Progesterone Regulates Superoxide Dismutase, Catalase, Malondialdehyde and Cholinesterase Activities in Trimethyltin-Induced Hippocampal Damage in Adult Male Wistar Rats

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ABSTRACT

Background: Reactive oxygen species (ROS), also known as free radicals, are normal by-products of mitochondrial respiratory chain activity. Overproduction of these free radicals can cause oxidative damage to bio-molecules, (lipids, proteins, DNA), which eventually can lead to chronic diseases like stroke, aging and other neurodegenerative diseases.

Objective: Hence, this study was designed to investigate the effect of progesterone (PROG) on antioxidant biomarkers such as superoxide dismutase (SOD), catalase (CAT) and malondialdehyde (MDA) as well as a biomarker in environmental study, cholinesterase (CHOL); following trimethyltin (TMT) induced neurotoxicity in the hippocampus of adult male Wistar rats.

Methods: Twenty four adult male Wistar rats were used in the study and divided into three groups. Group A as positive control was given 0.2 ml of normal saline (NS), group B, as negative control was administered 8 mg/kg (TMT) start dose only, while group C was administered 8mg/kg TMT start and 16 mg/kg of progesterone (TMT-PROG). All treatments were done intraperitoneally. The brains were excised and homogenized for enzyme analysis.

Results: The result of this study revealed that, in the TMT treated animals, SOD and CAT activities were significantly reduced while MDA, CHOL levels were significantly increased as compared to the animals that received only normal saline. In the TMT-PROG group, the activities of SOD and CAT were significantly increased while the activity of CHOL and MDA quantity were significantly reduced as compared to TMT-treated group. Values are presented as mean±SEM (*P<0.05).

Conclusion: After the biochemical analysis of the hippocampus of adult male Wistar rats, using superoxide dismutase, catalase, malondialdehyde, and cholinesterase to study oxidative, the study revealed that progesterone (16mg/kg) reduced the adverse effect caused in the hippocampus by trimethyltin (8mg/kg).

Keywords: Trimethyltin, Reactive oxygen species, Superoxide dismutase, Catalase, Malondialdehyde, Cholinesterase, Progesterone.

INTRODUCTION

Reactive oxygen species (ROS) are normal by-products of mitochondrial respiratory chain activity (DiMauro & Schon, 2008). Over production of ROS (oxidative stress) is a central feature of all neurodegenerative disorders and the
intrinsic mitochondrial apoptotic pathway which forms the major route of neurodegeneration (Lin & Beal, 2006). ROS concentration is mediated by mitochondrial antioxidants such as manganese superoxide dismutase and glutathione peroxidase. And also, neuronal biochemical composition is mainly susceptible to ROS since it involves pools of unsaturated lipids that are labile to peroxidation and oxidative modification (Butterfield et al., 2002). Some chemicals are known to increase the concentration of reactive oxygen species in biological environment, for the example is trimethyltin (Geloso et al., 2004).

Trimethyltin (TMT) is a colourless to white, sand-like solid with a strong, unpleasant odour, which Structural Formula (CH₃)₃Sn. It is widely used in agro-allied companies, primarily as an insect, bacteria and fungus control agent in preserving wood, textiles, leather, and paints. Trimethyltin (TMT) was used in this study to induce brain damage in the rats, because it has been known to be a potent neurotoxicant which produces a dose-dependent degeneration of neurons (Whittington et al., 1989). TMT induces its neurodegeneration ability in several ways, but it is majorly characterised by neuronal death in the limbic system, particularly in the hippocampus. The death to the neurons as a result of TMT intoxication can be followed by several other conditions, like reactive gliosis, epilepsy, neurobehavioral alterations increased production of reactive oxygen species (Geloso et al., 2004). Therefore progesterone a hormone which has been implicated to enhance damaged brain functions, where it is synthesized and secreted by nerve tissues (King & Brucker, 2010) can act to suppress the effects of trimethyltin.

Progesterone has been implicated in brain functions, where it is synthesized and secreted by nerve tissues (King and Brucker, 2010). A study by Zhang and his colleagues suggested that progesterone has regenerative potentials on the damaged neurons (Zhang, et al., 2010). Progesterone aids neuroregeneration by increasing the concentration of macrophages and microglia at injured sites (Schneider et al., 2003) and by increasing the circulation of endothelia progenitor cells in the brain (Espinoza and Wright, 2011). The hormone is also believed to reduce the concentration of oxygen free radicals in neuronal environment (Sriram, 2007).

Therefore this study focused on the use of progesterone to improve the activities of some enzymes of the brain after trimethyltin administration in adult male Wistar rats.

MATERIALS AND METHODS

Experimental Animals

Twenty four (24) adult male rats (Rattus norvegicus), weighing between 220-250g were used for this study. The rats were procured from the animal holdings in the Department of Zoology, University of Ilorin, and were allowed to acclimatize in the College of Health Sciences, University of Ilorin animal house, for 14 days prior to commencement of the treatments. The animals were housed in cages under normal light/dark cycle and at a normal room temperature/humidity. Food and water were available ad libitum.

Experimental Design

The rats were randomly divided into three (3) groups, with each group containing eight (8) animals. The animals in group A (NS) were administered 0.2 ml of normal saline intraperitoneally for 26 days, group B (TMT) animals were administered single dose of 8mg/kg of trimethyltin intraperitoneally and these animals were sacrificed after the 21st day of the administration. Group C (TMT-PROG) were given 8 mg/kg trimethyltin start, followed by 16 mg/kg of progesterone administration starting from 22nd day of trimethyltin administration. The administration of progesterone in this group lasted for the remaining five days of the treatments.

A single dose of trimethyltin was administered intraperitoneally to the rats to
induce the hippocampal cell damage (Brock and O'Callaghan, 1987). The dose (16 mg/kg) of Progesterone was determined from previous studies (Cutler, et al., 2006; Li et al., 2012; Gilmer et al., 2008; Chen et al., 2008). The first dose was given one (1) hour post injury for rapid absorption; the second dose was given six (6) hours later post 1st progesterone dose for gradual absorption and subsequent doses of progesterone was given every twenty-four (24) hours for five (5) days.

The animals were used in accordance to the Guidelines of national Research Council Guide for the care and Use of Laboratory Animals (National Research Council, 2011) and according to the principles of Good Laboratory Procedure (GLP) (WHO 1998).

Perfusion Fixation, Tissue Collection and Preparation of Brain Homogenate for Enzyme Studies: Transcardial perfusion fixation was done with 0.4% paraformaldehyde after which the brain was excised. Certain size (g), 1g from the individual brain tissues for enzyme study was homogenized in 4.0 ml of 5% sucrose solution (1:4), after which they were centrifuged for 10 minutes at 3000 rpm. The supernatants were kept frozen in the freezer at -4°C and were later used for the biomarkers (Superoxide dismutase, Catalase, Malondialdehyde and Cholinesterase) analysis.

Determination of Superoxide Dismutase (SOD) Activity: An aliquot of 0.2 ml of diluted tissue supernatant was added to 2.5 ml of 0.05 m carbonate buffer (pH 10.2) to equilibrate in the spectrometer and the reaction was started by addition of 0.3ml of freshly prepared 0.3mM epinephrine to the mixture which was quickly mixed by inversion. The reference curvet contained 2.5 ml of carbonate buffer, 0.3 ml epinephrine and 0.2 ml of H2O as blank while increase in absorbent was monitored every 30 seconds for 150 seconds.

Increase in absorbent per minute = \( \frac{A_3-A_0}{1.5} \)

\( A_3 \) = absorbance after 30 seconds

\( A_0 \) = absorbance after 150 seconds

% inhibition = \( \frac{100 - (100 \times \text{increase in absorbance for substrate})}{\text{increase in absorbent for blank}} \)

One unit of SOD activity is given as the amount of SOD necessary to cause 50 % inhibition of the oxidation of epinephrine (Misra and Fridovich, 1972).

Determination of Catalase (CAT) Activity: Catalase activity was assayed according to the method of Sinha (1972). To 0.4 ml of hydrogen peroxide (0.2 M), 1 ml of 0.01 M phosphate buffer (pH 7) was added, followed by the addition of 0.1 ml clear supernatant of the tissue homogenate (10 %w/v) and gently swirled at room temperature. The reaction of the mixture was stopped by adding 2 ml dichromate-acetic acid reagent (5 % K2Cr2O7 prepared in Glacial acetic acid). The change in the absorbance was measured at 620 nm and recorded after 3 minutes interval. Percentage inhibition was calculated using the equation.

\[ \text{% inhibition rate} = \left( \frac{\text{absorbance of blank} - \text{absorbance of sample}}{\text{absorbance of blank}} \right) \times 100 \]

(Sinha, 1972).

Determination of Malondialdehyde (MDA): The concentration of MDA was quantified according to the method of Beuge and Aust as outlined below; A portion of TBA reagent (2 ml of 0.7 % and 1 ml of TCA) were added to 2 ml of the sample. The mixture was thoroughly heated in a water bath at 100°C for 20 minutes. It was cooled and centrifuged at 4000 rpm for 10 minutes.

The absorbance of the supernatant was read at a wavelength of 540 nm against the reference blank of distilled H2O after centrifuging for another 10 minutes.

Concentration of MDA= \[ \frac{\text{Absorbance of sample}}{\text{extinction coefficient}} \]

Extinction coefficient of MDA= 1.56x10³nm⁻¹cm⁻¹. TBA: 0.7% i.e 0.7g in 100mls. TCA: 20% i.e. 2g in 100mls (Beuge and Aust, 1978)

Determination of Cholinesterase (CHOL): By Test Principle, butyrylthiocholine in the presence of cholinesterase gets converted to Butyrate and Thiocolchicol. The Thiocolcholin formed reacts with hexacyanoferrate (III) which is yellow and converts it into
Hexacyanoferrate (II) which is clear. The rate of this reaction is measured in a kinetic reaction.

The estimation was done at 450nm, after which working reagent 100 ul and sample 2 µl were pipetted into tests tubes with distilled water and incubated at 37 °C. The mixture was read at an initial absorbance after 90 seconds of incubation at the assay temperature. Stopwatch was started and the measurement was taken at 30, 60, 90 seconds intervals.

Calculation: Activity of cholinesterase \( \frac{\Delta \text{Abs}}{\text{minutes}} \times 55000.\Delta \text{Abs} = \text{change in absorbance} \) (Fortress Diagnostic, 2013).

**Ethical approval**

Ethical approval for this study was given by the Postgraduate School ethical review committee, University of Ilorin.

**Data Analysis**

The results were analyzed for statistical significance using one-way ANOVA followed by Dunnet S test (SPSS-15). Differences between groups were considered significant at \( p < 0.05 \) levels. All values were expressed as Mean ± SEM.

**RESULTS**

The determined biomarkers gave the results that the rats that received trimethyltin in both the negative controls and progesterone treated group showed swollen limbs as at the second day of administration which later disappeared. Also as at the second week of administration, the rats administered with normal saline maintained their body weight and some gained extra body weight but in the trimethyltin treated rats, both in the positive control and progesterone treated group reduced in body weights. Towards the end of the second week, some of the rats that collected trimethyltin alone became aggressive and other became very restless but in the progesterone treated group only one became aggressive and others were slow and sluggish.

Treated rats were given 8 mg/kg of Trimethyltin and 16 mg/kg of Progesterone and the enzyme status of the rats across the experimental groups as shown in figures 1, 2,& 4 as well as lipid peroxidation marker in figure 3 (Table 1).

The animals in group A (NS) that received normal saline showed high concentrations of superoxide dismutase which was significantly reduced to the concentration of superoxide dismutase in the hippocampus of the animals in group B (TMT) and C (TMT-PROG) (fig. 1.0). Also the concentration of superoxide dismutase in the hippocampus of animals in group C (TMT-PROG) was increased compared to the concentration of superoxide dismutase in hippocampus of animals in group B (TMT), (fig.1.0), but it was not statistically significant.

The animals in group B (TMT), (fig.2.0) showed decreased concentration of catalase in the hippocampus but the concentration of catalase was significantly increased in the hippocampus of the animals in group C (TMT-PROG).

The concentration of malondialdehyde in figure 3.0 in the hippocampus of animals in group B (TMT) was significantly increased as compared to the concentration of malondialdehyde in the hippocampus of animals in group C (TMT-PROG).

The concentration of cholinesterase in the hippocampus of animals in group B (TMT) was increased when compared to the concentration of cholinesterase in the hippocampus of animals in group C (TMT-PROG) (fig.4.0). This increment in concentration was not statistically significant.

**Table 1: Effect of Normal Saline, Trimethyltin and Progesterone Administrations on brain Enzymes.**

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>Superoxide Dismutase U/100mg wet weight</th>
<th>Catalase U/100mg wet weight</th>
<th>Malondialdehyde Nmol/100mg wet weight</th>
<th>Cholinesterase U/100mg wet weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>N/S (control)</td>
<td>88.326±3.68511</td>
<td>71.333±3.333</td>
<td>133.266±1.10202</td>
<td>143.333±29.96961</td>
</tr>
<tr>
<td>TMT</td>
<td>41.666±5.27046*</td>
<td>50.500±4.4026*</td>
<td>163.666±7.53820*</td>
<td>773.500±50.76137</td>
</tr>
<tr>
<td>TMT-PROG</td>
<td>54.583±6.87437*</td>
<td>78.333±3.6575</td>
<td>127.350±3.51101</td>
<td>587.166±79.63668</td>
</tr>
</tbody>
</table>

*\( P<0.05 \) Compared to NS
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DISCUSSION

Neurodegenerative disease is a condition in which neurons from brain and spinal cord lose their functional coordination of voluntary muscles (ataxia) or sensory dysfunction (dementia). This further leads to Mitochondrial (Mt) dysfunctions and excitotoxicity and finally apoptosis. Free radical generation and oxidative stress catalyzed by redox metals have been shown to play pivotal role in regulating redox reactions in-vivo contributing to reactive nitrogen species (RNS) and reactive oxygen species (ROS), which are major actors in neurodegeneration (Lin and Beal, 2006). Oxidative stress arises due to disturbed equilibrium between pro-oxidant/antioxidant homeostasis that further takes part in generation of ROS and free radicals which are potentially toxic for neuronal cells. Enzymatic antioxidants are exogenous or endogenous molecules that act against any form of oxidative stress. They neutralize ROS and other kinds of free radicals produced as consequence of oxygen species (OS) (DiMauro and Schon, 2008). In this study, the effect of progesterone against the neurotoxic effects of trimethyltin in the hippocampus of adult male Wistar rats was evaluated by checking the activities of the following antioxidant parameters like superoxide dismutase, catalase, malondialdehyde and cholinesterase.
Biomarkers of free radicals are the enzymes such as superoxide dismutase and catalase as well as malondialdehyde as a marker of lipid peroxidation. Cholinesterase is generally known as a biomarker in environmental and occupational medicine. Butyryl cholinesterase also referred to as pseudocholinesterase is found in the liver, pancreas, heart, serum and brain. The biological function of cholinesterase is unknown but it serves as an important marker for insecticide poisoning (Fortress Diagnostic, 2013).

Generally, the rats in group one (Normal Saline) and group two (Trimethyltin alone) served as negative and positive control groups respectively and these groups were used as reference points in this study. Figure 1.0 shows that there was significant reduction in the level of superoxide dismutase activity in both groups that was administered only trimethyltin and the group administered trimethyltin start followed by progesterone to the group that took normal saline, but there was no significant difference in activity of superoxide dismutase in the two groups that were treated with trimethyltin. This result suggests that progesterone was able to regulate partially the activities of superoxide dismutase. Progesterone might have an ameliorative effect but not statistically significant, in the hippocampus of adult male Wistar rats. This study corresponds with the works of Pajovic and Saicic, (2008); Li et al., (2012); Cai et al., (2015). Figure 2.0 revealed that the activity of catalase was significantly increased in the rats treated with trimethyltin start followed by progesterone as compared to the rats in the group that took normal saline, but there was no significant difference in activity of superoxide dismutase in the two groups that were treated with trimethyltin. This result suggests that progesterone was able to regulate partially the activities of superoxide dismutase. Progesterone might have an ameliorative effect but not statistically significant, in the hippocampus of adult male Wistar rats. This study supports the findings of Li et al., (2012); Cai et al., (2015); Oztekin et al., (2006). Butyrylcholinesterase (BuChE) or pseudocholinesterase, or plasma cholinesterase, is a nonspecific cholinesterase enzyme that hydrolyses many different choline-based esters (Bodur and Cokugras, 2005). In humans, it is made in the liver, found mainly in blood plasma, and encoded by the BCHEgene (Lockridge, 1988). The biological function of cholinesterase is unknown but it serves as an important marker for insecticide poisoning (Fortress Diagnostic, 2013). BuChE activity has been suggested to progressively increase in patients with Alzheimer's disease (Greig et al., 2002). Figure 4.0 revealed that cholinesterase activities of rats in the group administered with trimethyltin start followed by progesterone showed significant increase as compared to those of the rats in the group.
that received normal saline but showed reduction in levels to those in the group that took trimethyltin alone. In this study, progesterone’s performance as an ameliorative agent on cholinesterase activity in the hippocampus of adult male Wistar rats was minimal. It has been reported that estrogens regulate the cholinergic neurons that project to cerebral cortex and hippocampus, where they play an important role in cognitive function (McEwen, 2001).

In conclusion, progesterone was able to ameliorate the effect of trimethyltin by reducing the progression of free oxygen species in the hippocampus of adult male Wistar rats.

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