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In vivo antidiabetic evaluation of aloe vera in streptozotocin induced rats

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ABSTRACT

Aloe Vera has been used medicinally throughout history by many different cultures. Many compounds have been found in the exudates of the *Aloe Vera* plant that have been used medically by humans. We have examined the pharmacological hypoglycemic action of *Aloe Vera* in diabetic rats. *Aloe Vera* 250mg/kg (single dose study) reduced glucose, cholesterol, triglycerides, urea, creatinine, and lipids after treatment for 24 hrs. In chronic study (multiple dose study) also *Aloe Vera* reduced creatinine, urea, lipids, triglycerides and glucose after 15days and significantly reduced glucose levels at 15th day in diabetic rats. In glucose tolerance test in diabetic rats with *Aloe Vera* 250 mg/kg demonstrated glucose levels were found significantly less compared to the control group. *Aloe vera* serves as an important alternative source in the management of diabetes mellitus involved in reducing increased blood glucose during diabetes which should be examined further by oral hypoglycemic therapy.

Keywords*:* Diabetes, glucose, Aloe Vera, Cholesterol, Urea, Creatinine, Triglycerides.

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1. INTRODUCTION

Diabetes is a metabolic disease in which a person has high blood sugar, either because the body does not produce enough insulin, or because cells do not respond to the insulin that is produced. This high blood sugar produces the classical symptoms of Polyuria (frequent urination), polydipsia (increased thirst) and polyphagia (increased hunger). Based on the WHO recommendations hypoglycemic agents of plant origin used in traditional medicines are important [1]. Plant drugs and formulations are frequently considered to be less toxic and more free side effects than synthetic one [2].

Aloe Vera (L.) Burm. Fil. (Synonym A. barbadensisMiller) (Tamil – Southakathalai, Hindi – Ghikanvar), is cactus-like plant with green, dagger-shaped leaves that are fleshy, tapering, Spiny, marginated and filled with a clear viscous gel. The name was derived from the Arabic 'alloeh'meaning 'bitter', because of the bitter liquid found in the leaves. It is also known as 'lily of the desert', the 'plantof immortality', and the 'medicine plant' with qualities to serve as alternate medicine There have been several reports on the hypoglycemic activity of *Aloe*, which vary in regard to the plant species, the part of the plant used, and in the Preparation of extracts as well as the animal models. [3]

2. MATERIALS AND METHODS

Animals

Wistar rats of weight between 150 to 200 g obtained from NIN, Hyderabad, India, were used in the study. The animals were maintained under standard conditions in animal house of Vaageswari Institute of Pharmaceutical Sciences. The rats were males 8-10 weeks old with average weight of 150-200g. Animals were housed 3-4 per cage in a temperature-controlled (22±1) AC room, with a light/dark cycle of 12hr for a week following their arrival; the animals were allowed free access to the standard rat chow diet and tap water they were acclimating to the environment. Rats were also monitored daily and cages cleaned thrice weekly. At the start of the experiment animals were randomly distributed so that body weights, initial triglycerides (TG), total cholesterol (TC), other parameters in all the experimental groups were similar.

Collection of Plant material:

Purified Aloe Vera gel and extracts of Aloe Vera leaves were obtained from local area from Karimnagar, India. The gel were gotten from the leaves in to a clean container and used as such While the leaves from which the gel have been drained were air dried, ground and soaked in ethanol for 4 days. This was later filtered and the filtrates evaporated to dryness Using a rotary evaporator. The extracts were dissolved in sterile water and used for the Antimicrobial susceptibility testing.

Experimental protocol

The test samples were suspended in distilled water. Glibenclamide (2.5 mg/kg) was used as reference control during the study. All the test samples were administered through oral route [4].

***Single dose study (Acute study)*:**

In normoglycemic rats:

The rats were fasted for 18 h, but were allowed free access to water before and throughout the duration of experiment. At the end of the fasting period, taken as zero time (0 h), blood was withdrawn (0.1 ml) from the retro orbital route of each rat under mild ether anesthesia. Plasma was separated following centrifugation the glucose was estimated by using Glucose estimation kit from 'One touch ultra', Horizon. U.S.A. The normal rats were then divided into five groups of five rats each. Group I and II were noted as normal control and diabetic control. Groups III and IV received the test extract at a dose of 100 and 250 mg/kg, respectively, through oral route. Group V (standard) received glibenclamide (2.5 mg/kg) and served as reference control. All the test samples were administered in a similar manner. Blood glucose levels were examined after 1, 2, 4, 6, 8, 12 and 24 hrs of administration of single dose of test samples. ***Induction of Diabetes in Rats by using 60mg/kg of streptozocin*** (13)

After 2 weeks of feeding with high fat food the rats were fasted for a period of 18 hours before induction of diabetes, and were injected intra-peritonially with a single dose of Streptozocin 60 mg/kg (Sigma–Aldrich, St. Louis, MO, USA), freshly dissolved in normal saline solution. After the administration, the rats had free access to food (normal pellet diet) and water *ad libitum*. Diabetes in rats was identified by moderate polydipsia and marked polyuria. After 3 days i.e. 72hrs of injection, the fasting blood glucose levels were determined by following glucose oxidase/peroxidase GOD/POD method using a commercial glucose estimation kit with UV-Visible Spectrophotometer at 505nm. The rats showing fasting blood glucose more than 150 mg/dL were considered diabetic rats and selected for the grouping in experimentation.[5]

Experimental Design

Rats were considered diabetic when the blood glucose level was raised beyond 200 mg/100 ml of blood. This condition *was* observed at the end of 48 hrs after Alloxanisation. The rats were segregated into five groups of five rats in each [6].

Group I – Normal Control and rats received only vehicle that is distilled water.

Group II – Diabetic control and rats received only vehicle that is distilled water.

Group III – Rats received Ethanol Extract of Aloe Vera (100 mg/kg/day p.o) suspended in distilled water.

Group IV - Rats received Ethanol Extract of Aloe Vera (250 mg/kg/day p.o) suspended in distilled water.

Group V – Rats received Glibenclamide (2.5 mg/kg p.o) suspended in 2% v/v Tween 80 solution.

***Multidose study (Chronic Study)*:**

In streptozotocin induced diabetic rats

The selected rats were treated with similar test samples as above, but the blood glucose level was measured on 1, 3, 5, 7, 9 and 14 days of treatment. Glucose testing kit utilized for the measuring of plasma glucose levels was manufactured by Excel Diagnostic Pvt. Ltd.

Estimation of Lipid Profile:

Estimation of Lipid profile such as Total Cholesterol, Triglycerides, HDL, LDL, VLDL and serum glucose level was conducted appropriately as per specifications. Cholesterol- EGD test kit manufactured by Excel Diagnostics Pvt. Ltd. was used for this purpose. The test kit utilizes CHOD/ POD method for cholesterol analysis. Triglycerides testing kit utilized for measuring the triglycerides in the plasma was also manufacture by Excel Diagnostics Pvt. Ltd.

Estimation of Urea and Creatinine: Urea and Creatinine levels were also checked using the respective kits that were both manufactured by Excel Diagnostics Pvt. Ltd.

***Statistical Analysis*:**

The data were expressed as mean ± standard error mean (SEM). The Significance of differences among the group was assessed using one way and multiple way analysis of variance (ANOVA). The test followed by Dunnet's test p values less than 0.05 were considered as significance.

DATA ANALYSIS

All data are expressed as the standard error of the mean. Comparisons among the control and treatment groups were made using analysis of variance followed by a Student- Newman-Keuls t-test using the Graph pad instat statistical program. With all analyses, an associated probability (p value) of less than 5% ($P<0.05$) was considered significant.

3. RESULTS:

Upon administration of methanolic extract of Aloe Vera, significant changes were recorded in blood glucose levels, triglycerides, total cholesterol levels, urea and creatinine levels both in acute as well as in chronic study groups. It was observed that the higher dosage of Aloe Vera exhibited increased reduction in the values of parameters compared to low dosage administration. The values of the blood glucose levels observed by treating diabetes induced rats with methanolic Aloe Vera extract was comparable to the values obtained by treating with glibenclamide. Recorded values showed a dose dependant reduction of blood glucose levels, total cholesterol, triglycerides and urea levels in the alloxan induced diabetic rats treated with methanolic extract of Aloe Vera.

SINGLE DOSE STUDY:

Administration of single dose of Aloe Vera 100 mg/Kg and 250 mg/Kg, oral, each to two study groups which are diabetes induced by alloxan, significant reduction ($P>0.05$) in blood glucose levels was observed. The study period encompassed 24hrs. The results were significantly comparable to the standard drug glibenclamide. Aloe Vera at 250 mg/Kg exhibited better blood glucose level reduction compared to Aloe Vera administered at 100 mg/Kg and results shown in Table No.1,3,4,7,9.

CHRONIC STUDY:

During chronic study which encompassed a period of 15 days, the Aloe Vera (100 and 250 mg/kg, oral) produced a significant ($P>0.05$) in BGL of the diabetic rats compared to control. Fenugreek seed extract at the dose of 250 mg/kg body weight exhibited better BGL reduction than 100 mg/kg body weight and results shown in Table. No. 2, 5, 6,8,10.

GROUPS	DRUG	DOSE	0 Hr	1 Hr	2 Hr	4 Hr	6 Hr	8 Hr	12 Hr	24 Hr
I	Normal control	5% w/v Tween 80	111.5 ±6.34	113.8 ±7.43	114.9 ±8.84	111 ±9.67	165 ±7.73	113.6 ±6.30	111.6 ±6.42	111 ±5.9
II	Diabetic Control	5% w/v Tween 80	284.9 ±93.5	281.9 ±91.25	282.8 ±88.71	279 ±43.6	285.8 ±37.4	283.4 ±42.5	285.9 ±38.7	282.6 ±35.56
III	Diabetic Control+ A.V.	100 mg/Kg	287.4 ±13.2	281 ±18.5	262.8 ±20.8	238.3 ±24.5	190.8 ±29.5	131.3 ±14.8	102.6 ±8.74	86.6 ±10.6
IV	Diabetic Control+ A.V.	250 mg/Kg	288 ±66.4	278 ±68.3	259 ±78.9	232.6 ±86.3	186.8 ±75.3	128.4 ±75.0	99.4 ±48.8	84.5 ±68.45
V	Diabetic Control+ Glibenclamide	1.40 mg/kg	289.5 ±3.8	282.1 ±4.9	273 ±6.51	240.1 ±.96	191.9 ±5.4	133 ±4.89	103.9 ±.59	85.68 ±7.24

Table .No.1.Effect of ethanolic *Aloe Vera* on serum glucose levels in streptozotocin induced diabetic rats after single dose administration

GROUPS	DRUG	DOSE	1 day	3 day	5 day	7 day	14day
I	Normal control	5% w/v Tween 80	111.4 ±6.34	113.3 ±7.43	114.5 ±8.84	112± 9.97	165 ± 7.73
II	Diabetic Control	5% w/v Tween 80	284.9 ±93.55	283.9 ±91.25	279.8 ±88.71	283 ± 43.92	281.8 ±37.44
III	Diabetic Control+ A.V.	100 mg/Kg	287.4 ±13.2	263.4 ±18.5	198.2 ±20.8	152.6 ±24.54	133.8 ±29.4
IV	Diabetic Control+ A.V.	250 mg/Kg	289 ±66.2	261.4 ± 68.2	187.5 ±78.72	104.5 ±86.1	124.8 ±75.1
V	Diabetic Control+ Glibenclamide	1.40 mg/kg	289.4 ±3.8	258.9 ±4.9	163.3 ± 6.51	137.3 ± 3.96	125.9 ±5.4

Table. No.2. Effect of ethanolic *Aloe Vera* on serum glucose levels in streptozotocin induced diabetic rats after prolonged treatment

GROUPS	DRUG	DOSE	0 Hr	1 Hr	2 Hr	4 Hr	6 Hr	8 Hr	12 Hr	24 Hr
I	Normal control	5%w/v Tween 80	91.8 ±2.5	91.53± 2.8	91.69 ±3.1	92.75± 3.04	91.38± 3.4	91.95± 2.5	93.68± 2.5	92.93± 3.0
II	Diabetic Control	5%w/v Tween 80	180.3± 4.4	181.3± 4.4	179.5 ±4.2	179.53± 4.8	177.04 ±4.7	178.9± 4.5	182.93± 6.5	173.3± 4.8
III	Diabetic Control+ A.V.	100 mg/Kg	169.9± 4.5	167.1± 4.2	163. ±4.4	159.± 4.2	156.4± 4.3	152± 4.3	148.8± 3.7	140± 2.5
IV	Diabetic Control+ A.V.	250 mg/Kg	170.4± 1.5	168.2± 1.9	164.5± 3.7	161.8± 2.81	157.6± 3	153.7± 3.7	146.6± 2.1	143.4± 2.3
V	Diabetic Control+ Glibenclamide	1.4 mg/kg	170.9± 3.4	168.6± 3.3	165.2 ±3.2	162.51± 3.1	153.8 ±5.3	149.21± 2.5	147.48± 3.1	137.4± 3.01

Table.No.3: Effect of ethanolic *Aloe Vera* on triglyceride levels in serum in streptozotocin induced diabetic rats after single dose administration

GROUPS	DRUG	DOSE	0Hr	1 Hr	2 Hr	4 Hr	6 Hr	8 Hr	12 Hr	24 Hr
I	Normal control	5%w/v Tween 80	71.1 ±1.39	70.8 ±1.0	0.78 ±1.4	70.9 ±0.8	0.71 ±1.2	69.8 ±1.3	70.8 ±0.8	70.4 ±0.9
II	Diabetic Control	5%w/v Tween 80	298.1 ±3.9	298.1 ±3.2	297.5 ±3.2	297.5 ±2.7	293.4 ±2.7	296.4 ±0.24	294.5 ±1.8	298.31 ±1.76
III	Diabetic Control+ A.V.	100 mg/Kg	301.4 ±3.5	292.1 ±4.3	276.21 ±4.8	263.1 ±4.0	249.1 ±4.91	197.1 ±5.2	160.81 ±3.7	132.1 ±4.3
IV	Diabetic Control+ A.V.	250 mg/Kg	302 ±5.7	287.21 ±7.7	268.1 ±8.7	259.1 ±9.52	247.61 ±8.87	194.1 ±9.9	158.21 ±9.3	127.61 ±8.2
V	Diabetic Control+ Glibenclamide	1.40 mg/kg	299.71 ±3.2	278.01 ±3	237.11 ±3	217.11 ±3.4	196.81 ±2.5	172.51 ±2.7	143.21 ±1.8	121.11 ±1.7

Table.No.4: Effect of ethanolic *Aloe Vera* on total cholesterol in streptozotocin induced diabetic rats after single dose administration

GROUPS	DRUG	DOSE	1 day	3 day	5 day	7 day	14 day
I	Normal control	5% w/v Tween 80	91.1 ±2.81	91.5 ±2.81	91.6 ±3.1	92.71 ±3.04	91.31 ±3.4
II	Diabetic Control	5% w/v Tween 80	180.21 ±4.4	181.21 ±4.1	179.4 ±4.1	179.1 ±4.81	177.03 ±4.7

III	Diabetic Control+ A.V	100 mg/Kg	178.6 ±4.5	156.18 ±4.3	149.4 ±4.4	145.2 ±4.2	133.41 ±4.31
IV	Diabetic Control+ A.V	250 mg/Kg	177.41 ±1.5	151.21 ±1.9	147.1 ±3.7	141.4 ±2.8	131.61 ±3.0
V	Diabetic Control+Glib-enclamide	1.4 mg/kg	175.21 ±3.4	154.63 ±3.3	141.25 ±3.2	138.2 ±3.11	129.83 ±5.3

Table.No.5: Effect of ethanolic *Aloe Vera* on serum triglycerides levels in streptozotocin induced diabetic rats after prolonged treatment

GROUPS	DRUG	DOSE	1 day	3 day	5 day	7 day	14 day
I	Normal control	5% w/v Tween 80	71.18 ±1.39	70.88 ±1.0	70.76 ±1.4	70.92 ±0.8	70.78 ±1.2
II	Diabetic Control	5% w/v Tween 80	298.2 ±3.9	298.0 ±3.2	297.9 ±3.2	297.48 ±2.7	293.6 ±2.7
III	Diabetic Control+ A.V	100 mg/Kg	301.1 ±3.5	245 ±4.3	192.2 ±4.8	143 ±4.0	125.4 ±4.91
IV	Diabetic Control+ A.V	250 mg/Kg	302.2 ±5.7	238.2 ±7.7	187 ±8.7	139 ±9.52	121.6 ±8.87
V	Diabetic Control+ Glibenclamide	1.40 mg/kg	299.76 ±3.2	228.0 ±3	174.1 ±3	125.1 ±3.4	116.89 ±2.5

Table.No.6: Effect of ethanolic *Aloe Vera* on total cholesterol in streptozotocin induced diabetic rats after prolonged treatment

GROUPS	DRUG	DOSE	0 Hr	1 Hr	2 Hr	4 Hr	6 Hr	8 Hr	12 Hr	24 Hr
I	Normal control	5% w/v Tween 80	0.386 ±0.02	0.384 ±0.02	0.374 ±0.02	0.368 ±0.01	0.378 ±0.02	0.386 ±0.02	0.388 ±0.02	0.372 ±0.02
II	Diabetic Control	5% w/v Tween 80	6.22 ±0.31	6.38 ±0.32	6.26 ±0.30	6.33 ±0.33	6.28 ±0.3	6.31 ±0.3	6.36 ±0.23	6.25 ±0.19
III	Diabetic Control+ A.V	100 mg/Kg	6.24 ±0.35	6.18 ±0.17	5.94 ±0.19	5.64 ±0.27	5.24 ±0.24	4.76 ±0.11	3.56 ±0.20	2.62 ±0.19
IV	Diabetic Control+ A.V	250 mg/Kg	6.36 ±0.24	6.29 ±0.22	6.12 ±0.25	5.82 ±0.33	5.48 ±0.31	4.62 ±0.41	3.34 ±0.28	2.49 ±0.19
V	Diabetic Control+ Glibenclamide	1.40 mg/kg	6.32 ±0.15	6.24 ±0.17	6.02 ±0.02	5.68 ±0.2	5.32 ±0.21	4.51 ±0.19	3.12 ±0.29	2.14 ±0.31

Table.No.7: Effect of ethanolic *Aloe Vera* on serum creatinine levels in streptozotocin induced diabetic rats after single dose administration

GROUPS	DRUG	DOSE	1 day	3 day	5 day	7 day	14 day
I	Normal control	5% w/v Tween 80	0.386 ±0.02	0.384 ±0.02	0.374 ±0.02	0.368 ±0.01	0.378 ±0.02
II	Diabetic Control	5% w/v Tween 80	6.22 ±0.31	6.38 ±0.32	6.26 ±0.30	6.32 ±0.33	6.34 ±0.3
III	Diabetic Control+ A.V	100 mg/Kg	6.34 ±0.35	5.32 ±0.17	4.94 ±0.19	3.64 ±0.27	2.24 ±0.24
IV	Diabetic Control+ A.V	250 mg/Kg	6.36 ±0.24	5.28 ±0.22	4.64 ±0.25	3.58 ±0.33	2.18 ±0.31
V	Diabetic Control+ Glibenclamide	1.4 mg/kg	6.32 ±0.15	5.18 ±0.17	3.74 ±0.01	2.94 ±0.20	2.12 ±0.21

Table.No.8: Effect of ethanolic *Aloe Vera* on serum creatinine levels in streptozotocin induced diabetic rats after prolonged treatment

GROUPS	DRUG	DOSE	0 Hr	1 Hr	2 Hr	4 Hr	6 Hr	8 Hr	12 Hr	24 Hr
I	Normal control	5% w/v Tween 80	29.1 ± 1.0	28.4 ±1.6	28.6 ±6.89	28.9 ±1.4	29.4 ±1.4	30.2 ±1.3	29.2 ±1.3	29.6 ±1.5
II	Diabetic Control	5% w/v Tween 80	147.1 ±1.5	144.6 ±3.22	145.0 ±1.9	144.8 ±2.6	143.6 ±2.2	144.56 ±2.6	144.3 ±2.7	145.3 ±2.8
III	Diabetic Control+ A.V	100 mg/Kg	146.1 ±4.9	144.9 ±5.2	134.5 ±4.9	125.1 ±7.4	117.8 ±6.8	108.76 ±5.06	95.6 ±6.6	85.9 ±7.8
IV	Diabetic Control+ A.V	250 mg/Kg	147.3 ±13.4	143.0 ±14.1	130.8 ±40.8	120.9 ±11.6	115.2 ±7.2	106.9 ±7.06	93.1 ±7.6	82.4 ±7.2
V	Diabetic Control+Glibenclamide	1.40 mg/kg	148.1 ±8.6	142.4 ±9.8	123.5 ±12.9	117.3 ±12.6	111.1 ±12.44	102.4 ±9.4	92.4 ±9.9	78.5 ±7.3

Table.No.9: Effect of ethanolic *Aloe Vera* on urea levels in serum in streptozotocin induced diabetic rats after single dose administration

GROUPS	DRUG	DOSE	1 day	3 day	5 day	7 day	14 day
I	Normal control	5% w/v Tween 80	29.1 ± 1	28.4 ±1.6	28.6 ±6.89	28.9 ±1.4	29.4 ±1.4
II	Diabetic Control	5% w/v Tween 80	147.1 ± 1.5	145.6 ±3.22	145.3 ±1.9	146.8 ±2.6	144.6 ±2.2
III	Diabetic Control+ A.V	100 mg/Kg	146.1 ±4.9	141.91 ±5.2	134.5 ±4.9	125.1 ±7.4	82.8 ±6.8
IV	Diabetic Control+ A.V	250 mg/Kg	147.3 ±13.4	138.06 ±14.1	130.8 ±40.8	120.9 ±11.6	79.2 ±7.2
V	Diabetic Control+ Glibenclamide	1.40 mg/kg	148 ±8.6	134.4 ±9.8	123.5 ±12.9	117.3 ±12.6	72.1 ± 12.44

Table.No.10: Effect of ethanolic *Aloe Vera* on urea levels in serum in streptozotocin induced diabetic rats after prolonged treatment

4. DISCUSSION

Anxious reaction is an adaptive reaction of an individual when confronted with danger or threat. Behavioral and physiological responses accompanying anxiety prepare an individual to react appropriately to such situation. One of the most widely used animal models for screening putative anxiolytic is the elevated plus-maze[14]. The EPM is considered to be an etiologically valid animal model of anxiety because it uses natural stimuli, such as a fear of a new, brightly-lit open space and the fear of balancing on a relatively narrow raised platform, moreover it is known that anxiolytic agent increases the frequency of entries and time spent in open arm of the EPM [15]. In agreement with previously published reports, diazepam increased the percentage time spent on open arms and the number of entries on open arms [16]. Total number of open arm entries and number of closed arm entries are usually employed as measures of general activity. In the present study it is noted that administration of AEMC prolonged the time spent in the open arms and the number of entries into open arms.

The light/dark box is also widely used for rodents as a model for screening anxiolytic or

anxiogenic drugs, based on the innate aversion of rodents to brightly illuminated areas and on the spontaneous exploratory behavior of rodents in response to mild stressors, that is, a novel environment and light [17]. It has been reported that simply the measurement of the time spent in the light area, but not the number of transfers, is the most consistent and useful parameter for assessing an anxiolytic action [18]. The present study showed that AEMC could increase the time in the light area, suggesting again that AEMC possesses anxiolytic properties.

The hole-board test provides a simple method for measuring the response of an animal to an unfamiliar environment and is widely used to assess emotionality, anxiety and/or responses to stress in animals [19]. It has been shown that head-dipping behavior was sensitive to changes in the emotional state of the animal, and suggested that the expression of an anxiolytic state in animals may be reflected by an increase in headdipping behavior[20]. In the present

study AEMC increased head-dip counts and head-dip duration without changing locomotion. These results indicate that AEMC has a significant anxiolytic effect in this paradigm.

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