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Q1 Methyl jasmonate enhances memory performance through inhibition of oxidative stress and acetylcholinesterase activity in mice

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ABSTRACT

Aims: Current research effort focuses on the development of safer natural compounds with multipronged mechanisms of action that could be used to ameliorate memory deficits in patients with Alzheimer's disease, as cure for the disease still remains elusive. In this study, we evaluated the effect of methyl jasmonate (MJ), a naturally occurring bioactive compound on memory, acetylcholinesterase activity and biomarkers of oxidative stress in mice.
Main methods: Male Swiss mice were treated with intraperitoneal injection of MJ (10–40 mg/kg) alone or in combination with scopolamine (3 mg/kg) once daily for 7 days. Thirty minutes after the last treatment, memory functions were assessed using Y-maze and object recognition tests. Thereafter, acetylcholinesterase activity and levels of biomarkers of oxidative stress were assessed in mice brains using standard biochemical procedures.
Key findings: MJ significantly enhanced memory performance and reversed scopolamine-induced cognitive impairment in mice. MJ demonstrated significant inhibition of acetylcholinesterase activity suggesting increased cholinergic neurotransmission. It further decreased malondialdehyde concentrations in mouse brain indicating antioxidant activity. Moreover, MJ significantly increased glutathione levels and activity of antioxidant enzymes (catalase and superoxide dismutase) in mice brains. The increased oxidative stress; evidenced by elevated levels of malondialdehyde and decreased antioxidant defense systems in scopolamine-treated mice was attenuated by MJ.
Significance: The results of this study suggest that MJ may be useful in conditions associated with memory dysfunctions or age-related cognitive decline. The positive effect of MJ on memory may be related to inhibition of oxidative stress and enhancement of cholinergic neurotransmission through inhibition of acetylcholinesterase activity.

1. Introduction

Progressive loss of memory, which results in the inability to recall past events, is a major feature of Alzheimer's disease (AD). The incidence of AD increases with age and has been predicted to affect 1 in 85 people globally by the year 2050 [1]. AD is characterized by loss of neurons especially in brain regions associated with learning and memory [1]. AD is a complex disease of multiple pathologies associated with degeneration of several neuronal populations, especially central cholinergic pathways [2–3]. However, considerable data have accumulated over the years supporting the role of oxidative stress in the pathogenesis of AD [4–5]. Increased lipid peroxidation and decreased polyunsaturated fatty acids have been found in AD brains, which further support the role of oxidative stress in the pathology of the disease [3–4]. Oxidative stress occurs in brain tissues; whenever there is increased generation of reactive oxygen species (ROS) and impaired antioxidant defense systems [6]. Brain tissue in particular is more susceptible to the deleterious effects of ROS, because it has a high rate of oxygen consumption, and reduced antioxidant defense systems [2,7]. ROS produced lipid peroxidation, which triggers neuronal degeneration especially central cholinergic pathways [2,7]. Postmortem studies have confirmed elevated levels of malondialdehyde (MDA), an index of lipid peroxidation in AD brains, which confirmed the role of oxidative stress in the pathogenesis of the disease [8]. Moreover, scopolamine (SC)-induced memory loss has been linked to increased oxidative stress in the whole brain, as well as specific structures associated with memory and learning [9]. Also, SC has been reported to deplete antioxidant molecules like glutathione, and therefore further damage brain cells through increased lipid peroxidation [10]. Anti-cholinesterase agents like donepezil and rivastigmine, currently used for treatment of AD, are known to offer symptomatic relief; but not the underlying pathological abnormalities [4]. Thus, current research effort focuses on the development of natural products with multipronged mechanisms of action that could target various aspects of the pathologies of AD [11–12].

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Methyl jasmonate (MJ) is a bioactive compound obtained from jasmine, a perennial climbing plant that is well known for its sweet and highly scented flowers. Jasmine flower has been used in aromatherapy for depression, nervousness, tension, alertness and memory improvement [13]. MJ is one of the most well studied members of the jasmonate family and has gained international recognition as a potential agent for the treatment of cancer [14]. MJ was first isolated from essential oil of *Jasminum grandiflorum* by Demole in 1962 [15]. However, it has also been found in a variety of plant species especially fruits, which forms essential components of human diet [14]. Previous studies had shown that MJ selectively kills cancer cells without producing any toxic effects to normal cells [16]. MJ has been reported to have high lipid solubility, a property that has been ascribed to its ability to across various biological membranes including the blood brain barrier [14,17]. Our previous studies revealed that MJ exhibited a number of pharmacological activities including antidepressant, antinociceptive and anti-aggressive activities [18–20]. However, no detailed pharmacological investigations have been carried out to assess the effects of MJ on memory functions. Thus, this present study was carried out to investigate the effect of MJ on memory performance and the probable mechanisms underlying its action in mice.

2. Materials and methods

2.1. Experimental animal

Male Swiss mice (22–24 g; 6 weeks old) were obtained from the Central Animal House, University of Ibadan. The animals were housed in plastic cages at room temperature and had free access to commercial food pellets and water *ad libitum*. The animals were acclimatized for at least one week before commencement of experiments. The experimental procedures were carried out in compliance with National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985). Also, efforts were made to minimize the suffering of the animals.

2.2. Drugs and chemicals

Methyl jasmonate—MJ (Sigma, Germany), donepezil—DP (Pfizer, USA), scopolamine—SC (BDH Chemicals Ltd., England), trichloroacetic acid—TCA (Burgoyne Burbidges & Co., Mumbai, India), thiobarbituric acid—TBA (Guangdong Guanghua Chemical Factory Co., Ltd.) 5,5′-dithio-bis(2-nitrobenzoic acid)—DTNB (Aldrich, Germany) and Tris (hydroxymethyl)-amino-methane (Tris-buffer) (Hopkin & Williams Company, USA) were used in the study.

2.3. Drug preparation

Methyl jasmonate was dissolved in 95% ethanol and the solution was further diluted with distilled water. The final concentration of ethanol in the solution used for the study did not exceed 1%. Other drugs used in the study were dissolved in distilled water immediately before use. The doses of MJ used in the study were selected based on the results obtained from preliminary investigations.

2.4. Assessment of memory performance

2.4.1. Y-maze test

The effect of MJ on spatial working memory in mice was assessed using the Y-maze paradigm as previously described [21]. The animals were randomly distributed into various treatment groups ($n = 6$) and were given i.p. injection of MJ (10, 20, 40 mg/kg), DP (1 mg/kg) or vehicle (1% ethanol, 10 ml/kg) once daily for 7 days. Thirty minutes after the last treatment, each mouse was placed individually at the center of the Y-maze and allowed to explore all the three arms freely for 5 min. The number and sequence of arm entries were recorded and the apparatus was cleaned after each test. An entry was scored when the four paws of the animals were completely in the arm of the Y-maze. The percentage alternation, which is a measure of spatial working memory, was calculated by dividing the total number of alternations by the total number of arm entries, minus two and multiplied by 100 [21]. An alternation behavior was defined as consecutive entries into all three arms (*i.e.* ABC, CAB or BCA but not BAB) [21].

2.4.2. Object recognition test (ORT)

The effect of MJ on memory performance was also assessed using the ORT in an open-field chamber (60 cm × 50 cm × 40 cm) as previously described [22]. The discriminated objects (A, B and C) were identically sized (4.5 cm diameter and 11.5 cm height) cylindrical bottles. Objects A and B were white, whereas object C had a black and white pattern. The ORT consists of two phases; the trial phase and the test phase. Mice ($n = 6$) were treated with MJ (10, 20, 40 mg/kg), DP (1 mg/kg) or vehicle (1% ethanol, 10 ml/kg) daily for 7 days. The animals were acclimatized to the experimental condition for a period of 5 min. The trial phase was carried out by placing each mouse in the middle of two identical objects (A and B) on opposite sides (at a distance of 8 cm from the walls and 34 cm from each other) of the open-field chamber for 5 min. Thereafter, the animals were returned to their home cages for an interval of 4 h. In the test phase, object B was replaced with object C, which was novel to the mice and different from either object A or B. Mice were then left to explore objects A and C for a period of 5 min. The apparatus was cleaned after each test and the time spent (s) in exploring each of the objects was recorded in both phases. The discrimination index, which was used as a measure of non-spatial memory function was calculated as the difference in time exploring the novel and familiar object divided by the total amount of time spent with both objects [22].

2.4.3. Effect of MJ on scopolamine-induced memory impairment

The effect of MJ on SC-induced memory dysfunction was assessed utilizing the Y-maze and ORT paradigms. Mice were divided into various treatment groups ($n = 6$) and were pretreated either with SC (3 mg/kg) or in combination with MJ (10, 20, 40 mg/kg) once daily for 7 days. Thirty minutes after the last treatment, the animals were tested for memory function using the Y-maze and ORT paradigms as earlier described.

2.5. Biochemical assays

After testing for memory function, the animals were decapitated under ether anesthesia and the brains were immediately removed and kept in the refrigerator with ice block for 30 min. Thereafter, the whole brain was weighed and homogenized with 10% w/v phosphate buffer (0.1 M, pH 7.4). Each brain tissue homogenate was separated into various portions for the different biochemical assays.

2.5.1. Determination of acetylcholinesterase (AChE) activity in mice brain

Aliquots of homogenates of the individual mouse brain of the various treatment groups were taken and used to measure AChE activity, a marker for cholinergic neurotransmission [23]. Briefly, AChE activity in the homogenate was measured by adding 2.6 ml of phosphate buffer (0.1 M, pH 7.4), 0.1 ml of 5,5′-dithio-bis(2-nitrobenzoic acid) (DTNB) and 0.4 ml of the homogenate. Then 0.1 ml of acetylthiocholine iodide was added to the reaction mixture. The absorbance was read using a spectrophotometer at a wavelength of 412 nm and change in absorbance for 10 min at two minute interval was recorded. The rate of AChE activity was measured by following the increase of color produced from thiocholine when it reacts with DTNB. The change in absorbance per minute was determined and the rate of AChE activity was calculated and expressed as μmol/min/g tissue.

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