ORIGINAL ARTICLE



# Anti-oxidant, anti-inflammatory and anti-cholinergic action of Adhatoda vasica Nees contributes to amelioration of diabetic encephalopathy in rats: Behavioral and biochemical evidences

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Abstract 'Diabetic encephalopathy' refers to diabetes associat-ed cognitive decline (DACD), which involves oxidative-nitrosative stress, inflammation and cholinergic dysfunction. Current study was designed to investigate the effect of Adhatoda vasica , a known anti-inflammatory, antioxidant, anti-cholinesterase and anti-hyperglycemic plant, on diabetic enceph-alopathy. Streptozotocin (STZ)-induced diabetic Wistar rats were treated with Adhatoda vasica leaves ethanolic extract (AVEE) for 6 weeks at 100, 200 and 400 mg/kg/day dose. During fifth week of treatment, learning and memory was investigated in single Y-maze and passive avoidance test. At the end of the study biochemical parameters like acetylcholinesterase (AchE) activity, nitrite levels, tumor necrosis factor-alpha (TNF-α) and oxidative stress was measured from cerebral cortex and hippocampus regions of brain. AchE activity was found increased by 70 % in the cerebral cortex of diabetic rat brain. Lipid peroxidation (LPO) levels were increased by 100 % and 94 % in cerebral cortex and hippocampus of diabetic rats, respectively. Non-protein thiol levels, enzymatic activities of superoxide dismutase and catalase were found decreased in cerebral cortex and hippo-campal regions of diabetic rat brain. Nitrite levels in both regions of diabetic brain were increased by 170 % and 137 % respec-tively. TNF-α, a pro-inflammatory cytokine, was found signifi-cantly increased in diabetic rats. Conversely, animal groups treated with AVEE significantly attenuated these behavioral and biochemical abnormalities. The results suggest a protective role of Adhatoda vasica Nees against diabetic encephalopathy, which may be sum of its anti-oxidant, anti-cholinesterase, anti-inflammatory and glucose lowering action.

Keywords Diabetic encephalopathy · Adhatoda vasica Nees · Oxidative-nitrosative stress · Acetylcholinesterase (AchE) · Tumor Necrosis Factor-alpha (TNF-α)

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## Introduction

Diabetes a chronic metabolic disorder characterized by hyper-glycemia, affects over 300 million people worldwide and is expected to rise further [1]. Diabetic encephalopathy is a recently identified central nervous system (CNS) related com-plication of diabetes involving structural, functional, electro-physiological and neurochemical changes in brain [2–4], be-sides other known complications [2, 3, 5–10].

Diabetic encephalopathy appears to be a multifactorial pro-cess involving oxidative stress [11–17] and inflammation [14, 17–19]. Advanced glycation end products (AGEs), insulin and/ or C-peptide deficiency, impairment of insulin-like growth factor (IGF) system and amyloid-beta peptide (Aβ) levels also contrib-utes to diabetic encephalopathy [3, 9, 14, 20, 21].

Currently there is no target specific treatment for diabetic encephalopathy. However, it is a logical approach to utilize agents having anti-oxidant, anti-inflammatory and anti-hyperglycemic property [19, 22], such as herb or plant con-stituents Curcumin [15], lycopene [16], ginsenoside Re [17], sesamol [18], catalpol [19], tocotrienol [20], Rhizoma Anemarrhenae [21], gugulipid [23], aucubin [24] and resver-atrol [25] reported to reverse diabetic encephalopathy.

Adhatoda vasica Nees is plant drug in Ayurvedic system of medicine [26]. It possesses diverse number of pharmacological activities including antioxidant and radical scavenging activity [27–29], α-glucosidase inhibitory action [30], anticholinester-ase action [31] and anti-inflammatory property [32, 33]. No

report is available on Adhatoda vasica in diabetes with special reference to learning and memory in rats. Therefore, current study was designed to assess possible effect of AVEE on oxidative-nitrosative stress, hyperglycemia and inflammation induced cognitive decline in STZ-treated diabetic rats.

## Materials and methods

### Animals

Male Wistar rats (250–280 g) of 10–12 weeks age were obtained from the animal house facility JSS College of Pharmacy, Ooty, India. They were housed under standard laboratory conditions, maintained on a 12 h light and dark cycle. All animals had free access to food and water. Experimental protocols were approved from the Institutional Animal Ethical Committee (IAEC) and were conducted according to Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines, India.

### Collection of plant and Preparations of ethanol extract

Fresh leaves of Adhatoda vasica were collected during the month of June from local forest. Plant leaves were identified by Dr. Rajan S (Field botanist, Department of Ayush, Ministry of health and family welfare, Government of India, Emerald). Sample specimen was deposited at the Department of Phytopharmacy and Phytomedicine (TIFAC CORE), JSS College of Pharmacy, Ooty.

Shade-dried leaves of Adhathoda vasica were crushed to powder. 100 g of powdered sample was soaked in 250 ml of 95 % ethanol in a separating funnel for 24 h, with intermittent shaking. The plant extract then filtered through Whatman No. 1 filter paper and was concentrated using vacuum rotary evaporator at 50 °C until all solvent removed. Extract material was freeze-dried and stored in airtight desiccator at 4 °C [34].

### Drugs

Streptozotocin was purchased from Fluka Sigma-Aldrich (Mumbai, India). Rat TNF-α ELISA kit was purchased from R&D Systems (USA). All other chemicals used for biochem-ical estimations were of analytical grade.

### Induction and assessment of diabetes

Diabetes was induced in overnight fasted animals by a single intraperitoneal injection of streptozotocin, freshly dissolved in 0.1 M citrate buffer (pH 4.5) at a dose of 55 mg/kg of body weight. Control rats received an equal volume of citrate buffer. Diabetes was confirmed after 48 h of streptozotocin injection, by estimating plasma glucose levels. The rats having plasma glucose levels more than 250 mg/dl were selected for the present study [15].

### Treatment schedule

Selected animals were randomized into six groups (n =10) and received respective treatments for 6 weeks.

Group 1 Normal rats treated with distilled water (o.d., p.o.)
Group 2 Diabetic rats treated with distilled water (o.d., p.o.)
Group 3 Diabetic rats treated with 100 mg/kg (o.d., p.o.) of AVEE
Group 4 Diabetic rats treated with 200 mg/kg (o.d., p.o.) of AVEE
Group 5 Diabetic rats treated with 400 mg/kg (o.d., p.o.) of AVEE
Group 6 Normal rats treated with 400 mg/kg (o.d., p.o.) of AVEE

Doses were selected on the basis of available acute toxicity and pharmacological data on Adhatoda vasica leaf extract [27, 35]. Body weight and plasma glucose levels were measured at onset and at the end of the experiment. After 6 weeks treatment, blood samples were collected from anesthetized animals through retro-orbital puncture to obtain serum and plasma. Animals were sacrificed and brains were rapidly removed to isolate cerebral cortex and hippocampus. All samples were immediately stored at −80 °C until processed for biochemical estimations.

### Assessment of learning and memory

#### Y-maze test

Short-term spatial memory performance was assessed by recording spontaneous alternation behavior during a single session in a Y-maze [36]. This method is based on the tendency of rodents to enter an arm of a Y-maze that was not explored in the last two choices. Each rat, naive to the maze, was placed at the end of one arm and allowed to move freely through the maze during an 8-min session. The series of arm entries was recorded visually. Arm entry was considered to be completed when the base of the animal's tail had been completely placed in the arm. Alternation was defined as successive entries into the three arms on overlapping triplet sets. The effect was calculated as percent alternation according to the following formula:

$$\text{Percentage Alteration} = \frac{\text{Number of alterations}}{\text{Total number of arm entries} - 2} \times 100\ \%$$

#### Single trial passive avoidance test

This test was done 3 days after Y-maze task [37, 38]. The apparatus consisted of an illuminated chamber connected to dark chamber by a guillotine door. Electric shocks were

delivered to the grid floor by an isolated stimulator. On the first and second days of testing, each rat was placed on the apparatus and left for 5 min to habituate to the apparatus. On the third day, an acquisition trial was performed. Rats were individually placed in the illuminated chamber. After a habituation period (2 min), the guillotine door was opened and after the rat entering the dark chamber, the door was closed and an inescap-able scrambled electric shock (40 V for 3 s once) was delivered. In this trial, the initial latency (IL) of entrance into the dark chamber was recorded and rats with IL greater than 60 s were excluded from the study. Twenty-four hours later, each rat was placed in the illuminated chamber for retention trial. The inter-val between the placement in the illuminated chamber and the entry into the dark chamber was measured as step-through latency (STL, up to a maximum of 600 s as cut-off).

### Post mitochondrial supernatant preparation

Cerebral cortex and hippocampus were rinsed with ice cold saline and homogenized in chilled phosphate buffer (pH 7.4). The homogenates were centrifuged at 800×g for 5 min at 4 °C to separate the nuclear debris. The supernatant thus obtained was centrifuged at 10,500×g for 20 min at 4 °C to get the post mitochondrial supernatant, which was used to assay acetyl-cholinesterase, LPO, reduced glutathione, catalase, superox-ide dismutase (SOD) activity and nitrite levels.

### Acetylcholinesterase activity

Cholinergic dysfunction was assessed by measuring AchE levels in cerebral cortex and hippocampus [39]. The assay mixture contained 0.05 ml of supernatant, 3 ml of 0.01 M sodium phos-phate buffer (pH 8), 0.10 ml of acetylthiocholine iodide and 0.10 ml 5,5, dithiobis (2-nitro benzoic acid) (Ellman reagent). The change in absorbance was measured at 412 nm for 5 min. Results were calculated using molar extinction coefficient of chro-mophore ($1.36\times104\ M^{-1}\ cm^{-1}$) and expressed as mean±S.E.M.

### Assessment of oxidative stress

The malondialdehyde content, a measure of lipid peroxidation was assayed in the form of Thiobarbituric acid reactive sub-stances (TBARS) by the method of Wills [40], Non-protein by the method of Jollow et al. [41], cytosolic superoxide dismutase activity by the method of Kono [42] and catalase activity was assayed by the method of Claiborne [43].

### Nitrite estimation

Nitrite was estimated in the cortex and hippocampus regions using the Greiss reagent and served as an indicator of nitric oxide (NO) production. 500 μl of Greiss reagent (1:1 solution of 1 % sulphanilamide in 5 % phosphoric acid and 0.1 % napthaylamine diamine dihydrochloric acid in water) was added to 100 μl of post mitochondrial supernatant and absorbance was measured at 546 nm [44]. Nitrite concentration was calculated using a stan-dard curve for sodium nitrite. Nitrite levels were expressed as percentage of control.

### Estimation of tumor necrosis factor-alpha

TNF-α was estimated using rat TNF-α kit (R&D Systems). It is a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) using a microtiter plate reader at 450 nm. Concentrations of TNF-α were calculated from plotted stan-dard curve. TNF-α levels were expressed as mean±S.E.M.

### Statistical analysis

Results were expressed as mean±S.E.M. The inter group variation was measured by one way analysis of variance (ANOVA) followed by Tukey's test. Statistical significance was considered at $P < 0.05$. The statistical analysis was done using the Graphpad Prism Software version 5.

## Results

### Effect of AVEE on body weight and blood glucose levels

At the end of the study, highly elevated plasma glucose levels (413±5.42 mg/dl) were observed in diabetic animals as com-pared to the control rats (106±1.96 mg/dl). There was a marked decline in the body weights of streptozotocin-treated rats as compared to control rats (Table 1). Chronic AVEE treatment has significantly ($P<0.05$) and dose dependently improved the

Table 1 Effect of AVEE on body weight and plasma glucose level

| Group | Body weight (g) | | Plasma glucose (mg/dl) | |
|---|---|---|---|---|
| | Onset of study | End of study | Onset of study | End of study |
| Control | 259±2.10 | 310±4.94 | 111±1.29 | 106±1.96 |
| Diabetic | 265±2.42 | 161±6.80[a] | 109±1.33 | 413±5.42[a] |
| Diabetic+ AVEE 1 | 264±2.71 | 193±8.10[a,b] | 105±2.53 | 340±7.12[a,b] |
| Diabetic+ AVEE 2 | 254±3.98 | 248±5.77[a,b] | 115±2.21 | 235±6.63[a,b] |
| Diabetic+ AVEE 4 | 264±2.20 | 294±5.02[b] | 111±2.14 | 154±4.02[a,b] |
| AVEE 4 | 263±3.12 | 310±5.64 | 118±2.14 | 93±3.88 |

All values are expressed as mean±SEM. a=Different from control group; b=Different from diabetic group and one another ($P<0.05$)

AVEE 1=*Adhatoda vasica* ethanol extract 100 mg/kg; AVEE 2= *Adhatoda vasica* ethanol extract 200 mg/kg; AVEE 4=*Adhatoda vasica* ethanol extract 400 mg/kg

blood glucose levels and body weight of diabetic rats. AVEE per se had no effect on body weight, while slightly decreasing trend was observed in blood glucose levels.

## Effect of AVEE on learning and memory

### Y-maze test

In Y-maze test, significant lower (P <0.05) mean percentage alteration was observed in diabetic rats (44 %) as compared to control rats (71 %). However, groups treated with AVEE at 200 and 400 mg/kg/day dose showed significant (P<0.05) improvement in percentage alternation behavior (Fig. 1a). AVEE per se had no effect on percentage alteration.

### Single trial passive avoidance test

The diabetic rats developed significant impairment in acquisition and retention in passive avoidance test. In this respect, the mean IL which is indicative of acquisition in passive

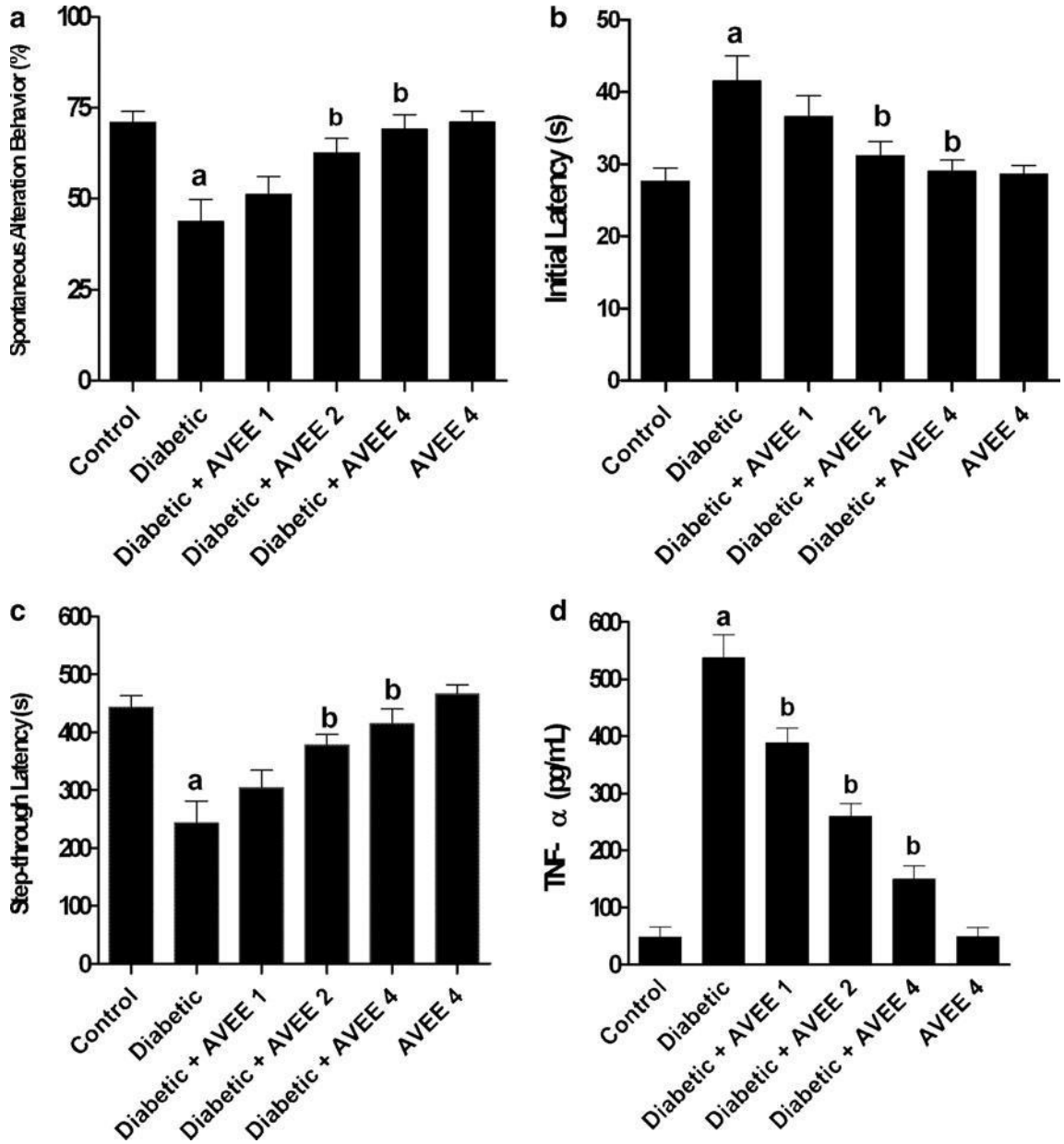


Fig. 1 a Effect of AVEE treatment on alteration behavior in a single session Y-maze. b Effect of AVEE treatment on IL in acquisition trial on passive avoidance test. c Effect of AVEE treatment on STL in retention trial on passive avoidance test. d Effect of AVEE treatment on TNF-α release in diabetic rats. Data are expressed as mean±SEM. a=Different from control group; b=Different from diabetic group and one another (P <0.05). AVEE 1=Adhatoda vasica ethanol extract 100 mg/kg; AVEE 2= Adhatoda vasica ethanol extract 200 mg/kg; AVEE 4=Adhatoda vasica ethanol extract 400 mg/kg

avoidance test showed a significant (P <0.05) increase in diabetic group (41 s) as compared to control group (27 s). However, 5 weeks AVEE treatment at 200 and 400 mg/kg/day dose significantly (P <0.05) decreased the IL of diabetic rats (Fig. 1b). AVEE per se had no effect on IL.

Retention of single trial passive avoidance training was compared in control, diabetic and AVEE treated rats (Fig. 1c). Regarding STL which indicates retention, there was a significant reduction (P <0.05) in diabetic group (250 s) as compared to control group (450 s). Chronic AVEE treatment (200 and 400 mg/kg/day) significantly (P<0.05) attenuated the STL of diabetic rats while AVEE per se had no effect on STL.

### Effect of AVEE on acetylcholinesterase activity

No significant change was observed in hippocampal AchE activity among all groups (Fig. 2a). While in diabetic group AchE activity was found increased by almost 70 % in cerebral cortex. Six weeks AVEE treatment at 200 and 400 mg/kg/day significantly (P<0.05) reversed the elevated AchE activity in the cerebral cortex. Moreover, AVEE per se had no effect on AchE activity in both regions of brain.

### Effect of AVEE on nitrosative stress

Nitrite levels were highly elevated in hippocampus (136 %) and cerebral cortex (165 %) of diabetic animals (Fig. 2b). Chronic AVEE treatment was able to alleviate increased nitrite level significantly (P<0.05) in hippocampus and cerebral cor-tex, while AVEE per se had no effect on brain nitrite level.

### Effect of AVEE on lipid peroxidation

Thiobarbituric acid reactive substance levels were increased significantly (P <0.05) in cerebral cortex (100 %) and hippocampus (94 %) of diabetic rats as compared to control group (Table 2). Chronic treatment with AVEE produced a significant (P <0.05) and dose dependent reduction in thiobarbituric acid reactive substance levels in cerebral cortex and hippocampus. AVEE per se did not show significant change in thiobarbituric acid reactive substances.

### Effect of AVEE on antioxidant profile

The reduced glutathione levels, enzyme activity of superoxide dismutase and catalase were significantly (P<0.05) decreased in the cerebral cortex and hippocampus of diabetic rats as compared to control group (Table 2). This reduction was significantly (P<0.05) and dose dependently improved by treatment with AVEE (100, 200 and 400 mg/kg/day) in both regions of brain. Moreover, AVEE per se did not alter the endogenous anti-oxidant profile in normal animals.

### Effect of AVEE on tumor necrosis factor-alpha

Serum TNF-α levels were found highly elevated (537.7±40) in diabetic rats as compared to control (48.17±19). Diabetic animals treated with AVEE at 100, 200 and 400 mg/kg/day dose, resulted in significant and dose dependent improvement in serum TNF-α level (Fig. 1d). However, AVEE per se had no effect on serum TNF-α level.

## Discussion

Alterations on insulin or glucose homeostasis, oxidative stress, formation of AGEs, C-peptide deficiency and increased

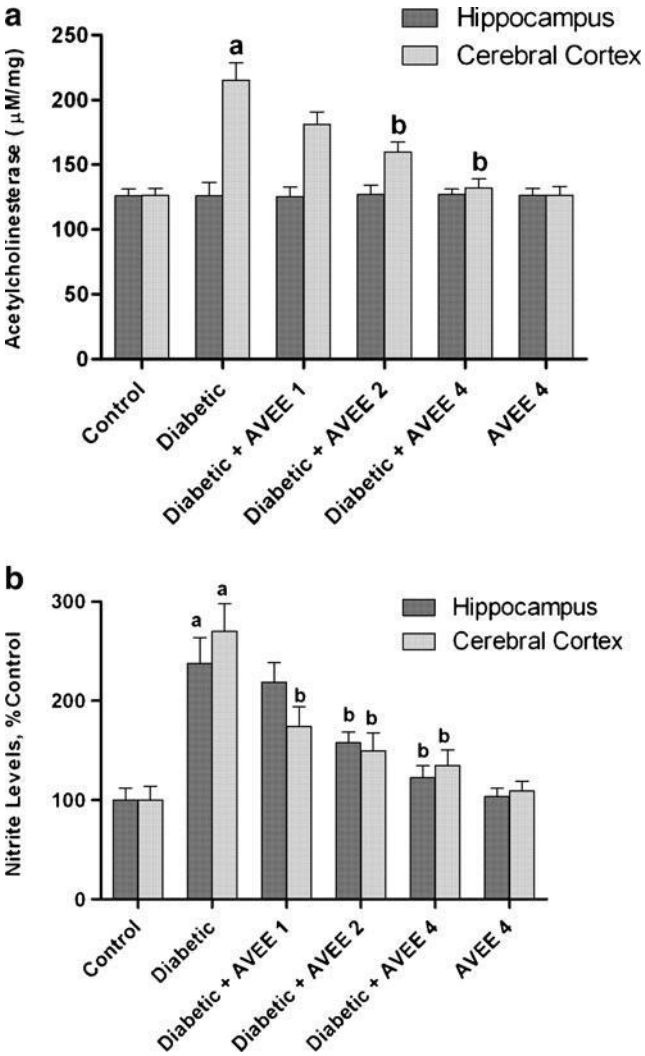


Fig. 2 a Effect of chronic AVEE treatment on acetylcholinesterase activity in hippocampus and cerebral cortex. b Effect of AVEE treatment on nitrite levels in cerebral cortex and hippocampus of diabetic rats. Data expressed as percentage of control. a=Different from control group; b= Different from diabetic group (P<0.05). AVEE 1=Adhatoda vasica ethanol extract 100 mg/kg; AVEE 2=Adhatoda vasica ethanol extract 200 mg/kg; AVEE 4=Adhatoda vasica ethanol extract 400 mg/kg

Table 2 Effect on AVEE treatment on lipid peroxidation, reduced glutathione, SOD and Catalase levels

| Group | | LPO (nmol/mg protein) | Non-protein thiols (mol) | SOD (units/mg protein) | Catalase (k/min$^{-1}$) |
|---|---|---|---|---|---|
| Control | Cerebral Cortex | 0.97±0.05 | 30.26±2.51 | 7.44±0.20 | 3.85±0.06 |
| | Hippocampus | 0.99±0.02 | 29.33±1.98 | 7.41±0.54 | 3.52±0.15 |
| Diabetic | Cerebral Cortex | 1.94±0.06 [a] | 13.68±1.66 [a] | 4.01±0.22 [a] | 0.90±0.10 [a] |
| | Hippocampus | 1.93±0.03 [a] | 14.20±2.23 [a] | 3.82±0.29 [a] | 0.97±0.04 [a] |
| Diabetic+AVEE 1 | Cerebral Cortex | 1.63±0.05 [b, c] | 22.33±1.65 [b] | 4.98±0.21 [b, c] | 1.45±0.13 [b, c] |
| | Hippocampus | 1.75±0.03 [b, d] | 21.98±1.25 [b] | 5.92±0.68 [b] | 1.48±0.07 [b, d] |
| Diabetic+AVEE 2 | Cerebral Cortex | 1.33±0.05 [b, c] | 26.12±1.44 [b] | 5.97±0.27 [b, c] | 2.30±0.16 [b, c] |
| | Hippocampus | 1.32±0.03 [b, d] | 25.18±1.24 [b] | 6.65±0.52 [b] | 2.10±0.02 [b, d] |
| Diabetic+AVEE 4 | Cerebral Cortex | 1.05±0.08 [b, c] | 28.25±1.30 [b] | 6.90±1.19 [b, c] | 3.14±0.15 [b, c] |
| | Hippocampus | 1.03±0.03 [b, d] | 27.33±1.16 [b] | 7.14±1.09 [b] | 3.37±0.07 [b, d] |
| AVEE 4 | Cerebral Cortex | 0.96±0.04 | 30.68±1.05 | 7.17±0.03 | 3.83±0.02 |
| | Hippocampus | 0.95±0.03 | 31.51±0.92 | 7.57±0.22 | 3.64±0.09 |

All values are expressed as mean±SEM. a=different from control; b=different from diabetic; c, d=different from one another ($P<0.05$)

AVEE 1=Adhatoda vasica ethanol extract 100 mg/kg; AVEE 2=Adhatoda vasica ethanol extract 200 mg/kg; AVEE 4=Adhatoda vasica ethanol extract 400 mg/kg

Aβ levels in diabetic brain are accelerating factors in development of cognitive decline in diabetic brain [9, 45–48].

Increased intracellular glucose oxidation leads to increase in reactive oxygen species (ROS) production [49, 50]. Overproduction of ROS leads to increase in oxidative stress and neuronal damage by promoting protein oxidation, DNA damage and peroxidation of membrane lipids [51, 52]. This glucose driven oxidative stress in neurons adversely affects the cognition and behavior [11, 53]. Oxidative stress caused by diabetes triggers inflammatory processes [14], well known inhibitors of neurogenesis. Considering these facts, it can be possible to treat diabetic encephalopathy with single or combination of anti-oxidants like melatonin, vitamin E, berberine, vitamin C, chromium picolinate, tocotrienol, N-acetylcysteine [20, 38, 53–55].

In present study, we found, increased lipid peroxidation levels and reduction in glutathione, superoxide dismutase and catalase activity in hippocampus and cerebral cortex of diabetic animals. Increased oxidative stress in diabetic brain is probably responsible for behavioral alterations observed in Y-maze and passive avoidance test. Six weeks treatment of AVEE mollified the levels of lipid peroxides, glutathione, superoxide dismutase and catalase in hippocampus and cerebral cortex in rats. Adhatoda vasica leaf has excellent anti-oxidant and radical-scavenging activity [27–29, 33]. It is a natural source of vitamin C, a known anti-oxidant [27]. Phytochemical screening showed abundant presence of alkaloids, flavanoids and phenol in AVEE [data not shown], which have beneficial effects related to anti-oxidant activity; particularly due to their ability to scavenge free radicals, donate hydrogen atoms or electrons, and chelate metal cations [56]. In present study, ameliorative affect of AVEE on oxidative stress in diabetic brain, is may be due to presence of vitamin C, phenol, flavanoid, vasicine, vasicinone.

Cholinergic neurotransmission plays role in learning and memory. AchE is the enzyme responsible for degradation of acetylcholine and terminating its physiological action; therefore cholinergic dysfunction is characterized by increased AchE activity [55]. Like other reported studies, we also observed significant rise in AchE activity in diabetic cerebral cortex which increase severity of diabetic encephalopathy [15, 16, 18, 57]. Vasicinone obtained from Adhatoda vasica is reported for its anticholinesterase activity [31]. It appears that AVEE may modulate of cholinergic neurotransmission in diabetic brain by exerting vasicinone mediated direct inhibitory action on AchE.

Moreover, down regulation of low-density lipoproteins receptor related protein 1 (LRP1) at blood–brain-barrier is responsible for Aβ accumulation, which accelerate cognitive decline in diabetic brain [21, 45, 46]. Increased Aβ is respon-sible for overproduction of NO due to increased expression of inducible nitric oxide synthase [45]. Physiologically, NO has been involved in neural signaling and synaptic plasticity, regulation of autonomic and osmotic functions, learning and memory. Over production of NO may result in formation of highly reactive peroxynitrite by reacting with free radicals. Highly reactive peroxynitrite is responsible for neuronal cell damage, nitration of synaptic proteins, cholinergic dysfunction and altered signal transduction pathways of cellular regulation [58–60]. It is known that NO plays a role in up-regulation of glucose transporters in neurons which might be detrimental where increased intracellular glucose leads to an oversupply of electrons in the mitochondrial transfer chain, resulting in mitochondrial membrane hyperpolarization and a further in-crease in free-radical production [61, 62]. Current study shows attenuation of nitrosative stress by AVEE in diabetic rats, which may be responsible for improved learning and memory in Y-maze and single passive avoidance test.

AGEs are components formed from non-enzymatic glycation of proteins and that are increased in diabetes through the polyol pathway. Their binding to respective receptors alters protein structure, functions and promotes oxidative stress [19]. The AGE-receptor ligation is also responsible for activation of transcription factor NF-κB, which leads to pro-inflammatory gene expressions and production of inflammatory cytokines like TNF-α, IL-1β, IL-2 and IL-6 [20, 63]. Increased TNF-α promotes inflammation mediated cognitive decline in diabetes by increasing microvascular permeability, hypercoagulability and nerve damage [64–66]. Vasicine is a quinazoline alkaloidal component of Adhatoda vasica and has anti-inflammatory property [33]. In present study, TNF-α inhibition is may be part of anti-inflammatory action of vasicine.

In conclusion, the results from present study demonstrated an impaired learning and memory coupled with increase in AChE, lipid peroxidation and decrease in antioxidant enzyme activity in diabetic rat brain. Chronic AVEE treatment alleviate behavioral and biochemical alterations in diabetic rats. This ameliorating effect of AVEE on diabetic encephalopathy is may be sum of its antioxidant, anti-inflammatory, anti-cholinesterase and glucose lowering activity.

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Conflicts of interest None

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