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Original Article

GABAergic effect of valeric acid from *Valeriana wallichii* in amelioration of ICV STZ induced dementia in rats

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ABSTRACT

Valeriana wallichii DC., Caprifoliaceae, is used to have anti-ulcer, anti-spasmodic, anti-epileptic, memory enhancer, anti-anxiety, anti-rheumatic, sedative, anti-asthmatic and diuretic activities. *V. wallichii* is reported to contain valpotriates, valeric acid, valerenic acid, valechlorine, valerianine, resins and alkaloids. Valeric acid, found in *V. wallichii* appears similar in structure to the neurotransmitter GABA. Valeric acid also acts as an NMDA-receptor antagonist. The aim of present study was to investigate the neuroprotective effect of *V. wallichii* containing valeric acid and its possible mechanism of action in amelioration of intracerebroventricular streptozotocin induced neurodegeneration in Wistar rats. The rhizomes of *V. wallichii* were powdered coarsely and extracted by percolation method using dichloromethane. Wistar rats (220–250 g) of either sex were divided into 5 groups, comprising 6 animals each. Valeric acid was isolated from plant extract and characterized using FT-IR. Picrotoxin (2 mg/kg) was used as GABA-A antagonist. Intracerebroventricular streptozotocin administration caused significant ($p < 0.05$) increase in escape latency, retention transfer latency on morris water maze on 17th, 18th, 19th and 20th day and elevated plus maze on 19th and 20th day respectively, as compared to normal untreated rats. Treatment with *V. wallichii* extract 100 and 200 mg/kg and valeric acid 20 and 40 mg/kg significantly decreased the escape latency and retention transfer latency, as compared to intracerebroventricular-streptozotocin group. Plant extract and valeric acid also decreased the level of lipid peroxidation and restored glutathione level in rat brains. Administration of picrotoxin significantly reversed the effects produced by plant extract and valeric acid in intracerebroventricular-streptozotocin treated rats. The findings may conclude that valeric acid present in *V. wallichii* has significant GABAergic effect in amelioration of experimental dementia.

Introduction

Dementia is a clinical syndrome which is due to degeneration of neurons in brain and spinal cord. It is associated with deterioration in cognitive ability and capacity for independent living (Fratiglioni et al., 2007). The prevalence of neurodegenerative disorders like Alzheimer's diseases (AD) has increased significantly as global population age (Chen and Zheng, 2012). Currently, the number of deaths caused by AD is severely high and projected to rise by 21.2 million in 2025 (Hirtz et al., 2007; Katzman, 2008). The characteristic feature of AD is vascular dementia, fronto-temporal dementia, and aggregation of amyloid, accumulation of neurofibrillary tangles, lewis body formation and neuronal death. There is the development of multiple cognitive deficits such as disturbances in executive functioning, apraxia, agnosia, aphasia and memory impairment (Fratiglioni et al., 2007) that interferes with daily social and professional brain outcomes. The most common symptoms of dementia are psychosis, aggression, insomnia, anxiety, depression, delirium, anger and sundowning (confusion in late afternoon or early evening) (Kuller and Lopez, 2008). The treatment of AD includes acetylcholinesterase inhibitor like Tacrine, Donepezil, Rivastigmine and Galantamine (Pokorski, 2002). Moreover, Food and Drug Administration has warned not to use atypical antipsychotics due to their increased mortality risk (Schneider et al., 2005).

AD is characterized by accumulation of amyloid beta peptide (Aβ) as fibrillary plaques and soluble oligomers in brain regions (Cole and Vassar, 2007). Tau protein is also identified as one of the main component of neurofibrillary tangles in various neurological diseases (Gendron and Petrucelli, 2009). In many areas of CNS, overactivation of NMDAR and subsequent influx or release of excessive Ca^{2+} has been noted to be predominant form of neurotoxicity. Ca^{2+} overload could ultimately lead to production of reactive oxygen species (ROS) and nitric oxide (NO) radicals,

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mitochondrial dysfunction, neuronal excitotoxicity and ultimately neuronal degeneration (Majdi and Chen, 2009). Recent researches have emerged with evidences that some pathogenic receptors are altered with lower levels of $GABA_AR$-subunits α1, α2, α4, δ, and β2, mRNA in prefrontal cortex and α1, α5, and β3 mRNAs in hippocampus were observed in AD (Rissman and Mobley, 2011).

Valeriana wallichii DC., Caprifioliaceae, is found abundantly at an altitude of 1300–3800 m in temperate Himalayas from Kashmir to and between 1250 and 1800 m in Khasi hills (Chauhan, 2006). It has got considerable reputation for its traditional use in pain (Vohora and Dandiya, 1992), epilepsy, insomnia, neurosis, sciatica (Nadkarni, 1976; Marder *et al.*, 2003). The plant is widely used in the treatment of anxiety and depression (Panijel, 1985; Ron *et al.*, 2000). It has been used as sedative in the treatment of insomnia and restlessness (Leathwood and Chauffard, 1985). *Valeriana* is reported to have antidepressant, anxiolytic, antispasmodic, anti-inflammatory and anticonvulsant activities (Sah et al., 2010). Valeric acid (VA) or pentanoic acid, a chief constituent of *V. wallichii* (Kokate et al., 2007), is a straight chain alkyl carboxylic acid with chemical formula $C_5H_{10}O_2$, molecular weight 102.15 and melting point: 34.5 °C. VA appears similar in structure to the neurotransmitter GABA but lacks amine functional group which contributes for the biological activities of GABA. It is reported as GABA-A agonist and is a very good relaxant. It also acts as an NMDA-receptor antagonist and showed anti-epileptic effect (Loeb et al., 1990). GABA-mediated postsynaptic inhibition in cultured mammalian neurons was observed on treatment with VA. It increases availability of synaptic GABA and/or enhances postsynaptic GABA responses, and thus enhances GABAergic activity. Keeping this in view, the present study was hypothesized to investigate GABAergic effect of VA from *V. wallichii* in amelioration of experimental dementia in intracerebroventricular streptozotocin model in rat brain.

Materials and methods

Collection and authentication of plant materials

The rhizomes of *Valeriana wallichii* DC., Caprifioliaceae, were collected from Kufri, Shimla, HP and duly taxonomically authenticated by Dr. R. Raina, Senior Scientist, Professor (Medicinal Plants), Dr. Y.S. Parmar University of Horticulture and Forestry, Nauni, Solan, HP, India and the sample was linked to UHF-Herbarium with field book number 12425.

Animals

Wistar Albino rats weighing 200–250 g of either sex were obtained from animal house (Reg No. 1541/PO/a/11/CPCSEA), Shoolini University, Solan, HP, India. The animals were allowed to acclimatize to the environment for 7 days prior to the experimental session. The animals were divided into different groups consist of six animals ($n = 6$) in each group. The study was conducted as per the guidelines of Committee for the purpose of control and supervision of experiments on animals (CPCSEA). The experimental study was duly been approved by Institutional Animal Ethical Committee (IAEC) vide protocol no. IACE/SU-PHARM/2/020. The animals were maintained at standard food pellet diet from Ashirwad Industries, Chandigarh, India, water ad libitum, temperature 25 ± 2 °C and humidity 45 ± 5%.

Extraction of plant material

Dried powdered rhizomes of *V. wallichii* (500 g) were taken and extracted by percolation method using dichloromethane as solvent. The powdered material was moistened with 150 ml of dichloromethane and allowed to stand for approximately 4 h in percolator. After completion of 4 h, 200 ml of dichloromethane was again added to form a shallow layer above the mass, and the mixture was allowed to macerate in closed percolator for 24 h. The outlet of percolator was opened after 24 h and the liquid contained therein was allowed to drip slowly. Additional dichloromethane was added until the percolate measures about three-quarters of required volume of finished product. After completion of extraction, solvent was then recovered and dried in rotary vacuum evaporator.

Isolation of valeric acid

The extract of *V. wallichii* was treated with methanol and sodium bicarbonate, which produces effervescence. Then extract solution was centrifuged, supernatant was separated and the solid mass was dissolved in water. It was completely dissolved in water by stir continuously and dilute hydrochloric acid was added dropwise to maintain pH 2. The extraction was done three to four times with methylene chloride in a separating funnel, concentrated the solution and thus obtained product was subjected to characterization (Houghton, 1988; Barnes, 2002; Gruenwald, 2004).

Fingerprint analysis of valeric acid

The fingerprint analysis of isolated VA in reference to standard sample was done using Agilent Technologies Cary 630 Fourier transform infrared spectrophotometry (FTIR) at range 4000–650 cm^{-1}.

Intracerebroventricular (ICV) administration of streptozotocin (STZ) for dementia

Rats were anaesthesized using ketamine hydrochloride (70 mg/kg *i.p.*) and xylazine (5 mg/kg, *i.p.*). The head of the animal was positioned in stereotaxic apparatus and a midline sagittal incision was made in the scalp. Two holes were drilled through the skull for placement of infusion cannula into lateral cerebral ventricles with the coordinates: 0.8 mm posterior to bregma; 1.5 mm lateral to saggital suture; 3.6 mm ventrally from the surface of the brain and the tooth bar was set at 0 mm. A Hamilton® syringe of 10 μl was attached through a skull hole to a stereotaxic apparatus and piston of the syringe was lowered manually into each lateral ventricle. STZ (3 mg/kg in two divided doses) dissolved in citrate buffer, pH 4.4, was slowly infused through intracerebroventricular route bilaterally (Tiwari et al., 2009). After ICV administration, the cut skin was sutured and the postoperative care were duly be made by applying povidone–iodine solution on wound.

The behavioral assessments were done using morris water maze test on 17^{th}, 18^{th}, 19^{th} and 20^{th} day and elevated plus maze on 19^{th} and 20^{th} day. On completion of protocol, the animals were sacrificed and brain was isolated.

Drug administration

The plant extract of *V. wallichii* was used in the dose range from 50 to 400 mg/kg in various investigations using rats (Subhan et al., 2009). Therefore, 100 and 200 mg/kg were selected as its submaximal dose to investigate the biological potential against experimentally induced neurodegeneration. The dosage of VA selected was 20 and 40 mg/kg due to its protective action against various experimental diseased conditions in rats (Loeb et al., 1990). *V. wallichii* extract 100 and 200 mg/kg, *p.o.* (suspended in 1% CMC solution) and valeric acid 20 and 40 mg/kg, *i.p.* (suspended in 1% Tween 80 solution) were given to animals treated with ICV-STZ