetic, STZ induced diabetic, Hydro alcoholic extract of *Vigna radiate*.

INTRODUCTION

Plants had been used for medicinal purposes long before recorded history. The worldwide prevalence of diabetes mellitus (DM) has risen dramatically over the past two decades; based on current trends, more than 360 million individuals will have diabetes by year 2030. In addition to oral agents and insulin therapy, phytotherapy is an alternative Herbal remedies are beneficial to patients with type 2 diabetes. *Vigna radaita* has nutritional factors which a good source of minerals, proteins, provitamin A and vitamin B complex. In Veterinary medicine *Vigna radaita* seed paste mixed with turmeric powder applied to treat dislocated bone of cattle. [CRC World Dictionary of Medicinal and Poisonous Plants] [1,2].

EXPERIMENTAL ANIMALS

Adult Female Wistar rats of weighing 180-220gms were used for this study. The inbred animals were procured from the animal house of C.L. BaidMetha College of Pharmacy, Thoripakkam, Chennai. They were housed five per cage under standard laboratory conditions at a room temperature at 22 ± 2^0 C with 12hr light/dark cycle. The animals were acclimatized to laboratory conditions one week and provided with standard pellet chow and water *ad libitum*. Ethical committee clearance was obtained from IAEC of CPCSEA. (IAEC/XLVI/08/CLBMCP/2015 dated: 20/08/2015).

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MATERIALS AND METHODS
Preparation of Plant Extract

The sprout seeds of *Vigna radaita* were collected from local source, Tamil Nadu in December. The plant material was identified and authenticated by Dr.P.Jayaraman., Retd. Professor, Presidency College,Chennai–600005, Tamilnadu. [PARC/2014/2095]. The powdered seed were extracted with water: ethanol (40:60) in Soxhlet's apparatus at room temperature for 24 hours. The extract was stored at 0-4°C. The yield of the hydro alcoholic extract was 6.84% w/w.

ACUTE ORAL TOXICITYSTUDIES:

The Acute Oral Toxicity Study was done according to the OECD guidelines 423 (Acute toxic class method). A single administration of 2000 mg/kg b.w /p.o of HAEVR was administrated to three adult female Wistar rats and observed for 14 days. There was no considerable change in body weight before and after treatment and no signs of toxicity were observed.

INDUCTION OF DIABETES

Diabetes was induced in rats byStreptozotocin at a dose of 55 mg/kg b.w i.p (dissolved in freshly prepared 0.01 M citrate buffer, pH 4.5.) After a period of 3 days blood glucose levels were checked. Rats showing the blood glucose levels more than 250 mg/dl was taken study [3].

EXPERIMENTAL DESIGN

Diabetes was induced in fasted Albino wistar rats (180-220 gms) by STZ 55 mg/kg b.wi.p except Group-I.
Group I- Control animals treated with 1% w/v SCMC.
Group II- STZ (55 mg/kg b.w., i.p) induced diabetic animals.
Group III- STZ (55 mg/kg b.w., i.p) induced diabetic animals treated with Glibenclamide 5mg/kg b.w/p.o
GROUP IV - STZ (55 mg/kg b.w., i.p) induced diabetic animals treated with HAEVR 200mg/kg b.w/ p.o
GROUP V - STZ (55 mg/kg b.w., i.p)induced diabetic animals treated with HAEVR 400mg/kg b.w/ p.o

Fasting blood glucose levels was measured before the administration of HAEVR. It was recorded as 1st day the 200 mg/kg/p.o and 400 mg/kg/p.o doses of the HAEVR along with the standard (Glibenclamide) were given for 28 days. The blood glucose levels were measured on 1th, 7th, 14th, 21th and 28th day of the treatment period [4].

Biochemical and Histopathology Analysis

On 28th day animal was sacrificed by decapitation, blood was collected and serum was separated to study the biochemical parameters. The serum Protein [5], serum lipids [6], cholesterol [7], triglyceride, HDL[8],VLDL, LDL [9], SGOT and SGPT[10] the plasma Creatinine [11], urea [12] and Histopathology studies of liver, kidney and pancreas were carried out by using standard procedure. (Figure 4).

RESULTS

The result of preliminary phytochemical analysis HAEVR showed presence of various such as, test for Alkaloids, phenols, flavonoids, and steroidswith absence of Carbohydrates, tannins, phenols, proteins, steroids and fixed oils

The Acute Oral Toxicity Study was done according to the OECD guidelines 423 (Acute toxic class method). A single administration of 2000 mg/kg b.w /p.o of HAEVR was administrated to three adult female Wistar rats and observed for 14 days. There was no considerable change in body weight before and after treatment and no signs of toxicity were observed (Table 1).

The body weight in group I was compared with II, III, IV andV were decreased significantly (p<0.001). The bodyweight in group II was compared with group III,(p<0.001). IV (ns) and V were increased significantly (p<0.001). (Table 2; Figure 1)

The blood glucose levels in group I was compared with group II, III, IV and V (p<0.001), were significantly increased. The blood glucose levels in group II was compared with group III, IV and V were significantly (p<0.001) decreased. (Table3; Figure 2)

The serum cholesterol level in group I was compared with group II, III, III and V weresignificantly increased (p<0.001). The serum creatinine level in group II was compared with III, IV and V were significantly decreased (p<0.001).(Table 4; Figure 3)

The serum HDL level in group I was compared with group II, III (p<0.01),IV and V (p<0.001) were significantly decreased. The HDL level in group II was compared with III, IV and V (p<0.001)significantly increased (Table 4; Figure 3).

The serum LDL level in group I was compared with group II, III (p<0.01), IV and V(p<0.001) were significantly increased. The serum LDL level in group II was compared with III, IV and V (p<0.001) were significantly decreased (Table 4; Figure 3).

The serum VLDL level in group I was compared with group II, III, IV and V (p<0.001) were significantly increased. The serum VLDL level in group II was compared with III, IV and V were significantly decreased (p<0.001). (Table 4; Figure 4).

The serum triglyceride level in group I was compared with group II, III, IV and V (p<0.001) were significantly increased. The serum triglyceride level in group II was compared with III, IV and V (p<0.001) were significantly decreased (Table 4; Figure 3).

The serum total protein level in group I was compared with group II (p<0.01) III, IV and V (ns) were significantly increased. The serum total protein level in group II was compared with group III, (0.001) group IV and group V were significantly decreased (p<0.01) (Table 5).

The serum creatinine level in group I was compared with group II, III, IV and V (p<0.001) were significantly increased. The serum creatinine level in group II was compared with III, IV and V were significantly decreased (p<0.001). (Table 5; Figure 4).

The serum urea level in group I was compared with group II, III, IV and V (p<0.001) were significantly increased. The serum urea level in group II was compared with group III, IV and V were significantly decreased (p<0.001).(Table 5; Figure 4).

The SGOT level in group I was compared with group II, III, IV and V (p<0.001) were significantly increased. The SGOT level in group II was compared with III, IV and V were significantly decreased (p<0.001).(Table 5; Figure 5).

The SGPT level in group I was compared with group II, III, IV and V were significantly increased (p<0.001). The SGPT level in group II was compared with III, IV and V were significantly decreased (p<0.001). (Table 5; Figure 5).

Table 1. Acute Oral Toxicity Study

Si. No.	Treatment group	Dose	Weight of animal in gms		Signs of toxicity	Onset of toxicity	Reversible or irreversible	Duration
			Before test	After test				
1.	*HAEVR*	2g/kg	180	180	No signs of toxicity	Nil	Nil	14 days
2.	*HAEVR*	2g/kg	175	175	No signs of toxicity	Nil	Nil	14 days
3.	HAEVR	2g/kg	190	190	No signs of toxicity	Nil	Nil	14 days

Table 2. Effect of HAEVR on body weight of STZ induced diabetic rats

Group	Body weight (gm.)				
	Day – 0	Day – 7	Day – 14	Day – 21	Day – 28
I	186.2±1.939	188.2±2.272	196.5±0.7638	204.0±1.183	210.2±1.400
II	183.2±4.110	152.5±1.432 a***	133.5±1.088 a***	124.7±0.8819 a***	121.3±1.542 a***
III	182.7±2.108	172.2±2.023 a*** b***	173.5±1.232a*** b***	181.7±0.8819 a*** b***	191.0±1.880 a*** b***
IV	180.0±3.651	160.7±2.246 a*** b ns	162.8±0.9458a*** b***	168.2±0.7923 a*** b***	175.0±1.238 a*** b***
V	184.0±3.317	166.3±1.994 a*** b***	169.8±0.9098a*** b***	175.8±1.400 a**** b***	181.2±1.447 a*** b***

Values are expressed as mean ± SEM of 6 animals. Comparisons were made between the following: a - Group I vs. II, III, IV and V, b - Group II vs. III, IV and V. Statistical Significance test for comparison was done by one way ANOVA followed by Dunnett's 't' test. Where *p< 0.05, **p< 0.01, ***P<0.001, ns-non significant

Table 3. Effect of HAEVR on blood glucose level in STZ induced diabetic rats

Group	Blood glucose (mg/dl)				
	Day – 0	Day – 7	Day – 14	Day – 21	Day – 28
I	91.00±3.120	83.50±1.668	95.83±3.646	91.33±2.996	95.50±3.828
II	100.3±5.649	250.8±1.922a***	285.2±2.613 a***	290.7±1.520 a***	297.8±1.701 a***
III	105.3±6.586	171.0±1.549a*** b***	142.0±1.461 a*** b***	121.3±1.783 a*** b***	103.3±1.856 a ns b***
IV	98.83±6.290	220.5±1.607 a*** b***	201.2±2.257 a*** b***	159.8±1.515 a*** b***	130.5±1.688 a*** b***
V	92.83±3.390	180.8±1.838a*** b***	161.0±1.528 a*** b***	120.0±1.065 a*** b***	109.8±2.272 a** b***

Table 4. Effect of HAEVR on Total cholesterol, HDL, LDL, VLDL, Triglycerides

Groups	Total cholesterol (mg/dl)	HDL (mg/dl)	LDL(mg/dl)	VLDL (mg/dl)	Triglycerides (mg/dl)
Group I	108.3±0.8242	60.60±0.7169	82.02±0.5598	16.34±0.2994	86.18±0.3630
Group II	206.3±0.6184 a***	33.46±0.3918 a***	141.6±0.8738 a***	40.87±0.4726 a***	170.5±0.5911 a***
Group III	119.3±0.4665 a*** b***	57.68±0.3218 a** b***	85.08±0.2794 a** b***	19.48±0.3522 a*** b***	90.37±0.4061 a*** b***
Group IV	159.3±0.9684 a*** b***	42.56±0.2840 a*** b***	111.0±0.4890 a*** b***	29.54±0.2956 a*** b***	123.6±0.4776 a*** b***
Group V	136.8±0.7151 a*** b***	54.88±0.5408 a*** b ***	91.93±0.4630 a*** b***	21.46±0.4489 a*** b***	96.34±0.4439 a*** b***

Table 5. Effect of HAEVR on Total protein, Creatinine, Urea, SGOT, SGPT

Groups	Total protein (mg/dl)	Creatinine (mg/dl)	Urea(mg/dl)	SGOT (U/L)	SGPT (U/L)
Group I	6.405±0.1967	0.4983±0.02822	19.08±0.2544	68.10±0.5008	41.44±0.5317
Group II	5.690±0.1119 a**	1.822±0.02774 a***	52.60±0.4275 a***	139.8±0.6129a***	98.96±0.4503 a***
Group III	6.635±0.1179a^{ns}b***	0.6667±0.01856 a***b***	23.29±0.2846 a*** b***	97.86±0.4566 a*** b***	60.86±0.6440 a*** b***
Group IV	6.442±0.1196a ^{ns}b**	1.272±0.02960 a*** b***	43.84±0.5212 a*** b***	89.37±0.4797 a*** b***	52.55±0.4378 a*** b***
Group V	6.512±0.1104a^{ns}b**	0.8817±0.01701 a***b***	27.28±0.5582a*** b***	82.49±0.5245 a*** b***	48.75±0.4090 a*** b***

Figure 1. Effect of HAEVR on body weight of STZ induced diabetic rats
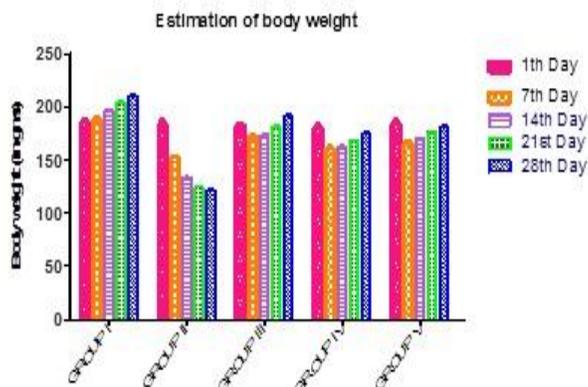

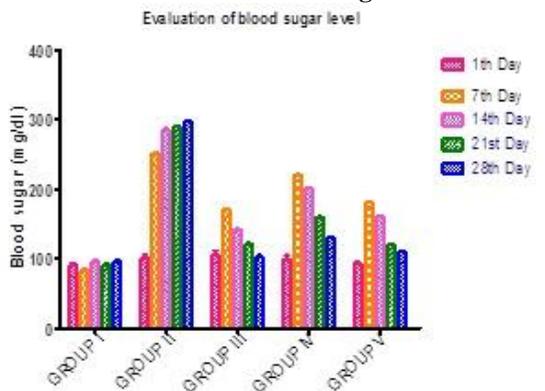**Figure 2. Effect of HEVR on blood glucose level in STZ**

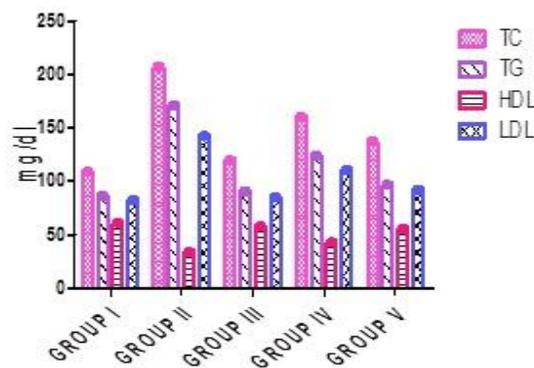**Figure 3. Effect of HEVR on TC, TG, HDL, LDL induced diabetic rats**

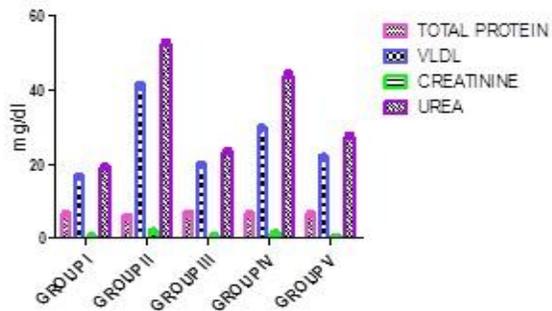**Figure 4. Effect of HEVR on total protein, VLDL, Creatinine & Urea**

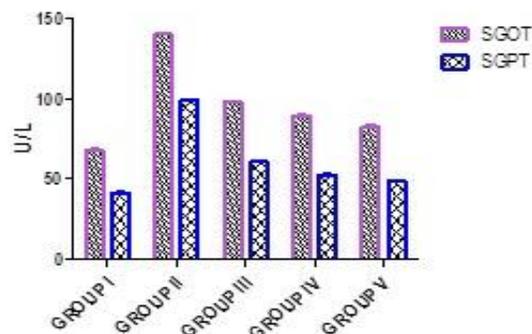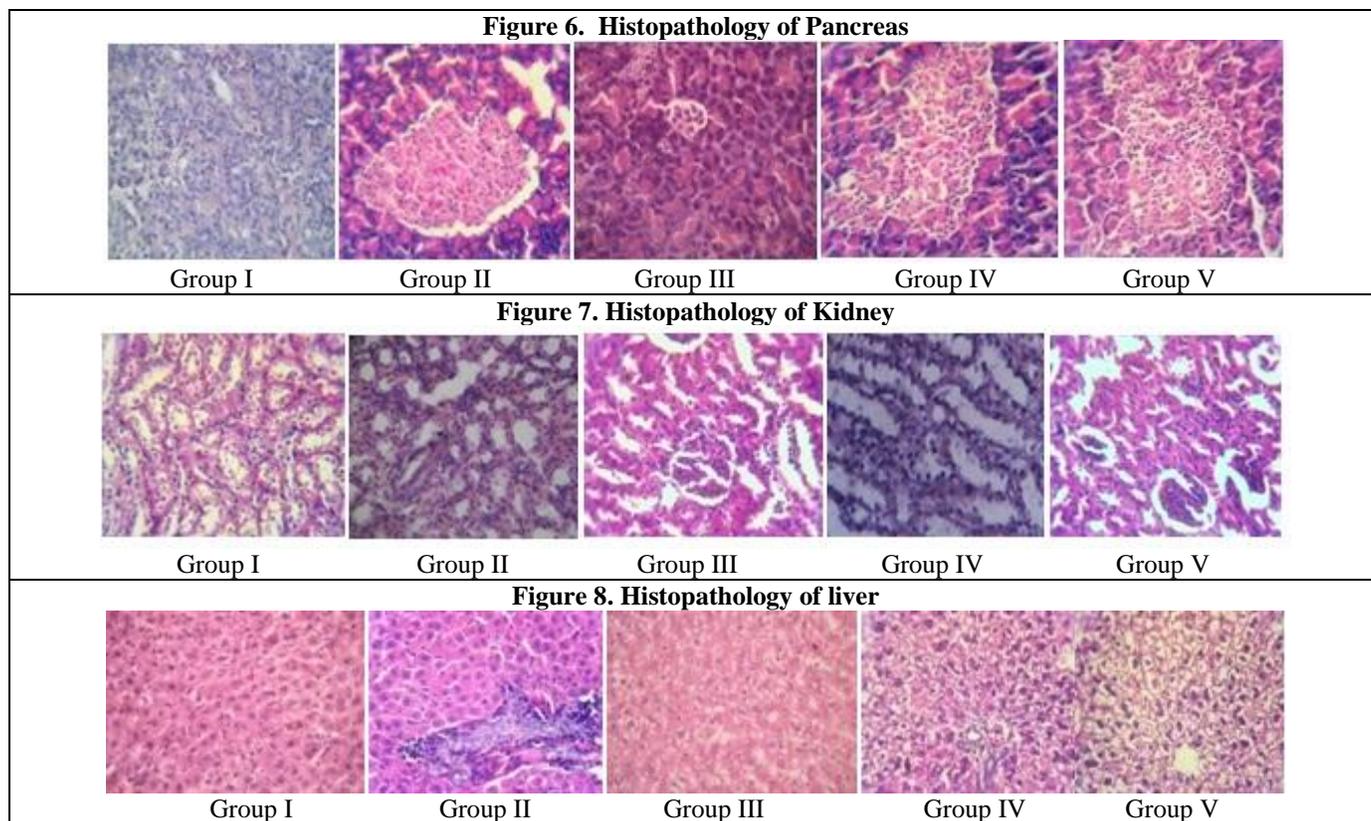**Figure 5. Effect of HEVR on SGOT & SGPT**

Figure 6. Histopathology of Pancreas

| Group I | Group II | Group III | Group IV | Group V |

Figure 7. Histopathology of Kidney

| Group I | Group II | Group III | Group IV | Group V |

Figure 8. Histopathology of liver

| Group I | Group II | Group III | Group IV | Group V |

DISCUSSION

Diabetes is the condition in which the body does not properly process food for use energy. The term diabetes mellitus describes a metabolic disorder of multiple aetiology characterized by chronic hyperglycemia with disturbances of carbohydrate, fat, and protein metabolism resulting from defects in insulin secretion insulin action, are both the chronic hyperglycemia of diabetes is associated with relatively specific long term micro vascular.

The increase in number of diabetic patients has motivated to find new methods to cure diabetes. Inspite of the presence of known anti-diabetic medicine in the pharmaceutical market, remedies from medicinal plants are used with success to treat this disease [13]. This attribute antihyperglycemic effects of these plants is due to their ability to restore the function of pancreatic tissues by causing an increase in insulin output or inhibit the intestinal absorption of glucose or to the facilitation of metabolites in insulin dependent processes. Hence, treatment with herbal drugs has an effect on protecting beta cells and smoothing out fluctuation in glucose levels [14]. The present study involved the evaluation of anti-diabetic activity of sprout of *Vigna radaita* in STZ induced diabetic rats.

Preliminary Phytochemical analysis showed the presence of flavonoids, which act as insulin secretagogues or insulin mimetic, probably by influencing the pleiotropic mechanisms to attenuate diabetic complications. Flavonoids may be responsible for the stimulation of glucose uptake in peripheral tissues and regulation of the activity and / or expression of the rate-limiting enzymes involved in carbohydrate metabolism [15]. Acute oral toxicity study of HAEVR did not exhibit mortality or any profound toxic reactions at a dose of 2000mg/kg/p.o

The mechanism by which STZ brings about its diabetic state include selective destruction of pancreatic insulin secreting beta cells, which make cells less active and lead to poor glucose utilization by tissues [16]. STZ induced diabetic model resembles type 1diabetes with final symptoms of insulin deficiency. Long term treatment (28 days) with active fraction of HAEVR resulted in mild improvement in plasma insulin levels. This suggests that *Vigna radaita* like glibenclamide stimulates insulin secretion from the remnant beta cells of islets of langerhans[17]or the drug may be mimicking one or more actions of insulin at the insulin receptor level or/and it may be influencing one or more post receptor events.

.   Experimental induction of hyperglycaemia with STZ is associated with the characteristic loss of body weight which is due to loss or degradation of structural proteins it leads to increased muscle wasting and due to loss of tissue protein, as the structural proteins are known to contribute to body weight. Diabetic rats treated with glibenclamide and HAEVR showed increased body weight when compared to untreated diabetic animals. It may be due to increased insulin secretion and glycaemic control of HAEVR.

The mechanism involved in suppressing blood glucose levels may be by the following possibilities. Reduced glucose transport or absorption from the gut, extra pancreatic action probably by stimulation of glucose utilization in peripheral tissues, increase in glycogenic or glycolytic enzyme activities in peripheral tissues, decrease in the secretion of counter-regulatory hormones like glucagon, growth hormones. The glibenclamide, stimulating insulin secretion from pancreatic β cells principally by inhibiting ATP sensitive K_{ATP} channels in the plasma membrane and decreases the blood glucose level [17]. Blood glucose level decreased significantly in glibenclamide and HAEVR treated diabetic rats and the histopathology of pancreas showed normal islets in pancreas with normal anatomy compared with normal rats which may be due to the anti-diabetic activity.

In diabetes, hyperglycaemia is accompanied with dyslipidemia [18] under normal circumstances; insulin activates the enzyme lipoprotein lipase, which hydrolyses triglycerides. However, in diabetic state lipoprotein lipase is not activated due to insulin deficiency, resulting in hyper triglyceridemia, and insulin deficiency is also associated with hyper cholesterolemia due to metabolic abnormalities. The dyslipidaemia is characterized by increase in TC, LDL, VLDL, TG and fall in HDL which is observed in STZ induced diabetic rats [19,20]. The diabetic rats treated with glibenclamide and HAEVR showed reduced severity of dyslipidaemia with decrease in TC, LDL, VLDL, TG and increase in HDL level.

Both SGOT and SGPT enzyme levels get elevated during liver damage which is more in diabetic rats [21,22]. The diabetic rats treated with glibenclamide and HAEVR reduced the SGOT and SGPT level. The liver histopathology of STZ induced diabetic rats showed centrilobular necrosis accompanied by fatty changes and ballooning degeneration in the heptocytes which was reversed in diabetic rats treated with glibenclamide and HAEVR which indicates that the liver damage is reduced in HAEVR treated group.

The diabetic hyperglycaemia induces elevation of the serum levels of urea and creatinine which are significant markers of renal dysfunction and reflecting a decline in the glomerular filteration rate [23] which is a complication in type II diabetes. Like glibenclamide the urea and creatinine level in HAEVR treated animals showed decreased serum urea and creatinine level. The kidney histopathology sections revealed almost no morphological difference in mean nuclear diameter of kidney cells from control it indicates that drenching HAEVR extract is safe on rat kidney. Hence it is an evident that HAEVR reduced the complication of diabetes.

CONCLUSION

The sprout of *Vigna radaita* reduces the blood glucose level and lipid profile like TC,TG, LDL, and VLDL, increase HDL. The *Vigna radaita* reduces SGOT, SGPT, total protein, creatinine and urea in STZ induced diabetic rats. Histopathology shows that the sprout of reduced liver and kidney damage which is common in diabetes.

Thus, it may be concluded that *Vigna radaita* produces significant anti-diabetic activity in Streptozotocin induced diabetic rats, which is comparable with that of Glibenclamide. Further work was necessary to elucidate the mechanism of action involved in the anti-diabetic activity of *Vigna radaita* with special references to phytochemical constituents.

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