ACUTE TOXIC EFFECTS OF METHYL ALCOHOL ON THE RAT BRAIN: THE PROTECTIVE EFFECTS OF CAFFEIC ACID PHENETHYL ESTER

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ABSTRACT

Background: Efficiency of caffeic acid phenethyl ester (CAPE) in reducing free radicals generated by oxidative stress has been previously reported. In the present study, the protective effect of CAPE on methyl alcohol (MeOH) induced oxidative damages on rat brain were presented.

Methods: The rats were randomly divided into four groups as follows: Control, methotrexate (MTX) alone, MTX+MeOH, and MTX+MeOH+CAPE (CAPE treatment). All animals except the control group were treated with MTX for 7 days. MTX was diluted in sterile saline and administered (0.3 mg/kg/day) intraperitoneally (ip). At the eighth day, MeOH was administered (3gm/Kg) (ip) in MeOH+MTX and CAPE treatment groups. Four hours after MeOH administration in the CAPE group rats were treated with 10 μmol/kg CAPE (ip), serum physiologic (i.p.) in MeOH+MTX group. After eight hours, rats were anaesthetized and sacrificed. Malondialdehyde (MDA) levels, paraoxonase-1 (PON-1) activity were measured on the cerebral tissue.

Results: MTX+MeOH group compared to the MTX alone group; a statistically significant increase in MDA levels (p= 0.042) were detected. In addition, MTX+MeOH group than MTX MTX alone group in led to a statistically significant decrease in PON-1 activity (p= 0.018). CAPE treatment, significantly decrease in MDA levels was compared with MeOH+MTX (p= 0.001). However, CAPE treatment caused an increase on PON-1 activity in MeOH group, which was statistically significant (p= 0.009).

Conclusion: Consequently, it was demonstrated for the first time that CAPE prevents acute MeOH intoxication induced brain injury by reducing the increase in lipid peroxidation, and elevating the decrease in PON-1 activity.

Key words: CAPE, methyl alcohol, neurotoxicity, rat brain.

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Introduction

Methyl alcohol, also known as methanol (MeOH), is an important public health concern because of the selective actions of its neurotoxic metabolite, formic acid, on the central nervous system¹. MeOH is a decoloured and relatively zestier than ethanol². It is used in the industrial fabrication of numerous synthetical organic compositions.

Methanol is inexpensive and readily obtainable, thus, it is used in the manufacture of unlawful liquor in several countries³. MeOH by itself is has little toxicity. However, MeOH is converted in the liver into formol and afterwards formic acid, very toxic metabolites. Metabolic acidosis is the major biologic characteristic of MeOH intoxication.

Formic acid inhibits cytochrome oxidase activity in the mitochondrial inner membrane, leading to cytotoxic hypoxia followed by cell death⁴. The production of the toxic metabolites of MeOH are accompanied by an increase in nicotinamide adenine dinucleotide (NADH) level and the formation of superoxide anion that may be involved in lipid peroxidation and oxidative stress⁵,⁶.

Tissue toxicity is often accompanied by lipid peroxidation, indicated by elevated levels of malondialdehyde (MDA), reactive oxygen species, and diminished cellular antioxidant status⁷.

In humans, formic acid accumulates, and its metabolism is folic acid dependent. In MeOH intoxication, model rats do not develop metabolic acidosis, as their high liver folate content and rapid formic acid metabolism is able to appropriately metabolize formic acid; humans lack this capability.

Thus, exposure of rats to methotrexate (MTX) enable us to create a rat model to study MeOH toxicity. Folate-dependent formic acid metabolism can be impaired by MTX treatment⁸.
The administration of MeOH intoxication contain: the recension of metabolic acidemia, the management of folinic acid, and the procurance of a counterpoison to inhibit the metabolism of MeOH to formic acid. Even though both fomepizole and ethanol are efficient, fomepizole is the favoured antidote for MeOH intoxication (8).

MeOH has been commonly noted as a harmful toxin in humans and is recognised to damage both the optic tract and brain. It bring about classic neuropathological alterations for instance striatal necrosis, and loss of myelinated axons of the deep white matter. Symptoms variety from dizziness, headache and changes in consciousness, epileptic seizures, and mortality in serious cases.

MeOH exposure has been shown to cause increased apoptosis in neuronal cells (9). The pathophysiology of MeOH intoxication occurs by formic acid inhibiting mitochondrial function, leading to increased oxidative stress (6, 10). Oxidative stress is an imbalance between free radical production and antioxidant defense (11).

The brain is extremely impressionable to oxidative injury, due to an excessive rate of oxidative metabolic activity, high lipid ingredient, and a few concentration of free radical scavenger (10). MDA is an end product of lipid peroxidation. Moreover, it is a frequently measured biomarker of oxidative stress (12). Paraoxonase-1 (PON-1) is a serum enzyme, bound to high density lipoproteins (HDL) (11, 13). PON-1 plays a key role in the protection against oxidative damage of lipoprotein and biological membranes, as it hydrolyses lipid peroxides (14).

Antioxidant and neuroprotective agents were reported to be effective in reducing free radicals generated by oxidative stress (10). The protective effect of lipoic acid on MeOH induced free radical changes, and oxidative damage, in the rat cerebrum have been reported (19). Caffeic acid phenethyl ester (CAPE) is a neuroprotective molecule and a component of propolis, a mixture collected by honeybees. It has antioxidant, anti-inflammatory, and immunomodulatory properties and has been shown to inhibit lipoxygenase activities as well as suppress lipid peroxidation (13, 15). However, to the best of our knowledge, there is no experimental study examining the protective effects of CAPE against neuronal damage in the intoxicated rat brain induced by a toxic dose of MeOH.

We hypothesize that CAPE can be used as an antidote in MeOH poisoning. Thereby, the aim of this study is to evaluate the effect of CAPE against MeOH induced brain toxicity in a rat model. We evaluated the MDA levels and PON-1 activity to assess lipid peroxidation.

**Methods**

**Chemicals**

MTX was obtained from Koçak Farma, Istanbul, Turkey and diluted in saline. MeOH, and CAPE were purchased from Sigma Chemical Co. MeOH was diluted in saline, and administered as a 20% w/v solution. CAPE was dissolved in methylsulfinylmethane and diluted in saline.

**Animals**

We hereby declare that the experiments reported here comply with the current laws and regulations of the Turkish Republic on the care and handling of experimental animals. The study protocols were reviewed and approved by Institutional Animal Ethical Committee (Approval no: 2011/52).

Male Sprague Dawley rats (weight between 320 and 340 gm), used in the present experiment were maintained under standard laboratory conditions and were fed rat chow with water ad libitum. The rats were randomly divided into four groups as follows: Control, MTX alone, MTX+MeOH and MTX+MeOH+CAPE (CAPE treatment).

Each group consisted of eight animals. All animals except the control group were treated with MTX; we aimed to create a rat model of acute MeOH intoxication (19). MTX was diluted in sterile serum physiologic and administered (0.3 mg/Kg/day) (ip) for 7 days.

The control group was given an equal amount of vehicle only. On the eight day, MeOH (3 gm/Kg) was administered (ip) in MeOH, and CAPE groups. Four hours after MeOH treatment, 10 μmol/Kg CAPE (ip) was injected in the CAPE group; an equal amount of saline was injected in the MTX alone, MTX+MeOH, and control groups. After eight hours, rats were anaesthetized with (ip) an injection of ketamine (50 mg/Kg), decapitated, then the brains of the rats were removed meticulously and stored in a 10% formalin solution.

**Biochemical measurements**

The brain of the rats were excised meticulously and instantly stored at -50°C for biochemical research. Assays were carried out on the supernatants from cerebral tissue homogenates, designed at 14.000 rpm for 30 minute at +4°C.
The protein concentration of the tissue samples were measured by the method of Lowry(20), MDA levels was measured according to method of Ohkawa et al(21), PON-1 activity was measured spectrophotometrically by the modified Eckerson method(22).

**Statistical analyses**

Statistical analysis was carried out by using Statistical Package for the Social Sciences version 11.5 (SPSS 11.5 for Windows, Chicago, IL, USA). The results are expressed as mean ± standard deviation. The one-way analysis of variance (ANOVA) and post hoc multiple comparison tests (LSD) were performed on the data of biochemical variables to examine the difference among the groups. A p-value of < 0.05 was considered to be statistically significant.

**Results**

Biochemical values of the study are presented in Table 1.

<table>
<thead>
<tr>
<th>Groups</th>
<th>PON-1 (U/mg protein)</th>
<th>MDA (nmol/g wet tissue)</th>
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<tbody>
<tr>
<td>Control (I)</td>
<td>14.13±2.59</td>
<td>216.20±22.38</td>
</tr>
<tr>
<td>MTX alone (II)</td>
<td>11.84±2.49</td>
<td>312.35±50.76</td>
</tr>
<tr>
<td>MTX+MeOH (III)</td>
<td>7.79±4.70</td>
<td>357.87±59.76</td>
</tr>
<tr>
<td>MTX+MeOH+CAPE treatment (IV)</td>
<td>12.32 ±12.93</td>
<td>256.21±28.21</td>
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</tbody>
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<table>
<thead>
<tr>
<th>P values</th>
<th>I – II N.S.</th>
<th>0.001</th>
</tr>
</thead>
<tbody>
<tr>
<td>II – III</td>
<td>0.018</td>
<td>0.042</td>
</tr>
<tr>
<td>III – IV</td>
<td>0.009</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Data are presented as mean ± Standard deviation for 8 animals in each group. N.S: not significant, MDA: malondyaldehyde, PON: paraoxonase, MTX: methotrexate, CAPE: caffeic acid phenethyl ester, MeOH: methyl alcohol.

Table 1: Effects of MeOH on rat brain tissue MDA levels and PON-1 activities.

The MTX alone group (312.35±50.76 nmol/gm wet tissue) was compared with the control group (216±22.38 nmol/gm wet tissue), a statistically significant increase in MDA levels (p= 0.001) were detected; however, there was not any statistically significant decrease in PON-1 activity (p> 0.05).

The MTX+MeOH group (357.87±59.76 nmol/gm wet tissue) were compared to MTX alone group (312.35±50.76 nmol/gm wet tissue) and a statistically significant increase in MDA levels (p= 0.042) were found. In addition, the MTX+MeOH group (7.79±4.70 U/mg protein), compared to the MTX alone group (11.84±2.49 U/mg protein), had a statistically significant decrease in PON-1 activity (p= 0.018). In the CAPE treatment group (256.21±28.21 nmol/gm wet tissue), a statistically significant decrease in MDA levels was observed when compared with the MeOH+MTX group (357.87±59.76 nmol/gm wet tissue) (p= 0.001).

Additionally, in the CAPE treatment group (12.32 ±12.93 U/mg protein), an increase in the PON-1 activity, when compared to the MeOH group (7.79±4.70 U/mg protein), was statistically significant (p= 0.009).

**Conclusions**

This study is the first to evaluate the oxidative status and protective effect of CAPE, by determining the levels of the antioxidant enzyme PON-1, along with MDA, in rat cerebral tissue, when exposed to MeOH. The enzyme like PON-1 plays an important role in free radical and peroxide metabolism, and is responsible in part for protecting cells against oxidative stress(14).

MDA is the catabolite of several main chain reactions that lead to oxidation of unsaturated lipids; thus, it serves as a recognized indicator of oxidative damage. It is recognized that enhanced levels of MDA serve as a marker of lipid oxidation extent in the cerebrum(23). In a previous study, it was demonstrated that exposure to MeOH caused an increased production of free radicals and increased oxidative damage to proteins throughout the brain and optic nerve(24).

In our study, when we compared the group with MTX + MeOH, to the MTX alone group, we found increased MDA levels, and decreased PON-1 activity, in rat cerebral tissue. In this study, as in a previous study(24), oxidative stress and lipid peroxidation is induced by MeOH in rat brain tissue.

The increase in the levels of MDA demonstrate enhanced lipid peroxidation, leading to tissue damage, and an inability of antioxidant defense mechanisms to prevent the formation of excess free radicals. First, we demonstrated that MeOH intoxication leads to a decrease in PON-1 activity in rat cerebral tissue. PON-1 is a pivotal endogenous antioxidant. Peroxidative role for paraoxonase inhibits high-density lipoprotein oxidation; therefore, it can protect low density lipoproteins from free radical induced oxidation(25). PON-1 expression may be down regulated by an oxidative stress related phenomena. Therefore, a decrease of PON-1 activity in rat cerebral tissue as a result of MeOH induced neurotoxicity is related to oxidative stress.
The most characteristic magnetic resonance imaging and pathological finding in MeOH toxicity is bilateral putaminal necrosis, which may have varying degrees of hemorrhage\textsuperscript{(26,27)}.

One of the limitations of our study was that histopathology was unable to be included. Bilateral cerebral ischemia of the basal ganglia is the most well known finding of MeOH intoxication\textsuperscript{(27,28,29)}. Injury to the brain likely represents a direct toxic effect of MeOH metabolites, anoxia, and acidosis\textsuperscript{(27,28)}.

Oxidative stress contributes directly to necrosis and apoptosis through a number of pathways in ischemic and anoxic tissue\textsuperscript{(28,29)}. The affected area of the brain receives insufficient oxygen and glucose to maintain cell function, thus altering cellular activity at the electrochemical and metabolic level, with an accompanying release of toxic products such as MDA\textsuperscript{(28,29)}.

CAPE has powerful antiapoptotic, anti-inflammatory, and antioxidant properties; therefore, CAPE has a potential protective role against hypoxic ischemic and toxic injury\textsuperscript{(30,31)}. CAPE is one of the most powerful lipophilic antioxidants. CAPE prevents glutamate, MTX and neonatal hypoxic ischemic injury induced neurotoxicity. Uzar et al. have shown that CAPE treatment decreased cerebral ischemic damage in rats\textsuperscript{(31-34)}.

In this study, CAPE substantially diminished the MeOH stimulated fatty acid oxidation in the rat cerebrum. Considering the prevention of oxidative injury with CAPE treatment, numerous researchers attributed the preventive activities of CAPE to its antioxidative function and anti inflammatory properties\textsuperscript{(18,20)}. In addition, the PON-1 activity in rats given CAPE and MeOH was significantly higher than the group treated with MeOH. This finding suggests that CAPE may prevent a reduction in PON-1 activity with MeOH poisoning. CAPE prevents the down regulation of PON-1 by destroying free radicals. This finding is another indication that CAPE prevents lipid peroxidation in the rat cerebrum. These results indicate that CAPE might be a new agent to preserve cerebral tissue from oxidative damage due to MeOH induced neurotoxicity in rat cerebral tissue. In conclusion, the findings of the present study demonstrate when acute MeOH is administered as a 3 gm/Kg dose, neurotoxicity is observed in a rat brain, likely the result of reactive oxygen species. It was demonstrated for the first time that CAPE prevents an acute MeOH induced brain injury by preventing an increase in lipid peroxidation, and further increasing PON-1 activity.

References


Acute toxic effects of methanol alcohol on the rat brain: the protective effects of caffeic acid


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