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Effect of hydroalcoholic extract of *Vitex negundo* Linn. leaves on learning and memory in normal and cognitive deficit mice

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ABSTRACT

Objective: To demonstrate the improvement in learning and memory by hydroalcoholic extract of *Vitex negundo* Linn. leaves (HEVN). **Methods:** The leaves were macerated and percolated using 70 % ethanol to obtain dark green colored semisolid mass of HEVN. Effects of HEVN were evaluated in normal as well as in scopolamine- induced cognitive deficit mice paradigms using elevated plus maze (EPM) and object recognition test (ORT). Additionally, the effect of HEVN on acetyl-cholinesterase level (AchE) and oxidative stress in mice brain and in sodium nitrite induced respiratory arrest in mice was evaluated. **Results:** Preliminary phytochemical analysis revealed the presence of alkaloids, flavonoids, proteins, and carbohydrates in the HEVN. Administration of HEVN (250 and 500 mg/kg/day, p.o.) for 8 days significantly increased inflexion ratio in EPM, discrimination index in ORT, and decreased brain AchE in both paradigms and prolonged the onset of time of death in sodium nitrite induced respiratory arrest in mice. Furthermore, HEVN (250, 500, and 1 000 mg/kg/day, p.o.) decreased brain lipid peroxidation and HEVN (500 and 1000 mg/kg/day, p.o.) increased brain reduced glutathione in scopolamine- induce cognitive deficit mice. **Conclusions:** The present study revealed the effectiveness of HEVN in improving learning and memory processes in both paradigms. The effect might be due to AchE inhibition, antioxidant effect, and/or increase in cholinergic transmission.

1. Introduction

Vitex negundo Linn. (Family: Verbenaceae) (VN) is commonly known as five leaved chaste tree (English), Nirgudi (Marathi), Nirgundi (Hindi), Indrani (Sanskrit)[1]. VN is large, aromatic, shrub or a small, slender tree with an irregular trunk growing up to 4.5 m in height. Its stem and branches are covered with thin, grey bark, which becomes almost black and scaly when old. It occurs wild in most parts of India near moist places[2]. Although all parts VN are used as medicine in the indigenous system of medicine, the leaves are the most potent for medicinal use[3]. It has been employed in Indian traditional medicinal system for the treatment of various ailments including brain tonic and to improve memory[4,5]. The effectiveness of VN has been scientifically reported for various activities such as anti-inflammatory[6]; gastroprotective[7]; anti-cancer[3]; antioxidant[8,9]; central nervous system (CNS) depressant[10]; anticonvulsant[11]; etc. VN leaves contains monoterpenoids iridoids (2-p-hydroxybenzoyl mussaenosidic; nishindaside; negundoside), triterpenoids (betulinic acid; ursolic acid), flavonoids (gardenin A; gardenin B; corymbosin; vitexicarpin; 5-hydroxy-3,6,7,3,4-penta-metoxyflavone; 3,5-dihydroxy-6,7,3,4 tetramethoxyflavanol), phenolic acid (p-hydroxybenzoic acid; 3,4-dihydroxybenzoic acid), and essential oil (sabinene, 4-terpineol, β-caryophyllene, and viridiflorol)[2]. Lignans, one class of natural compounds present in VN, showed anti-cholinesterase *in vitro* activity[12].

Recently, Kanwal *et al.* was demonstrated the improvement in learning and memory tasks in the shuttle-box by using the scopolamine- induced dementia with the aqueous herbal extract of plant VN at a dose of 300 mg/kg through inhibiting lipid peroxidation (LP), augmenting endogenous antioxidant enzymes and decreasing brain acetyl-cholinesterase (AChE) activity[12]. The higher concentration of most of the phytoconstituents, could be extracted using aqueous ethanol hence we used hydroalcoholic extract of VN leaves (HEVN).

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Our study was aimed to demonstrate the improvement of learning and memory by HEVN in normal as well as in scopolamine- induced cognitive deficit mice.

2. Materials and methods

2.1. The plant material and extraction

The fresh leaves of VN were collected from Bhor region (Dist. Pune, India) in the month of September 2009 and were authenticated and specimen was deposited at the Botanical Survey of India, Pune (Voucher Specimen No. BSI/WRA/Tech/545). The leaves were shade dried, coarsely powdered, and the powder material (200 g) was macerated for 24 h and percolated using 70% ethanol and the menstrum collected was concentrated till dry to obtain 48 g dark green coloured semisolid mass of HEVN (Yield 24%). HEVN was suspended in 1% gum acacia in distilled water[13,14].

2.2. Drug and chemicals

Piracetam (Nootropil® suspension, UCB Pvt. Ltd., India); scopolamine (Buscopan® injection, Cadila Pharma, India); acetylthiocholine iodide (Himedia, India); and other chemicals of analytical grade were procured from the local vendors of Pune, India. Nootropil® was suspended in 1 % gum acacia in distilled water; Buscopan® was diluted with distilled water; and sodium nitrite was dissolved in distilled water to prepare appropriate respective doses. All solutions were prepared freshly.

2.3. Animals

Adult Swiss albino mice (18-25 g) of either sex (Grade II), procured from National Toxicology Center, Pune, India were used for the studies. Animals were housed in groups of 5-6 in standard polypropylene cages with wire mesh top at standard environmental condition of temperature (25 ±2) ℃ and relative humidity of 45%-55% under 12 h: 12 h light: dark cycle in the institutional animal house. Animals had free access to standard pellet rodent diet (Lipton India Ltd., Mumbai, India) and water was provided ad libitum. All experiments were carried out between 08:00 to 16:00. The experimental protocol was approved by the Institutional Animal Ethics Committee of Rajgad Dnyanpeeth's College of Pharmacy, Bhor, India constituted as per the rules and guidelines provided by the Committee for the Purpose of Control and Supervision of Experiments on Animals, Chennai, India (Approval No. RDCOP/ IAEC/12/09).

2.4. Dose selection

All drugs and vehicle (1% gum acacia in distilled water) were administered in the volume of 5 mL/kg and doses were calculated according to the body weight of animals. The doses of HEVN selected in the present study were according to the previously reported LD_{50} (7580 mg/kg, p.o.)[15] and that were used in the previous study[11].

2.5. Preliminary phytochemical analysis of HEVN

The preliminary phytochemical analysis was performed using various standard phytochemical tests for the qualitative estimation of presence of various phytochemicals in HEVN[16,17].

2.6. Experimental design

Swiss albino mice (18-25 g) of either sex were divided into 10 groups (n=6) and treated for 8 days as follows- Group I (Normal control): vehicle (5 mL/kg/day, p.o.); group II (Piracetam): piracetam (200 mg/kg/day, p.o.); group III, IV, and V (HEVN 250, 500, and 1000): HEVN (250, 500, and 1000 mg/kg/day, p.o.) respectively; group VI (Control): vehicle (5 ml/kg/day, p.o.); group VII (Piracetam+scop): piracetam (200 mg/kg/day, p.o.); group VIII, IX, and X (HEVN 250+scop, 500+scop, and 1000+scop): HEVN (250, 500, and 1000 mg/kg/day, p.o.) respectively. Additionally, on 8th day, in EPM test 45 minutes after the respective treatments and in ORT 30 minutes before the respective treatments, scopolamine (0.4 mg/kg, i.p.) was administered to groups VI, VII, VIII, IX, and X to induce cognitive deficit in mice. To evaluate the effect on learning and memory in normal mice (without inducing cognitive deficit) groups I, II, III, IV, and V were used and that in scopolamine- induced cognitive deficit mice groups VI, VII, VIII, IX, and X were used.

2.7. Elevated plus maze (EPM) test

The apparatus consisted of two open arms (16 cm× 5 cm) and two enclosed arms (16 cm × 5 cm × 12 cm). The arms extended from a central platform (5 cm × 5 cm) and the maze was elevated to a height of 25 cm from the floor. On the 8th day after the last treatment, each mouse was placed at the end of an open arm, facing away from the central platform. The transfer latency (TL), the time taken by mouse with all its four legs to move into one of the enclosed arm, was recorded as L0. If the animal did not enter into one of the enclosed arms within 90 s, it was gently pushed into one of the two enclosed arms and the TL was assigned as 90 s. The mouse was allowed to explore the maze for another 10 s and then returned to its home cage. The retention of this learned task was examined 24 h after the 8th day trial i.e. on 9th day and the TL was recorded as L1. The effect on TL was expressed by inflexion ratio (IR). Increase in IR after 24 h indicated improved retention of learned task. IR was calculated using the formula:

$IR = (L0 - L1) / L1$

Where L0 = initial TL (s) on 8th and L1 = TL after 24 hr of first day trial i.e. on 9th day[18-20].

2.8. Object recognition test (ORT)

The apparatus consisted of a white colored plywood box (70 × 60 × 30 cm) with a grid floor that could be easily cleaned with hydrogen peroxide after each trial. The apparatus was