PCB (AROCLOR 1254) INDUCES OXIDATIVE DAMAGE IN RAT BRAIN REGIONS: PROTECTIVE IMPACT OF MELATONIN SUPPLEMENTATION

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BACKGROUND

PCBs are a family of halogenated aromatic hydrocarbons with 209 possible congeners. Half-life: 2-8 yrs (Philips et al., 1989).

PCBs are lipophilic and resistant to biological decomposition and can accumulate in higher tropic levels through the food chain (Kamrin and Ringer, 1994).

Most of the systemic toxic and biological effects of PCBs are mediated by the aryl hydrocarbon receptor (AhR) (Tilson and Kodavanti, 1998).

Aroclor 1254 is a commercial mixture of polychlorinated biphenyls.
PCBs induced toxic manifestations are associated with the production of ROS (Tharappel et al., 2002).

Aroclor 1254 induces oxidative stress in ventral prostate, testis, lung, kidney (Sridhar et al., 2004; Murugesan et al., 2005; Krishnamoorthy et al., 2005; Banudevi et al., 2006).

The brain like many other tissues has a range of antioxidant defenses, which help to maintain a balance redox status (Pajovic et al., 2003).

ROS are closely involved in Parkinson’s disease, schizophrenia and Alzheimer’s disease (Smythies, 1999).
Mechanism of Action of PCB

- **L** interacts with **AhR** in the **CYTOSOL**
- **AhR+L** transformation
- **ARNT** activates the receptor
- **AhR+L** in the **NUCLEUS**
- **XRE** regulatory gene
- **TRANSCRIBED mRNA**
- **TRANSLATION**
- **CYTOCHROME P-450 & OTHER PROTEINS**
- **MULTIPLE BIOLOGICAL RESPONSE**
H₂O₂ + O₂ → H₂O + O₂

Cellular Respiration

ONOO⁻ → H₂O₂

Catalase

O₂⁻ → H₂O₂

SOD

Environmental factors

Oxidative Burst

O₂⁻ → NO⁻

DNA Damage

GSH → GSSG

GSH

SOD

Oxidized protein

Lipid Peroxidation

GRd

MDA

4-HNE

Other by-products

GSH

GRd

(Reiter, 2006)
The Cu/ Zn SOD (SOD1, the most affected antioxidant enzyme during neurodegeneration (Olanow, 1993; Kim et al., 2000), catalyzes the dismutation of superoxide into hydrogen peroxide.

Cu-Zn SOD is mainly expressed in neurons (Peluffo et al., 2005).

In neuronal cells, endogenous Cu-Zn SOD is normally expressed but is rapidly down regulated after several types of acute brain insults (DeKosky et al., 2004; Peluffo et al., 2005) rendering the brain more susceptible to oxidative stress.

GPX4 is the only major antioxidant enzyme known to directly reduce phospholipid hydroperoxides within membranes and lipoproteins (Yant et al., 2003).
GPx4 shows a unique cellular distribution in the brain compared to GPx1 (Savaskan et al., 2007).

(GPx-4/ phGPx) is a unique membrane of the selenium dependent enzyme in mammals with a pivotal role in brain development and function. GPx4 protects cortical neurons from oxidative injury and amyloid toxicity (Ran et al., 2006).

During postnatal development, GPx-4 mRNA is mainly distributed in cortex, hippocampus and cerebellum, indicating a neuronal rather than glial origin.

In fully mature adult brain, GPx-4 is expressed in all neuronal cell layers and most prominently in the hippocampus (Savaskan et al., 2007).
\[ 2O_2^- + 2H^+ \xrightarrow{\text{ecSOD}} H_2O_2 + O_2 \]

**Extracellular space**

**Cytoplasm**

Membrane bound phGPx

\[ \text{LOOH} + 2\text{GSH} \xrightarrow{\text{GRd}} \text{GSSG} + \text{LOH} + O_2 \]

\[ 2O_2^- + 2H^+ \xrightarrow{\text{Cu/Zn SOD}} H_2O_2 + O_2 \]

\[ 2H_2O_2 + 2\text{GSH} \xrightarrow{\text{GPx}} H_2O + O_2 + \text{GSSG} \]

**Mitochondrion**

\[ 2O_2^- + 2H^+ \xrightarrow{\text{MnSOD}} H_2O_2 + O_2 \]

\[ 2H_2O_2 + 2\text{GSH} \xrightarrow{\text{GPx}} H_2O + O_2 + \text{GSSG} \]

**Peroxisome**

\[ 2H_2O_2 \xrightarrow{\text{CAT}} H_2O + O_2 \]

**Nucleus**

\[ 2O_2^- + 2H^+ \xrightarrow{\text{Cu/Zn SOD}} H_2O_2 + O_2 \]

\[ 2H_2O_2 + 2\text{GSH} \xrightarrow{\text{GPx}} H_2O + O_2 + \text{GSSG} \]

\[ \text{GRd} \]
The **cerebellum** is involved in the coordination of movement and balance. Many areas of the **cerebral cortex** process sensory information or coordinate motor output necessary for control of movement. The **hippocampus** has long been implicated in memory function in humans and other animals (Squire, 1992).

Cerebellum, Cerebral cortex and hippocampus are highly sensitive to oxidative stress (Gomez et al., 2005; Esparza et al., 2005).
Neurotoxic effects of PCB

Aroclor 1254 alter the synaptic transmission and plasticity in hippocampus of the rat (Gilbert and Liang, 1998).

PCBs are neuroendocrine disrupting chemicals (Kester, 2000; Gore, 2001). PCBs have been shown to reduce tryptophan hydroxylase activity and 5-HT concentration in rat brain regions (Chu et al., 1996; Khan and Thomas, 2004).

PCBs affect hippocampal function in different ways— alters dendritic growth and actin cytoskeleton (Tanq et al., 2007; Lein et al., 2007).

PCB modulates activities of Membrane bound ATPases by inducing the levels of free radicals in hippocampus and hypothalamus (Muthuvel et al, 2006; Sridevi et al., 2007).
CREATINE KINASE

CK (EC 2.7.3.2) are a family of enzymes that catalyze the reversible transfer of a phosphoryl group between ATP and Creatine (Lott and Abbot, 1989).

CK is used as a reliable marker in the assessment of myocardial, muscular and cerebral damage (Bell and Khan, 1999).


(CK) BB plays a key role in regulation of ATP level in neural cells.
Acetylcholinesterase

The cholinergic system plays a crucial role in cognitive function, in which choline esterases are ubiquitous constituents.

Vincent et al. (1992) reveals that the PCBs exposure affect the cholinergic system in experimental animals.

Evidence in the literature showing that the activity of AchE inhibited by free radical formation (Tsakiri et al., 2000).
Amyloid Precursor Protein

Integral membrane glycoprotein expressed in many tissues and concentrated in the synapses of neurons and APP695 is exclusive to neurons (Butterfield, 2004).

Proteolysis generates Amyloid β, a 39-43 amino acid peptide

Aβ1-42 leads to an influx of Ca\(^{2+}\) in to the neuron resulting in loss of intracellular Ca\(^{2+}\) homeostasis, mitochondrial dysfunction, and ultimately cell death (Butterfield and Kimball, 2004)

Oxidative stress and Aβ-production are proportionally linked to each other because amyloid β induces oxidative stress invivo and invitro (Tabner et al., 2005) and oxidative stress induces the Aβ (Tamagno et al., 2005; 2008).
Oxidative stress promotes intra cellular accumulation of Aβ through enhancing the amyloidogenic pathway in SHSY5Y neuroblastoma cells (Misonou et al., 2006).

Expression and the activity of BACE1 is increased by oxidants and lipid peroxide productants HNE (Tonge et al., 2005; Tamagno et al., 2007)

\[
\text{A\beta(1-42):}
\]

\[
\text{H}_2\text{N-Asp}^1\text{-Ala}^2\text{-Glu}^3\text{-Phe}^4\text{-Arg}^5\text{-His}^6\text{-Asp}^7\text{-Ser}^8\text{-Gly}^9\text{-Tyr}^{10}\text{-Glu}^{11}\text{-Val}^{12}\text{-His}^{13}\text{-His}^{14}\text{-Gln}^{15}\text{-Lys}^{16}\text{-Leu}^{17}\text{-Val}^{18}\text{-Phe}^{19}\text{-Phe}^{20}\text{-Ala}^{21}\text{-Glu}^{22}\text{-Asp}^{23}\text{-Val}^{24}\text{-Gly}^{25}\text{-Ser}^{26}\text{-Asn}^{27}\text{-Lys}^{28}\text{-Gly}^{29}\text{-Ala}^{30}\text{-Ile}^{31}\text{-Ile}^{32}\text{-Gly}^{33}\text{-Leu}^{34}\text{-Met}^{35}\text{-Val}^{36}\text{-Gly}^{37}\text{-Gly}^{38}\text{-Val}^{39}\text{-Val}^{40}\text{-Ile}^{41}\text{-Ala}^{42}\text{-COOH}
\]

**Methionine 35** has been show to be a critical residue in Aβ-1-42 mediated oxidative stress and neurotoxicity
A  Amyloidogenic pathway

B  Non-Amyloidogenic pathway

Barron et al., 2006
The main secretory product of pineal gland

Lowering circulating levels of melatonin also exaggerates the oxidative damage to tissues that are subjected to increased oxidative stress (Reiter, 1999).
(i) Direct free radical scavenger

(ii) Indirect antioxidant when stimulating antioxidant enzymes

(iii) Stimulates the synthesis of glutathione

(iv) Increase the efficiency of mitochondrial electron transport chain, thereby lowering electron leakage and reducing free radical generation (Gomez et al., 2005; Hardeland et al., 2006)

Upon oxidation, melatonin converts to a number of antioxidant compounds cyclic 3-hydroxymelatonin, N1-acetyl-N2-formyl-5-methoxykynuramine and N1-acetyl-5-methoxykynuramine (Hardeland et al., 2006).
(a) Binding to plasma membrane localized melatonin receptors
(b) Binding to nuclear receptors of the orphan family
(c) Binding to intracellular proteins such as calmodulin
(d) Antioxidative effects
Fig. 1. Proposed melatonin mechanism of action against oxidative stress. When cells are subjected to an oxidative challenge, there is an increment of ROS production which alters the cellular redox state (1). I-κB is phosphorylated and NF-κB translocates into the nucleus (2) and binds to its κB response elements; some of these are located in the promoter regions of the major antioxidant enzymes (3). To maintain this antioxidant pathway, melatonin inhibits the RORα route. The indoleamine blocks the RORα activity through, at least, two different mechanisms. One is the direct melatonin interaction with calmodulin (4), which in turn leads to the inactivation of the calmodulin-dependent kinases (5); this step would repress RORα transcriptional activity on the I-κB gene, allowing the maintenance of the NF-κB pathway (6). Furthermore, melatonin could also restrain RORα constitutive activity through its membrane receptor, m1 (7). Likewise, melatonin is able to counteract oxidative stress, by means of its direct scavenging activity (8).
**HYPOTHESIS**

Melatonin could protect the toxic effects induced by PCB (Aroclor 1254) in selected brain regions of adult rats

**Aim**

To study the protective role of melatonin on PCB induced toxic effects in cerebellum, cerebral cortex and hippocampus of adult rats
CHAPTER - I

Effect of melatonin on PCB (Aroclor 1254) induced changes in antioxidant system in cerebellum, cerebral cortex and hippocampus of adult rats
MATERIALS AND METHODS

Adult male albino rats of wistar strain *Rattus norvegicus* (age 90 days)

All animal procedures were approved by our Institute Ethical Committee *(Reg. No. IAEC No. 03/010/04)*

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>Corn oil vehicle for 30 days.</td>
</tr>
<tr>
<td>Group II</td>
<td>Aroclor 1254 (2 mg/kg bw/ day i.p) for 30 days.</td>
</tr>
<tr>
<td>Group III</td>
<td>Aroclor 1254 (2 mg/kg bw/ day i.p) with simultaneous administration of melatonin (5mg/ kg bw/ day i.p) for 30 days.</td>
</tr>
<tr>
<td>Group IV</td>
<td>Aroclor 1254 (2 mg/kg bw/ day i.p) with simultaneous administration of melatonin (10 mg/ kg bw/ day i.p) for 30 days.</td>
</tr>
<tr>
<td>-----------------</td>
<td>---------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Group V</td>
<td>Melatonin (5 mg/kg bw/ day/ i.p) for 30 days.</td>
</tr>
<tr>
<td>Group VI</td>
<td>Melatonin (10 mg/kg bw/ day/ i.p) for 30 days.</td>
</tr>
</tbody>
</table>

The dosage and duration of PCB was selected according to our previous studies (Kaya et al., 2002; Venkataraman et al., 2004). The dose level of melatonin was selected according to Gomez et al., 2005; Feng and Zhang, 2005.
Effect of PCB (Aroclor 1254) and melatonin on body weight of adult male rats

Each bar represents the mean ± SEM of 6 animals.
Significance at P<0.05 followed by SNK,
a: Control Vs others b: PCB Vs other groups.
24 hours after last treatment, the animals were sacrificed and brain was immediately removed and washed in ice-cold physiological saline repeatedly and dissected over ice-cold glass slides to the following regions:

Cerebellum (C), Cerebral cortex (Cc) and Hippocampus (H) (Glowinski and Iverson, 1966).

Regions from each of the brain tissue were blotted, weighed accurately, and placed in chilled 0.1 mol/L Tris–HCl buffer, pH 7.4. The samples were homogenized to produce 10% homogenates.
Effect of PCB (Aroclor 1254) and melatonin on cerebellum, cerebral cortex and hippocampus weight of adult male rats

Each bar represents the mean ± SEM of 6 animals.
Significance at P<0.05 followed by SNK,
a: Control Vs others b: PCB Vs other groups.
<table>
<thead>
<tr>
<th>Enzymatic Antioxidants</th>
<th>Non Enzymatic Antioxidants</th>
<th>Reactive oxygen species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total SOD, Cu/Zn SOD &amp; Mn SOD <em>(Del Maestro &amp; Mc Donald, 1986)</em></td>
<td>GSH <em>(Moron et al., 1979)</em></td>
<td>Lipid peroxidation <em>(Devasagayam &amp; Tarachand, 1987)</em></td>
</tr>
<tr>
<td>Catalase <em>(Sinha, 1972)</em></td>
<td>Vitamin C <em>(Omaye et al., 1979)</em></td>
<td>Hydrogen peroxide <em>(Pick &amp; Keisari, 1981)</em></td>
</tr>
<tr>
<td>Glutathione Reductase <em>(Stall et al., 1969)</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glutathione–s–transferase <em>(Habig et al., 1973)</em></td>
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</tr>
</tbody>
</table>

Reactive oxygen species include:
- Lipid peroxidation *(Devasagayam & Tarachand, 1987)*
- Hydrogen peroxide *(Pick & Keisari, 1981)*
- Hydroxyl radical *(Puntarulo & Cederbaum, 1988)*
mRNA Expression

(i) Cu/Zn Superoxide dismutase (SOD-1)

(ii) Glutathione peroxidase–4 (phGPx)

**STATISTICAL ANALYSIS**

The data were analyzed using one-way ANOVA followed by Student’s Newman Keul’s (SNK) test was used to assess the statistical significance of each group.
Effect of melatonin on Total superoxide dismutase activity in selected brain regions of PCB (Aroclor 1254) exposed rats

Each bar represents the mean ± SEM of 6 animals.
Significance at P<0.05 followed by SNK,
a: Control Vs others b: PCB Vs other groups.
Effect of melatonin on Mn SOD activity in selected brain regions of PCB (Aroclor 1254) exposed albino rats

Each bar represents the mean ± SEM of 6 animals. Significance at P<0.05 followed by SNK, a: Control Vs others b: PCB Vs other groups.

Effect of Melatonin on Catalase activity in selected brain regions of PCB (Aroclor 1254) exposed rats
Effect of melatonin on Glutathione peroxidase activity in selected brain regions of PCB (Aroclor 1254) exposed rats

Each bar represents the mean ± SEM of 6 animals. Significance at P<0.05 followed by SNK, a: Control Vs others b: PCB Vs other groups.
Effect of melatonin on Glutathione Reductase activity in selected brain regions of PCB (Aroclor 1254) exposed rats

Each bar represents the mean ± SEM of 6 animals.
Significance at P<0.05 followed by SNK,
a: Control Vs others b: PCB Vs other groups.
Effect of Melatonin on vitamin C concentration in selected brain regions of PCB (Aroclor 1254) exposed rats

Each bar represents the mean ± SEM of 6 animals.
Significance at P<0.05 followed by SNK,
a: Control Vs others b: PCB Vs other groups.
Effect of melatonin on H$_2$O$_2$ generation in selected brain regions of PCB (Aroclor 1254) exposed rats

Each bar represents the mean ± SEM of 6 animals. Significance at P<0.05 followed by SNK,
a: Control Vs others b: PCB Vs other groups.

Int J Dev Neurosci, 2008: 26: 585-591
Effect of melatonin on lipid peroxidation (LPO) level in selected brain regions of PCB (Aroclor 1254) exposed rats

![Graph showing the effect of melatonin on lipid peroxidation in different brain regions.]

Each bar represents the mean ± SEM of 6 animals.
Significance at P<0.05 followed by SNK,
a: Control Vs others b: PCB Vs other groups.

*Int J Dev Neurosci, 2008: 26: 585-591*
Cu-Zn SOD (447 bp)
Gene bank accession no. for Cu-Zn SOD is XO5634

Sense : (nt.58-77) : 5’-GCAGAAGGCAAGCGGTGAAC-3’
Antisense: (nt.504-485) : 5’-TAGCAGGACAGCAGATGAGT-3’

GPx4 (461 bp)
Gene bank accession no. for GPx4 is D87896

Sense : (nt.265-285) : 5’-ATGCACGAATTCTCAGCCAAG -3’
Antisense: (nt.725-709): 5’-GGCAGGTCCTTCTCTTCTAT -3’

Limaye *et al.*, 2003; Nam *et al.*, 2003
Cu/Zn SOD mRNA Expression - Cerebellum

A - 100 bp ladder; B - Control; C - PCB; D - PCB + Melatonin (5 mg); E - PCB + Melatonin (10 mg); F - Melatonin (5 mg); G - Melatonin (10 mg)

Each bar represents the mean ± SEM of 3 independent observations. Significance at P<0.05 followed by SNK, a: Control Vs others b: PCB Vs other groups.
GPx-4 mRNA Expression - Cerebellum

A – 100 bp ladder; B – Control; C – PCB; D – PCB + Melatonin (5 mg)
E - PCB + Melatonin (10 mg); F - Melatonin (5 mg); G – Melatonin (10 mg)

Each bar represents the mean ± SEM of 3 independent observations.
Significance at P<0.05 followed by SNK,
a: Control Vs others  b: PCB Vs other groups.
Cu/Zn SOD mRNA Expression – Cerebral Cortex

A – 100 bp ladder; B – Control; C – PCB; D – PCB + Melatonin (5 mg)
E - PCB + Melatonin (10 mg); F - Melatonin (5 mg); G – Melatonin (10 mg)

Each bar represents the mean ± SEM of 3 independent observations.
Significance at P<0.05 followed by SNK,
a: Control Vs others  b: PCB Vs other groups.
GPx-4 mRNA Expression – Cerebral cortex

A – 100 bp ladder; B – Control; C – PCB; D – PCB + Melatonin (5 mg)
E - PCB + Melatonin (10 mg); F - Melatonin (5 mg); G – Melatonin (10 mg)

Each bar represents the mean ± SEM of 3 independent observations.
Significance at P<0.05 followed by SNK,
a: Control Vs others  b: PCB Vs other groups.
Cu/ Zn SOD mRNA Expression – Hippocampus

A – 100 bp ladder; B – Control; C – PCB; D – PCB + Melatonin (5 mg)
E - PCB + Melatonin (10 mg); F - Melatonin (5 mg); G – Melatonin (10 mg)

Each bar represents the mean ± SEM of 3 independent observations.
Significance at P<0.05 followed by SNK,
a: Control Vs others  b: PCB Vs other groups.
GPx-4 mRNA Expression – Hippocampus

A – 100 bp ladder; B – Control; C – PCB; D – PCB + Melatonin (5 mg)
E - PCB + Melatonin (10 mg); F - Melatonin (5 mg); G – Melatonin (10 mg)

Each bar represents the mean ± SEM of 3 independent observations.
Significance at P<0.05 followed by SNK,
a: Control Vs others  b: PCB Vs other groups.
CHAPTER – II

Effect of melatonin on PCB (Aroclor 1254) induced changes in membrane bound ATPases, creatine kinase system and acetylcholinesterase in cerebellum, cerebral cortex and hippocampus of adult male rats
Membrane bound ATPases

<table>
<thead>
<tr>
<th>ATPase Type</th>
<th>Reference (Year)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na(^+)K(^+) ATPase</td>
<td>Bonting, 1970</td>
</tr>
<tr>
<td>Ca(^{2+}) ATPase</td>
<td>Hjerten and Pan, 1983</td>
</tr>
<tr>
<td>Mg(^{2+}) ATPase</td>
<td>Ohinashi et al., 1982</td>
</tr>
<tr>
<td>Inorganic phosphorus</td>
<td>Fiske and Subbarao, 1925</td>
</tr>
</tbody>
</table>

Creatine kinase (Okinaka et al., 1964).

Serum and tissues CK isoforms were separated by Polyacrylamide gel electrophoresis (Smith, 1972).

Acetylcholine esterase (Ellman et al., 1961).
Effect of melatonin on Na⁺K⁺ATPase activity in selected brain regions of PCB (Aroclor 1254) exposed adult rats

Effect of Melatonin on Ca²⁺ATPase activity in selected brain regions of PCB (Aroclor 1254) exposed adult rats

Each bar represents the mean ± SEM of 6 animals.
Significance at P<0.05 followed by SNK,
a: Control Vs others b: PCB Vs other groups.

Int J Dev Neurosci, 2008: 26: 585-591
Effect of Melatonin on Mg$^{2+}$ ATPase activity in selected brain regions of PCB (Aroclor 1254) exposed adult rats

Each bar represents the mean ± SEM of 6 animals. Significance at P<0.05 followed by SNK, a: Control Vs others b: PCB Vs other groups.

Int J Dev Neurosci, 2008: 26: 585-591
Effect of Melatonin on creatine kinase activity in selected brain regions of PCB (Aroclor 1254) exposed adult rats

Effect of Melatonin on serum creatine kinase levels in PCB (Aroclor 1254) exposed adult rats

Each bar represents the mean ± SEM of 6 animals.
Significance at P<0.05 followed by SNK,
a: Control Vs others b: PCB Vs other groups.

Basic & clinical Pharmacology & Toxicology, 2009, 105: 92-97
Effect of Melatonin on serum creatine kinase isoforms in PCB exposed rats

Each bar represents the mean ± SEM of 3 independent observations.

A – Control
B – Aroclor 1254
C – Aroclor 1254 + Melatonin (5 mg)
D – Aroclor 1254 + Melatonin (10 mg)

Basic & clinical Pharmacology & Toxicology, 2009, 105: 92-97
Effect of melatonin on CK-BB levels in selected brain regions of PCB (Aroclor 1254) exposed rats

Each bar represents the mean ± SEM of 3 independent observations.
Effect of melatonin on acetylcholinesterase (AchE) activity in selected brain regions of PCB (Aroclor 1254) exposed adult rats

Each bar represents the mean ± SEM of 6 animals. Significance at P<0.05 followed by SNK, a: Control Vs others b: PCB Vs other groups.

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CHAPTER III

Effect of melatonin on PCB (Aroclor 1254) induced changes in Amyloid β protein expression in cerebellum, cerebral cortex and hippocampus of adult male rats
<table>
<thead>
<tr>
<th>Technique</th>
<th>Steps involved</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gel electrophoresis</td>
<td>Transfer of proteins to the membrane</td>
</tr>
<tr>
<td>Blocking non-specific binding</td>
<td>Antibody incubation</td>
</tr>
<tr>
<td>Protein detection</td>
<td></td>
</tr>
</tbody>
</table>

| Primary | : Beta Amyloid – Mouse monoclonal IgG2a (1: 1000) |
| Secondary | : Rabbit antimouse IgG peroxidase conjugate (1:5000) |
| Primary | : Beta Actin – Rabbit polyclonal (1: 1000) |
| Secondary | : Goat Anti rabbit (1: 5000) |
Each bar represents the mean + SEM of 3 observations. Significance at P<0.05 followed by SNK, a: Control Vs others, b: PCB Vs other groups.
Effect of melatonin on PCB (Aroclor 1254) induced beta amyloid protein expression in cerebral cortex of adult rats

Each bar represents the mean + SEM of 3 independent observations. Significance at P<0.05 followed by SNK, a: Control Vs others, b: PCB Vs other groups.
Effect of melatonin on PCB (Aroclor 1254) induced beta amyloid protein expression in hippocampus of adult rats

Each bar represents the mean + SEM of 3 observations. Significance at P<0.05 followed by SNK, a: Control Vs others, b: PCB Vs other groups.
Melatonin levels in serum and brain regions of treated animals
Trunk blood was collected in clean, dry test tubes and allowed to clot at room temperature and then centrifuged at 1500 x g for 10 min and the serum was removed and used for Melatonin assay by ELISA.

The level of melatonin in serum and selected brain regions from control and experimental animals was determined with melatonin ELISA kit (IBL, Hamburg, Germany) according to the method described by Lahiri et al., (2004).

**Sensitivity**: <1.6 pg/ml

**Cross reactivity**: N-Acetyl-Serotonin 1.6%

**Intra assay**: 8.8 – 151.7 pg/ml

**Inter assay**: 5.6 -134.3 pg/ml
Serum melatonin levels in PCB (Aroclor 1254) and exogenous melatonin treated adult rats

Each bar represents the mean ± SEM of 6 animals. Significance at P<0.05 followed by SNK, a: Control Vs others b: PCB Vs other groups.

Int J Dev Neurosci, 2008: 26: 585-591
Determination of tissue melatonin level

A fixed amount of (50mg) of tissue sample was suspended in a 1x homogenizing buffer (25mM Tris-Hcl, pH 7.4, 1mM EDTA, 1mM EGTA), homogenized and centrifuged at 11,000x g for 30 min at 4°C.

Tissue extracts were assayed for protein concentrations using Lowry method (1951), and a known amount of tissue extract was added to measure levels of melatonin.
Melatonin levels in selected brain regions of PCB (Aroclor 1254) and exogenous melatonin treated adult rats.

Each bar represents the mean ± SEM of 6 animals. Significance at P<0.05 followed by SNK, a: Control Vs others b: PCB Vs other groups.
CHAPTER – IV

Histomorphological changes in cerebellum, cerebral cortex and hippocampus of PCB (Aroclor 1254) adult rats: Impact of melatonin

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>PCB</th>
<th>PCB + Melatonin (5 mg)</th>
<th>PCB + Melatonin (10 mg)</th>
<th>Melatonin (5 mg)</th>
<th>Melatonin (10 mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CNC</td>
<td>CNC</td>
<td>CNC</td>
<td>CNC</td>
<td>CNC</td>
<td>CNC</td>
<td>CNC</td>
</tr>
</tbody>
</table>

Cerebral cortex (10x)

CNC - Cortical Neuronal cells

Haematoxylin and eosin staining
Cerebral Cortex – 40x

Control

PCB

PCB + Melatonin (5 mg)

PCB + Melatonin (10 mg)

Melatonin (5 mg)

Melatonin (10 mg)

NNC – Normal Nerve Cells

PNS – Peri Neuronal Spaces

PN – Pyknotic Nucleus

NS – Neuronal shrinkage

DN – Degenerative neurons
**Hippocampus – 10x**

- **Control**
- **PCB**
- **PCB + Melatonin (5 mg)**
- **PCB + Melatonin (10 mg)**
- **Melatonin (5 mg)**
- **Melatonin (10 mg)**

**HL – Hippocampal layer**
Hippocampus – 40x

- **Control**: NNC
- **PCB**: DN
- **PCB + Melatonin (5 mg)**: DN
- **PCB + Melatonin (10 mg)**: NNC
- **Melatonin (5 mg)**: NNC
- **Melatonin (10 mg)**: NNC

NNC – Normal nerve cells  
HL – Hippocampal layer  
DN – Degenerative neurons
Cerebellum (10x)

Control

PCB

PCB + Melatonin (5 mg)

PCB + Melatonin (10 mg)

Melatonin (5 mg)

Melatonin (10 mg)

PCL - Purkinje cell Layer
GCL – Granular cell layer
Cerebellum (40x)

PC – Purkinje cells  GC – Granular cells
NNC – Normal nerve cells  DN – Degenerative neurons
Senile plaques

ROS

Membrane-bound AR Pase
Aroclor 1254 → (+) Oxidative stress → (-) Melatonin

(+)

Amyloid deposition → (+) Neurodegeneration

(+) Creatine kinase System and ATPases & Cholinergic system
Conclusion

PCBs are neurotoxic compounds which induce the production of free radicals leading to oxidative stress.

Oxidative stress and formation of free radicals are the major factors of the cytopathology of many neurodegenerative disorders.

Melatonin, which possesses characteristics of an antioxidant, is the leading candidate for the prevention and treatment of neurological disorders.

This study proves the usefulness of melatonin in disorders affecting the brain free radical formation.
Effect of melatonin on PCB (Aroclor 1254) induced neuronal damage and changes in Cu/Zn superoxide dismutase and glutathione peroxidase-4 mRNA expression in cerebral cortex, cerebellum and hippocampus of adult rats

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Protective role of melatonin on PCB (Aroclor 1254) induced oxidative stress and changes in acetylcholine esterase and membrane bound ATPases in cerebellum, cerebral cortex and hippocampus of adult rat brain

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Oxidative Stress Alters Creatine Kinase System in Serum and Brain Regions of Polychlorinated Biphenyl (Aroclor 1254)-Exposed Rats: Protective Role of Melatonin

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PCB (Aroclor 1254) enhances oxidative damage in rat brain regions: Protective role of ascorbic acid

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Neurotoxins: Free Radical Mechanisms and Melatonin Protection

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POLYCHLORINATED BIPHENYLS

The neurotoxic effects of polychlorinated biphenyls (PBCs) are a consequence of their ability to produce free radicals. The proteins embedded in membranes which control ionic gradients across both plasma and organellar membranes are especially easily damaged when oxidized by PBCs. When rats were treated with PBC (Aroclor 1254; 2 mg/kg daily for 30 days) neural levels of lipid peroxidation products along with concentrations of •OH and H₂O₂ were elevated. Conversely, GSH concentrations as well as the activities of a variety of enzymes (Na⁺K⁺ATPase, Ca²⁺ATPase, Mg ²⁺ATPase and acetylcholinesterase) were diminished [255]. Giving melatonin (either 5 or 10 mg/kg daily) in combination with the PCB reversed the effects of PBC. In a follow-up study where Aroclor 1254 was used to induce neuronal damage and suppress CuZnSOD and GPx-4 mRNA expression, melatonin again relieved the effects of the PCB [256]. In this study, the benefits of melatonin against PCB neurotoxicity were seen in the cerebral cortex, the hippocampus and cerebellum. In both studies, the protective actions of melatonin were attributed to its antioxidative actions.


Publications


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Melatonin

\[
\text{H}_3\text{C}-\text{O}-\text{C}-\text{H}_2\text{N}-\text{CH}_3
\]

\[\text{•OH} \rightarrow \text{Indolyl radical}\]

\[\text{H}_2\text{O}_2 \rightarrow \text{N}^1\text{-acetyl-N}^2\text{-formyl 5-methoxykynuramine}\]

\[\text{•OH} \rightarrow \text{Cyclic 3-hydroxy melatonin}\]

\[\text{Catalase} \rightarrow \text{N}^1\text{-acetyl-5-methoxykynuramine}\]

\[\text{Urinary excretion}\]