ISSN Print: 2394-7500
ISSN Online: 2394-5869
Impact Factor: 5.2
IJAR 2017; 3(10): 366-371
www.allresearchjournal.com
Received: 22-08-2017
Accepted: 25-09-2017

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Anti-diabetic activity of isolated fractions of *Vigna radiata* and *Allmania nodiflora* in streptozotocin induced diabetic rats

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Abstract
The present study focuses on the determination of the anti-diabetic activity of the extracted fractions of the seeds of *Vigna radiata*; Fabaceae family and whole plant of *Allmania nodiflora*; Amaranthaceae family in rats with diabetes induced through STZ. The 20 mg/kg and 40 mg/kg doses of *Vigna radiate* and *Allmania nodiflora* were given to the rats for 28 days. The antidiabetic activity was compared to the standard pioglitazone at a dose of 2.7 mg/kg, by measuring blood glucose through accu-chek active test meter. The results revealed that *Vigna radiata* had significant anti-diabetic activity. Also, the *Vigna radiata* remained safe till 300 mg/kg in acute toxic and 1000 mg/kg in sub-acute toxic studies and had photochemically. From these findings it can be inferred that *Vigna radiata* has lowered FBG in experimentally induced diabetic rats.

Keywords: Anti-diabetic activity, *Vigna radiata*, *Allmania nodiflora*, STZ, FBG-fasting blood glucose

Introduction
Diabetes mellitus is a metabolic condition with several etiology featured by chronic hyper glycaemia and defective insulin secretion leading to disturbed carbohydrate, fat and protein metabolism, insulin action, or both. Middle aged people are most often affected by type II diabetes whereas 55% of deaths are observed in women[1].The estimation of the world wide prevalence of diabetes was 2.8% in 2000 which may rise to 4.4% in 2030 [2]. Conservative medical rehearse, side effects are observed with current therapies of diabetes mellitus. Effective, safe and cheap drugs can be made through medicinal plants that were used by humans to prevent or cure diseases including diabetes since the dawn of civilization [3]. Developments in molecular biology and information technology have enhanced the understanding of the mechanism of action of many herbal drugs and the associated phytomedicines which differ in many respects from that of synthetic drugs or single chemical entities [4]. *Vigna radiata* and *Allmania nodiflora* had different medicinal uses but no proven anti-diabetic activity.
The mung bean *Vigna radiate* (L.) is a legume cultivated for its edible seeds and sprouts across Asia. The mung bean plant is an annual, erect or semi-erect, reaching a height of 0.15-1.25 m [5]. It is slightly hairy with a well-developed root system. During the past few decades, flavonoids, phenolic acids, organic acids and lipids have been identified from the seeds and sprouts of mung beans and have been shown to contribute to its pharmaceutical activities [6].Seeds used for paralysis, rheumatism, coughs, fevers and liver ailments.
Allmania nodiflora is an annual herb, erect or rising, reaching a size of 10-50 cm in height. It has branched stems from near the base. It is found in tropical region sandy soils, in China and Asia [7]. Fruits of *Allmania nodiflora* are used for constipation and dysentery and the leaves are used as febrifuge, Hypolipidemic. Nutritive, appetizer [8].
The present study investigates the anti-diabetic property of mung seeds and *Allmania nodiflora* plant.

Materials and Methods
Plant Material
The whole of *Vigna radiata* and *Allmania nodiflora* plants were collected from fields of Guntur in February and hilly areas of Vishakhapatnam in December respectively.

The plant material was found and validated by Dr. P. Jayaraman, Professor, Presidency College, Chennai–600005, Tamil Nadu [PARC/2012/2095, PARC/2012/2096]. A token specimen was submitted at the pharmacognosy department of the college.

Extraction

The seeds of *Vigna radiata* and whole plant of *Allmania nodiflora* were dried under shade and are ground into coarse powder. The powders (500g each) were subjected to serial exhaustive extraction with several solvents basing on their polarity, such as chloroform, methanol, petroleum ether and water serially, the solvent was then evaporated and the concentrate was dried off through rotavac with temperature set at 40-50 ^{0}C. The produced marc with considerable blood sugar lowering effect were selected for further fractionation in various steps, solvent separation using methanol, chloroform and n-butanol serially, the bioactive fraction is subjected to column separation and partitioned with mixture of two solvents with near polarity at various ratios as depicted in the scheme below, the fraction with good biological activity is subjected to flash chromatography and the resulting bioactive fractions are isolated using preparative TLC, with 20 X 20 cm glass plates (0.5 mm) which are silica gel G coated. The fractions were tested for reduction of the blood glucose levels in normal rats through the elution with the chloroform and the fractions that are biologically active were taken into the study [9]. Phytochemical Tests were performed to identify the phytoconstituents [10].

Animals

Swiss Albino male mice weighing 25 – 30 g for acute and sub chronic toxicity studies and adult Wistar rats of either sex weighing 180-220 g were used for antidiabetic study. The inbred animals were procured from the animal house of Mahaveer Enterprises, Hyderabad. They were housed five per cage under standard lab conditions with a room temperature at 22 ± 2 ^{0}C with 12 hr light/dark cycle. The animals were adjusted to lab conditions one week and given standard pellets chow and water ad libitum. Ethical committee clearance was obtained from IAEC of CPCSEA.

Toxicity study [11]

The procedure was followed as per OECD guidelines (Organization of Economic Cooperation and Development) 423 (Acute oral toxicity - Acute toxic class method). Three male albino mice weighing between 20-25 gm were taken into the study. 300 mg/kg body weight p. o. is taken as the starting dose level of the isolated fractions. Dose was administered accordingly to the overnight fasted mice with water ad libitum, food was not given till 3-4 hours post drug administration and seen for the evidence of toxicity.

Body weights of the mice were taken at the start and end of the treatment, monitored for any alterations in eyes, skin, fur and mucous membranes and any systems like circulatory, respiratory, central, autonomic nervous systems, behavior pattern and locomotor activity and signs like convulsions, tremors, salivation, lethargy, diarrhea, sleep and coma were took a note. Both the onset and signs of toxicities if any were observed for 14 days.

Sub-chronic toxicity study [12]

The below experimental procedure was used to determine the sub-chronic toxicity of VRCM and ANCM in mice.

Group I: Control animals received 10% tween 20, 2 ml/kg/p. o. for 28 days

Group II: Isolated fractions of VRCM and ANCM at a dose level of 1000 mg/kg/p. o. suspended in 10% tween 20, 2 ml/kg/p. o. for 28 days

Food-water intakes and body weight were noted twice per day with subsequent review for any toxic modulation and mortality. All animals were immolated by the end of 28 day treatment period, under anesthesia using over dose ether. Blood was taken from the jugular vein in anticoagulant pretreated tubes and shaken gently and was used for estimation of hemogram and leukogram using fully automatic hematology analyzer. Liver, spleen, brain, heart, kidney, lung, testis and ovaries were separated and preserved for histopathologic study using 10% formalin.

Induction of diabetes

Streptozotocin 90 mg/kg (Acetate buffer 0.1M freshly made, having pH 4.5) was given intraperitonially to the neonatal rats of 10-12 g weight on day five, postnatally (n5-STZ) [13] Freshly prepared buffer which serves as control was also given in the same way to the neonatal rats. After four weeks, all these rats were segregated from their mothers, provided with standard pellet feed (Rayan's Biotech, Hyderabad) along with water ad libitum.

Experimental design

Grouping of animals

Group I - Normal Rats (vehicle control)
Group II - Rats serve as negative control

Pretreated set

Group III - Rats given VRCM 20 mg/kg
Group IV - Rats given VRCM 40 mg/kg
Group V - Rats given ANCM 20 mg/kg
Group VI - Rats given ANCM 40 mg/kg
Group VII - Rats given Pioglitazone 2.7 mg/kg

Post treated set

Group VIII - Rats given VRCM 20 mg/kg
Group IX - Rats given VRCM 40 mg/kg
Group X - Rats given ANCM 20 mg/kg
Group XI - Rats given ANCM 40 mg/kg
Group XII - Rats given Pioglitazone 2.7 mg/kg

Rats were categorized into two sets, one is pre-treatment and other is post-treatment (i.e. after taking streptozotocin, they remain untreated for 12 weeks), both have five groups (n = 10) each, of the pre-treatment groups, administration of drugs starts from 4th week of STZ administration till 21st day after 12 weeks whereas in the case of post treated groups, fractions are given after 12th week of taking streptozotocin for 21 days. Group I is to serve as control, group II as negative control, takes only vehicle. Pre-treated set has five groups from group III to group VII, which were treated in the way as explained above. VRCM, ANCM and pioglitazone were given as suspension in 10% tween 20 (vehicle) p. o. Dilutions were made as such to give 0.2ml/100g intra-gastrically. Negative control group received vehicle alone. Post treated set also has five groups (n = 10), but they remain untreated till 12th week after streptozotocin is given. All treatments were given intra-gastrically.

Oral glucose tolerance test (OGTT)

OGTT was done in both the pre-treated and post treated groups on 7th and 12th week after the streptozotocin treatment. An extra four groups of normal rats with similar age were used to study the effects of these treatments on OGTT in normal rats. The effect of the fractions on glucose overloaded hyperglycemia was learned in all the groups. Normal rats kept under fasting overnight nearly 12h, were taken into 6 groups (n = 6) of which group I being a control, group II, III, IV, V and VI were given VRCM 20, 40, ANCM 20, 40 mg/kg p. o. respectively, group VII rats were given 2.7 mg/kg of pioglitazone intra-gastrically. Group III to VII are pre-treated set whereas post-treated set remain untreated. Zero hour sample was measured for blood glucose levels by tail vein puncture. Animals were given oral glucose (4g/kg BW) after half an hour past drug administration and the blood glucose levels were measured at 0.5, 1, 2 and 3 h past glucose administration [14]. Blood glucose levels were read through a glucometer (Accu-chek Active™ Test meter).

Hypoglycemic effect in n5-STZ rats after chronic administration

After OGTT was done on 12th week after taking streptozotocin, both pre and post treatment rats were used to find the effect on the levels of blood glucose. Rats having more than 150 mg/dL blood glucose concentrations were regarded diabetic and taken into the study (n = 6) [15]. All the rats were given isolated fractions and pioglitazone as stated before. Blood glucose levels were measured through glucometer (Accu-chek Active™ Test meter) by tail vein puncture on days 1, 7, 14 and 21, 30 min past drug administration [16].

Effect on diabetes

Induction of diabetes mellitus in experimental animals [17]

Diabetes was produced in male wistar albino rats of 2–3 months age (180–200 g body weight) by giving streptozotocin (single dose of 55 mg/kg B.W.) intraperitoneally, made by dissolving in freshly prepared 0.01 M citrate buffer with pH 4.5.

After taking STZ, the animals were given food and water ad libitum and 5% glucose given with drinking water for the initial 24 hours to balance any hypoglycemia. The generation of diabetes was established past 72 hours of STZ injection, under light anesthesia the blood was drawn by cutting the tip of tail of each rat and the blood glucose concentration was measured. Animals with > 200 mg/dl blood glucose were regarded diabetic and divided into groups accordingly.

Grouping of animals

Group I - Normal Rats (vehicle control).
Group II - Rats served as negative control

Pretreated groups

Group III - Rats given VRCM 20 mg/kg
Group IV - Rats given VRCM 40 mg/kg
Group V - Rats given ANCM 20 mg/kg
Group VI - Rats given ANCM 40 mg/kg
Group VII - Rats given Pioglitazone 2.7 mg/kg

Experimental design [18]

The animals were sorted into seven groups each having six rats. Group I were normal rats, Group II were STZ (55 mg/kg b.w., i.p) induced diabetic rats. Group III and group IV were given VRCM 20 and 40 mg/kg and group V and group VI rats were given ANCM 20 and 40 mg/kg, group VII rats were given pioglitaozone (PIO) 2.7 mg/kg for 28 days.

Blood glucose concentrations under fasted state were noted during the pre-administration of fractions on 1st, 7th, 14th, 21st and 28th days of treatment period. Blood was collected by making an incision on the rat tail. Blood glucose concentrations were measured through a glucometer (Accu-chek Active™ Test meter). Effect on liver glycogen and glucose-6-phosphatase was measured accordingly glycogen was analyzed in fresh isolated livers of anesthetic state rats (sodium thiopental, 50 mg/kg). Parts of nearly 2 g were done homogenization and extraction with 8 ml of 6% $HClO_4$. The floating liquid in the upper part was under neutralization with 5 N K_2CO_3 and taken into the enzymatic glycogen assay.

Statistical analysis

All the values were expressed as mean ± standard error (SEM). One way analysis of variance followed by Dunnet's test comparing with p less than 0.05 were noted significant among the groups.

Results

The preliminary phytochemical studies indicate the presence of phenols, tannins and flavanoids in the chloroform and methanolic extract of the seeds of mung bean and *Allmania nodiflora* plants. In acute toxicity study, *Vignaradiata* and *Allmania nodiflora* did not produce any lethality up to the dose level of 300 mg/kg.

Table 1: Acute oral toxicity studies

S. No.	Drug treatment	Dose	Weight of animal group		Signs of toxicity	Onset of toxicity	Reversible or irreversible	Duration
			Before treatment (1st day)	After treatment (14th day)				
1.	VRCM	300 mg/kg	20	24	No signs of toxicity	NIL	NIL	14 days
2.	VRCM	300 mg/kg	25	31	No signs of toxicity	NIL	NIL	14 days
3.	VRCM	300 mg/kg	20	23	No signs of toxicity	NIL	NIL	14 days
As no toxicity or death has been observed for these dose levels the same dose level was tried again								
4.	ANCM	300 mg/kg	26	30	No signs of toxicity	NIL	NIL	14 days
5.	ANCM	300 mg/kg	31	35	No signs of toxicity	NIL	NIL	14 days
6.	ANCM	300 mg/kg	27	32	No signs of toxicity	NIL	NIL	14 days

Table 2: Hematological parameters of mice after sub chronic toxicity studies

Hematological parameters	Control	VRCM 1000 mg/kg p. o.	ANCM 1000 mg/kg p. o.
Erythrocytes (x1012/l)	5.92 ± 0.32	5.75 ± 0.41ns	5 ± 0.5ns
Leukocytes (x109/l)	3.4.3 ± 0.17	3.7 ± 0.14ns	3.32 ± 0.4ns
Hematocrit (%)	0.43 ± 0.03	0.4 ± 0.03ns	0.54 ± 0.01ns
Hemoglobin (g%)	13.2 ± 1.42	13.31 ± 2.13ns	13.12 ± 1.22ns
Differential Count per/cmm			
Neutrophils (x109/l)	2.33 ± 0.31	2.48 ± 0.23ns	2.44 ± 0.2ns
Eosinophils (x109/l)	0.08 ± 0.001	0.08 ± 0.004ns	0.08 ± 0.002ns
Lymphocytes (x109/l)	3.12 ± 0.17	3.41 ± 0.84ns	3.12 ± 0.66ns
Monocytes (x109/l)	0.11 ± 0.03	0.16 ± 0.05ns	0.14 ± 0.03ns
Basophils (x109/l)	0.03 ± 0.0016	0.04 ± 0.0015ns	0.03 ± 0.001ns

Table 3: Histopathological report of control, VRCM and ANCM treated mice tissues

S. No.	Organ	Groups		
		Control	VRCM 1000 mg/kg	ANCM 1000 mg/kg
1.	Liver	Shows normal liver with central vein with cords of hepatocytes	Shows normal liver with central vein with cords of hepatocytes	Shows normal liver with central vein with cords of hepatocytes
2.	Kidney	Normal kidney glomeruli and tubules	Normal kidney glomeruli and tubules	Normal kidney glomeruli and tubules
3.	Heart	Normal cardiac fiber	Normal cardiac fiber	Normal cardiac fiber
4.	Testis	Shows normal testicular tubules with normal spermatogenesis	Shows normal testicular tubules with normal spermatogenesis	Shows normal testicular tubules with normal spermatogenesis
5.	Lungs	Shows normal lung tissue with bronchi and alveoli	Shows normal lung tissue with bronchi and alveoli	Shows normal lung tissue with bronchi and alveoli
6.	Brain	Shows normal brain tissues with astrocytes and nerve fibers	Shows normal brain tissues with astrocytes and nerve fibers	Shows normal brain tissues with astrocytes and nerve fibers
7.	Pancreas	Shows normal pancreatic tissue	Shows normal pancreatic tissue	Shows normal pancreatic tissue
8.	Ovary	Shows normal ovary with maturing follicles	Shows normal ovary with maturing follicles	Shows normal ovary with maturing follicles

Table 4: Effect of isolated fractions on prediabetes in pretreated rats

Treatment	Blood glucose levels (mg/dL) at various intervals (days) after 12th week			
	1	7	14	21
Control	64.3 ± 4.9 $^{a\#}$	68 ± 5.2 $^{a\#bns}$	60.5 ± 7.1 $^{a\#bns}$	62 ± 7.1 $^{a\#bns}$
Negative control	237.1 ± 10.2	248.2 ± 5.7 ^{bns}	233.4 ± 16.2 ^{bns}	226.5 ± 8.9^{bns}
VRCM 20 mg/kg	167.8 ± 6.4 ^{a*}	148.5 ± 3.6 ^{a**bns}	133.2 ± 4.1 $^{a\#b*}$	112.6 ± 3.3 $^{a\#b**}$
VRCM 40 mg/kg	142.2 ± 3.1 ^{a**}	130.1 ± 2.2 $^{a\#bns}$	123.4 ± 8.6 $^{a\#b*}$	108.4 ± 4.1 $^{a\#b**}$
ANCM 20 mg/kg	163.4 ± 12.1 ^{a*}	130.6 ± 10.4 $^{a\#b*}$	124.3 ± 11.3 $^{a\#b*}$	103.8 ± 9.3 $^{a\#b**}$
ANCM 40 mg/kg	154.4 ± 10.3 ^{a**}	112.1 ± 7.9 $^{a\#b**}$	103.2 ± 7.8$^{a\#b**}$	92.4 ± 14.2 $^{a\#b\#}$
PIO 2.7mg/kg	158.3 ± 6.8 ^{a**}	128.7 ± 6.8 $^{a\#b*}$	110 ± 10.2 $^{a\#b**}$	112.3 ± 9.6 $^{a\#b**}$

Data represents mean ± SEM of blood glucose levels. a = represents comparison of blood glucose levels of all the groups (n = 6) with that of negative control, b = blood glucose levels on day 7, 14, 21 compared with blood glucose levels on day 1 using one way ANOVA followed by Dunnett's test. *p<0.05; **p<0.01; #p<0.001, ns-non significant.

Table 5: Effect of isolated fractions on prediabetes in post-treated rats

Treatment	Blood glucose levels (mg/dL) at various intervals (days) after 12th week			
	1	7	14	21
Control	71.5 ± 4.3 $^{a\#}$	68.2 ± 6.1 $^{a\#bns}$	66.1 ± 4.2 ^{a*bns}	72 ± 6.8 ^{a*bns}
Negative control	211 ± 6.9	204.6 ± 10.7 ^{bns}	207.3 ± 10.5 bns	216.6 ± 9.6 ^{bns}
VRCM 20 mg/kg	204.3 ± 5.3 $^{a\#}$	182.3 ± 8.4 ^{ansbns}	160.3 ± 6.4 ^{a*b*}	138.1 ± 5.9 $^{a\#b\#}$
VRCM 40 mg/kg	221.4 ± 9.7 $^{a\#}$	184.3 ± 7.3 ^{ansbns}	155.2 ± 7.4 ^{a**b**}	124.2 ± 4.3 $^{a\#b\#}$
ANCM 20 mg/kg	216.3 ± 11.8 $^{a\#}$	184.4 ± 14.3 ^{ansbns}	168.4 ± 9.3 ^{a*b*}	154.1 ± 8.7 ^{a**b**}
ANCM 40 mg/kg	198.4 ± 14.3 $^{a\#}$	164.6 ± 11.3 ^{a*b*}	141.7 ± 6.7 ^{a**b**}	102.3 ± 5.4$^{a\#b\#}$
PIO 2.7mg/kg	204.2 ± 12.1 $^{a\#}$	168.3 ± 6.8 ^{a*b*}	132.4 ± 8 $^{a**b\#}$	98.4 ± 7.5 $^{a\#b\#}$

Data represents mean ± SEM of blood glucose levels. a = represents comparison of blood glucose levels of all the groups (n=6) with that of negative control, b = blood glucose levels on day 7, 14, 21 compared with blood glucose levels on day 1 using one way ANOVA followed by Dunnett's test. *p<0.05; **p<0.01; #p<0.001, ns-non significant.

Table 6: Effect of isolated fractions on STZ induced diabetis in rats

Treatment	Blood glucose levels (mg/dL) at various intervals (days)				
	1	7	14	21	28
Control	71.5 ± 4.3 a#	68.2 ± 6.1 a#	66.1 ± 4.2 a#	72 ± 6.8 a#	70 ± 4.3 a#
Negative control	216.2±11.9	218.2±12.2 bns	221.5±8.7 bns	204.0±13.1 bns	210.2±7.4 bns
VRCM 20 mg/kg	203.2±8.1ans	192.5±7.4 ansbns	133.5±8a**b*	124.7±6.8 a#b*	121.3±5.5 a#b**
VRCM 40 mg/kg	219.7±7.1ans	182.2±2.0 ansbns	133.5±7.2 a**b**	121.7±10.8 a#b**	111.0±11.8 a#b#
ANCM 20 mg/kg	214.0±13.6ans	170.7±4.2 ansbns	162.8±5.9 a*bns	158.2±6.7 a*b*	145.0±8.2 a**b*
ANCM 40 mg/kg	204.0±9.3ans	176.3±3.9 ansbns	169.8±5.4 a*b*	145.8±8.4 a**b**	131.2±10.4 a**b#
PIO 2.7mg/kg	212.3 ± 8.4ans	147.2 ± 4.8a*b**	122.1 ± 6.3 a#b#	113.1 ± 4.4 a#b#	93.7 ± 3.2 a#b#

Data represents mean ± SEM of blood glucose levels in diabetic rats. a = represents comparison of blood glucose levels of all the groups (n = 6) with that of negative control, b = blood glucose levels on day 7, 14, 21 and 28 compared with blood glucose levels on day 1 using one way ANOVA followed by Dunnett's test. *p<0.05; **p<0.01; #p<0.001, ns-non significant.

Discussion

In this study the hypoglycemic activity of the VRCM and ANCM fractions were evaluated in streptozotocin induced diabetic rats. Both the fractions significantly reduced the blood glucose amounts compared to the standard drug.

Acute and sub-acute toxicities of the fractions were tested and LD_{50} cut off mg/kg B.W was seen as above 300mg/kg b.w. and globally harmonized system (GHS) classes also come in category 4, as the dose studies are limited to 300 mg/kg b.w. Drug treated mice does not exhibit any haematological alterations compared to normal control animals. Histopathological examination of internal organs did not exhibit changes in their normal architecture suggesting no damage caused by both the fractions.

Pretreatment with VRCM 20 mg/kg shows a marked decrease in blood glucose concentrations on first (p<0.05), 7th (p<0.01), 14th (p<0.001) and 21st day (p<0.001) compared to the blood glucose concentrations of negative control group, while the decrease in blood glucose concentrations were prominent on 14th (p<0.05) and 21st (p<0.01) days only, compared to blood glucose concentrations of day one. VRCM 40 mg/kg exhibited a marked decrease in blood glucose concentrations on 1ST (p<0.01), 7th (p<0.001), 14th (p<0.001) and 21st days (p<0.001) compared to blood glucose concentrations of negative control group, while the decrease in blood glucose concentrations were prominent on 14th (p<0.05) and 21st (p<0.01) days only, compared to blood glucose concentrations of day one. ANCM 20 mg/kg pre-treatment exhibited a marked decrease in the blood glucose concentrations on 1ST (p<0.05), 7th (p<0.001), 14th (p<0.001) and 21st days (p<0.001) compared to blood glucose concentrations of negative control group while the decrease in blood glucose concentrations were prominent on 7th (p<0.05), 14th (p<0.05) and 21st (p<0.01) days. ANCM 40 mg/kg pre-treatment exhibited a marked decrease in blood glucose concentrations on 1ST (p<0.01), 7th (p<0.001), 14th (p<0.001) and 21st days (p<0.001) compared to blood glucose concentrations of negative control group while the decrease in blood glucose concentrations were marked on 7th (p<0.01), 14th (p<0.01) and 21st (p<0.001) days compared to blood glucose concentrations of day one.

Basal blood glucose concentrations were high in post treatment groups compared to pre-treatment rats on day one. Post treated VRCM 20 mg/kg did not exhibit a marked decrease in the blood glucose concentrations on 14th and 21st days (p<0.05) and p<0.001 respectively compared to negative control group and 14th (p<0.05) and 21st (p<0.001) days compared to basal blood glucose concentrations on day one and treatment with VRCM 40 mg/kg did not show a marked reduction on 7th (p<0.05), 14th (p<0.01) and 21st day (p<0.001), compared to negative control group and 7th (p<0.05), 14th (p<0.01) and 21st days (p<0.001), compared to basal blood glucose concentrations on day one. ANCM 20 mg/kg exhibited a marked reduction in the blood glucose amounts on 14th and 21st days (p<0.05 and p<0.01 respectively, compared to negative control group and 14th (p<0.05) and 21st (p<0.01) compared to basal blood glucose amounts on day one and ANCM 40 mg/kg exhibited a marked reduction on 7th (p<0.05), 14th (p<0.01) and 21st days (p<0.001), compared to negative control group and 7th (p<0.05), 14th (p<0.01) and 21st days (p<0.001), compared to basal blood glucose amounts on day one. The results are comparable with that of standard treated groups.

In the post treatment rats, the basal blood glucose amounts were more than those seen in rats which take isolated fractions and pioglitazone from 4th week, whose protective action on pre-treated was described through the certainty that there causes the pancreatic β-cells disruption, there will be insulin sensitivity, evidencing the less basal blood glucose amounts in them. In conclusion, utilizing these drugs as prophylactic in basal hyperglycemic stage persons could decrease the risk of progressing into T2DM and also have therapeutic importance in the treating T2DM.

In the study on the effect of stz induced diabetes, diabetic rats with blood glucose levels above 175mg/dl were taken, treatment with the isolated fractions of VRCM 20 mg/kg exhibited a marked decrease in the blood glucose amounts on 14th (p<0.05), 21st (p<0.05) and 28th day (p<0.01) compared to blood glucose amounts on day one, whereas VRCM 40 mg/kg, exhibited a marked decrease in blood glucose amounts on 14th (p<0.01), 21st (p<0.01) and 28th day (p<0.001). Treatment with 20 mg/kg of *Allmania nodiflora* fraction ANCM, exhibited a marked decrease in blood glucose amounts on 21st (p<0.05) and 28th day (p<0.05). ANCM 40 mg/kg also exhibited a marked reduction on 14th (p<0.05), 21st (p<0.01) and 28th days (p<0.001) compared to blood glucose amounts on day one and is comparable to the standard.

Longer term treatment (28 days) with active fraction of VRCM and ANCM produced mild advancement in plasma insulin amounts. This proposes that *Vigna radiata*as like glibenclamide initiates insulin secretion from the residual beta cells of islets of Langerhans [19] or the drug might imitate one or more activities of insulin at the receptor level or/and it might impact one or more post receptor events.

Conclusion

The isolated VRCM and ANCM fractions of *Vigna radiata* and *Allmania nodiflora* shown marked hypoglycemic activity on STZ induced diabetes.

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