EVALUATION OF PERIPHERAL NEUROPATHIC EFFECT OF ALOE VERA LEAVES EXTRACT IN DIABETIC RAT

Anil Shrestha¹*, N. C. Nagalakshmi² and Shiva Kumar Swamy²

¹Department of Pharmacology, Kantipur Dental College General Hospital & Research Center, Kathmandu, Nepal.
²Department of Pharmacology, Mallige College of Pharmacy, Bangalore-90, India.

ABSTRACT

Aims: To evaluate the diabetic peripheral neuropathic effect of hydro-alcoholic leaves extract of Aloe vera in Streptozotocin (STZ) induced diabetic rat models. Study design: Diabetic peripheral neuropathic effect was carried out by dividing animal randomly into five groups, each containing 6 animals. Place and Duration of Study: Department of Pharmacology, Mallige College of Pharmacy, Bangalore-90, between August 2014 and April 2015. Methodology: Diabetes was induced by a single i.p. injection of 50 mg/kg streptozotocin. Grip strength, Eddy’s hotplate, Formalin test, Tail immersion test (Hot & cold water) and Pin prick test were performed to determine the extent of neuropathic pain in diabetic rats. Imipramine (10.5mg/kg), Duloxetine (20mg/kg), Aloe vera (300mg/kg) and Aloe vera (450mg/kg) was administered orally for 21 consecutive days starting after 4th week in STZ induced diabetic rats., Total cholesterol ,Triglycerides, HDL, LDL, Thiobarbituric acid reactive substance (TBARS), superoxide dismutase (SOD) and Catalase in serum were also performed to access the oxidative stress. Results: Chronic treatment with Aloe vera for three weeks starting after fourth week in STZ induced diabetic rat’s attenuated hyperalgesia. Treatment with Aloe vera at doses of 300 and 450 mg/kg p.o. daily significantly restored the reduced body weight, elevated blood sugar, reduced grip strength and lipid profiles. It also ameliorated diabetic induced raised level of TBARS and decreased level of SOD and Catalase. Conclusion: It was concluded that antidepressant, antidiabetic and antioxidant effect of Aloe vera may be responsible for observed antinociceptive and protective role against damage to the neurons in STZ induced diabetic peripheral neuropathic rat models with P<0.001.
KEYWORDS: Aloe vera; Diabetic peripheral neuropathy; Streptozocin; Imipramine; Duloxetine.

1. INTRODUCTION

Diabetic peripheral neuropathy (DPN) is a micro-vascular complication of diabetes mellitus associated with considerable mortality, morbidity, substantially impair quality and expectancy of life which is characterized by pain, sensory loss and paresthesia, it affects 50% of patients with diabetes.[1] Up to 50% of DPN may be asymptomatic and 80% of amputations follow a foot ulcer or injury, early recognition of at-risk individuals, provision of education and appropriate foot care may result in a reduced incidence of ulceration and consequently amputation.[2] Therefore, it causes a huge burden on both society and individuals, represents a major public health problem.

Hyperglycemia is the main factor for prevalence of diabetic neuropathy induces oxidative stress through various cellular pathways such as increasing aldose reductase activity,[3] altering protein kinase C activity[4] and increasing glycation end-products.[5] Exposure of high blood glucose levels over an extended period of time causes producing a large amount of Reactive Oxygen Species (ROS) can damage mitochondrial DNA in dorsal root ganglia resulting to peripheral nerves dysfunction.[6] Several studies have been proposed that oxidative stress is one of the major factor impairing dorsal root ganglia and sensory nerve fibers.[7,8]

A Number of classes of drugs are found effective for symptomatic relief of mechanical hyperalgesia in clinical studies. Tricyclic anti-depressants,[9] Selective Serotonin Reuptake Inhibitors,[10] Anticonvulsants,[11] Gabapentin,[12] have been beneficial to inhibit neuropathic pain and give symptomatic relief.


However, till date no scientific evaluations are conducted for confirming its role in diabetic peripheral neuropathy. Thus the present study is designed for evaluation of peripheral neuropathic effect of Aloe vera leaves extract in diabetic rat.
2. MATERIAL AND METHODS

2.1. Animals
Male albino rats of Wistar strain weighing 160-200 g were included for the study. The experimental protocol was approved by Institutional Animal Ethics Committee (MCP/IAEC-030/2014-15) and animals were maintained under standard conditions in the animal house approved by Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA).

2.2. Drugs and chemicals
Streptozotocin purchased from Sigma-Aldrich Mumbai India. Imipramine and Duloxetine were obtained as gift sample from Torrent pharmaceuticals, Ahmadabad. Other reagents and chemicals were of analytical grade and purchased from local suppliers of Bangalore, India.

2.3. Collection and Authentication of Plant Material
The leaves of Aloe vera were collected from the local garden of Karnataka Bangalore. The plant was identified and authenticated by botanist at FRLHT (Foundation for Revitalization of Local Health Traditions) Jarakabande Kaval, post Attur, Yelahanka, Bangalore (560106). A herbarium specimen was preserved in the college museum for future reference.

2.4. Extraction methodology
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 95°mixture (1:1) for 72 h. The solvents were evaporated to obtain a dark hydro-alcohol extract that contained tannins, flavonoids and alkaloids as revealed by photochemical screening. In the process of maceration, 10g of crushed plant part was dissolved in 100 ml of organic solvent i.e. ethanol, and distilled water[20]

2.5. Preparation of drug solutions
Aloe vera extract was prepared in distilled water and STZ was dissolved in cold citrate buffer (pH 4.5). All drug solutions were prepared freshly.

2.7. Induction and assessment of diabetes
Diabetes was induced in overnight fasted adult Wistar Albino rat by a single intra-peritoneal injection of 50mg/kg STZ[21] Blood glucose levels were measured after 72 h of STZ injection. Blood samples were obtained from tail vein by subjecting the rats to light ether
anesthesia. Glucose concentration was measured by using glucometer with glucoseoxidase impregnated strips. Animals with blood glucose levels >250 mg/dl was considered diabetic and selected for further study.

2.8. Experimental design

Hypersensitivity to pain stimuli was well developed after fourth week after diabetes induction, treatment was started after fourth week and continued end of up to seventh week. The treatment period was for 21 days. Animals were randomly divided into five groups, each containing 6 animals; group I Normal control received saline, group II diabetic control, group III received Duloxetine (20mg/kg, p.o. daily) for grip strength, pain threshold, cold and hot immersion test and pin prick test, where as in formalin test group received Imipramine (10.5 mg/kg, p.o. daily), group IV received Aloe vera leaves extract (300 mg/kg, p.o. daily) & group V received Aloe vera leaves extract (450 mg/kg p.o. daily). Body weight and blood glucose levels were measured throughout the experiment every week during treatment period. For the determination of blood glucose, tail vein was punctured with sharp needle to ooze out a drop of blood and the blood glucose was measured by a commercial glucometer. For biochemical estimations, at the end of experiment blood was withdrawn from retro-orbital plexus of overnight fasted rats by using a micro-capillary technique under light anesthesia.

2.9. Behavioral parameters

2.9.1. Grip strength

Grip strength was evaluated on fourth and seventh weeks in normal and in streptozotocin diabetic control and drug treated groups with the help of rota rod apparatus in which the rats were placed on a horizontal rod rotating at a speed of 25 rpm. The rats which were capable of remaining on the top for 25 sec or more, in three successive trials were selected for the study. The test was carried out to evaluate the muscular strength or neuromuscular function.

2.9.2. Eddy’s Hot plate test

Thermal Hyperalgesia was evaluated on fourth week and seventh week in normal and in streptozotocin diabetic control and drug treated groups. Paw withdrawal latency (cut-off time: 10 s) of each rat was determined using Eddy’s hot plate, constant temperature maintained at 55±1°C.
2.9.3. Formalin test

The formalin test was carried out according to the method of Courteix et al., 1993. Formalin challenge was done once in all the groups. Formalin (0.1 ml 10%) was administered to the dorsal surface of the left hind paw in both normal and diabetic rats. Each animal was then placed in a plexi glass chamber and observed for licking and duration of paw elevation. Mirrors were placed in each chamber to enable unhindered observation. Peripheral neuropathic pain behaviour was quantified as the numbers of flinches of the injected paw during 1-min periods every 5 min, up to 60 min after injection. Flinching was readily discriminated and was characterized as rapid and brief withdrawal, or as flexing of the injected paw. The initial acute phase (0–5 min) was followed by a relatively short quiescent period (10 - 20 min), which was then followed by a prolonged tonic response (25–60 min). Changes in duration of paw licking and paw elevation was observed after seventh week of streptozotocin administration.[22]

2.9.4. Cold and hot water immersion test

Cold and hot immersion tests were carried out according to the method described by Sharma et al., 2008. In the cold immersion test, the tail of the rat was immersed in cold water maintained at 10°C, while in the hot immersion test; the tail was immersed in water maintained at 45°C. In both the tests, basal tail flick latency (withdrawal response of tail) or signs of struggle was observed. The cut off time was 15 seconds. Cold and hot immersion tests were carried out on fourth and seventh week in normal and in streptozotocin diabetic control and drug treated groups. Changes in tail flick latency in all groups were compared. At the end of the treatment period the rats were sacrificed under anesthesia and the sciatic nerve was excised to assess the oxidative stress.[25]

2.9.5. Pin prick test

The mechanical hyperalgesia were assessed by the pin prick test as described by Erichsen and Blackburn-Munro. The surface of the injured hind paw was touched with the point of the bent gauge needle (at 90° to the syringe) at intensity sufficient to produce a reflex withdrawal response in normal non-operated animals, but at an intensity which is insufficient to penetrate the skin. The duration of the paw withdrawal was recorded in seconds with a stopwatch. A cut-off time of 20 seconds was maintained. Pin prick test was carried out on 4th and 7th week in normal and in streptozotocin diabetic control and drug treated groups.[26] At the end of the
treatment period the rats were sacrificed under anesthesia and the sciatic nerve was excised, structural integrity of sciatic nerve was accessed by histopathological studies.

2.10. Estimations Biochemical parameters
After 21 days of treatment blood was withdrawn through retro orbital route and total cholesterol, triglycerides, HDL, LDL were estimated.$^{[27,28]}$

2.11. Estimation Oxidative stress parameters
After 21 days of treatment blood was withdrawn through retro orbital route and centrifuged at 5000rpm for 20 min and serum was separated.

2.11.1. Thiobarbituric acid reactive substance (TBARS)
375 mg of TBA was dissolved in 2 ml of 0.25 N hydrochloric (HCl), followed by 15 g of trichloroacetic acid (TCA) for a total volume of 100ml. The solution was heated in a water bath at 50ºC to dissolve TBA properly. Then, 1 ml of serum was combined with 2 ml of TCA—TBA—HCl and mixed thoroughly. The solution was heated for 15 min in a boiling water bath. After cooling, the flocculant precipitate was removed by centrifugation. Sample absorbance was then determined at 535 nm against a blank that contained all reagents except the serum sample. Serum MDA concentration was expressed as nmol/ml.$^{[29]}$

2.11.2. Superoxide dismutase (SOD)
100 µl of Serum sample in 0.2 M sucrose in phosphate buffer (0.25 M, pH 7.4) was taken in test tube to this a mixture containing 1 ml of sodium carbonate, 0.4ml of Nitro Blue Tetrazolium (NBT) and 0.2 ml of EDTA was added and zero minute reading will be taken at 560nm. The reaction was initiated by addition of 0.4 ml of 1 mM hydroxylamine hydrochloride to the test tube. The reaction mixture was incubated at 25ºC for 5 minutes; the reduction of NBT was measured at 560 nm.$^{[20]}$

2.11.3. Catalase
100 µl of Serum sample in 0.15 M KCl buffer was added to 1.9 ml of phosphate buffer (0.25 M, pH 7) and absorbance was measured at 240 nm. To the above reaction mixture 1 ml of Hydrogen Peroxide solution was added and the absorbance measured after allowing stand for 1 minute at 240 nm using phosphate buffer as blank solution. One international unit of catalase utilized is that amount which catalyzes the decomposition of 1mM hydrogen peroxide per minute at 37ºC and expressed in terms of units/mg of protein.$^{[30]}$
2.12. Statistical analysis
The results were expressed as mean ± standard error of mean. Each group contained six rats (n=6). The data were analyzed by one-way analysis of variance (ANOVA) followed by Tukey’s multiple comparison tests and two-way ANOVA followed by Bonferroni post test using Graph Pad Prism Software. \( P \leq 0.05 \) was considered statistically significant.

3. RESULTS AND DISCUSSION
3.1. Effects of Aloe vera extract in body weight
The body weight decreased rapidly in STZ treated diabetic rats. The body weight increased normally in control rats, while STZ induced diabetic rats (Diabetic control) showed a significant decrease in body weight during post-STZ injection. A progressive loss of body weight was noted and maximum decrease in body weight was observed after seventh weeks STZ-injection (pre 163.3±1.520 to 134.8±2.104, \( P<0.001 \)). Significant decrease in body weight of the animals of Imipramine 10.5 mg/kg and Duloxetine 20 mg/kg treated groups was observed as similar to diabetic control group. The weight of the animals of other groups was also decreased significantly till day 28 days as compared to normal control group. On seventh week the animals treated with Aloe vera 300 mg/kg and Aloe vera 450 mg/kg were observed with significant increase in their body weight as compared to Diabetic control (Table 1).

3.2. Effects of Aloe vera extract in Blood glucose levels
The blood glucose levels increased rapidly after STZ injection. The blood glucose level of all experimental groups, except normal control group was increased significantly after the STZ injection till fourth week. On the seventh week of diabetes induction, in the diabetic control blood glucose levels increased to the maximum measurable value of 342.0±8.262 mg/dl and found to be significant increased \( (P<0.001) \) compared to the value of first day was 82.33±0.2018 mg/dl. In contrast, normal control animals remained normoglycaemic during the entire testing period of 21 days. The animals treated with Imipramine 10.5 mg/kg, Duloxetine 20 mg/kg, were observed with significant increase \( (P<0.001) \) in blood glucose level as similar to Diabetic control group during the entire testing period of treatment (Table 2).

3.3. Effects of Aloe vera extract in grip strength
The diabetic control and diabetic rats treated rats with Duloxetine 20 mg/ kg were showed significant reduction in grip strength as compared with the normal rats on the fourth and
seventh weeks that \( P<0.001 \). Further treatment with *Aloe vera* extracts (300 and 450 mg/kg) significantly increased grip strength on the seventh week (Fig. 1).

### 3.4. Effects of Aloe vera extract in Eddy’s hot plate test

The diabetic rats were showed significant reduction in paw withdrawal latency as compared with the normal rats on the fourth and seventh weeks indicating that the diabetic rats had thermal hyperalgesia \( P<0.001 \). Further treatment with *Aloe vera* extracts (300 and 450 mg/kg) and Duloxetine 20 mg/kg significantly reduced thermal hyperalgesia on the seventh weeks (Fig. 2).

### 3.5. Effects of Aloe vera extract in formalin induced pain

Diabetic animals showed hyperalgesia to chemical stimuli in all three phases. Groups treated with Imipramine 10.5 mg/kg, *Aloe vera* 300mg/kg, *Aloe vera* 450 mg/kg, significantly \( P<0.001 \) decreased sum of flinches in first phase when compared to diabetic control. Imipramine 10.5 mg/kg \( P<0.001 \), *Aloe vera* 300mg/kg \( P<0.001 \) and *Aloe vera* 450 mg/kg \( P<0.001 \), were significantly decreased flinches when compared to control groups. All treatments were significantly \( P<0.001 \) decreased sum of flinches in Q phase when compared to diabetic control and normal control. In third phase groups treated with Imipramine 10.5 mg/kg \( P<0.001 \), *Aloe vera* 300mg/kg \( P<0.001 \), *Aloe vera* 450mg/kg \( P<0.001 \), were significantly decreased flinches when compared to diabetic as well as normal control groups (Fig. 3).

### 3.6. Effects of Aloe vera extracts in cold and hot water immersion test

Tail withdrawal latencies of diabetic rats were significantly \( P<0.001 \) less than non-diabetic rats indicative of hyperalgesia. After three weeks of treatment, Tail withdrawal latency of groups treated with Duloxetine 20 mg/kg, *Aloe vera* 300mg/kg, *Aloe vera* 450 mg/kg were increased significantly \( P<0.001 \) when compared to diabetic control. Comparative analysis revealed that there was no significant difference found in latency period between *Aloe vera* and standard drug treated groups (Fig. 4).

### 3.7. Effect of Aloe vera extract in Paw withdrawal latency in Pin Prick test

After three weeks of treatment *Aloe vera* (300mg/kg and 450mg/kg), Duloxetine 20 mg/kg, treated groups significantly \( P<0.001 \) increased the paw withdrawal latency when compared to diabetic control. Comparative analysis revealed that there was no significant difference in
paw withdrawal latency period between *Aloe vera* (300mg/kg and 450mg/kg) and the standard (Fig. 5).

### 3.8 Effects of *Aloe vera* extract on serum levels of total cholesterol, triglyceride, HDL and LDL

Significant increased in the levels of serum total cholesterol triglyceride and LDL (*P*<0.001), and decreased HDL levels (*P*<0.001) were observed in the diabetic rats when compared with the normal control. The diabetic rats treated with *Aloe vera* extract (300 and 450 mg/kg) for three weeks showed significantly decreased levels of serum total cholesterol triglyceride and LDL (*P*<0.001) and increased level of total HDL (*P*<0.001) (Table 3).

### 3.9. Effects of *Aloe vera* extract on oxidative stress markers

*Aloe vera* 300mg/kg (*P*<0.001), *Aloe vera* 450 mg/kg (*P*<0.001) treated groups significantly showed decrease in TBARS when compared to diabetic control. Imipramine 10.5 mg/kg, Duloxetine 20 mg/kg treated groups and diabetic group showed significant (*P*<0.001) increase in TBARS when compared to normal control (Table 4).

*Aloe vera* 300mg/kg (*P*<0.001), *Aloe vera* 450 mg/kg (*P*<0.001) treated groups were significantly shown increase in serum SOD and catalase when compared to diabetic control. Imipramine and Duloxetine treated groups showed significant (*P*<0.001) decrease in SOD and catalase when compared to normal control (Table 4).

**Table 1. Effects of *Aloe vera* extract in body weight**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Change in the body weights on different days</th>
<th>1 day</th>
<th>4th week</th>
<th>5th week</th>
<th>6th week</th>
<th>7th week</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td></td>
<td>165.3±2.108</td>
<td>208.7±6.146</td>
<td>2267±5.481</td>
<td>240±4.472</td>
<td>253±3.159</td>
</tr>
<tr>
<td>Diabetic control</td>
<td></td>
<td>163.3±1.520</td>
<td>154.7±1.978***</td>
<td>150.3±0.954***</td>
<td>143.7±1.202***</td>
<td>134.8±2.104***</td>
</tr>
<tr>
<td>Imipramine 10.5 mg/kg</td>
<td></td>
<td>170.3±3.480</td>
<td>163.3±3.602***</td>
<td>158.7±3.291***</td>
<td>153.2±3.291***</td>
<td>143.7±2.940***</td>
</tr>
<tr>
<td>Duloxetine 20 mg/kg</td>
<td></td>
<td>171.7±1.745</td>
<td>160.3±0.954***</td>
<td>154.3±2.499***</td>
<td>146.2±2.688***</td>
<td>135.7±2.275***</td>
</tr>
<tr>
<td><em>Aloe vera</em> 300mg/kg</td>
<td></td>
<td>163.3±1.520</td>
<td>155.3±1.520***</td>
<td>162.3±2.028***</td>
<td>166±2.129***</td>
<td>172.0±862***</td>
</tr>
<tr>
<td><em>Aloe vera</em> 450mg/kg</td>
<td></td>
<td>172.0±3.055</td>
<td>164.7±2.776***</td>
<td>171.7±2.445***</td>
<td>178.3±2.445***</td>
<td>185.0±2.236***</td>
</tr>
</tbody>
</table>

*All the values are mean ± SEM, n=6, evaluated by two way ANOVA followed by Bonferroni post test, where *** is *P*<0.001 when compared to normal control group and a= *P*<0.001 when compared to diabetic control group.*
Table 2. Effects of \textit{Aloe vera} extract in Blood glucose levels

<table>
<thead>
<tr>
<th>Groups</th>
<th>Change in the blood glucose level on different days</th>
<th>1 day</th>
<th>4\textsuperscript{th} day</th>
<th>4\textsuperscript{th} week</th>
<th>5\textsuperscript{th} week</th>
<th>6\textsuperscript{th} week</th>
<th>7\textsuperscript{th} week</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td></td>
<td>82.33±2.27</td>
<td>79.33±1.11</td>
<td>80.33±1.08</td>
<td>80.83±1.10</td>
<td>80.33±0.80</td>
<td>81.33±0.80</td>
</tr>
<tr>
<td>Diabetic control</td>
<td></td>
<td>82.33±0.20</td>
<td>301.5±6.13***</td>
<td>318.3±4.92**</td>
<td>330±6.46**</td>
<td>334.7±6.59**</td>
<td>342.0±8.26***</td>
</tr>
<tr>
<td>Imipramine 10.5 mg/kg</td>
<td></td>
<td>79.17±0.54</td>
<td>276.8±10.41***</td>
<td>291.0±10.94***</td>
<td>298.0±11.6***</td>
<td>301.5±12.21***</td>
<td>307.7±11.88***</td>
</tr>
<tr>
<td>Duloxetine 20 mg/kg</td>
<td></td>
<td>80.33±0.95</td>
<td>300.7±3.15***</td>
<td>324.7±5.96***</td>
<td>334.7±4.90***</td>
<td>345.7±5.73***</td>
<td>354±7.30***</td>
</tr>
<tr>
<td>\textit{Aloe vera} 300mg/kg</td>
<td></td>
<td>79.50±0.61</td>
<td>285.0±9.73***</td>
<td>293.2±9.57***</td>
<td>164.5±2.91***</td>
<td>151.0±1.67***</td>
<td>134.0±1.46***</td>
</tr>
<tr>
<td>\textit{Aloe vera} 450mg/kg</td>
<td></td>
<td>79.67±0.61</td>
<td>290.5±7.92***</td>
<td>306.5±5.82***</td>
<td>172.2±2.78***</td>
<td>148.3±5.12***</td>
<td>126.2±3.67***</td>
</tr>
</tbody>
</table>

All the values are mean ± SEM, n=6, evaluated by two way ANOVA followed by Bonferroni post test, where *** is $P<0.001$ when compared to normal control group, and $a= P<0.001$ when compared to diabetic control group.
Fig. 1. Effects of *Aloe vera* extract in grip strength

*All the values are mean ± SEM, n=6, evaluated by two way ANOVA followed by Bonferroni post test, where *** is P<0.001 when compared to normal control group, and a= P<0.001 when compared to diabetic control group.*

![Grip strength graph](image1)

Fig. 2. Effects of *Aloe vera* extract in eddy’s hot plate test

*All the values are mean ± SEM, n=6, evaluated by two way ANOVA followed by Bonferroni post test, where *** is P<0.001 when compared to normal control group, and a= P<0.001 when compared to diabetic control group.*

![Paw withdrawal latency graph](image2)
Fig. 3. Effects of Aloe vera extract in Formalin induced pain

All the values are mean ± SEM, n=6, evaluated by two way ANOVA followed by Bonferroni post test, where *** is P<0.001 when compared to normal control group, and a= P<0.001 when compared to diabetic control group.

Fig. 4. Effects of Aloe vera extracts in cold water immersion test

All the values are mean ± SEM, n=6, evaluated by two way ANOVA followed by Bonferroni post test, where *** is P<0.001 when compared to normal control group, and a= P<0.001 when compared to diabetic control group.
Fig. 5. Effects of Aloe vera extracts in hot water immersion test

All the values are mean ± SEM, n=6, evaluated by two way ANOVA followed by Bonferroni post test, where *** is P<0.001 when compared to normal control group, and a= P<0.001 when compared to diabetic control group.

Fig. 6. Effect of Aloe vera extract on Paw withdrawal latency in Pin Prick test

All the values are mean ± SEM, n=6, evaluated by two way ANOVA followed by Bonferroni post test, where *** is P<0.001 when compared to normal control group, and a= P<0.001 when compared to diabetic control group.
Table 3. Effects of Aloe vera extract on serum levels of total cholesterol, triglyceride, HDL and LDL

<table>
<thead>
<tr>
<th>Groups</th>
<th>Total Cholesterol (µg/dl)</th>
<th>Triglycerides (mg/dl)</th>
<th>High density lipoproteins (mg/dl)</th>
<th>Low density lipoproteins (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>61.17±1.078</td>
<td>38.83±0.3073</td>
<td>40.33±0.6667</td>
<td>46.0±0.926</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>166.2±3.156***</td>
<td>165.8±4.408</td>
<td>24.0±0.5164</td>
<td>82.17±1.302***</td>
</tr>
<tr>
<td>Imipramine 10.5 mg/kg</td>
<td>132.5±0.8851***</td>
<td>163.7±2.290**</td>
<td>29.0±0.7303***</td>
<td>77.33±1.926***</td>
</tr>
<tr>
<td>Duloxetine 20 mg/kg</td>
<td>135.5±1.204**</td>
<td>150.0±1.653***</td>
<td>30.50±1.360***</td>
<td>75.33±0.6667***</td>
</tr>
<tr>
<td>Aloe vera 300mg/kg</td>
<td>117.0±2.620***,a</td>
<td>128.5±1.057***,a</td>
<td>35.17±0.7923***,a</td>
<td>60.0±0.5774***,a</td>
</tr>
<tr>
<td>Aloe vera 450mg/kg</td>
<td>94.33±1.961***,a</td>
<td>50.50±2.094***,a</td>
<td>38.33±0.6667***,a</td>
<td>50.33±1.174***,a</td>
</tr>
</tbody>
</table>

All the values are mean ± SEM, n=6, evaluated by one way ANOVA followed by Tukeys Multiple Comparison Test, where *** is P<0.001 when compared to normal control group, and a= P<0.001 when compared to diabetic control group.

Table 4. Effects of Aloe vera extract on oxidative stress markers

<table>
<thead>
<tr>
<th>Groups</th>
<th>TBARS (ng/mg of protein)</th>
<th>SOD (U/mg of protein)</th>
<th>CATALASE (U/mg of protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Control</td>
<td>0.08957±0.001540</td>
<td>17.99±0.08648</td>
<td>1.719±0.01615</td>
</tr>
<tr>
<td>Diabetic Control</td>
<td>0.1972±0.001004***</td>
<td>7.075±0.06505***</td>
<td>0.3688±0.006419***</td>
</tr>
<tr>
<td>Imipramine 10.5mg/kg</td>
<td>0.2158±0.001816***</td>
<td>6.977±0.03211***</td>
<td>0.3688±0.009378***</td>
</tr>
<tr>
<td>Duloxetine 20mg/kg</td>
<td>0.234±0.002592***</td>
<td>6.905±0.04566***</td>
<td>0.3530±0.006518***</td>
</tr>
<tr>
<td>Aloe vera extract 300mg/kg</td>
<td>0.1599±0.001015***,a</td>
<td>9.833±0.07251***,a</td>
<td>0.9611±0.01270***,a</td>
</tr>
<tr>
<td>Aloe vera extract 450mg/kg</td>
<td>0.1365±0.00292***,a</td>
<td>13.12±0.1242***,a</td>
<td>1.342±0.1138***,a</td>
</tr>
</tbody>
</table>

All the values are mean ± SEM, n=6, evaluated by one way ANOVA followed by Tukeys Multiple Comparison Test, where *** is P<0.001 when compared to normal control group, and a= P<0.001 when compared to diabetic control group.

3.10. Histopathology of sciatic nerve

Structural changes in sciatic nerves of all the groups of experiments were examined. In diabetic control, there was multifocal loss of both large and small myelinated fibers. Small myelinated fiber loss was found to be more prominent than large diameter fiber loss. Thickened and hyalinised endoneurial vessel when compared to normal control and Aloe vera extracts (300 mg/kg and 450 mg/kg) treated groups rats.
Normal control

Diabetic control

Imipramine 10.5 mg/kg
The present study has been carried out to evaluate diabetic peripheral neuropathic effect of *Aloe vera* leaves extract (300 mg/kg and 450 mg/kg) in diabetic rat. The STZ induced diabetic rat is the most commonly employed animal model of painful diabetic neuropathy. STZ injection caused diabetes mellitus probably due to destruction of the β cells of the islets of langerhans of the pancreas; over-production of glucose and decreased utilization by the tissues is responsible for induction of diabetes. Hyperglycemia accompanied by weight loss.
were seen in adult rats treated with STZ which were stable for seven weeks, which indicates the irreversible destruction of β cells of the islets of langerhans of pancreas.

Diabetic neuropathy is a long-term complication of diabetes that develops early in the course of the disease and is observed in 60–70% of all diabetic patients. It is known that diabetic neuropathy is a nerve degenerative disease characterized by axonal degeneration, nerve fiber demyelination, and a reduction in the number of medium to large diameter nerve fibers, particularly in peripheral nerves. Diabetic neuropathic pain involves injury or alteration of the normal sensory and modulatory nervous systems to produce a set of symptoms that are often difficult to treat. Multiple changes occur in the injured neural structures & in areas which are not directly injured. The resulting pain includes spontaneous “burning” sensations, with intermittent sharp lightening like stabbing and lancinating pain. Increased sensitivity and pain may be elicited by minimal stimulation such as a light touch, or even a breeze (allodynia), or temperature changes (thermal hyperalgesia).

Diabetic neuropathy is triggered by hyperglycemia, which leads to a persistent accelerated flux of glucose through the polylol pathway. The rate limiting enzyme in this pathway is aldose reductase. The increased flux through the polylol pathway is followed by abnormal protein kinase C metabolism, oxidative stress, accelerated glycation and decreased endoneural capillary perfusion, leading eventually to nerve degeneration.

The degree and duration of hyperglycemia plays a key role in development of diabetic neuropathy and is responsible for the intensity and extent of the functional and anatomical abnormalities. Acute hyperglycemia decreases nerve function. Chronic hyperglycemia is associated with the loss of myelinated and unmyelinated fibers, wallerian degeneration and blunted nerve-fiber reproduction. Approaches to prevent or to treat neuropathy include the intensive treatment of hyperglycemia and various symptomatic treatments.[31]

In diabetic peripheral neuropathy, symptomatic treatment involves use of antidepressants and anticonvulsants. Antidepressants are effective in relieving neuropathic pain. Many clinicians prescribe antidepressants rather than anticonvulsants as first-line in neuropathic pain, either because of perceived greater chance of benefit or lower chance of adverse effects.[32] The supra-spinal and spinal serotonin (5-HT) pathways are involved in pain perception.[33] But the mode of action of antidepressants is based on its interference with reuptake of monoamines (Nor-epinephrine and 5-HT).
Induction of diabetes with STZ was associated with a characteristic loss of body weight, which is due to increased muscle wasting, and loss of tissue proteins. Diabetic rats treated with aloe vera showed an increase in body weight after fourth week as compared to the diabetic control, which may be due to its effect in controlling muscle wasting.

The hypoglycemic effect was observed with the treatment of Aloe vera in STZ induced hyperglycemic rats, with the maximum effects seen in Aloe vera 450mg/kg group, which may be due to its antioxidant property and due to active constituents reported in the both extracts. The grip strength evaluation provides a non-invasive method of assessing the functioning status of the sciatic nerve fibers during diabetic neuropathy, proper grip strength requires for coordinated functions involving motor response, sensory input and cortical integration. The occurrence and severity of diabetic peripheral neuropathy has been associated with significant decrease in muscle grip strength or neuromuscular function in diabetes mellitus. In this present study, significant improvement in muscle grip strength after 21 days treatment with Aloe vera extracts (300 and 450 mg/kg) was observed.

Formalin induced pain test has shown that administration of Imipramine blocked the tonic flinch responses in formalin test. As reported earlier for a drug of the similar treatment category, Amitriptyline (a nonselective nor-epinephrine and 5-HT reuptake inhibitor), Desipramine (a relatively selective nor-epinephrine reuptake inhibitor) blocked tonic flinch responses in the formaldehyde solution test. Aloe vera extract inhibited formalin test induced flinch responses in the acute phase as well as tonic phase. It is known that the tonic phase of the formaldehyde solution test reflects an injury induced spinal sensitization of dorsal horn neurons which is fundamental to the development of neuropathic pain.

In Eddy’s hot plate test and Tail immersion test (Hot and Cold immersion test), animals were treated with Duloxetine increased the tail withdrawal latency which indicated that the animals developed an increased pain threshold level during this treatment. Studies have provided evidence of interaction of antidepressant with opioid receptor which could account for their analgesic action. Aloe vera has previously been reported to have antidepressant activity. In our present study, Aloe vera leaves extract has significantly reduced the tail withdrawal latency in both cold and hot immersion test.

In pin prick test (Noxious mechanical hyperalgesia), animals treated with Duloxetine, Aloe vera leaves extract showed a significant increase in paw withdrawal latency compared to the
Diabetic group. This is because the high threshold fiber Aδ and C fibers would have increased threshold levels of pain due to the treatment. The activation of opioid receptors may also be a cause for this increase in pain threshold.

Diabetic oxidative stress induced free radicals are involved in vascular endothelial damage of epineural arterioles of the sciatic nerve in diabetic rats. Impaired blood flow contributes to noxious stimulus hypersensitivity and vasodilators have been demonstrated to reduce allodynia in diabetic rats. Dietary antioxidant by scavenging reactive oxygen species has improved vascular resistance in diabetic rats.

Lipid profile abnormalities are the most common complications in diabetes mellitus found in 40% of diabetes. Hyper-triglyceridemia is a common finding in patients with diabetes and in insulin resistant states which results from accumulation of very low-density lipoprotein particles, either by decreased catabolism or overproduction, or both. The increased in serum triglyceride level in STZ-induced diabetes was observed in our study may be due to lack of insulin, normally activates the enzyme lipoprotein lipase. Aloe vera has previously been reported to have hypo-lipidaemic activity. In our present study, Aloe vera leaves extract has showed a significant control in triglycerides and cholesterol levels.

Diabetic rats exhibited a significant increase in TBARS, an index of lipid peroxidation and reduction in antioxidant enzyme activity. These parameters regained to normal levels when treated with Aloe vera. Aloe vera is known to modulate the oxidative stress markers of the body. The extracts of A. vera skin, by supercritical carbon dioxide extraction and ethanol, showed stronger antioxidant activities and free radical scavenging activities. Hence, Aloe vera could play a vital role in attenuating diabetic oxidative stress induced changes in the nerve physiology and may be useful to treat symptoms of diabetic neuropathic pain.

Histopathology of sciatic nerve in the untreated diabetic rats showed derangement of myelin with disconnected layers and nerve fibre loss. Demyelination and nerve fiber loss do play a crucial role in Diabetic peripheral neuropathy progression in long-term diabetes. Aloe vera treated groups showed preserved density of myelinated fibers and minimal axonal degeneration in sciatic nerve and the vessels were not thickened indicating. Aloe vera could play a vital role in attenuating diabetic oxidative stress induced changes in the nerve physiology.
4. CONCLUSION
The significant protective role of *Aloe vera* observed in the present investigation could be the result of synergistic/potentiative action of its constituents, since they contain a diverse array of active principles which are able to target multiple mechanisms involved in the pathophysiology of diabetes. *Aloe vera* has shown significant increase in body weight and pain sensitivity. This indicates its protective role against damage to the neurons and prevents progression of diabetic neuropathy. Therefore, it can be concluded that antidepressant, anti-diabetic and antioxidiant effect of *Aloe vera* may be responsible for observed anti-nociceptive and protective role against damage to the neurons in STZ induced diabetic peripheral neuropathic rat models. Hence, it could be helpful in treating the diabetic patient having the complication of diabetic neuropathy Although Imipramine and Duloxetine showed better results, *Aloe vera* can be considered as an alternative herbal therapeutic option in the treatment of diabetic neuropathic pain.

ETHICAL APPROVAL
All authors hereby declare that the experimental protocol was approved by Institutional Animal Ethics Committee (MCP/IAEC-030/2014-15) and animals were maintained under standard conditions in the animal house approved by Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA).

REFERENCES


