Original Article
DOI: 10.4274/tjps.galenos.2020.04379

Protective effect of *Dalbergia sissoo* extract against amyloid-β (1-42) induced memory impairment, oxidative stress and neuroinflammation in rats

Protective effect of *D. sissoo* against amyloid-β induced neurotoxicity

Shikha Raheja[1], Amit Girdhar[1], Anjoo Kamboj[2], Viney Lather[3], Deepti Pandita[4*]
[1]IKG Punjab Technical University, Jalandhar, Kapurthala-144603, Punjab, India; Jan Nayak Ch. Devi Lal Memorial College of Pharmacy, Sirsa-125055 Haryana, India
[2]Chandigarh College of Pharmacy, Landran, Mohali-140307, Punjab, India
[3]Amity Institute of Pharmacy, Amity University, Sector-125 Noida- 201313, India
[4]Amity Institute of Molecular Medicine & Stem Cell Research, Amity University, Sector-125, Noida- 201313, India

Abstract
Objectives: *Dalbergia sissoo* (*D. sissoo*), a common medicinal plant for gastric and skin problems also has brain revitalizing effect as reported in ayurvedic literature but its neuroprotective effect has not been reported so far in amyloid β (Aβ) 1-42 model of Alzheimer disease (AD). The current study describes the protective effect of ethanolic extract of *D. sissoo* leaves (EEDS) against Aβ (1-42) induced cognitive deficit, oxidative stress and neuroinflammation in rats.
Materials and Methods: EEDS (300 mg/kg and 500 mg/kg) was administered orally in rats for two weeks prior to intracerebroventricular (i.c.v.) Aβ (1-42) administration. The neuroprotective effect of EEDS was assessed by evaluating behavioural, biochemical and neuroinflammatory parameters in rat hippocampus. Memory function was assessed in Morris water maze task two weeks after Aβ (1-42) administration. After three weeks of surgery, all biochemical parameters were evaluated and histopathological examination of tissues was carried out.
Results: EEDS improved the cognitive ability of Aβ (1-42)-administered rats in Morris water maze (MWM) task. It reduced oxidative stress by significantly declining the levels of nitrite and malondialdehyde (MDA) while elevating the levels of catalase and reduced glutathione (GSH) in rat brain. Further, EEDS mitigated neuroinflammation in rats by decreasing the concentrations of neuroinflammatory markers in a dose dependent manner.
Conclusion: The study reveals that *D. sissoo* leaf extract has a beneficial role in alleviating cognitive deficits in AD by modulating cholinergic function, oxidative stress and neuroinflammation.
Key words: Alzheimer's disease, *Dalbergia sissoo*, cognitive deficit, oxidative stress, amyloid β (1-42)

deeptipandita@yahoo.co.uk
+91 7015976090
0000-0002-3644-7045
09.10.2019

INTRODUCTION

Dalbergia sissoo, Roxb. ex DC. (*D.sissoo*) commonly known by the names Indian rosewood; Sheesham; and Shinshapa (family fabaceae), is a perennial tree belonging to the Indian subcontinent and Southern Iran. The leaves and bark of *D.sissoo* have been extensively used in traditional medicine for various gastric and skin problems including dysentry, dyspepsia and leucoderma.[1,2] The juice of *D.sissoo* leaves has been used in senility and as a nervine tonic to revitalize the brain function.[3] *D. sissoo* is known to possess diverse phytoconstituents including biochanin A, tectorigenin, mesoinisitol, isocaviumin, tectorigenin, dalbergin, dalberginone, tannins, fixed oils and essential oils.[4] A number of studies have reported anti-inflammatory, anti-oxidant, anti-spermatogenic, memory enhancing, cardioprotective and gastroprotective activities of this plant.[5-10] Recently, extract of *D. sissoo* leaves has shown neuroprotective effect in 3-nitropropionic acid induced neurodegeneration in rats.[11] In addition, biochanin A, major isoflavone glycoside present in *D. sissoo* leaves has shown to exhibit anti-oxidant and neuroprotective effect in different studies.[12,13] However, no study has been reported so far to evaluate the beneficial effect of *D. sissoo* against amyloid-β (Aβ) 1-42 induced memory impairment, oxidative stress and neuroinflammation in rats.

Alzheimer's disease (AD) is a major neurodegenerative disorder and a form of dementia which has afflicted around 50 million people worldwide and is now being recognized as a public health challenge globally.[14] AD is characterized by gradual decline of memory function and impaired ability to learn, think, communicate and make judgements. During the course of disease, there is cholinergic neuronal degeneration and dysfunction primarily in cerebral cortex, hippocampus and amygdala which ultimately results in memory impairment. It is evident that neuropathological changes in AD brain are manifested by deposition of Aβ senile plaques and neurofibrillary tangles.[15] Accumulation of pathogenic Aβ peptide in the aggregated form (dimmers or oligomers) in specific regions of brain cause synaptic loss and breaking of neuronal circuits which results in neuronal dysfunctioning.[16] Administration of aggregated Aβ (i.c.v.) in rodents mimics the pathological features of AD and induces amnesic effects resulting in memory impairment.[17]

It is widely believed now that increased oxidative stress is regarded as one of the crucial factors in progression of AD.[18] Recent evidence indicates that Aβ induced neurotoxicity may be associated with increased oxidative stress.[19] It was confirmed by studies in which antioxidant treatment improved learning abilities in Aβ-treated rats and delayed the clinical progression of disease.[20-22] Further, increased oxidative stress has also been linked with neuronal inflammation and apoptosis.[23] Implication of oxidative stress and neuroinflammation in pathogenesis of various neurodegenerative diseases including AD has made treatment with agents having anti-oxidant and anti-inflammatory potential, a promising approach for treatment of AD.

Various approaches for treatment of this debilitating disorder have been investigated in recent years including cholinesterase inhibitors, gene therapy, immunotherapy, modulation of Aβ and tau deposition and modulation of inflammation and oxidative damage.[24] Symptomatic treatment with galantamine, donepezil and rivastigmine (cholinesterase inhibitors) and memantine (NMDA receptor antagonist) is currently approved therapy for AD. However none of these target the underlying disease mechanism which necessitates the search for new drugs that can prevent or delay the disease progression. Currently, plant based medicines are attractive targets for treatment of diseases in which the major underlying cause is oxidative

stress. Therefore, current study was attempted to explore the protective role of *D. sissoo* against Aβ-(1-42) induced oxidative stress and cognitive dysfunction in rats.

MATERIALS AND METHODS

Drugs and chemicals

Aβ (1-42), donepezil and commercial kits were obtained from Sigma-Aldrich, USA. Biochanin A was purchased from Clearsynth Labs Ltd., Mumbai. All the reagents used in the experimental work were of analytical grade.

Plant extraction

The green leaves of Indian rosewood were obtained from medicinal garden of JCDM College of Pharmacy, Sirsa, India. A specimen voucher was submitted at Raw Materials Herbarium and Museum at National Institute of Science Communication and Information Resources (NISCAIR), New Delhi, India and was authenticated (Ref no.- NISCAIR/RHMD/Consult/2017/3104-53-2). After proper washing and drying under shade for one week, the leaves were grinded and defatted with hexane. Powdered leaves were then macerated with ethanol in a beaker and kept at 25±2°C for 5- 6 days. The extract was filtered and then evaporated under reduced pressure to completely remove the solvent. The final 8 g of dried extract was obtained and stored at 2 - 4°C in a dark area till further studies.

Standardisation of extract

Standardization of EEDS was carried out using high performance liquid chromatography (HPLC) with biochanin A as a standard compound as it is the major constituent present in the leaves of *D. sissoo*.[4,25] HPLC instrument (Shimadzu) was supplied with SPD-10AVP UV-visible detector, reciprocating LC-10 ATVP pumps, phenomenex C-18 column (250 mm × 4.6 mm, 5 µm) and a rheodyne injector. The data was acquired and processed using LC-solution software, version 6.42. The solvents acetonitrile and ammonium acetate (10 Mm) were used as mobile phase which was fluxed at a flow rate of 1ml/min. The chromatogram was recorded at 200 nm and volume injected was 20µl. The standard calibration curve of biochanin A was prepared using five different concentrations from 1 µg/ml to 5 µg/ml. Stock solutions of standard and sample were prepared in methanol (10 mg/ml), filtered by 0.22 µm filter paper and sonicated for 10 min.

Animals

Male rats of Wistar strain (350-400 g) were acquired from Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, Haryana and were housed in the animal house maintained with standard laboratory conditions (temp: 24 ± 2°c, relative humidity: 60-70 % and a natural 12 h light and 12 h dark cycle) and provided water and food as required. The research protocol was accorded by Institutional Animal Ethics Committee, JCDM College of Pharmacy. Ethical guidelines were followed during experimentation on rats.

Grouping of animals and drug treatment

Wistar rats were assigned to 5 groups at random with eight animals in each group. Group I: sham control group; rats were administered vehicle (4µl) i.c.v., Group II: Aβ group; rats were injected Aβ (1-42) (4µl) i.c.v., Group III: Aβ + EEDS (300 mg/kg) group; Aβ model rats were pre-treated with EEDS (300 mg/kg) for two weeks, Group IV: Aβ + EEDS (500 mg/kg) group; Aβ model rats were pre-treated with EEDS (500 mg/kg) for two weeks, Group V: standard group; Aβ model rats were treated with standard anti-alzheimer drug donepezil (5mg/kg). Doses of *D. sissoo* extract were selected based on previous reports.[5,8] The drug treatment schedule is represented in Fig. 1.

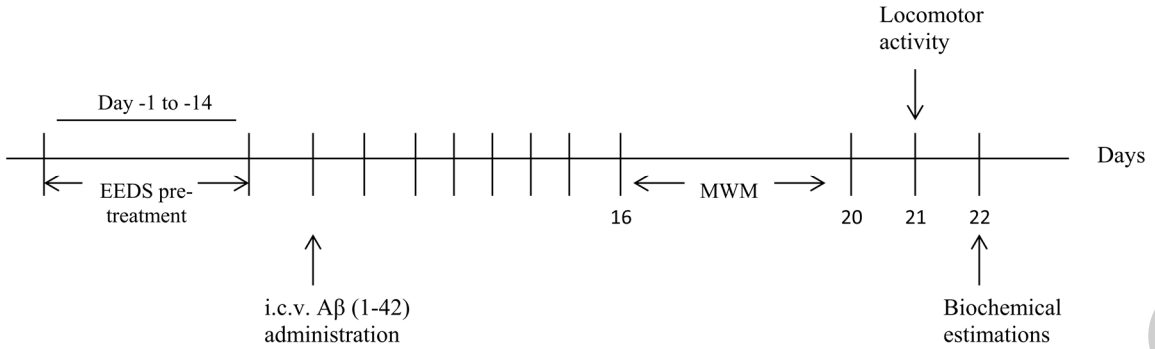

Figure 1. Schematic representation of drug treatment schedule.

Administration of i.c.v. Aβ (1-42)

Stereotaxic surgery was performed in rats for i.c.v. administration of Aβ (1-42) to induce AD. Anaesthesia was induced in rats by intraperitoneal injection of ketamine (100 mg/kg). The scalp was shaved and positioned in stereotaxic frame and dissected from midline to expose the skull. Burr holes were drilled 2 mm posterior to bregma, 1.5 mm lateral to the midline on either side of the skull and 1.0 mm below the cortical surface for entry of cannula into hippocampus. Aβ (1-42) was dissolved in saline (1µg/µl) and 4 µl was infused slowly in the hippocampal region through holes using Hamilton syringe. The sham group animals were treated with same surgical procedure and received the same volume (4 µl) of vehicle after surgery. As a post surgical care, animals were administered gentamycin (5mg/kg, i.p.) to prevent infection.

Assessment of behavioural parameters

Morris water maze (MWM) task

Two weeks after induction of AD in rats, memory acquisition and retention was evaluated by MWM apparatus that was equipped with a water tank of diameter 130 cm and height 60 cm. The water (23±1°c) was filled in the tank up to a height of 35 cm and tempera paint was added to make it opaque. The tank was partitioned into 4 quadrants of same size and a platform was positioned in one of the quadrants for escape of animals such that its surface was 2 cm below the water surface. Animals were given training sessions for 4 days in succession from day 16 to 19 (session 1 to 4) with four trials per day. During training sessions, animals were successively left in pool for 60 s to site the platform's location and once it was located, rats were guided to sit there for 30 s before the next trial. Escape latency was documented after every trial. On day 20, probe trial was conducted in which the platform was taken off from the pool and animals were randomly released into the tank and time spent by the animal in target quadrant was recorded.

Assessment of locomotor activity

On day 21, motor activity of the rats was measured by using digital actophotometer (IMCORP, India). Each animal was placed in actophotometer equipped with infrared light sensitive photo cells for 10 minutes and number of motor counts displayed on digital counter were recorded as measure of motor performance.

Assessment of biochemical parameters

Sample preparation and measurement of total protein

Wistar rats were sacrificed by decapitation for biochemical estimations on day 22 following surgery. The brains were cleansed with ice-cold saline and dissected to isolate hippocampus. The dissected hippocampal tissue was then homogenized with ice-cold phosphate buffer (0.1 mM/L, *p*H=7.4) and ultracentrifuged (Remi cold centrifuge) at 3000 rpm for 15 min. Clear supernatant obtained was stored at -80°c and used for the further biochemical assays. The

amount of protein in sample was determined by Lowry's method.[26] In this estimation bovine serum albumin was taken as a standard. Total protein content was represented in mg.

Measurement of oxidative stress markers

Assay of GSH was performed by following the method previously reported by Ellman (1959).[27] The concentration of GSH was represented as µmol/mg protein. MDA was assessed in tissue samples by the procedure previously reported by Ohkawa et al., 1979.[28] The standard curve was prepared and concentration of MDA was estimated and expressed as nmol/mg protein. In the supernatant collected, nitrite content was measured by using Griess reagent in colorimetric assay.[29] The standard curve of sodium nitrite was plotted and amount of nitrite was determined and presented as µmol/ mg protein. Further, catalase activity was measured by previously reported Aebi's method and absorbance was recorded at 240 nm.[30] The enzymatic activity was represented as nmoles of H_2O_2 consumed/min/mg of protein.

Measurement of acetylcholinestrase (AChE)

The AChE activity was estimated by the procedure previously demonstrated by Ellman et al., 1961 and was represented as nmol/mg protein.[31]

Measurement of neuroinflammatory markers

The concentrations of TNF-α and IL-6 in samples were measured by commercial Quantikine rat assay kits of TNF-α and IL-6 (Becton Dickinson Biosciences, India Pvt. Ltd.)

Histology of brain tissue

The brains of animals were removed and stored in 10 % formalin solution. Before histopathological examination, brains were cut into thin sections. The sections were dehydrated and embedded in paraffin blocks. Further, blocks were cut using microtome into 5-6 µm thin slices which were stained with hematoxylin-eosin.

Statistical analysis

All data was analysed by using Graph Pad Prism software (San Diego, CA, USA). One-way ANOVA was applied for analysing the data of biochemical measurements. Tukey's post hoc test was applied for multiple comparisons among the groups. Two-way ANOVA was applied for evaluating the data of escape latency. The results were represented as mean ± S.D. The value of $P < 0.05$ was considered significant.

RESULTS

Quantity of biochanin A in EEDS

HPLC analysis revealed that the content of biochanin A, the major constituent in EEDS, was 63.262 µg/mg of dried extract. Peaks of EEDS (sample) and standard biochanin A are presented in Fig. 2a, b.

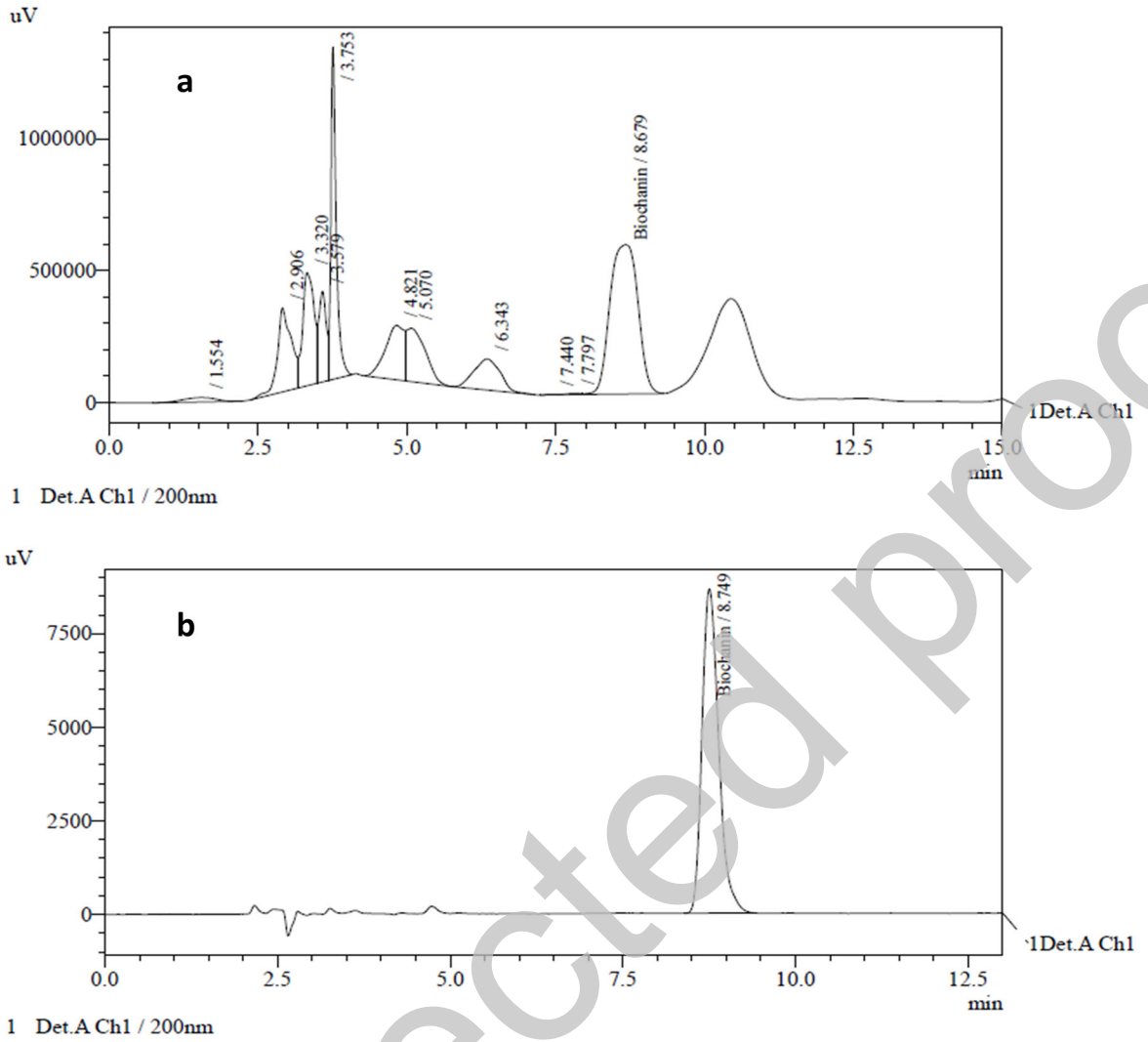

Figure 2. HPLC chromatograms of **a.** *D. sissoo* ethanolic leaf extract **b.** standard biochanin A.

EEDS reversed Aβ (1-42) induced memory dysfunction in MWM task

Two-way ANOVA showed that there was no significant decrease in escape latencies during four consecutive sessions as compared to session 1 (day 1) in Aβ (1-42) lesioned animals. However, in EEDS pre-treated (300 and 500 mg/kg) group escape latencies were significantly (P<0.001) decreased as compared to session 1 indicating improvement in cognitive performance. Further, there was no significant difference in memory retaining effect of EEDS (500 mg/kg) and the standard drug donepezil (Fig. 3a). In probe trial, Aβ (1-42) injected animals were unable to locate the platform and time for which animals remained in target quadrant was significantly less (P<0.001, F = 593.7, df = 39) compared with sham operated animals (Fig 3b).

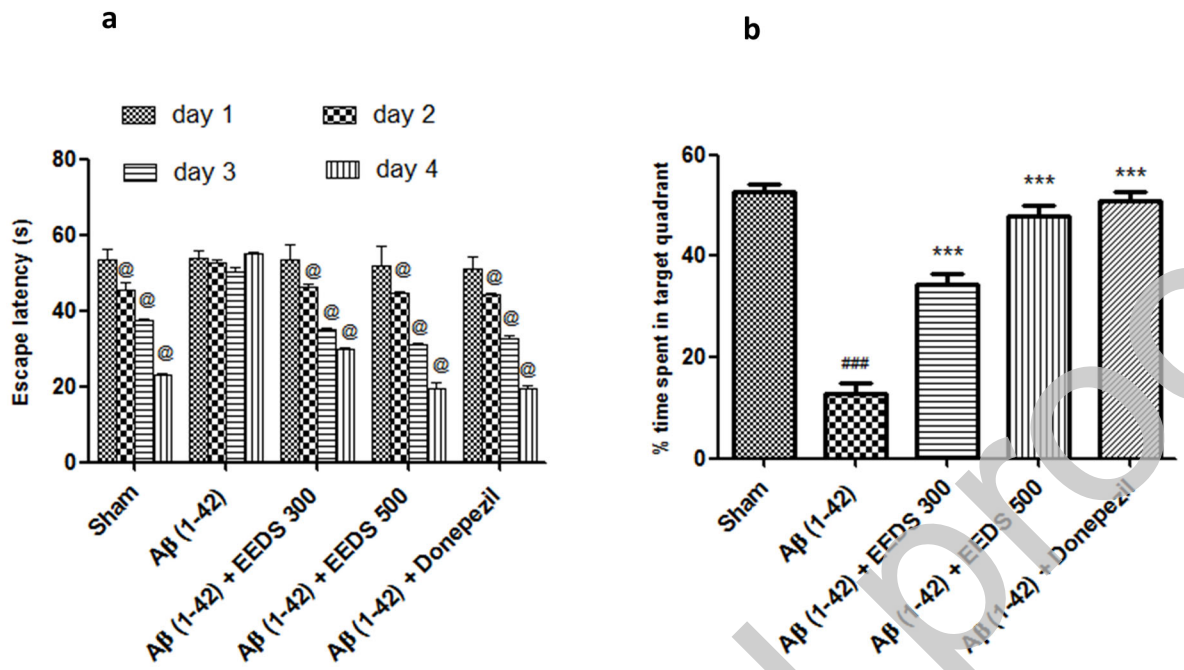

Figure 3. Results of MWM task. Effects of EEDS (300 & 500 mg/kg) on escape latency (**a)** in Aβ (1-42) injected rats. $^{@}P<0.001$ compared to day 1; % time spent in target quadrant (**b**). $^{\#\#\#}P< 0.001$ compared to sham control, $^{***}P< 0.001$ compared to Aβ (1-42) control. Data were expressed as mean ± S.D., (n=8).

Effect of EEDS on oxidative stress markers

A significant rise was observed in concentrations of MDA and nitrite while diminution of catalase and GSH concentrations in Aβ (1-42) injected rats as compared to sham operated rats ($P<0.001$). However, EEDS (300 mg/kg and 500 mg/kg) administration significantly attenuated oxidative stress by reducing the elevated MDA ($P<0.001$, $F = 231.9$, $df = 29$), nitrite levels ($P<0.001$, $F = 123.0$, $df = 29$) and restoring GSH ($P<0.001$, $F = 555.5$, $df = 29$) and catalase levels ($P<0.001$, $F = 119.1$, $df = 29$) compared to lesioned group. Further, the effect EEDS (500 mg/kg) on oxidative stress parameters was not significant compared to that of donepezil (Table 1).

Effect of EEDS on AChE activity

The AChE activity was augmented significantly in hippocampus of Aβ (1-42) injected rats compared to sham operated animals ($P<0.001$). However, EEDS administration in rats (300 and 500 mg/kg) significantly attenuated the AChE activity ($P<0.001$, $F = 366.2$, $df =29$) (Table 1).

Table 1. Effect of EEDS (300 and 500 mg/kg) on various biochemical parameters

Group	GSH (µM/mg protein)	MDA (nmol/mg protein)	Nitrite (µg/mg protein)	Catalase (nmoles of H_2O_2 consumed/mg protein)	AChE (nmol/min/mg protein)
Sham	11.15 ± 0.4148	4.042 ± 0.6871	21.33 ± 2.805	35.89 ± 3.262	20.77 ± 1.465
Aβ (1-42)	1.528 ± 0.3620$^{\#}$	17.28 ± 0.7286$^{\#}$	47.83 ± 2.927$^{\#}$	11.80 ± 1.795$^{\#}$	62.20 ± 1.819$^{\#}$

Aβ (1-42) + EEDS 300	3.917 ± 0.4286[c]	11.97 ± 0.7575[c]	42.83 ± 2.927[a]	17.24 ± 2.811[b]	57.08 ± 4.524
Aβ (1-42) + EEDS 500	10.25 ± 0.6797[c]	7.892 ± 0.4769[c]	25.67 ± 2.582 [c]	32.52 ± 1.951 [c]	30.69 ± 1.534
Aβ (1-42) + Donepezil	10.88 ± 0.3765[c]	6.733 ± 1.281[c]	22.67 ± 2.338 [c]	33.42 ± 2.067 [c]	22.42 ± 1.813

[#]$P < 0.001$ compared to sham control, [a]$P < 0.05$ compared to Aβ (1-42) control, [b]$P < 0.01$ compared to Aβ (1-42) control, [c]$P < 0.001$ compared to Aβ (1-42) control. Data were expressed as mean ± S.D.

Effect of EEDS on locomotor activity

EEDS administration (300 and 500 mg/kg), had no significant difference in locomotor activity among all the groups ($P > 0.05$) (Fig. 4).

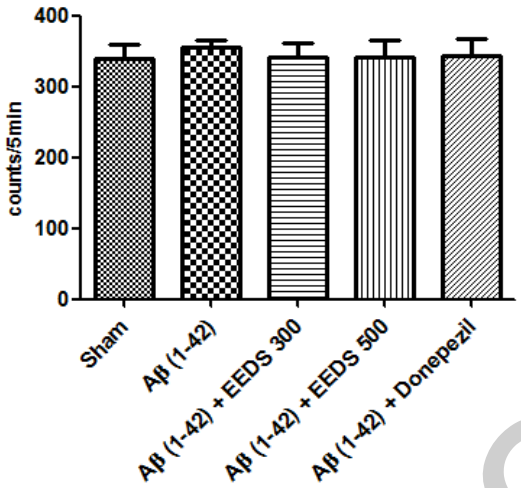

Figure 4. Effect of EEDS (300 & 500 mg/kg) on motor performance.

Effect of EEDS on neuroinflammatory markers

Aβ (1-42) infusion significantly raised TNF-α and IL-6 levels in hippocampus compared to sham operated rats ($P<0.001$). However, pre-treatment with EEDS (300 mg/kg and 500 mg/kg) significantly attenuated TNF-α ($P<0.001$, $F = 990.0$, $df = 29$) and IL-6 ($P<0.001$, $F = 480.8$, $df = 29$) levels dose dependently compared to Aβ (1-42) model group. Further, no significant difference was seen between effects of EEDS (500 mg/kg) and donepezil (5 mg/kg) (Fig. 5a, b).

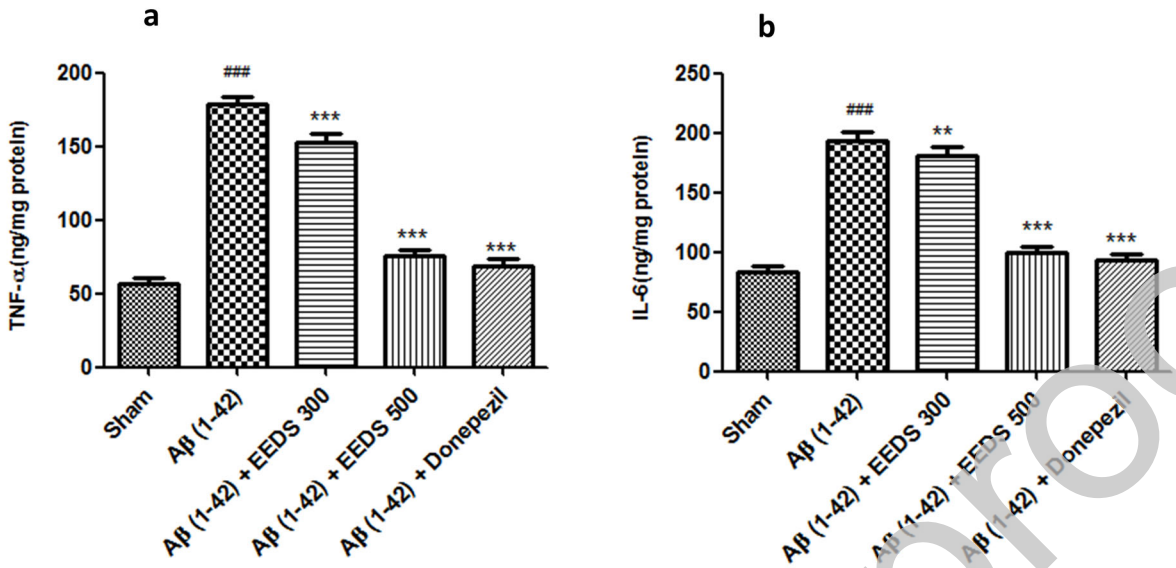

Figure 5. Effect of EEDS (300 & 500 mg/kg) on TNF-α in Aβ (1-42) model group **(a)**, IL-6 in Aβ (1-42) model group (**b**). $^{###}P< 0.001$ compared to sham control, $^{**}P< 0.01$ compared to Aβ (1-42) control, $^{***}P< 0.001$ compared to Aβ (1-42) control. Data were expressed as mean ± S.D

Histopatholgical studies

The findings of histopathological studies suggest that there was severe neuronal degeneration in Aβ (1-42) treated group. The neurons of Aβ (1-42) administered brains were reduced in size and had irregular shape; white patches were observed (Fig. 6). However, in EEDS (300 mg/kg) treated group, neurodegeneration was reduced; nuclei were clear and further in EEDS (500 mg/kg) treated group, neurons retained their original shape and structure; neuronal degeneration was not visible.

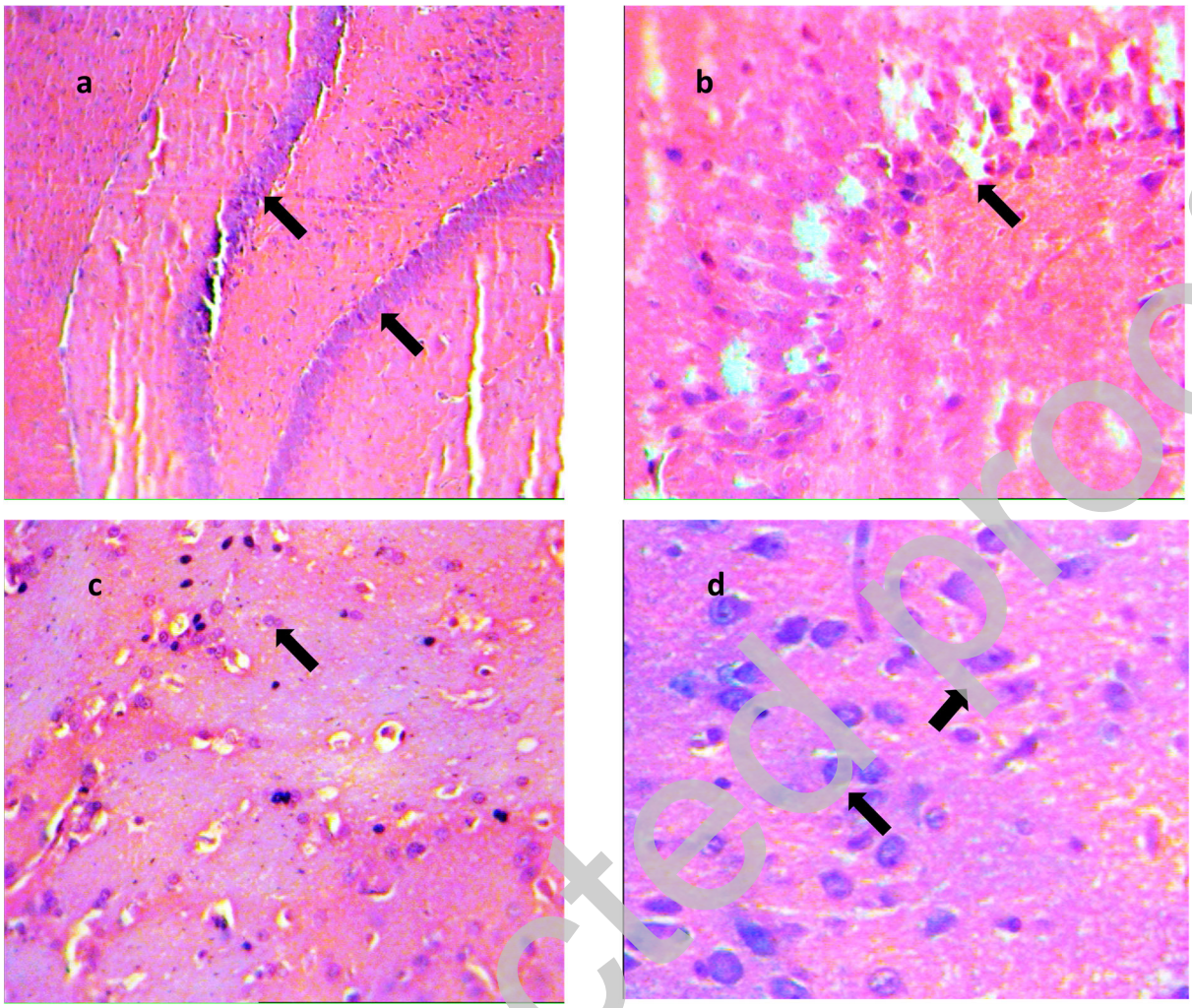

Figure 6. Microscopic sections showing effect of Aβ (1-42) and EEDS on neuronal degeneration in hippocampus of rat brain (hematoxylin-eosin staining). Sham control **(a)**, Aβ control **(b)**, Aβ + EEDS 300 mg/kg **(c)**, Aβ + EEDS 500 mg/kg **(d)**.

DISCUSSION

In AD, a progressive decline in memory function occurred which has been associated with deposition of hyperphosphorylated tau proteins and senile plaques in neuronal cells. Since Aβ has pathological roles in progression of AD, Aβ-injected rat model is regarded as the most reliable model for understanding the pathophysiology and pathogenesis of AD. It has been found in the previous studies that i.c.v. Aβ (1-42) infusion in rodents causes marked reduction in acetylcholine in hippocampus and cerebral cortex and dopamine release in striatum. Further, administration of Aβ (1-42) in rats causes impairment of learning and memory function.[32] It also aggravates production of the free radicals causing oxidative stress and neuronal cell apoptosis. These findings suggest that there is an involvement of multiple neuronal pathways in Aβ (1-42) induced neurotoxicity. In the current study, we observed decline in memory function after i.c.v. infusion of Aβ (1-42) in rat brain which is in accordance with previous findings.[22,33]

Plant-derived natural products are of great interest to researchers owing to their versatile applications. Medicinal plants and their phyto-constituents have tremendous potential for development as effective anti-alzheimer drugs. Various plant extracts and bioactive molecules with anti-oxidant property have shown protective effect against Aβ (1-42)-induced neurotoxicity.[33-35] Recently, the protective effect of *Tetraclinis articulata* essential oil has been observed in Aβ (1-42) induced oxidative stress and cognitive deficits in rats.[36]

Phytochemical studies on *D.sissoo* leaves had revealed the presence of biochanin A as a major phytoconstituent which is reported to have anti-oxidant and neuroprotective effect in different studies. Biochanin- A has shown to attenuate the production of reactive oxygen species, TNF-α and IL-1β in lipopolysaccharide-treated rats.[37] Further, biochanin-A has also shown neuroprotective effect against glutamate induced cytotoxicity in PC12 cell lines.[38]
The present study was thus planned to study the protective effect of *D.sissoo* extract against oxidative damage and memory dysfunction in rats. It was noticed that Aβ (1-42) treatment caused memory deficit in MWM task as indicated by increase in escape latencies. Further, Aβ (1-42) administration in rats also resulted in increased AChE activity indicating cholinergic deficit. Moreover, there was a significant augmentation of nitrite and MDA levels and diminution of GSH levels and catalase activity in Aβ (1-42) injected animals resulting in increased lipid peroxidation indicating the role of oxidative stress in Aβ-induced neurotoxicity. Furthermore, Aβ (1-42) infusion in hippocampus also elevated the levels of inflammatory mediators TNF- α and IL-6, suggesting that Aβ (1-42) induced neurotoxicity is associated with neuroinflammation.
However, EEDS pre-treatment decreased the latency time and improved the performance of rats exposed to Aβ in MWM task in a dose dependent manner. EEDS pre-treatment also decreased the AChE activity and improved cholinergic function in Aβ (1-42) treated rat brains. Further, EEDS administration significantly depleted the levels of MDA and nitrite while raised GSH levels and increased catalase activity in Aβ (1-42) lesioned rats. In addition, the amount of neuroinflammatory mediators; IL-6 and TNF- α was significantly reduced by EEDS pre-treatment implying its ameliorative effect on neuroinflammation.
The outcome of the present study thus revealed that *D. sissoo* attenuated oxidative stress and memory dysfunction induced by Aβ (1-42) in rats. In addition, *D. sissoo* also reduced AChE activity and neuroinflammation. Therefore, it is suggested that *D. sissoo* has neuroprotective effect against Aβ (1-42) induced neurotoxicity which may be attributed to the presence of phytoconstituent, biochanin A.

CONCLUSION

EEDS was found to ameliorate the effect of Aβ (1-42) on cognitive function and further attenuated the oxidative stress and neuroinflammation in rats. The present study findings thus conclude that *D. sissoo* may be useful in prevention or treatment of AD.

ACKNOWLEDGEMENT

The authors sincerely thank JCDM College of Pharmacy, Sirsa and IKG Punjab Technical University, Jalandhar, India for providing the support and technical assistance for completion of this research work.

CONFLICTS OF INTEREST: The authors declare no conflict of interest.

REFERENCES

1. Nadkarni KM. KM Nadkarni's Indian materia medica: with Ayurvedic, Unani-Tibbi, Siddha, allopathic, homeopathic, naturopathic & home remedies, appendices & indexes. 1 (Vol. 1). Popular Prakashan; 1996.
2. Chopra RN, Nayar SL. Glossary of Indian medicinal plants. Council of Scientific and Industrial Research; New Delhi; 1956.
3. Sharma PV. Dravyaguna vijnana (Audbhida aushada dravya) Vol-2. Chaukhamba bharati academy Varanasi; 2003. p. 806-808.
4. Al-Snafi AE. Chemical constituents and pharmacological effects of Dalbergia sissoo-A review. IOSR J Pharm 2017;7(2):59-71. doi: 10.9790/3013-0702015971

5. Hajare SW, Chandra S, Sharma J, Tandan SK, Lal J, Telang AG. Anti-inflammatory activity of Dalbergia sissoo leaves. Fitoterapia. 2001;72(2):131-9. https://doi.org/10.1016/S0367-326X(00)00272-0
6. Roy N, Laskar RA, Sk I, Kumari D, Ghosh T, Begum NA. A detailed study on the antioxidant activity of the stem bark of Dalbergia sissoo Roxb., an Indian medicinal plant. Food Chem. 2011;126(3):1115-21. https://doi.org/10.1016/j.foodchem.2010.11.143
7. Vasudeva N, Vats M. Anti-spermatogenic activity of ethanol extract of Dalbergia sissoo Roxb. stem bark. J Acupunct Meridian Stud. 2011;4(2):116-22. https://doi.org/10.1016/S2005-2901(11)60017-4
8. Sau S, Handral M. Evaluation of memory enhancing activity of leaf extract of Dalbergia sissoo in mice. Int J Pharm Sci Drug Res. 2015;7:263-9.
9. Kasa JK, Singh TU, Parida S, Addison MP, Darzi SA, Choudhury S, Kandasamy K, Singh V, Dash JR, Shanker K, Mishra SK. Assessment of Indian rosewood (Dalbergia sissoo) standardized leaf extract on isoproterenol-induced myocardial injury in rats. Cardiovasc Toxicol. 2015;15(3):250-60. https://doi.org/10.1007/s12012-014-9292-9
10. Khan MI, Khan MR. Gastroprotective potential of Dalbergia sissoo roxb. stem bark against diclofenac-induced gastric damage in rats. Osong Public Health Res Perspect. 2013;4(5):271-7. https://doi.org/10.1016/j.phrp.2013.09.006
11. Swaroop TV, Banerjee S, Handral M. Neuroprotective evaluation of leaf extract of Dalbergia sissoo in 3-nitropropionic acid induced neurotoxicity in rats. Int J Pharm Sci Drug Res. 2014;6(1):41-7.
12. Biradar SM, Joshi H, Chheda TK. Biochanin A ameliorates behavioural and neurochemical derangements in cognitive-deficit mice for the betterment of Alzheimer's disease. Hum Exp Toxicol. 2014;33(4):369-82. https://doi.org/10.1177%2F0960327113497772
13. Wang J, Wu WY, Huang H, Li WZ, Chen HQ, Yin YY. Biochanin A protects against lipopolysaccharide-induced damage of dopaminergic neurons both in vivo and in vitro via inhibition of microglial activation. Neurotox Res. 2016;30(3):486-98. https://doi.org/10.1007/s12640-016-9648-y
14. World Alzheimer Report. The state of the art of dementia research: New frontiers, published by Alzheimer's disease International, London; 2018.
15. Singh SK, Srivastav S, Yadav AK, Srikrishna S, Perry G. Overview of Alzheimer's disease and some therapeutic approaches targeting Aβ by using several synthetic and herbal compounds. Oxid Med Cell Longev. 2016. http://dx.doi.org/10.1155/2016/7361613
16. Palop JJ, Mucke L. Amyloid-β–induced neuronal dysfunction in Alzheimer's disease: from synapses toward neural networks. Nat Neurosci. 2010;13(7):812. https://dx.doi.org/10.1038%2Fnn.2583
17. Nitta A, Itoh A, Hasegawa T, Nabeshima T. β-Amyloid protein-induced Alzheimer's disease animal model. Neurosci Lett. 1994;170(1):63-6.
18. Huang WJ, Zhang XI, Chen WW. Role of oxidative stress in Alzheimer's disease. Biomed Rep. 2016;4(5):519-22. https://doi.org/10.3892/br.2016.630
19. Butterfield DA, Swomley AM, Sultana R. Amyloid β-peptide (1–42)-induced oxidative stress in Alzheimer disease: importance in disease pathogenesis and progression. Antioxid Redox Signal. 2013;19(8):823-35. https://doi.org/10.1089/ars.2012.5027
20. Bagheri M, Joghataei MT, Mohseni S, Roghani M. Genistein ameliorates learning and memory deficits in amyloid β (1–40) rat model of Alzheimer's disease. Neurobiol Learn Mem. 2011;95(3):270-6. https://doi.org/10.1016/j.nlm.2010.12.001

21. Wang N, Chen X, Geng D, Huang H, Zhou H. Ginkgo biloba leaf extract improves the cognitive abilities of rats with D-galactose induced dementia. J Biomed Res. 2013;27(1):29. https://dx.doi.org/10.7555%2FJBR.27.20120047
22. Singh A, Kumar A. Microglial inhibitory mechanism of coenzyme Q10 against Aβ (1-42) induced cognitive dysfunctions: possible behavioral, biochemical, cellular, and histopathological alterations. Front Pharmacol. 2015;6:268. https://doi.org/10.3389/fphar.2015.00268
23. Rosales-Corral S, Reiter RJ, Tan DX, Ortiz GG, Lopez-Armas G. Functional aspects of redox control during neuroinflammation. Antioxid Redox Signal. 2010;13(2):193-247. https://doi.org/10.1089/ars.2009.2629
24. Yiannopoulou KG, Papageorgiou SG. Current and future treatments for Alzheimer's disease. Ther Adv Neurol Disord. 2013;6(1):19-33.
25. Farag SF, Ahmed AS, Terashima K, Takaya Y, Niwa M. Isoflavonoid glycosides from Dalbergia sissoo. Phytochemistry 2001;57(8):1263-8. https://doi.org/10.1016/s0031-9422(01)00195-9
26. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein estimation by Lowry's method. J Biol Chem. 1951;193:265. https://doi.org/10.1177%2F175628561246167 9
27. Ellman GL. Tissue sulfhydryl groups. Arch Biochem Biophys. 1959;82(1):70-7.
28. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem. 1979;95(2):351-58. https://doi.org/10.1016/0003-2697(79)90738-3
29. Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JS, Tannenbaum SR. Analysis of nitrate, nitrite, and [15N] nitrate in biological fluids. Anal Biochem. 1982;126(1):131-38. https://doi.org/10.1016/0003-2697(82)90118-X
30. Aebi H. Catalase in Methods of Enzymatic Analysis. In: Bergmayer, H.U., Ed., Chemie, 2nd Edition, Vol. 2, FRG, Weinheim; 1974. p. 673-84.
31. Ellman GL, Courtney KD, Andres Jr V, Featherstone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol. 1961;7(2):88-95. https://doi.org/10.1016/0006-2952(61)90145-9
32. Nabeshima T, Nitta A. Memory impairment and neuronal dysfunction induced by β-amyloid protein in rats. Tohoku J Exp Med. 1994;174(3):241-49. https://doi.org/10.1620/tjem.174.241
33. Ali T, Yoon GH, Shah SA, Lee HY, Kim MO. Osmotin attenuates amyloid beta-induced memory impairment, tau phosphorylation and neurodegeneration in the mouse hippocampus. Sci Rep. 2015;5:11708. https://doi.org/10.1038/srep11708
34. Huang SH, Lin CM, Chiang BH. Protective effects of Angelica sinensis extract on amyloid β-peptide-induced neurotoxicity. Phytomedicine. 2008;15(9):710-721. https://doi.org/10.1016/j.phymed.2008.02.022
35. Shi SH, Zhao X, Liu B, Li H, Liu AJ, Wu B, Bi KS, Jia Y. The effects of sesquiterpenes-rich extract of Alpinia oxyphylla Miq. on amyloid-β-induced cognitive impairment and neuronal abnormalities in the cortex and hippocampus of mice. Oxid Med Cell Longev. 2014: 451802. http://dx.doi.org/10.1155/2014/451802
36. Sadiki FZ, El Idrissi M, Cioanca O, Trifan A, Hancianu M, Hritcu L, Postu PA. Tetraclinis articulata essential oil mitigates cognitive deficits and brain oxidative stress in an Alzheimer's disease amyloidosis model. Phytomedicine. 2019;56:57-63. https://doi.org/10.1016/j.phymed.2018.10.032
37. Wu WY, Wu YY, Huang H, He C, Li WZ, Wang HL, Chen HQ, Yin YY. Biochanin A attenuates LPS-induced pro-inflammatory responses and inhibits

the activation of the MAPK pathway in BV2 microglial cells. Int J Mol Med. 2015;35(2):391-8. https://doi.org/10.3892/ijmm.2014.2020
38. Tan JW, Tham CL, Israf DA, Lee SH, Kim MK. Neuroprotective effects of biochanin A against glutamate-induced cytotoxicity in PC12 cells via apoptosis inhibition. Neurochem Res. 2013;38(3):512-8. https://doi.org/10.1007/s11064-012-0943-6