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Effect of caffeine, caffeic acid and their various combinations on enzymes of cholinergic, monoaminergic and purinergic systems critical to neurodegeneration in rat brain—*In vitro*

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ABSTRACT

Caffeine and caffeic acid are two bioactive compounds that are present in plant foods and are major constituent of coffee, cocoa, tea, cola drinks and chocolate. Although not structurally related, caffeine and caffeic acid has been reported to elicit neuroprotective properties. However, their different proportional distribution in food sources and possible effect of such interactions are not often taken into consideration. Therefore, in this study, we investigated the effect of caffeine, caffeic acid and their various combinations on activities of some enzymes [acetylcholinesterase (AChE), monoamine oxidase (MAO) ecto-nucleoside triphosphate diphosphohydrolase (E-NTPase), ecto-5[1]-nucleotidase (E-NTDase) and Na^+/K^+ ATPase relevant to neurodegeneration *in vitro* in rat brain. The stock concentration of caffeine and caffiec acid and their various proportional combinations were prepared and their interactions with the activities of these enzymes were assessed (*in vitro*) in different brain structures. The Fe^{2+} and Cu^{2+} chelating abilities of the samples were also investigated. The results revealed that caffeine, caffeic acid and their various combinations exhibited inhibitory effect on activities of AChE, MAO, E-NTPase and E-NTDase, but stimulatory effect on Na^+/K^+ ATPase activity. The combinations also exhibited Fe^{2+} and Cu^{2+} chelating abilities. Considering the various combinations, a higher caffeine to caffeic acid ratio produced significantly highest enzyme modulatory effects; these were significantly lower to the effect of caffeine alone but significantly higher than the effect of caffeic acid alone. These findings may provide new insight into the effect of proportional combination of these bioactive compounds as obtained in many foods especially with respect to their neuroprotective effects.

1. Introduction

Caffeine is a well-known plant alkaloid found in leaves, seeds and nuts of a number of plants. It is a major constituent of coffee, cocoa, tea, cola drinks and chocolate. Caffeine is a central nervous system (CNS) stimulant that belongs to the methylxanthine class (Ding et al., 2014), and most widely consumed psychoactive food bioactive compound in the world (Nehlig et al., 1992). Research has shown that caffeine can induces a broad spectrum of cellular and pharmacological effects, including central nervous system (CNS) stimulation (Fredholm et al., 1999), potential cognitive enhancement properties (Abreu et al., 2011), and antioxidant properties (Noschang et al., 2009; Shi et al., 1991), among others. Evidence suggests that in humans, there is a relationship between chronic coffee/caffeine consumption and cognitive function (Ritchie et al., 2007; Santos et al., 2010). Acevedo et al. (2016) recently reported that caffeine exposure stimulates motor activity in the mouse spinal cord. Nevertheless, it has been reported that higher doses of caffeine induces negative effects such as anxiety, restlessness, insomnia, and tachycardia (Herz, 1999).

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Caffeic acid is a natural phenolic compound found in many plants and occurs in diets as part of fruits, vegetables, tea and wine (Clifford, 1999). Clifford (2000) reported that the absorption of this compound is directly associated with the amount of coffee consumed, being able to achieve 500 to 800 mg/day in individuals with high coffee intake (Clifford, 2000). It has been reported to have broad spectrum of bioactive activities including antidiabetic, antihypertensive, antioxidant, immunomodulatory, anti-inflammatory and neuroprotective properties (Chiou et al., 2017; Chan and Ho, 1997; Tota et al., 2010; Know et al., 2010). Studies have also revealed that caffeic acid exerts a protective effect against hydrogen peroxide-induced oxidative injury in the brain (Know et al., 2010), ameliorates cerebral ischemia (Zhou et al., 2006) and protect against brain damage as well as behavioral and biochemical changes caused by aluminium-induced toxicity (Lee et al., 2007). These bioactive phytochemicals that occur naturally in plants have begun to receive much attention as safe antioxidants, since they may be used to protect humans from deleterious effect of oxidative stress (Scalbert et al., 2005). On the other hand, the use of antioxidants may reduce the progression of neuronal degeneration such as Alzheimer's disease (AD) (Dokuyucu et al., 2016; Habtemariam, 2016; Atta-ur-Rahman and Choudhary, 2001).

Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) are key enzymes of the cholinergic system that play critical role in the pathogenesis of neurodegenerative diseases especially Alz AD. AChE is responsible for the hydrolysis of acetylcholine at the synaptic cleft of cholinergic neurons (Lendvai and Vizi, 2008). Impairment of the cholinergic neuron have been implicated in the pathogenesis of AD and major therapeutic strategy for managing this disease involves the use of cholinesterase inhibitors (Lendvai and Vizi, 2008).

Furthermore, ecto-nucleoside triphosphate diphosphohydrolase (E-NTPase) are class of enzyme well characterized in the central nervous system (Rocha et al., 1993; Schetinger et al., 2007). It hydrolyzes ATP and ADP into nucleoside monophosphates. The importance of E-NTPDase in the tissues is related closely to the presence of purinoreceptors on the cells (Beaudoin et al., 1997). Na^+/K^+-ATPase is an enzyme of the plasma membrane responsible for the active transport of sodium and potassium ions in the nervous system regulating the ionic gradient of neuronal cells. The enzyme is present in high concentration in brain cellular membranes, consuming about 40–50% of the ATP generated in this tissue (Ericinska and Silver, 1994) and plays a pertinent role in brain development and function. Studies have shown that inhibition of Na^+/K^+ATPase activity induces the release of neurotransmitters including glutamate, which is proposed to play a major role in neuronal death after excitotoxic and ischemic insults (Lees, 1991).

Monoamine oxidases (MAOs) are an integral proteins which are found in the outer mitochondrial membrane and are responsible for the regulation and metabolism of monoamine neurotransmitters in the brain and peripheral tissues (Walker et al., 1971). One of their primary role is the regulation of the levels of biogenic amines in the brain and peripheral tissues by catalyzing their oxidative deamination. Recent studies have established that MAOs are associated with psychiatric and neurological disorders, including depression, Parkinson's disease (PD) and AD. Inhibition of MAOs has been reported to possess therapeutic activities such as neuroprotective properties and functions as antidepressants and antianxiety agents and thus, could increase the level of neurotransmitters in the central nervous system (Saura et al., 1994).

Over the years, establishing therapeutic safe dose for caffeine has been quite challenging due to its CNS stimulatory effect and its ability to induce addiction. In lieu of this, recent research works are focusing on combining caffeine intake with other bioactive compounds with potential neuro-protective properties. This is aimed to offer a potential therapeutic synergy as well as ameliorate the possible detrimental effects that may be associated with caffeine consumption (Dorostghoal et al., 2012). Hence, this present study sought to investigate the effects of caffeine, caffeic acid and its proportional combinations on enzymes of cholinergic, monoaminergic and purinergic systems relevant to neurodegeneration in different brain structures – *in vitro*.

2. Materials and methods

2.1. Chemicals and reagents

Chemicals and reagents used such as ATP, AMP, ouabain, and malachite green were procured from Sigma-Aldrich, Inc., (St Louis, MO). Trichloroacetic acid (TCA), 5,51-dithiobisnitrobenzoic acid were sourced from Sigma-Aldrich, Chemie GmbH (Steinheim, Germany); methanol, pyrocatechol 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.

2.2. Sample preparation

The stock concentration of caffeine and caffiec acid (1 mg/ml) were prepared according to the method of Kantamala et al., 1990 and then kept at −4 °C for subsequent analysis. Thereafter, various combinations of caffeine and caffeic acid (75:25, 50:50, 25:75) were prepared and used. For the combination, caffeine was first added to each experimental analysis before caffeic acid.

2.3. Experimental animals

Wistar strain albino rats weighing 200–300 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.

2.4. Preparation of tissue homogenate

The rats were decapitated under ketamine and xylazine anaesthesia and rapidly dissected. The tissues; cerebral cortex (CC) and whole brain minus cerebral cortex (WBMC) were rapidly isolated, rinsed with cold saline, placed on ice and weighed. These tissues were singly 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 were centrifuged for 10 min at 3000 x *g* in a refrigerated centrifuge (KX3400C, KENXIN Intl. Co., Hong Kong) at 4 °C to yield a pellet that was discarded, and a supernatant, which was used for all enzyme assays (Akomolafe et al., 2016). The protein content of the homogenates were determined by the method of Lowry et al. (1951).

2.5. Acetylcholinesterase (AChE) activity assay

The acetylcholinesterase (AChE) enzymatic assay was determined by modification of the spectrophotometric method of Ellman et al. (1961) as previously described by Rocha et al. (1993). The reaction mixture (2 ml final volume) contained 100 mM K^+-phosphate buffer, pH 7.5 and 1 mM 5,5[1]-dithiobisnitrobenzoic acid (DTNB). The method is based on the formation of the yellow anion, 5,5[1]-dithio-bis-acid-nitroben-zoic, measured by absorbance at