Administration of Cannabis extracts causes alteration in brain and plasma nitric oxide concentration in rats

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ABSTRACT: Nitric oxide is a biological messenger molecule that plays important roles in controlling neurotransmitter release, neurodevelopment, memory function, and regulation of gene expression. In this study, the time course effects of varying dose of Cannabis extract on nitric oxide in the brain and plasma was investigated. Twenty four hours after the last treatment, animals were sacrificed under anaesthesia, the brain of the animals were harvested, mopped dry and stored on ice. Nitric oxide activity was assayed according to Griess reaction assay. In the plasma, the results shows that at 4 weeks, there was a significant increase (p < 0.05) in the concentration of nitric oxide in all doses with compared with control. 25 mg/kg dose has a higher concentration compared with 12.5 mg/kg and 50 mg/kg body weight dose. However, at 8 weeks, there was a decrease in concentration of nitric oxide in all the doses compared to 4 weeks, but when compared with the control group, only 12 mg/kg dose significantly decreased. In the brain however, 12.5 mg/kg dose shows no significant difference in NO concentration through the duration of exposure compared to the control group. However, 25 mg/Kg and 50 mg/Kg body weight dose of Cannabis significantly decrease NO concentration in the brain of rat. The results suggest that at high doses, NO is reduced in the brain and at low dose its increased. The decrease in NO concentration may affect the regulatory function of NO in the brain as nitric oxide affect serotonergic transmission which could explain the imopaired cognitive function in chronic Cannabis users.

KEYWORDS: Nitric oxide, Cannabis, Neurodevelopment, Cognitive function.
INTRODUCTION

Nitric oxide (NO) is synthesized by nitric oxide synthases (NOS), which plays a critical role in a number of physiological and pathological processes in humans. The physiological roles of NO depend on its local concentrations, as well as its availability and the nature of downstream target molecules (Jose et al., 2015). Its double-edged sword action has been linked to neurodegenerative disorders. Excessive NO production, as those evoked by inflammatory signals, has been identified as one of the major causative reasons for the pathogenesis of several neurodegenerative diseases. Moreover, excessive NO synthesis under neuroinflammation leads to the formation of reactive nitrogen species and neuronal cell death (Jose et al., 2015). There is an intimate relation between NO and neuroinflammation in the human brain.

The role of NO in neuroinflammation has been defined in animal models where this neurotransmitter can modulate the inflammatory process acting on key regulatory pathways, such as those associated with excitotoxicity processes induced by glutamate accumulation and microglial activation (Jose et al., 2015). Activated glia express inducible NOS and produce NO that triggers calcium mobilization from the endoplasmic reticulum, activating the release of vesicular glutamate from astroglial cells resulting in neuronal death. This change in microglia potentially contributes to the increased age-associated susceptibility and neurodegeneration (Jose et al., 2015).

The participation of oxidative stress in the development of several neurodegenerative disorders has been largely documented (Calabrese et al., 2007), with NO suggested as a starring character (Chabrier et al., 1999). The augmented nitration of proteins can be initiated by an increase in the production of NO during neuroinflammation and the generation of free radicals by dysfunctional mitochondria, which are commonly observed in various neurodegenerative disorders (Guix et al., 2005; Pacher et al., 2007).

Cannabis is a widely used substance that is illegal in some countries. Its non-medical consumption is of concern to regulatory agencies across the globe. Studies have shown that Cannabis causes neurotransmitter imbalances.

Since NO is a major regulatory biological messenger that plays a major role in control of neurotransmitters involved in cognitive function, we investigated the effect of Cannabis consumption on NO concentration in the brain and plasma of experimental rats.

MATERIALS AND METHODS

Experimental Design

A total of sixty male rats with body weight ranging from 80 to 90 g were used for the experiment. The rats were in good health condition and were kept in a plastic-wired animal cage. They were divided into twelve groups of five animals each. Each group were housed in separate cages and allowed to acclimatize for two weeks before the commencement of Cannabis exposure.

Collection and Extraction of Cannabis

Fresh Cannabis was obtained from the National Drug Law Enforcement Agency (NDLEA). It was air-dried at room temperature and pulverized using a clean, dry electric blender. Milled Cannabis (250 g) were soaked in 1000ml of petroleum ether in a round bottom flask for 24 hours, and decanted; the filtrate was kept and fresh 500ml of petroleum ether was added to the residue. This was repeated until the filtrate is colourless. The filtrate was concentrated using a rotary evaporator. The concentrated extract was dissolved in olive oil at 50mg/ml and kept in a dark bottle to prevent photolysis.

Blood and Tissue Collection

Each group of animals were sacrificed after an overnight fast using diethyl-ether anaesthesia at the end of the exposure time. The blood was collected by cardiac puncture into lithium-heparin tubes. The whole blood samples were centrifuged immediately for 10 minutes at 4000 rpm to separate plasma from red blood cells. The brain was removed for biochemical analysis. All other samples were stored at −20 °C until analysed.

Determination of Nitric oxide Concentration in Plasma

Nitric oxide activity was determined using the Griess reaction assay. The nitric oxide (NO) assay determines the production of NO from samples by estimating the concentrations of the end products of nitric oxide production (nitrite). Plasma sample (200 µl) was diluted to 500 µL with distilled water and 500 µL of 0.3 N NaOH was added to the sample. Samples were incubated at room temperature for 5 minutes. To the sample, 250 µl of 10% ZnSO4 was added to deproteinize and centrifuged at 4,000 for 30 minutes. A sample of the supernatant (400 µl) was taken and added to 400 µl of Griess reagent (Prepared freshly by mixing equal volume of Griess reagent A and Griess reagent B). Absorbance was read at 540nm after 30 min of incubation. The calibration curve used for determining NO concentration in the samples is shown in Figure 1.
Statistical Analysis

The results obtained are expressed as Mean ± standard error of mean (SEM). One-way analysis of variance (ANOVA) followed by turkey’s multiple range test was used to analyse the results. Values with p<0.05 were regarded as being significant, using the Statistical Package for Social Sciences (SPSS) version 17.0.

RESULTS AND DISCUSSION

In this study, we investigated the possibility of Cannabis consumption on interference with neurotransmitter function via alteration of NO metabolism. To do this, we investigated the time course effects of varying dose of Cannabis extract on nitric oxide in the brain and plasma.

Administration of Cannabis extract at 12.5 mg/kg dose shows no significant difference in NO concentration in the brain through the duration of exposure compare to the control group (Table 1). However, 25 mg/kg and 50 mg/kg body weight dose of Cannabis significantly decrease NO concentration in the brain of rats. Also in the plasma there is a significant increase in NO concentration in all doses at 4 weeks (Table 2).

At 8 weeks, decrease in NO concentration was observed compared to 4 weeks but the concentration still higher compared to control except 12.5 mg/kg dose, which is significantly lower than the control group. At 12 weeks, a further decrease was also observed in all doses but not significantly different from the control group except at 12.5 mg/kg dose.

These results suggest that at high dose NO is reduced in the brain and at low dose its increased. The decrease in NO concentration may affect the regulatory function of NO in the brain as NO control the release of neurotransmitter in the brain, which could explain the impaired cognitive function in chronic Cannabis users.

The role of NO in neuroinflammation has been determined in animal models associated with excitotoxicity processes induced by glutamate accumulation and microglial activation (Kim et al., 2009). NO released from activated microglia acts at the presynaptic site blocking the reuptake of glutamate, thus inducing the activation of N-Methyl-D-aspartate (NMDA) receptors and facilitating neuronal death (Rao et al., 2007, 2012). Selective neuronal death is typical of most neurodegenerative diseases including Parkinson’s disease, Alzheimer Disease, Amyotrophic Lateral Sclerosis and Multiple Sclerosis (Guix et al., 2005). Most neurodegenerative diseases including Parkinson’s disease, Alzheimer Disease, Amyotrophic Lateral Sclerosis and Multiple Sclerosis (Guix et al., 2005). The participation of oxidative stress in the development of several neurodegenerative disorders has been largely documented (Calabrese et al., 2007), with NO suggested as a starring character (Chabrier et al., 1999).

The augmented nitration of proteins can be initiated by an increase in the production of NO during neuroinflammation and the generation of free radicals by dysfunctional mitochondria, which are commonly observed in various neurodegenerative disorders (Guix et al., 2005; Pacher et al., 2007). Moreover, it has been demonstrated that NO is able to activate molecular elements, such as cyclooxygenase (COX) (Mollace et al., 2005), which is typically up-regulated in brain cells under inflammatory conditions (Mancuso et al., 2007). In addition, the combination of NO and free radicals like the superoxide anion will result in the formation of highly reactive peroxynitrite. Peroxynitrite can then nitrate tyrosine residues on proteins to 3-nitrotyrosine, induce lipid peroxidation, and cause DNA damage (Ischiropoulos and Beckman, 2003).

It has been suggest that NO interact with serotonergic neurotransmission by suppressing the overflow of the serotonin (Whitton et al., 1994). Nitric oxide regulates serotonin overflow by decreasing the level of hippocampal serotonin and dopamine by inhibiting the rate limiting enzyme tryptophan hydroxylase in serotonin metabolism (Gregers et al., 2000). Therefore decreases in NO observed in this study suggest possible increase in hippocampal serotonin in the experimental rats. There is increasing evidence showing that alterations in the NO signalling may be related with different diseases as it plays a key role in diverse neurodegenerative-
associated processes such as neuronal death, necrosis, apoptosis and autophagy (Calabrese et al., 2007). Nitric oxide is a biological messenger molecule which mediates diverse physiologic roles. NO mediates blood vessel relaxation by endothelium, immune activity of macrophages and neurotransmission of central and peripheral neurons (Dawson and Dawson, 1998). In the central nervous system, NO play important roles in neurotransmitter release, neurotransmitter reuptake, neurodevelopment, synaptic plasticity, and regulation of gene expression (Dawson and Dawson, 1998). The results obtained in this study therefore indicates that chronic exposure to Cannabis can impair the release and regulation of neurotransmitters mediated by NO which could be a trigger to neurodegenerative disorders in Cannabis users.

REFERENCES


Table 1: Nitric Oxide concentration (μM) in the brain. Values are expressed as Mean ± standard error of mean; n = 5. Significantly different (p < 0.05)

<table>
<thead>
<tr>
<th>Groups</th>
<th>4 Weeks</th>
<th>8 Weeks</th>
<th>12 Weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>41.83 ± 2.93</td>
<td>36.00 ± 1.36</td>
<td>34.33 ± 0.58</td>
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<tr>
<td>12.5 mg/Kg body weight</td>
<td>48.08 ± 4.43</td>
<td>38.08 ± 3.87</td>
<td>39.75 ± 5.06</td>
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<td>25 mg/Kg body weight</td>
<td>37.67 ± 2.09</td>
<td>40.58 ± 5.11</td>
<td>25.58 ± 1.25</td>
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<td>50 mg/Kg body weight</td>
<td>33.92 ± 1.58</td>
<td>37.67 ± 1.17</td>
<td>20.58 ± 2.19</td>
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Table 2: Nitric Oxide concentration (μM) in the Plasma. Values are expressed as Mean ± standard error of mean; n = 5. Significantly different (p < 0.05)

<table>
<thead>
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<th>Groups</th>
<th>4 Weeks</th>
<th>8 Weeks</th>
<th>12 Weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>9.33 ± 0.68</td>
<td>8.5 ± 0.48</td>
<td>7.67 ± 0.68</td>
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<tr>
<td>12.5 mg/Kg body weight</td>
<td>18.50 ± 1.98</td>
<td>6.83 ± 1.73</td>
<td>2.67 ± 0.68</td>
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<tr>
<td>25 mg/Kg body weight</td>
<td>30.17 ± 1.42</td>
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<td>6 ± 1.52</td>
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<tr>
<td>50 mg/Kg body weight</td>
<td>20.17 ± 5.79</td>
<td>10.17 ± 1.08</td>
<td>6 ± 1.52</td>
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