



ORIGINAL RESEARCH ARTICLE

EVALUATION OF ALOE VERA LEAVES EXTRACT IN STREPTOZOTOCIN- INDUCED DIABETIC NEPHROPATHY IN RAT

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ABSTRACT

Having an anti-diabetic activity, Aloe Vera (*Aloebarbadensis*) has been used for medicinal purposes in several cultures for millennia. It has also been proved that aloe vera is having protective activity in nephrotoxic rats. The objective of the present study was to evaluate the effect of Aloe vera leaves extract on diabetic nephropathy in Streptozotocin-induced diabetic rat. Diabetes was induced by Streptozotocin (60 mg/kg-i.p.). Four weeks later rats were randomly selected and divided into 5 groups (n=6). Group I: normal control. Group II: diabetic nephropathy (DN) control. Group III: DN+AVE (250 mg/kg). Group IV: DN+AVE (500 mg/kg). Group V: DN+AMG (1 mg/1ml in drinking water). The treatment was given for 8 weeks. Various parameters like physiological, antidiabetic, renal function, antioxidants and histopathology were measured. Administration of aloe vera extract showed significant decrease in body weight, food and water intake, kidney weight, blood glucose level, serum creatinine, BUN, protein in urine, serum uric acid and lipid peroxidation. It also significantly increases the plasma insulin level, liver glycogen content, urine volume, GFR, SOD, catalase, Reduced glutathione. Histopathological studies confirmed that administration of AVE prevented kidney damage, which provided structural support for the renal shielding effect. The significant effect of aloe vera extract on diabetic nephropathy could be due to the inherent antihyperglycemic, antioxidant, improvement of renal function parameters. In the near future AVE could constitute a lead to the discovery of a novel drug for the treatment of diabetic nephropathy.

Key words: *Aloebarbadensis*, *Amino guanidine hydrogen carbonate*, *Diabetic nephropathy*, *Renal function test*, *Streptozotocin*.

INTRODUCTION

Diabetic nephropathy is a major underlying cause of both mortality and morbidity in type 1 and type 2 diabetic mellitus (DM).¹ The chronic complication can be divided as vascular and non-vascular.² Vascular complications are subdivided into micro-vascular retinopathy, nephropathy, neuropathy and macro-vascular complications are peripheral vascular disease, coronary artery disease and cerebrovascular disease.³ Non vascular complication include problems such as gastroparesis, skin changes

and sexual dysfunction.²

Previous report suggests that 43% of the chronic renal failure patients on dialysis have diabetic nephropathy, 60% death cases of diabetic mellitus patients are due to diabetic nephropathy and death case of diabetic mellitus patients due to renal failure are 17 times more as compared to non-diabetic mellitus patients.^{4,5}

Aloe Vera (*Aloebarbadensis*) has been used for medicinal purposes in several cultures for millennia; Greece, India, China, Japan, Egypt and Mexico.⁶ The herb is used in various conditions like digestive problems, asthma, immune system enhancement, peptic ulcer, externally in skin irritation, burns, scalds, sunburn wounds, eczema, acne, psoriasis, dermatitis, ulcers.^{7,8} Aloe Vera also having anti-diabetic activity.^{9,10} There are established evidences on elevated levels of serum creatinine and urea being reduced by aloe vera in combination with standard antidiabetic drugs.¹¹ It has also been proved that aloe vera is having protective activity in nephrotoxic rats.¹² So, from all above evidence shows aloe vera is having nephroprotective activity.

However, no work is reported regarding the activity of aloe vera in diabetic nephropathy. Therefore, the present study was designed to investigate the effect of aqueous extract of aloe vera on diabetic nephropathy in streptozotocin induced experimental diabetic wistar rats.

MATERIALS AND METHODS

Collection and authentication of plant material

The leaves of Aloe vera (*Aloe barbadensis*) belonging to the family Xanthorrhoeaceae were collected from the local garden of Bangalore Karnataka. The plant was identified and authenticated by Dr. K. Ravikumar, senior botanist at FRLHT (Foundation for Revitalisation of Local Health Traditions) Jarakabande Kaval, post Attur, Yehalanka, Bangaluru (560106). A herbarium voucher specimen (11/15) was preserved in the college museum for future reference.

Extraction procedure

The Aloe vera leaves were sterilized properly. Fresh Aloe vera leaf with gel was dried in the oven at 80°C for 48 hours and then powdered (with electric

mill). The powder was cold extracted in water/ethanol mixture (1:1) for 72 h. The solvents were evaporated to obtain a dark hydro-alcohol extract whose contained tannins, flavonoids and alkaloids as revealed by phytochemical screening. In the process of maceration, 10g of the crushed plant part was dissolved in 100 ml mixture of ethanol, and distilled water.

Experimental animals

Male Albino Wistar Rats weighing 180-200 g were used for the present study. The animals were collected from Raghavendra Enterprises, Bangalore. The animals were maintained under controlled conditions of temperature ($25 \pm 2^\circ\text{C}$), humidity ($50 \pm 5\%$) and 12 hrs. light-dark cycles. All the animals were acclimatized for seven days before the study. The animals were housed in sanitized polypropylene cages containing sterile paddy husk as bedding. They had free access to standard pellets as basal diet and water ad libitum. All the studies conducted were approved by the Institutional Animal Ethical Committee (IAEC) of Raghavendra Institute of Pharmaceutical Education and Research (RIPER), Ananthapuramu. Approval No. 878/ac/05/CPCSEA/025/2015, according to prescribed guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

Induction of Diabetes and Experimental Design

In before overnight fasted wistar rats received streptozotocin (60mg/kg, i.p), freshly prepared in 0.1M cold citrate buffer (pH 4.5). Normal control rats received citrate buffer only. 48 hrs after STZ administration, blood samples were collected from retro orbital plexus and plasma glucose was measured by using a glucometer with glucose oxidase impregnated strips. The induction of diabetes mellitus was confirmed by determination of plasma glucose level (≥ 300 mg/dl) and selected for further

Nephropathy study.

Diabetic nephropathy will be well developed after 4 weeks of diabetes induction, treatment was started after 4th week and continued end of up to 8th week. Rats were randomly grouped into 5 groups (6 rats/group) and received the following treatment for 8 weeks.

Group 1: Normal control received normal saline (1ml/100g/day, p.o)

Group 2: DN control received streptozotocin (60 mg/kg, i.p)

Group 3: DN rats received Aloe vera 250mg/kg, p.o (DN+TEST 1)

Group 4: DN rats received Aloe vera 500mg/kg, p.o (DN+TEST 2)

Group 5: DN rats received aminoguanidine hydrogen carbonate (1 mg/1ml in drinking water) (DN+AMG)

Measurement of Physiological Parameters

Measurement of Body Weight, Kidney Weight

The body weight was recorded at 0th week and final day of 8th week and kidney weight of each animal was recorded in grams on the final day of 8th week using a digital balance.

Measurement of Food Intake and Water Intake The food intake in grams/rat/day was recorded at 0th week and final day of the 8th week. Water intake in ml/rat/day was recorded at 0th week and final day of the 8th week.

Measurement of Anti Diabetic Parameters

Estimation of Blood Sugar³¹: Serum glucose levels were determined by the glucose oxidase – peroxidase (GOD-POD) method.

Estimation of plasma insulin³: Plasma insulin was estimated by RIA kit method.

Estimation of Liver Glycogen Content⁶: Liver

glycogen content was measured by the scheme of vander vies. 200mg of Liver tissue was thinly ground with 20 ml of 5% Trichloroacetic acid in a Homogenizer and protein precipitate was filtered. 2ml of clear supernatant was pipette out into a 20ml capacity calibrated test tube and after that 2ml of 10N KOH was added. This tube was sited in a hot water bath for 1 h. later than cooling, to counteract the excess of alkali 1 ml of glacial acetic acid was added and fluid brought equal to the mark with water. Slowly, Test tube containing 4 ml of anthrone reagent, 2ml solution was added from the previous step which was positioned in cold water to stop too much heating. After systematic integration, the tube was to be found in a hot water bath for perfectly 10 min for the improvement of colour and cooled with running tap water. The optical density was interpreted within 2 h. in a spectrophotometer at 650 nm against a blank.

Renal Function Parameters

Blood urea nitrogen¹³: $\text{Urea} + 2\text{H}_2\text{O} \rightarrow 2\text{NH}_4^+ + \text{CO}_2$
 $\text{NH}_4^+ + 2\text{-oxoglutarate} + \text{NADH} \rightarrow \text{L-glutarate} + \text{NAD} + \text{H}_2\text{O}$; Calculation: - BUN = $\frac{\Delta A \text{ of test}}{\Delta A \text{ of standard}} \times \text{concentration of standard (mg/dl)}$

Estimation of Protein urine (Biuret Method)¹⁴:

Prepare blank, test and standard solution as follows

Blank solution: (3ml of Biuret reagent), Standard solution: (Add 30 μ l of the total Protein standard solution to 3 ml of Biuret reagent), Test Solution: (Add 30 μ l urine to 3ml of Biuret reagent). Mix well and incubate at 37°C. Read the absorbance at 578 nm against reagent blank.

Calculations: Total Protein (gm/dl) = $\frac{\text{Abs. of Test}}{\text{Abs. of Std.}} \times 6.5$

Estimation of Uric Acid²¹

Calculations: Uric acid (mg/dl) = Absorbance of test/ Absorbance of standard x 5

Serum Creatinine¹⁷**Measurement of Glomerular Filtration Rate**²⁷:

Glomerular filtration rate was calculated using the formula

$$\text{GFR (ml/min)} = \frac{\text{Urinary creatinine} \times \text{urine volume}}{\text{Serum creatinine} \times 1440}$$

Estimation of Kidney Antioxidant Parameters

Estimation of Superoxide Dismutase (SOD)²: SOD was predictable by the Method of Misra and Fridovich (1967)

Estimation of Catalase (CAT)¹: Catalase was measured by Hugo E. Aebi Method, 1974.

Estimation of Reduced Glutathione¹⁰: Reduced glutathione was estimated by Ellman method

Estimation of lipid peroxidation²⁴

Histopathology of Kidneys: At the end of the protocol the animals were sacrificed and their kidneys were isolated. The isolated left kidney was cut into small pieces and after removal of extra tissues preserved in 10% formalin solution for 24 hours. Specimens were cut in sections of 3-5µm in thickness by microtome and stained by hematoxyline-eosin (H&E) stain and. The stained samples were observed under microscope and analysed by using a light microscope.

Results

Effect of Aloe vera extract (AVE) on Physiological parameters

As shown in table no. 1 and 2 Streptozotocin -induced diabetic nephropathy rats exhibited a significant decrease in the body weight and significant increase in kidney weight, food and water intake when compared to normal control. Oral administration of AVE and standard amino guanidine hydrogen carbonate significantly increases in body weight and significantly decreases in kidney weight, food and water intake when compared to diabetic nephropathy control group

Table 1: Effect of Aloe vera Extract on Physiological parameters

Group	Body weight(g) at 0 th Week	Body weight(g) at 8 th Week	Food intake (g/rat/day) at 0 th Week	Food intake(g/rat/day) at 8 th Week	Water intake(ml/rat/day) at 0 th Week	Water intake(ml/rat/day) at 8 th Week
Normal	191.0±2.30	197.3±1.20	18.3±0.48	19.6±1.20	80.3±4.21	78.6±2.02
DN control	189.7±3.18 ^{ns}	157.7±4.33 [*]	20.5±1.50 ^{ns}	38.0±1.52 [*]	78.4±2.05 ^{ns}	94.0±1.15 [*]
DN+TEST 1	184.3±2.18 ^{ns}	180.0±2.88 [#]	19.3±0.56 ^{ns}	21.0±1.73 ^{##}	82.6±3.51 ^{ns}	80.3±2.40 [#]
DN+TEST 2	183.0±2.08 ^{ns}	181.0±3.51 [#]	19.8±2.21 ^{ns}	19.0±0.57 ^{##}	76.9±1.04 ^{ns}	78.0±1.52 ^{##}
DN+AMG	182.3±3.38 ^{ns}	189.7±2.90 ^{##}	18.1±1.76 ^{ns}	22.0±2.51 ^{##}	75.8±3.67 ^{ns}	76.3±1.45 ^{##}

Each value represents the mean ±S.E.M (n=6). One-way ANOVA followed Tukey's sposthoc test. Ns & * indicates non-significant and p<0.001 respectively when compared to normal group. # & ## indicates p<0.01 & p<0.001 respectively when compared to diabetic nephropathy control group.

Table 2. Effect of Aloe vera Extract on Kidney weight

Group	Kidney weight(g)
Normal	1.82±1.56
DN Control	2.71±2.42*
DN+TEST 1	2.42±0.15 ^{ns}
DN+TEST 2	1.98±9.53 #
DN+AMG	2.01±0.23 #

Each value represents the mean ±S.E.M (n=6). One-way ANOVA followed Tukey's spsthoctest. * indicates p<0.01 when compared to normal group. ns& # indicates non- significant & p<0.01 respectively when compared to diabetic nephropathy control group.

Effect of Aloe vera extract (AVE) on Anti Diabetic Parameters

As shown in table no. 3, 4 and figure no. 2, 3, 4 STZ-induced diabetic nephropathy rats exhibited a significant increase in blood glucose level and significant decrease in plasma insulin and liver glycogen content when compared to normal control. Oral administration of AVE and amino guanidine hydrogen carbonate significantly decreases in blood glucose level and significantly increases in plasma insulin and liver glycogen content when compared to diabetic nephropathy control group.

Table 3. Effect of Aloe vera Extract on Blood glucose level

Group	2 nd week	8 th week
Normal	78±1.06	87.4±2.34
DN Control	289±6.95*	278±4.67*
DN+TEST1	170±4.07#	139±3.94#
DN+TEST2	145±2.94#	113±5.61#
DN+AMG	214±6.93#	203±3.89#

Each value represents the mean ±S.E.M (n=6). One-way ANOVA followed Tukey's spsthoctest. *

indicates p<0.001 when compared to normal group. # indicates p<0.001 when compared to diabetic nephropathy control group.

Table 4. Effect of Aloe vera Extract on Plasma Insulin and Liver Glycogen content

Group	Plasma insulin (µIU/ml)	Liver glycogen content (g/100 g of liver tissue)
Normal	13.4±0.56	2.04±0.42
DN Control	5.2±0.32*	0.55±0.18*
DN+TEST 1	11.4±0.87#	1.97±3.54 #
DN+TEST 2	12.9±0.13#	1.67±2.24 #
DN+ AMG	10.5±3.98#	1.22±0.16#

Each value represents the mean ±S.E.M (n=6). One-way ANOVA followed Tukey's spsthoctest. * indicates p<0.001 when compared to normal group. # indicates p<0.001 when compared to diabetic nephropathy control group.

Effect of Aloe vera extract (AVE) on Renal Function Parameters

Each value represents the mean ±S.E.M (n=6). One-way ANOVA followed Tukey's spsthoctest. * indicates p<0.001 when compared to normal group. # & ## indicates p<0.01 & p<0.001 respectively when compared to diabetic nephropathy control group.

As shown in table no. 5, 6 and figure no. 5 & 6 Streptozotocin-induced diabetic nephropathy rats exhibited a significant increase in BUN, protein urea, uric acid, serum creatinine and significant decrease in urine volume and GFR when compared to normal control. Oral administration of AVE and amino guanidine hydrogen carbonate significantly decreases in BUN, protein urea, uric acid, serum creatinine and significantly increases in urine volume and GFR when compared to diabetic nephropathy control group.

Table 5. Effect of Aloe vera Extract on Renal Function Tests

Group	Blood urea nitrogen (mg/dl)	Protein in urine (mg/day)	Serum uric acid (mg/dl)
Normal	12.6±0.88	0.58±0.68	1.89±0.63
DN Control	24.6±1.45*	20.45±0.94*	5.67±0.38*
DN+TEST 1	16.6±1.45 [#]	0.95±0.68 [#]	2.72±0.23 ^{##}
DN+TEST 2	16.0±1.15 [#]	0.87±0.59 [#]	1.69±0.18 ^{##}
DN+ AMG	16.3±0.88 [#]	0.69±0.49 [#]	2.89±0.04 ^{##}

Each value represents the mean ±S.E.M (n=6). One-way ANOVA followed Tukey's sposthocstest. * indicates p<0.001 when compared to normal group. # & ## indicates p<0.01 & p<0.001 respectively when compared to diabetic nephropathy control group.

Table 6. Effect of Aloe vera Extract on Renal Function Tests

Group	Urine volume (ml/rat/day)	Serum creatinine (mg/dl)	GFR (ml/kg bw/min) (or) Creatinine clearance
Normal	12.8±0.39	0.99±0.76	0.63±0.01
DN Control	6.7±0.45*	2.91±0.45*	0.03±0.02*
DN+TEST 1	11.6±1.56 [#]	1.01±0.15 [#]	0.51±0.06 [#]
DN+TEST 2	11.9±1.93 [#]	0.90±0.12 [#]	0.49±0.08 [#]
DN+ AMG	10.8±1.69 [#]	0.89±0.32 [#]	0.52±0.05 [#]

Each value represents the mean ±S.E.M (n=6). One-way ANOVA followed Tukey's sposthocstest. * indicates p<0.001 when compared to normal group. # indicates p<0.001 when compared to diabetic nephropathy control group.

Effect of Aloe vera extract (AVE) on kidney anti-oxidant parameters.

As shown in table no. 7 Streptozotocin -induced diabetic nephropathy rats exhibited a significant decrease in SOD, CAT, Reduced glutathione and significant increases in lipid peroxidation when compared to normal control. Oral administration of AVE and amino guanidine hydrogen carbonate significantly increases in SOD, CAT, Reduced glutathione and significantly decreases lipid peroxidation in when compared to diabetic nephropathy control group.

Table 7. Effect of Aloe vera Extract on Kidney Antioxidants

Group	Superoxide dismutase (SOD) (U/mg protein)	Catalase (µmol of H ₂ O ₂ decomposed/min/mg protein)	Reduced glutathione (µg/g tissue)	Lipid peroxidation (µ mol MDA/mg protein)
Normal	33.1±1.24	63.5±1.41	82.8±2.56	16.4±1.35
DNControl	13.2±1.75**	22.4±1.74**	31.5±3.42*	76.8±3.15**
DN+TEST 1	19.5±2.51 [#]	49.5±1.65 ^{##}	49.9±3.56 [#]	28.3±2.56 ^{##}
DN+TEST 2	29.9±1.72 ^{##}	58.4±3.15 ^{##}	76.5±3.42 ^{##}	20.8±1.65 ^{##}
DN+ AMG	27.6±0.26 ^{##}	52.8±5.45 ^{##}	74.9±1.75 ^{##}	22.4±1.41 ^{##}

Each value represents the mean ±S.E.M (n=6). One-way ANOVA followed Tukey's sposthocstest. * & ** indicates p<0.01 & p<0.001 respectively when compared to normal group. # & ## indicates p<0.01 & p<0.001 respectively when compared to diabetic nephropathy control group.

Histopathology of kidney: As shown in figure 1 histological examination of the kidney tissues showed progressive histopathological alteration shown by the focal haemorrhage between the degenerated tubules

at the corticomedullary function in the streptozotocin treated group. The histological abnormalities in renal tissues were markedly improved after administration of AVE shown by the normal appearance with normal tubular diameter.

DISCUSSION

Diabetic nephropathy, a frequent and major microvascular complication of diabetes mellitus, is the most common cause of end-stage renal disease.¹³ Chronic Diabetes Mellitus causes multiple complications like diabetic nephropathy, neuropathy, retinopathy and myopathy. Several factors, such as hyperglycaemia, Hyperlipidaemia, oxidative stress and inflammatory cytokines, contribute to the progression of renal damage in diabetic nephropathy.¹⁴

In the present study aloe vera extract (AVE) shows significant antidiabetic, hypolipidemic and nephroprotective effects in STZ induced diabetic nephropathy. Streptozotocin -induced diabetes characterized by a severe loss in body weight, which is connected with characteristic symptoms of diabetes. Diabetic nephropathy rats treated with AVE, the weight loss was found to be reversed and normalized the food and water intake.

In this study oral administration of AVE at doses 250&500mg/kg b.w. significantly decreased the kidney weight. Fasting blood glucose level in diabetic rats is a vital basal parameter for monitoring diabetes.¹⁵

In the present study, diabetes was induced by Streptozotocin . This produces hyperglycaemia by selective cytotoxic effects on pancreatic β cells. One of the intracellular phenomenon for its cytotoxicity is through the production of free radicals.¹⁶ It has been resulted in decreased insulin production. In DN. Glycogenesis in liver was mainly regulated by insulin.

The decreased levels of liver glycogen were observed in diabetic nephropathy rats. This may be due to the diminutive levels of insulin in diabetic nephropathy state or oxidative stress by diabetes may inactivate the glycogen synthase.¹⁷ Oral administration of AVE significantly increases the insulin level and liver glycogen content.

Reduction in antioxidants like SOD, Catalase, GSH and increased levels of MDA were observed in STZ-induced diabetic rats. Oral administration of AVE significantly restored those parameters.

DN characterized by elevated levels of serum creatinine, BUN, urinary albumin excretion rate, & decreased of urine volume, GFR.¹⁸ Oral administration of AVE significantly decreased the levels of serum creatinine, blood urea nitrogen and significantly increased the urine volume and GFR.

Proteinuria is measured as a main sign of diabetic nephropathy.¹⁹ In the present study proteins appeared in the urine DN rats. Oral administration of AVE significantly decreased the levels of urinary protein excretion that shows the development of renal functions.

Here administration of aminoguanidine ameliorated the renal dysfunction as assessed by reduced above parameters. Our results are in harmony to those observed by other investigators.^{20, 21}

Here in the histopathological studies we found that Streptozotocin caused an important damage in renal structure showing noticeable glomeruli and tubular damages, haemorrhagic conditions. This was most likely due to the generation of reactive radicals and to succeeding lipid peroxidation. Oral administration of AVE improves the histological alterations induced by STZ.

CONCLUSION

The result of the present study showed that aloe vera extract has a better renoprotective effect in diabetic rats. The administration of AVE showed significant decrease in body weight, food and water intake, kidney weight, blood glucose level, serum creatinine, BUN, protein in urine, serum uric acid and lipid peroxidation. It also increases the plasma insulin level, liver glycogen content. Urine volume, GFR, SOD, catalase, Reduced glutathione. The result obtained from histopathological study confirmed that administration of AVE prevented kidney damage, which provided structural support for the renal shielding effect. Further study is required to approach the new insight for the possible mechanism of action.

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