NeuroToxicology 56 (2016) 107–117

Contents lists available at ScienceDirect

NeuroToxicology

Full length article

Alkaloid extracts from Jimson weed (*Datura stramonium* L.) modulate purinergic enzymes *in* rat brain

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ARTICLE INFO

Article history:
Received 14 January 2016
Received in revised form 27 May 2016
Accepted 24 June 2016
Available online 21 July 2016

Keywords:
Neuromodulation
Alkaloid extracts
Jimson weed
Purinergic signaling

ABSTRACT

Although some findings have reported the medicinal properties of Jimson weed (*Datura stramonium* L.), there exist some serious neurological effects such as hallucination, loss of memory and anxiety, which has been reported in folklore. Consequently, the modulatory effect of alkaloid extracts from leaf and fruit of Jimson weed on critical enzymes of the purinergic [ecto-nucleoside triphosphate diphosphohydrolase (E-NTPDase), ecto-5′-nucleotidase (E-NTDase), alkaline phosphatase (ALP) and Na^+/K^+ ATPase] system of neurotransmission was the focus of this study. Alkaloid extracts were prepared by solvent extraction method and their interaction with the activities of these enzymes were assessed (*in vitro*) in rat brain tissue homogenate and *in vivo* in rats administered 100 and 200 mg/kg body weight (*p.o*) of the extracts for thirty days, while administration of single dose (1 mg/kg body weight; *i.p.*) of scopolamine served as the positive control. The extracts were also investigated for their Fe^{2+} and Cu^{2+} chelating abilities and GC–MS characterization of the extracts was also carried out. The results revealed that the extracts inhibited activates of E-NTPDase, E-NTDase and ALP in a concentration dependent manner, while stimulating the activity of Na^+/K^+ ATPase (*in vitro*). Both extracts also exhibited Fe^{2+} and Cu^{2+} chelating abilities. Considering the EC_{50} values, the fruit extract had significantly higher ($P < 0.05$) modulatory effect on the enzymes' activity as well as metal chelating abilities, compared to the leaf extract; however, there was no significant difference ($P > 0.05$) in both extracts' inhibitory effects on E-NTDase. The *in vivo* study revealed reduction in the activities of ENTPDase, E-NTDase, and Na^+/K^+ ATPase in the extract-administered rat groups compared to the control group, while an elevation in ALP activity was observed in the extract-administered rat groups compared to the control group. GC–MS characterization revealed the presence of atropine, scopolamine, amphetamine, 3-methyoxyamphetamine, 3-ethoxyamhetamine cathine, spermine, phenlyephirine and 3-piperidinemethanol, among others in the extracts. Hence, alterations of activities of critical enzymes of purinergic signaling (*in vitro* and *in vivo*) by alkaloid extracts from leaf and fruit of Jimson weed suggest one of the mechanisms behind its neurological effects as reported in folklore.

1. Introduction

Over thirty years ago, the concept of purinergic neurotransmission was initially proposed by Burnstock (1978). Thereafter, the roles of several nucleoside di- and triphosphates (ADP, UDP, ATP, UTP) in defined neurotransmission signaling mechanisms in the central nervous system has been elucidated (Yegutkin, 2008). In addition, purinergic signaling have also been identified in several other tissues to mediate signal transduction such as in the regulation of epithelial cell responses (Bucheimer and Linden, 2004); gastrointestinal, myocardium and liver functions (Roman and Fitz, 1999; Vassort, 2011) non-adrenergic, non-cholinergic smooth muscle contractility and neuron-glia interactions (Vassort, 2011).

At the completion of their signal transduction roles, extracellular nucleotides are inactivated by hydrolyses to adenosine, mediated by sequential set of hydrolyzing ecto-enzymes (Yegutkin, 2008). These enzymes include; ecto-nucleoside triphosphate diphosphohydrolase (E-NTPDase) family; ecto-nucleotide pyrophosphatase/phosphodiesterase (E-NPP) family; ecto-5′-nucleotidase (E-NTDase); and alkaline phosphatases (AP) (Yegutkin, 2008). Adenosine produced by these reactions mediates attenuation of inflammation and tissue damage as well as several cardioprotective, neuroprotective, vasodilatatory and angiogenic

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http://dx.doi.org/10.1016/j.neuro.2016.06.012
0161-813X/

responses (Yegutkin, 2008). Hence, the impairment in these enzymes' activities could significantly influence neuronal functions.

The Na^+/K^+ activated adenosine 5′-triphosphatase (Na^+/K^+ATPase), or Na^+/K^+ pump is a membrane bound protein, concentrated at pre-synaptic nerve endings (Ravindran et al., 2012) and is involved in maintaining the electrochemical gradient across the cell membranes (de Lores Arnaiz and Ordieres, 2014). This enzyme mediates the equilibrium of Na^+/K^+ across neuronal membrane by coupling the hydrolysis of one molecule of ATP to the entry of two K^+ with simultaneous exit of three Na^+ from cells (de Lores Arnaiz and Ordieres, 2014). A distortion of this equilibrium results in the depolarization of nerve endings with the entry of Ca^{2+} into cells followed by the release of neurotransmitters and neuronal swelling (de Lores Arnaiz and Ordieres, 2014). Physiologically, the transportation of ions by the sodium/potassium pump is critical for the movement of electrolyte across epithelial cells; therefore, dysfunction of this pump can lead to neuronal dysfunction, renal dysfunction, heart disease and hypertension (Ravindran et al., 2012).

Jimson weed (*Datura stramonium* L.) is an alkaloid-rich annual herbaceous plant, which has several pharmacological properties (Soni et al., 2012; Ademiluyi et al., 2016). The leaf and fruit have been reported to have highest abundance of alkaloids, especially the tropane alkaloids (Steenkamp et al., 2004). Although there are some reports on the plant's medicinal properties (Soni et al., 2012; Altameme et al., 2015), several deleterious neurological effects such as loss of memory, hallucination, anxiety, and so on, has been reported in folklore. Furthermore, clinical case reports and eye-witness accounts have reported intentional use of this plant, predominantly among adolescents for its 'mind-altering' effects (Adegoke and Alo, 2013). Also, cases of accidental cases of Datura-induced toxicity in humans mostly *via* consumption of contaminated farm produce have been reported (Soni et al., 2012). However, despite these deleterious effects of this plant on neuronal function, there is dearth of information on the effect of its phytoconstituents on critical enzymes essential for proper neuroactivity. Therefore, we have designed this study to investigate the effect of crude alkaloid extracts from leaf and fruit of Jimson weed on the activities of key enzymes of the purinergic (E-NTPDase, E-NTDase, ALP and Na^+/K^+ATPase), systems of neurotransmission *in vitro* and *in vivo* in rat brain.

2. Materials and methods

2.1. Collection and preparation of samples

Jimson weed (*Datura stramonium* L.) plant was harvested at the stage of opening of first capsule, from local farm settlement in Akure, Ondo State (South West) Nigeria, during the late raining season (August) of year 2014. The plant was authenticated at the Forest Research Institute of Nigeria (FRIN) Ibadan, Oyo State (South West) Nigeria. A sample voucher was deposited at the institute's herbarium (voucher number FHI 110111). Leaves and fruits of the plant were carefully separated, washed with water to remove dirt, and dried under shade for several days until a constant weight was obtained. Thereafter, the dried samples were pulverized in an electronic stainless steel blender, and stored in air-tight dark containers in the refrigerator at 4 °C for alkaloid extraction.

2.2. Chemicals and reagents

Chemicals and reagents used such as, ATP, ADP, AMP, ouabain, scopolamine (SCOP), malachite green, were procured from Sigma-Aldrich, Inc., (St Louis, MO); trichloroacetic acid (TCA) was sourced from Sigma-Aldrich, Chemie GmbH (Steinheim, Germany); methanol and acetic acid were sourced from BDH Chemicals Ltd., (Poole, England). All other chemicals were of analytical grade while the water used for all analysis was glass distilled.

2.3. Preparation of alkaloid extracts

Alkaloid extract of samples were prepared according to the method of Harborne (1998), with slight modifications (Ademiluyi et al., 2016). Briefly, pulverized samples were defatted with *n*-hexane for 24 h. Thereafter, 10 g of defatted samples were extracted with 100 mL of 10% acetic acid in ethanol for 24 h. This was followed by filtration, first using Muslin cloth and then filter paper (Whatman no. 1). The clear filtrate obtained was concentrated under vacuum at 45 °C in a rotary evaporator (Laborota 4000 Efficient, Heidolph, Germany). Subsequently, the filtrate was precipitated using concentrated ammonium hydroxide. The whole solution was allowed to settle and the precipitate was collected and rinsed with dilute ammonium hydroxide to obtain the alkaloid extracts. The extracts were collected and stored in the refrigerator at 4 °C and used for all analysis. For each experimental analysis, aqueous solution of the extracts were prepared and used.

2.4. Experimental animals

Wistar strain albino rats weighing 200–210 g were purchased from the breeding colony of Department of Veterinary Medicine, University of Ibadan, Nigeria. Rats were maintained at 25 °C, on a 12 h light/12 h dark cycle, with free access to food and water. They were acclimatized under these conditions for 1–2 week before the experiment. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

2.5. Preparation of tissue homogenate

The rat was immobilized by cervical dislocation and the whole brain tissue was rapidly isolated, rinsed with cold saline, placed on ice and weighed. This tissue was subsequently homogenized in appropriate Tris-HCl buffer (pH 7.4) (1/5 w/v) with about 10-up- and −down strokes at approximately 1200 rev/min in a Teflon glass homogenizer (Mexxcare, mc14 362, Aayu-shi Design Pvt. Ltd. India). The homogenate was centrifuged for 10 min at 3000 × *g* in a refrigerated centrifuge (KX3400C, KENXIN Intl. Co., Hong Kong) at 4 °C to yield a pellet that was discarded, and a supernatant (homogenate), which was used for all enzyme assays (Adefegha et al., 2015). The protein content of the brain homogenate was determined by the method of Lowry et al. (1951).

2.6. E-NTPDase activity assay

The effect of the alkaloid extracts on E-NTPDase activities (using both ATP and ADP as substrates) activities were determined in rat brain tissue homogenate incubated with appropriate dilutions of the extracts as described by Schetinger et al. (2007). The assay mixture consist of 1.5 mM $CaCl_2$, 5 mM KCl, 0.1 mM EDTA, 10 mM glucose, 225 mM sucrose,45 mM Tris-HCl buffer (pH 8.0), in a final volume of 200 mL. Twenty microliters of tissue homogenate (8–12 μg of protein) was added to the reaction mixture and pre-incubated at 37 °C for 10 min. The reaction was initiated by the addition of ATP or ADP as substrate to obtain a final concentration of 1.0 mM, followed by incubation for 20 min. The reaction was stopped by the addition of 200 μL of 10% trichloroacetic acid (TCA) to obtain a final concentration of 5%. Thereafter, the reaction mixture were chilled on ice for 10 min. The released inorganic phosphate (Pi) was assayed by the method of Chan et al. (1986). The E-NTPDase activities were thereafter, expressed as percentage inhibition of the reference thus: