

EVALUATION OF ANTIDIABETIC AND ANTIHYPERLIPIDEMIC EFFECT OF *VERNONIA DIVERGENS* IN STREPTOZOTOCIN-INDUCED DIABETIC RATS

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Received: 31 January 2019, Revised and Accepted: 19 March 2019

ABSTRACT

Objective: The current investigation for antidiabetic activity of the plant *Vernonia divergens* (DC.) Edgew. has not been reported till date. However, to enlighten the folkloric claim of the plant, the study was carried out on various animal models such as albino mice, albino rabbits, Wistar rats, rabbits, hamsters, dogs, and monkeys.

Methods: The whole plant of *V. divergens* was studied on various animal models. Screening methods generally have been carried out on rodents and non-rodents, respectively. Various biochemical and hematological parameters such as serum glucose, plasma insulin, lipid profile and activities of liver enzymes, red blood cells, white blood cells, hemoglobin, and differential counts were measured to assess the antihyperglycemic and antihyperlipidemic activities as well as safety profile of the extract.

Results: Among all experimental extracts of *V. divergens*, it was found that the aqueous and methanolic extracts had maximum control of blood glucose in diabetic Wistar rats. While comparing with normoglycemic animals, it was observed that reduction of blood sugar level and increase in plasma insulin level are maximum with test extract. Among the study, the effects of the methanolic extract of *V. divergens* (MEVD) and aqueous extract of *V. divergens* (AEVD) were done through oral route in both the models, i.e., normoglycemic and hyperglycemic animal models. The safety profile was evaluated by toxicological evaluation and observed that, even at a higher dose level of 3000 mg/kg body weight, the MEVD and AEVD were safe and retain normal physiological and behavioral effect. The whole protein, whole cholesterol, aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase enzyme activity of streptozotocin-administered rats showed significantly higher than normal rats, and the test extract-treated rats significantly reduced the elevated levels.

Conclusion: It is concluded that the MEVD and AEVD (DC.) Edgew. might be beneficial in effectively reducing the blood glucose concentration and managing the various complications of diabetes. However, in comparison between both the extracts, the methanol extract was found to be significantly more potent than that of the A.E. in all aspects.

Keywords: *Vernonia divergens* (DC.) Edgew., Normoglycemic, Hyperglycemic, Wistar rats.

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INTRODUCTION

The term diabetes mellitus (DM) describes as a metabolic cum vascular syndrome of more than one etiology characterized by chronic hyperglycemia with resultant abnormalities in metabolism of protein fat and carbohydrate ensuing from impaired secretion and defected action of insulin or both. The outcomes of DM include long-term casualty, i.e., damage, dysfunction, and failure of several organs. DM may represent with attribute signs and symptoms such as polyphagia, polydipsia, polyuria, blurring of vision, and weight loss [1]. This is affecting nearly 25% of the population [2]. The prevalence of diabetes according to the World Health Organization projections is likely to increase by 35% [3]. Currently, the incidence of diabetes is nearly 150 million globally, and by the year 2025, this is likely to increase to 300 million or more [3,4]. While reviewing the statistical projection status of India, we can come across the fact that the incidence of diabetics is increasing and will rise from 15 million in 1995 to reach up to 57 million in the year 2025 which can make the country highest prevalent of diabetics worldwide [3]. Therefore, it is necessary to look for new solutions to manage this health problem. Many herbal products have been described for DM in ancient literature of Ayurveda in India [5]. The belief in traditionally used herbal drugs for curing various disease state is very strong in Nigeria and many other poor African countries [6,7].

India is one of the highest populated diabetogenic countries. In human population, DM remains one of the major contributors among all

other diseases. Due to enhancing awareness now-a-days the concern of medicinal plant in health care system is increasing and interest in local plant remedies for treating of various disease is growing in many developed as well as developing countries. *Vernonia divergens* is commonly known as insulin plant having a potent effect against glucose reduction belonging to family Asteraceae which is used as an outstanding drug for DM described earlier [8]. Thus, the rationale of the present study was to ascertain the truth of the claim about antidiabetic activity on the plant *V. divergens* (DC.) Edgew. so that novel compound against the treatment of diabetes can be identified.

METHODS**Collection and authentication of plant**

The plant *V. divergens* appears in the month of March and November. The whole plant was collected from Keonjhar district, a forest at Daitari, field No. 1, (Odisha). Before their use, they were carefully identified by Dr. Pratap Chandra Panda, Principal Scientist, Regional Plant Resource Centre, Bhubaneswar.

Preparation of plant extracts

The plants collected were dried at room temperature by the method of shade drying. The plants were spreaded over a polythene cover under fan. After drying, pulverizing was done with the help of mechanical grinder to get the powder form of plant material. The powder was then passed through a fine mesh sieve to get a fine powder. The powdered

material (600 g) was extracted with different solvents separately of non-polar to polar, for example, petroleum ether (pet. ether), chloroform, methanol, and water by the help of Soxhlet apparatus. The liquid extracts were concentrated under vacuum to yield dry extracts and preserved in a desiccator until further experiments.

Percentage yield of the extracts

The percentage yield of the plant *V. divergens* was found to be 2.66%, 1.66%, 14.66%, and 12.66% with pet. ether, chloroform, methanol, and water, respectively. The percentage yield of the methanolic extract of *V. divergens* (MEVD) was found to be greater (14.66%) than extracts with other solvents.

Preparation of drug solution

The semisolid extracts of *V. divergens* were dissolved in sufficient quantity of solvent (tween and normal saline), and the aqueous extracts (A.Es) were dissolved in normal saline for administration. Glibenclamide 5 mg/kg was dissolved in a sufficient quantity of normal saline and used in the treatment [9]. Streptozotocin 40 mg/kg was dissolved in the buffer solution and used to induce diabetes in rats [10].

Selection of animals

For both toxicological and antidiabetic assessments, adult Wistar albino rats of healthy status weighing about 150–200 g were selected after getting the Institutional Animal Ethics Committee approval (Approval No-10/15/SPS/IAEC/SOAU). The selected animals have been housed in acrylic cages in well-known environmental conditions (temperature: 20–25°C and relative humidity: 45–55% under 12 h light/dark cycle). To acclimate the laboratory condition, animals were fed with standard rodent diet for 1 week and water *ad libitum* [11].

Induction of diabetes

In the present study, a single dose of streptozotocin 40 mg/kg in normal saline was administered intraperitoneally [10]. After 1 week assessment of gradual development, diabetes was done. For the determination of blood glucose concentration, an experiment was performed on the 7th day. The animals with blood glucose levels between 200 and 250 mg/dl were chosen for antidiabetic screening and they were considered as diabetic rats.

Experimental procedure

The study was carried out as mentioned below to check whether the MEVD is acting as an insulin secretagogue (stimulation of insulin secretion from the pancreatic β -cell and to check the utilization) activity as well as to check whether the MEVD possesses antidiabetic activity and hypoglycemic activity.

Overnight fasted animals were grouped in six groups of six animals each group.

Group	Animals used	Sex	Body weight (g)	Treatment
I	Wistar albino rats	Male	150–200	Served as control (Normal saline). Once daily
II	Wistar albino rats	Male	150–200	Served as standard (Glibenclamide 5 mg/kg). Once daily
III	Wistar albino rats	Male	150–200	Test drug I. Dose 1. once daily
IV	Wistar albino rats	Male	150–200	Test drug I. Dose 2. once daily
V	Wistar albino rats	Male	150–200	Test drug II. Dose 1. once daily
VI	Wistar albino rats	Male	150–200	Test drug II. Dose 2. once daily

Analytical procedure

Blood sample was drawn into tubes at different time intervals. Serum was separated out by centrifugation and used in different

estimations. Diabetes was induced by a single dose of Streptozotocin administration. Blood glucose level, which is elevated in association with hyperglycemia, was measured to assess whether the plant extract has antidiabetic/hypoglycemic effect. To estimate plasma insulin level insulin kit was used. Radioimmunoassay was performed by employing double antibody technique to get the final result [12]. A significant body weight reduction is associated with diabetes hence the body weight was recorded before, during and after the treatment. Acute toxic study was performed to find out toxic symptoms of the extract and gross behavioral changes were also noticed. Sub-acute toxicity study was performed to reveal the influence of the extract on biochemical, hematological and histopathological findings. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) etc. were assayed in serum. Serum lipids like total cholesterol (TC), high density lipoprotein (HDL), Low density lipoprotein (LDL), Very LDL (VLDL) and triglyceride (TG) were estimated. Serum total protein and albumin were analyzed. Blood parameters like hemoglobin (Hb), red blood cells (RBC), white blood cells (WBC) etc. counts were determined. Clotting time was also determined.

Toxicity studies

Acute toxicity studies

To rule out any toxicity of the extracts, acute oral toxicity test was performed on 12 h fasted healthy Wistar albino rats according to the principles of Organization for economic co-operation and development (OECD) (OECD guidelines 423) [13]. This study was performed with the major aim of therapeutic index establishment and also for the purpose of primary screening. Therapeutic index meant the ratio of pharmacologically effective dose to the lethal dose. A group of 10 mice have been fasting overnight was administered the A.E. of *V. divergens* (AEVD) once orally at 4 dose levels (500, 1000, 2000, 3000 mg/kg). The initial observation of test drug treated mice was continuously recorded and then further reading was taken occasionally for 4 h. Final recording of overnight mortality was done [14]. In the course of duration of study, spontaneous activity reduction and behaviors of the mice were attentively observed [15].

Sub-acute toxicity studies

The purpose of this study is to determine the maximum tolerated dose and daily dose for 3–4 weeks and to indicate the nature of toxic reaction of the drug. In present study, the influence of *V. divergens* extract on hematological parameters, a pathological change on 21 days dosing in streptozotocin induce diabetic rats were carried out. The diabetic rats were grouped into 6 groups of 6 animals each. Group I animals received solvent, Group II animals received standard drug, Group III (100 mg/kg body weight) and Group IV (200 mg/kg body weight) was received MEVD and Group V (100 mg/kg body weight) and Group VI (200 mg/kg body weight) was received AEVD once daily for 21 days. At the end of the experimental period the blood was collected, serum was separated and subjected to hematological and biochemical examination [14].

Statistical analysis

The values are represented as mean \pm standard error mean. The data obtained from various studies were subjected to one-way analysis of variance (ANOVA) [16]. Dunnett's t-test was also performed after ANOVA for establishing statistical significance.

RESULTS

In Table 1 Preliminary antidiabetic effect of various extracts of *V. divergens* on single dose treated diabetic rats induced by streptozotocin was shown. It revealed that pet. ether, chloroform, M.E. and A.E. registered 6.81%, 16.50%, 52.76% and 33.25% decrease in fasting blood glucose levels respectively at the dose level of 100 mg/kg in oral route at the end of 8 h. However at the same time solvent control (distilled water) and pet. ether showed gradual increase in blood sugar level. The standard drug glibenclamide 5 mg/kg possess 72.84% decrease in blood glucose level at same duration. The A.E. significantly decreases the blood sugar level at the end of 4 h and 8 h in the level of $p < 0.01$, while the methanol

extract (M.E.) showed significant decrease of blood sugar level starting from 2 h to the end of 8 h at the level of $p < 0.05$ to $p < 0.001$ with respect to solvent control. However the standard drug caused decrease of blood sugar level beginning from 1 h ($p < 0.05$) up to 8 h ($p < 0.001$) when compared with solvent.

Table 2 summarizes that the M.E. and A.E. at the dose level of 100 mg/kg and 200 mg/kg registered 19.33% and 30.72% and 14.54% and 25.08% reduction in glucose concentration of blood after 4 h, respectively, while the standard drug glibenclamide (5 mg/kg) reduces blood glucose level up to 48.20% at the same time. At the end of 4 h, the test extracts at two dose levels bear a significant ($p < 0.001$) decrease of blood glucose compared to solvent control, while the standard drug holds the same significance ($p < 0.001$) from the beginning of 2 h.

The data recorded in Table 3 showed that the M.E. and A.E. possess 25.79% and 36.81% and 19.44% and 33.31% decrease in blood glucose level at the dose level of 100 mg/kg and 200 mg/kg, respectively, by oral route at the end of 8 h, while the standard drug registered 48.45% reduction at the same time. However, the solvent control group showed a negligible reduction of blood glucose level at the end of 8 h. The two

extracts at the dose level of 100 mg/kg showed a significant decrease ($p < 0.01$) at 8 h, and at the same time, the dose level 200 mg/kg showed more significant ($p < 0.001$) when compared to solvent control.

The result expressed in Table 4 revealed that the M.E. and A.E. in the dose level of 100 mg/kg and 200 mg/kg possess 48.54% and 55.00% and 34.10% and 50.09% reduction in the blood glucose level, respectively, at the end of 8 h, while the standard drug registered 65.03% decrease in blood sugar level at the same time.

Table 5 shows the hypoglycemic effect of MEVD and AEVD on multidose treated normoglycemic rats. The purpose of the study is to establish the therapeutic validity of the test extracts in long-term use. The data showed that there was a decrease in blood sugar level to the extent of 25.56% and 34.14% in case of M.E. and 19.14% and 30.09% in case of A.E. at the dose level of 100 mg/kg and 200 mg/kg, respectively, on the 21st day of treatment.

The purpose of antidiabetic study is to confirm the antidiabetic effect of the test extracts on longer duration of treatment, and the data are shown in Table 6. The M.E. and A.Es are registered 50.68% and 57.68% and 40.55%

Table 1: Preliminary antidiabetic effect of various extracts of *Vernonia divergens* on single dose treated streptozotocin-induced diabetic rats

Group and treatment	Blood glucose levels (mg/dl)					
	0 h	1 h	2 h	4 h	8 h	% decrease at the end of 8 h
Solvent (tween+water)	251.2±11.5	256.5±11.3	274.3±9.3	278.7±8.4	282.3±8.4	-
Standard glibenclamide (5 mg/kg)	274.3±13.9	201.2±11.6 ^c	149.2±9.0 ^c	101.7±9.6 ^c	74.5±4.6 ^c	72.84
Pet. ether extract (100 mg/kg)	266.7±11.4	275.3±11.9	264.3±10.9	254.7±9.2	248.5±6.5 ^c	6.81
Chloroform extract (100 mg/kg)	264.3±11.7	253.2±9.8	243.3±9.6	224.5±8.9 ^c	220.7±6.2 ^c	16.50
Methanol extract (100 mg/kg)	259.7±11.6	241.2±9.6	200.5±9.7 ^c	148.3±10.9 ^c	122.7±6.4 ^c	52.76
Aqueous extract (100 mg/kg)	271.7±13.8	261.2±11.6	223.5±10.3 ^b	192.3±9.6 ^c	181.3±7.9 ^c	33.25
F values	1.31	5.36**	21.94**	49.04**	133.62**	

Values are explicated in the form of mean±SEM. To analyze the statistical significance, Dunnett's t-test and one-way ANOVA were executed. (F-value indicates statistical significance at * $p < 0.05$, ** $p < 0.01$. t-value stands for statistical significance at ^a $p < 0.05$, ^b $p < 0.01$ and ^c $p < 0.001$, respectively, when compared to Group I). SEM: Standard error mean, ANOVA: Analysis of variance, Pet. ether: Petroleum ether

Table 2: OGTT of MEVD and AEVD in single dose treated glucose-loaded hyperglycemic rats

Group and treatment	Blood glucose levels (mg/dl)					
	60 min	0 h	1 h	2 h	4 h	% decrease at the end of 4 h
Solvent (tween+water)	104.2±4.6	107.5±4.1	123.3±2.4	136.5±2.0	102.3±2.6	-
Standard glibenclamide (5 mg/kg)	104.5±4.4	90.2±3.8	78.7±2.7 ^c	66.5±2.9 ^c	54.2±2.7 ^c	48.20
MEVD (100 mg/kg)	104.3±4.5	102.2±2.4	92.5±2.7 ^c	88.7±2.9 ^c	84.16±2.1 ^c	19.33
MEVD (200 mg/kg)	104.7±4.3	95.3±2.1 ^b	84.2±2.2 ^c	78.2±2.4 ^c	72.5±2.4 ^c	30.72
AEVD (100 mg/kg)	105.5±4.0	104.5±3.9	98.5±2.8 ^c	93.3±2.8 ^c	90.2±2.8 ^b	14.54
AEVD (200 mg/kg)	105.7±3.9	99.3±2.8	90.7±2.5 ^c	85.2±2.2 ^c	79.3±1.8 ^c	25.08
F value		3.63*	37.14**	89.25**	45.30**	

Values are explicated in the form of mean±SEM. To analyze the statistical significance, Dunnett's t-test and one-way ANOVA were executed. (F-value indicates statistical significance at * $p < 0.05$, ** $p < 0.01$. t-value stands for statistical significance at ^a $p < 0.05$, ^b $p < 0.01$, and ^c $p < 0.001$, respectively, when compared to Group I). OGTT: Oral glucose tolerance test, SEM: Standard error mean, ANOVA: Analysis of variance, MEVD: Methanolic extract of *Vernonia divergens*, AEVD: Aqueous extract of *Vernonia divergens*

Table 3: Hypoglycemic effect of MEVD and AEVD on single dose treated normoglycemic rats

Group and treatment	Blood glucose levels (mg/dl)					
	0 h	1 h	2 h	4 h	8 h	% decrease at the end of 8 h
Solvent (tween+water)	103.3±2.4	103.7±2.0	104.3±2.2	106.3±2.4	105.2±1.8	-
Standard glibenclamide (5 mg/kg)	102.2±2.9	90.5±3.1 ^b	81.7±2.5 ^c	74.3±1.2 ^c	52.7±1.3 ^c	48.45
MEVD (100 mg/kg)	104.7±2.8	104.5±2.2	97.2±1.9 ^a	94.2±1.9 ^c	77.7±2.7 ^c	25.79
MEVD (200 mg/kg)	102.3±2.4	102.5±2.4	95.5±1.4 ^b	85.3±1.5 ^c	64.7±1.5 ^c	36.81
AEVD (100 mg/kg)	103.7±2.7	103.2±2.5	99.5±1.5 ^a	96.2±1.3 ^b	83.5±4.5 ^c	19.44
AEVD (200 mg/kg)	103.7±1.2	103.5±1.2	96.7±1.4 ^a	90.2±1.6 ^c	70.2±2.1 ^c	32.31
F value	1.05	5.31**	16.56**	39.97**	54.81**	

Values are explicated in the form of mean±SEM. To analyze the statistical significance, Dunnett's t-test and one-way ANOVA were executed. (F-value indicates statistical significance at * $p < 0.05$, ** $p < 0.01$. t-value stands for statistical significance at ^a $p < 0.05$, ^b $p < 0.01$, and ^c $p < 0.001$, respectively, when compared to Group I). SEM: Standard error mean, ANOVA: Analysis of variance, MEVD: Methanolic extract of *Vernonia divergens*, AEVD: Aqueous extract of *Vernonia divergens*

and 52.59% of decrease of blood glucose level at the tested dose level of 100 mg/kg and 200 mg/kg, respectively, on the 21st day of treatment, while the standard drug showed 60.81% reduction on the same day.

Plasma insulin levels significantly ($p < 0.001$) increased in case of both M.E. and A.Es at both 100 mg/kg and 200 mg/kg dose level. Both extracts at both the dose level indicated a rise in insulin concentration of

133.43 and 173.37 and 126.51 and 156.16 $\mu\text{U/ml}$, respectively, on 14th day, while standard drug glibenclamide showed highest concentration of plasma insulin, i.e., 181.94 $\mu\text{U/ml}$ at the end of 14th day and the data are depicted in Table 7.

The content of Table 8 indicated that there was a decrease in percentage loss of body weight when measured on 3rd, 7th, 14th, and 21st

Table 4: Antidiabetic activity of MEVD and AEVD in single dose treated streptozotocin-induced diabetic rats

Group and treatment	Blood glucose levels (mg/dl)					
	0 h	1 h	2 h	4 h	8 h	% decrease at the end of 8 h
Solvent (tween+water)	252.5±10.6	258.7±10.2	272.7±9.4	288.5±8.9	297.7±7.1	–
Standard glibenclamide (5 mg/kg)	273.2±11.4	259.3±9.7	219.2±8.2 ^c	129.7±6.6 ^c	95.5±5.5 ^c	65.03
MEVD (100 mg/kg)	263.3±9.7	254.7±9.5	226.3±8.8 ^b	171.2±5.9 ^c	135.5±4.4 ^c	48.54
MEVD (200 mg/kg)	261.2±9.5	245.6±10.1	211.7±9.0 ^c	148.3±6.1 ^c	117.5±6.4 ^c	55.00
AEVD (100 mg/kg)	273.7±10.2	266.2±10.3	246.5±8.6	200.6±5.5 ^c	180.3±5.3 ^c	34.10
AEVD (200 mg/kg)	271.5±9.7	258.7±9.6	227.3±8.8 ^b	168.7±5.9 ^c	135.5±4.1 ^c	50.09
F value	0.38	0.46	6.37**	72.31**	171.09**	

Values are explicated in the form of mean±SEM. To analyze the statistical significance, Dunnett's t-test and one-way ANOVA were executed. (F-value indicates statistical significance at * $p < 0.05$, ** $p < 0.01$. t-value stands for statistical significance at * $p < 0.05$, ^b $p < 0.01$, and ^c $p < 0.001$, respectively, when compared to Group I). SEM: Standard error mean, ANOVA: Analysis of variance, MEVD: Methanolic extract of *Vernonia divergens*, AEVD: Aqueous extract of *Vernonia divergens*

Table 5: Hypoglycemic effect of MEVD and AEVD on multidose treated normoglycemic rats

Group and treatment	Blood glucose levels (mg/dl)				
	Day 0	Day 7	Day 14	Day 21	% decrease at the end of day 21
Solvent (tween+water)	104.2±2.2	104.8±2.4	104.7±2.1	105.5±2.6	–
Standard glibenclamide (5 mg/kg)	106.2±2.9	86.16±2.8 ^c	68.5±2.7 ^c	52.3±2.0 ^c	50.70
MEVD (100 mg/kg)	104.3±2.5	101.5±2.2	93.3±2.4 ^b	77.7±2.6 ^c	25.56
MEVD (200 mg/kg)	104.5±2.4	98.2±2.5	84.7±2.9 ^c	68.5±2.0 ^c	34.34
AEVD (100 mg/kg)	105.3±2.5	103.5±2.2	97.5±2.0	85.16±2.1 ^c	19.14
AEVD (200 mg/kg)	104.7±2.3	100.3±2.1	89.7±1.9	73.2±2.2 ^c	30.09
F value		8.09**	27.59**	60.61**	

Values are explicated in the form of mean±SEM. To analyze the statistical significance, Dunnett's t-test and one-way ANOVA were executed. (F-value indicates statistical significance at * $p < 0.05$, ** $p < 0.01$. t-value stands for statistical significance at * $p < 0.05$, ^b $p < 0.01$, and ^c $p < 0.001$, respectively, when compared to Group I). SEM: Standard error mean, ANOVA: Analysis of variance, MEVD: Methanolic extract of *Vernonia divergens*, AEVD: Aqueous extract of *Vernonia divergens*

Table 6: Antidiabetic activity of MEVD and AEVD on multidose treated streptozotocin-induced diabetic rats

Group and treatment	Blood glucose levels (mg/dl)					
	Day 0	Day 3	Day 7	Day 14	Day 21	% decrease at the end of day 21
Solvent (tween+water)	275.3±4.2	265.5±4.1	253.7±4.1	238.5±6.1	238.5±6.1	–
Standard glibenclamide (5 mg/kg)	274.3±3.6	227.2±4.2 ^c	182.5±4.2 ^c	156.3±4.4 ^c	107.5±4.3 ^c	60.81
MEVD (100 mg/kg)	279.2±4.2	263.3±4.0	233.2±5.4 ^a	186.2±4.2 ^c	137.7±4.2 ^c	50.68
MEVD (200 mg/kg)	280.5±3.8	261.7±4.8	226.2±4.7 ^b	173.2±3.1 ^c	118.7±4.5 ^c	57.69
AEVD (100 mg/kg)	278.7±4.7	272.2±3.3	250.7±5.1	210.2±4.7 ^a	165.7±4.2 ^c	40.55
AEVD (200 mg/kg)	279.5±4.3	267.7±4.3	230.7±5.1 ^b	181.5±4.7 ^c	132.5±4.2 ^c	52.59
F value	0.36	15.63***	28.44***	40.13***	108.00***	

Values are explicated in the form of mean±SEM. To analyze the statistical significance, Dunnett's t-test and one-way ANOVA were executed. (F-Value indicates statistical significance at * $p < 0.05$, ** $p < 0.01$. t-value stands for statistical significance at * $p < 0.05$, ^b $p < 0.01$, and ^c $p < 0.001$, respectively, when compared to Group I). SEM: Standard error mean, ANOVA: Analysis of variance, MEVD: Methanolic extract of *Vernonia divergens*, AEVD: Aqueous extract of *Vernonia divergens*

Table 7: Effect of MEVD and AEVD on plasma insulin levels in streptozotocin-induced diabetic rats

Group and treatment	Plasma insulin level ($\mu\text{U/ml}$)			
	Day 0	Day 7	Day 14	Day 21
Solvent (tween+water)	22.66±3.75	21.28±2.80	26.87±3.54	23.58±2.79
Standard glibenclamide (5 mg/kg)	33.84±3.67 ^c	169.95±11.61 ^c	181.94±9.58 ^c	113.86±7.59 ^c
MEVD (100 mg/kg)	23.56±2.87 ^b	94.38±9.58 ^c	133.43±11.49 ^c	115.29±9.13 ^c
MEVD (200 mg/kg)	32.89±3.51 ^c	154.18±11.83 ^c	173.37±10.53 ^c	121.93±8.19 ^c
AEVD (100 mg/kg)	24.36±3.73 ^b	89.66±7.56 ^c	126.5±9.14 ^c	113.58±9.41 ^c
AEVD (200 mg/kg)	32.66±2.65 ^c	145.51±12.53 ^c	156.16±10.25 ^c	117.84±6.23 ^c
F value	3.61*	44.34**	73.21**	87.32**

Values are explicated in the form of mean±SEM. To analyze the statistical significance, Dunnett's t-test and one-way ANOVA were executed. (F-value indicates statistical significance at * $p < 0.05$, ** $p < 0.01$. t-value stands for statistical significance at * $p < 0.05$, ^b $p < 0.01$, and ^c $p < 0.001$, respectively, when compared to Group I). SEM: Standard error mean, ANOVA: Analysis of variance, MEVD: Methanolic extract of *Vernonia divergens*, AEVD: Aqueous extract of *Vernonia divergens*

days of treatment in both tested dose levels. The M.E. at 100 mg/kg possesses 6.72% loss of body weight on 21st day, while on the 3rd day, the percentage loss of body weight registered 12%. Similarly at a dose level of 200 mg/kg on 21st day measured 5.55% and on 3rd day 10% loss of body weight. The A.E. at 100 mg/kg possesses 7.42% loss of body weight on 21st day, while on the 3rd day, the percentage loss of body weight registered 11%. Similarly at dose level of 200 mg/kg on 21st day measured 5.8% and on 3rd day 10% loss of body weight.

The data presented in Table 9 demonstrated that the glycogen content of the liver and kidney of DM animals treated with M.E. and A.Es of leaves of *V. divergens* and standard drug was significantly increased

Table 8: Percentage loss of body weight in MEVD and AEVD in streptozotocin-induced diabetic rats

Group and treatment	Percentage loss of body weights			
	Day 3	Day 7	Day 14	Day 21
Solvent (tween+water)	10	16	18.66	25.33
Standard glibenclamide (5 mg/kg)	8	6.66	6.66	3.33
MEVD (100 mg/kg)	12	10.11	8.0	6.72
MEVD (200 mg/kg)	10	8.23	6.38	5.55
AEVD (100 mg/kg)	11	10.15	9.0	7.42
AEVD (200 mg/kg)	10	8.15	6.66	5.8

The values indicating the percentage loss in body weights are calculated from the mean body weights of animals (n=6), in the respective groups on the respective days. MEVD: Methanolic extract of *Vernonia divergens*, AEVD: Aqueous extract of *Vernonia divergens*

Table 9: Effect of MEVD and AEVD on the glycogen concentration in the liver and kidney

Group and treatment	Liver glycogen mg/g tissue	Kidney glycogen mg/g tissue
Solvent (tween+water)	19.16±2.21	11.48±1.50
Standard glibenclamide (5 mg/kg)	33.66±2.76**	17.50±1.80*
Normal	35.67±2.70	19.40±1.40
MEVD (100 mg/kg)	28.46±1.19*	13.60±2.20
MEVD (200 mg/kg)	31.80±4.40**	15.67±1.67
AEVD (100 mg/kg)	27.66±2.65*	12.60±2.06
AEVD (200 mg/kg)	30.16±2.72**	14.5±1.19
F value	5.09 ^b	3.10 ^a

Values are explicated in the form of mean±SEM. To analyze the statistical significance Dunnett's t-test and one-way ANOVA were executed. (F-Value indicates statistical significance at *p<0.05, **p<0.01. t-value stands for statistical significance at *p<0.05, ^bp<0.01, and ^cp<0.001, respectively, when compared to Group I). SEM: Standard error mean, ANOVA: Analysis of variance, MEVD: Methanolic extract of *Vernonia divergens*, AEVD: Aqueous extract of *Vernonia divergens*

Table 10: Effect of MEVD and AEVD on lipid profile in subacute toxicity

Group and treatment	Lipid profile				
	TC (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
Solvent (tween+water)	226.8±5.4	180.0±6.7	34.2±4.1	162.8±6.3	42.7±3.0
Standard glibenclamide (5 mg/kg)	160.3±4.7 ^c	101.8±3.1 ^c	46.7±3.3 ^a	84.7±5.3 ^c	23.8±2.6 ^c
Normal	137.8±4.1	87.2±1.7	50.7±3.3	65.2±4.7	17.2±3.0
MEVD (100 mg/kg)	194.2±4.1	134.8±5.0 ^b	38.2±4.6	134.7±5.1	31.7±2.4 ^a
MEVD (200 mg/kg)	185.5±5.5 ^b	114.3±4.6 ^c	40.2±3.5	114.8±4.1 ^b	28.3±2.1 ^b
AEVD (100 mg/kg)	205.2±5.0	145.5±5.1	36.5±5.4	148.2±5.4	35.8±3.1
AEVD (200 mg/kg)	191.7±4.1	124.3±5.0	39.6±4.1	129.7±4.7	30.2±3.0
F value	56.27**	54.53**	2.76*	57.79**	13.51**

Values are explicated in the form of mean±SEM. To analyze the statistical significance, Dunnett's t-test and one-way ANOVA were executed. (F-value indicates statistical significance at *p<0.05, **p<0.01. t-value stands for statistical significance at *p<0.05, ^bp<0.01, and ^cp<0.001, respectively, when compared to Group I). SEM: Standard error mean, ANOVA: Analysis of variance, MEVD: Methanolic extract of *Vernonia divergens*, AEVD: Aqueous extract of *Vernonia divergens*, TC: Total cholesterol, TG: Triglyceride, HDL: High-density lipoprotein, LDL: Low-density lipoprotein, VLDL: Very low-density lipoprotein

when compared with solvent control after the completion of 21 days of drug exposure, even the test extracts and standard drug approach the content of glycogen toward normal level.

The data observed in Table 10 present a significant (p<0.01) reduction of TC in serum in the groups treated with test extraction and standard drug when compared with solvent control. The extent of reduction in both the close level of test extracts was dose dependent. In case of TGs, there was a significant reduction with both test extract and standard drug when compared with solvent control on the 21st day of treatment. The value of HDL in case of test extract at tested dose levels and standard drug showed significant increase than that of solvent control group, while the LDL and VLDL levels significantly (p<0.001) decrease in all drug-treated groups when compared with solvent control.

The hematological parameters exhibited in Table 11 show that the animals treated with standard drug glibenclamide and test extracts in the tested dose levels bear normal in RBC count and Hb, whereas the clotting time slightly elevated. However, the diabetic rats treated with solvent showed a decrease value of RBC, WBC, and Hb. The neutrophil count appears to nearly equal with that of normal value. The other hematological parameters such as eosinophil, lymphocyte, and monocytes in case of the standard drug and test extract-treated animals did not show any alteration.

From Table 12, it was observed that AST and ALT enzyme activity of streptozotocin-induced diabetic rats was significantly higher than normal rats. The test extracts and treated rats at tested dose had reduced enzyme activity when compared with diabetic rats. The total protein and albumin content of serum was significantly lowered in diabetic rats when compared with solvent control, but in the drug-treated diabetic animals, it returned to nearly normal.

DISCUSSION

Diabetes is a chronic disease that occurs when either insulin deficiency or resistance or both exists. The hyperglycemic condition causes increased glycosylation leading to various complications such as neuropathy, retinopathy, nephropathy, and cardiomyopathy [17]. The present study was carried out to investigate and validate the antidiabetic activity of the plant *V. divergens* (DC.) Edgew. The blood glucose lowering property of MEVD and AEVD is comparable with the standard drug glibenclamide (5 mg/kg body weight). Among the tested extract, the M.E. and A.E. at the dose level of 100 mg/kg possess a marked reduction in blood glucose level through oral route. Therefore, the M.E. and A.E. were selected for the evaluation of hypoglycemic and/or antidiabetic activity. It is observed that the MEVD and AEVD effectively control the glucose concentration of blood in normoglycemic as well as hyperglycemic rats in single unit dose treated model. The background mechanism of such effect might be due to a decrease in the initial absorption rate when glucose and plant fiber given together orally [18]. Hence, it may be presumed that the glucose lowering effect

Table 11: Effect of MEVD and AEVD on hematological parameters in subacute toxicity

Group and treatment	RBC (millions/ml)	WBC (millions/ml)	Hb (g/dl)	Clotting time (min)	Neutrophil (%)	Eosinophil (%)	Lymphocyte (%)	Monocyte (%)
Solvent (tween+water)	2.7±0.4	7.9±0.9	7.3±1.5	1.4±0.01	21.2±3.7	4.7±0.7	72.7±4.5	4.5±0.7
Standard glibenclamide (5 mg/kg)	4.9±0.6 ^a	6.9±1.1	10.6±1.2	1.1±0.08	27.8±2.3	1.9±0.5 ^c	69.8±3.3	2.7±0.7
Normal	5.2±0.6	7.2±1.2	11.8±1.4	1.1±0.07	32.7±1.5	1.5±0.07	68.7±3.3	1.8±0.2
MEVD (100 mg/kg)	3.7±0.8	6.2±0.4	8.3±1.5	1.4±0.07	24.3±2.1	2.5±0.3 ^b	71.4±4.1	3.3±0.9
MEVD (200 mg/kg)	4.6±0.7	6.6±0.7	8.8±1.2	1.3±0.07	26.2±1.6	2.1±0.4 ^b	70.3±3.9	3.1±0.3
AEVD (100 mg/kg)	3.1±1.0	5.9±1.1	8.2±1.1	1.4±0.8	23.1±1.1	2.7±0.7	71.7±3.5	3.9±0.6
AEVD (200 mg/kg)	4.1±0.5	6.4±0.9	8.5±1.2	1.3±0.7	25.2±1.2	2.3±0.2	70.8±3.3	3.2±0.5
F value	2.8*	1.05	2.02	3.88*	3.2*	8.50**	0.95	2.17

Values are explicated in the form of mean±SEM. To analyze the statistical significance, Dunnett's t-test and one-way ANOVA was executed. (F-value indicates statistical significance at *p<0.05, **p<0.01. t-value stands for statistical significance at *p<0.05, ^bp<0.01, and ^cp<0.001, respectively, when compared to Group I). SEM: Standard error mean, ANOVA: Analysis of variance, MEVD: Methanolic extract of *Vernonia divergens*, AEVD: Aqueous extract of *Vernonia divergens*, RBC: Red blood cells, WBC: White blood cells

Table 12: Effect of MEVD and AEVD on biochemical parameters in subacute toxicity

Group and treatment	AST (μl)	ALT (μl)	ALP (μl)	Albumin (g/dl)	Total Protein (g/dl)
Solvent (Tween+water)	36.16±3.8	48.67±4.1	290.50±4.5	5.82±0.8	4.76±0.8
Standard glibenclamide (5 mg/kg)	25.17±3.2 ^a	30.34±2.3 ^b	270.84±4.9 ^a	4.20±0.8	6.26±1.0
Normal	22.84±3.9	28.34±3.4	262.17±6.1	3.40±0.4	8.08±0.5
MEVD (100 mg/kg)	35.84±3.9	38.84±3.0 ^a	282.17±5.0	5.16±0.8	5.18±0.8
MEVD (200 mg/kg)	30.67±3.1	34.67±2.2 ^b	276.16±4.5 ^a	4.70±0.7	5.66±0.7
AEVD (100 mg/kg)	37.66±3.4	39.84±3.9	284.33±6.1	5.66±0.7	5.01±0.6
AEVD (200 mg/kg)	32.16±3.2	35.67±2.3	278.66±5.1 ^a	4.90±0.8	5.33±0.5
F value	3.66*	7.14**	4.65**	1.61	2.68*

Values are explicated in the form of mean±SEM. To analyze the statistical significance, Dunnett's t-test and one-way ANOVA were executed. (F-value indicates statistical significance at *p<0.05, **p<0.01. t-value stands for statistical significance at *p<0.05, ^bp<0.01, and ^cp<0.001, respectively, when compared to Group I). SEM: Standard error mean, ANOVA: Analysis of variance, MEVD: Methanolic extract of *Vernonia divergens*, AEVD: Aqueous extract of *Vernonia divergens*, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, ALP: Alkaline phosphatase

of the extract was achieved by an extraintestinal action. The two extracts at the dose level of 100 mg/kg showed a significant decrease (p<0.01) in blood glucose at 8 h, while at the same time, the dose level 200 mg/kg showed more significant (p<0.001) when compared to solvent control. The test extracts showed a significant decrease (p<0.001) of blood glucose level starting from 4 h onward in the tested dose levels, while standard drug possesses the same rate of significance beginning from 2 h while compared with solvent control. The observed data results of the experiments suggested that both the MEVD and AEVD are able to maintain the hypoglycemic effect up to 21st day and no behavioral changes are observed during the treatment periods.

This study further supports the antidiabetic effect of the test extracts whose effectiveness persists up to 21 days and the blood sugar level decreases gradually during the observed days, which presumed that the test extracts contain some antidiabetic active principle responsible for this effect. The hypoglycemic active principle acts by initiating the release of insulin from the pancreatic cell of hyperglycemic animal (sulfonylurea like effect) [19]. The proper mechanism of action is further supported by oral glucose tolerance test, in which glucose level reduces in the response of test extract. While insulin level concerned in comparison with standard drug, it was observed that there was an increased plasma insulin level and hence concluded the insulinotropic effect [11]. Therefore, it is credible that glucose-lowering properties of the extracts strive a direct effect in hyperglycemic rat probably by a similar mechanism to insulin which was substantiated by an extrapancreatic mode of action. The experiment revealed that M.E. and A.E. of *V. divergens* (200 mg/kg) significantly (p<0.001) decrease the glucose level on hyperglycemic animal. The glucose lowering activity observed in the diabetic animal may be due to the stimulation of β-cells in the pancreatic islets.

It has been reported that diabetes is a heterogeneous metabolic disorder, in which one of the symptomatic characters is loss in body weight [20]. It has been stated that DM results deficiency to glucose

utilization for energy leads to increased utilization & decreased storage of proteins. By the above-stated reason, depletion of protein takes place resulting in reduced body weight [21]. The results of the study presented that the decreased loss of body weight might be contributed by increased use of glucose by the tissue.

The glycogen content of the liver and kidney of DM animals treated with M.E and A.E of leaves of *V. divergens* and standard drug was significantly increased when compared with solvent control after the completion of 21 days of treatment, even the test extracts and standard drug approach the content of glycogen toward normal glycogen level. It was reported that hyperglycemia results in decreased glucose utilization and glycogen synthesis [22]. The glycogen content in the liver and kidney increases in the extract-treated group compared to the diabetic rats indicating participation of the extract components in glycogen synthesis similar to insulin [23]. Dyslipidemia is a known complication associated with diabetes [24]. The abnormally high concentration of serum TG, TC, LDL, and low HDL observed in diabetic rats compared to control rats is in consonant with reports from previous studies [24-27], indicating that an increase in glucose level on induction of diabetes results in an equivalent rise in blood lipids. It has been reported that, in the diabetic patients those are untreated or under treatment, hypertriglyceridemia and hypercholesterolemia often occur due to the increased generation of VLDL and deficiency of lipoprotein lipase [28]. The result revealed that the test extract reduces VLDL, TC and TG actively. Hence, it might be surmised that, similar to insulin, the test extract is the prime cause for the endowment of the transcription of lipoprotein lipase.

The result of hematological parameters evidenced that the test extracts have no significant effect on the same and is evident for the safely used at the MEVD and AEVD for a longer duration time. The experimental approach has been conducted for the test extracts to elicit tonic response over an exposure period of 21 days' alteration in the marker enzymes such as AST, ALT, and ALP. The data obtained from this study also indicated that the MEVD significantly decreased

the levels of AST, ALT, and ALP. The increased activity of transaminases, which are active in the absence of insulin due to increased availability of amino acids in diabetes, is believed to be responsible for the increased gluconeogenesis and ketogenesis observed in the disease [29]. Alanine and aspartate transaminase activities are used as an indicator of hepatocyte damage [30]. Elevation of serum ALP concentration in patients with DM has been observed for several years, but the source and reasons are unknown [31].

CONCLUSION

The findings of the current study indicated that MEVD and AEVD may get a place in the treatment of DM as hypoglycemic and/or antidiabetic agent. The MEVD and AEVD (DC.) Edgew. might be beneficial in effectively reducing the blood glucose concentration and managing of various complications of diabetes. However, in comparison between both the extracts, the M.E. was found to be significantly more potent than that of the A.E. in all aspects. Clinical studies are required to establish whether the administration of MEVD and AEVD can potentiate the antidiabetic effect of conventional agents.

ACKNOWLEDGMENT

The authors are contented to the Department of Pharmacology of School of Pharmaceutical Sciences, Siksha 'O' Anusandhan Deemed to be University for rendering adequate facility in the laboratory for the purpose of this research, and also to Dr. Manoj Ranjan Nayak for his kind support and cooperation.

AUTHORS' CONTRIBUTION

Laxmidhar Maharana: Design of protocol of the study, Manoj Kumar Sethi: Preparation and correction of the manuscript. Rudra Narayan Dash: Performance of whole experiments, Snigdha Pattnaik: Supervision of experiments.

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