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.

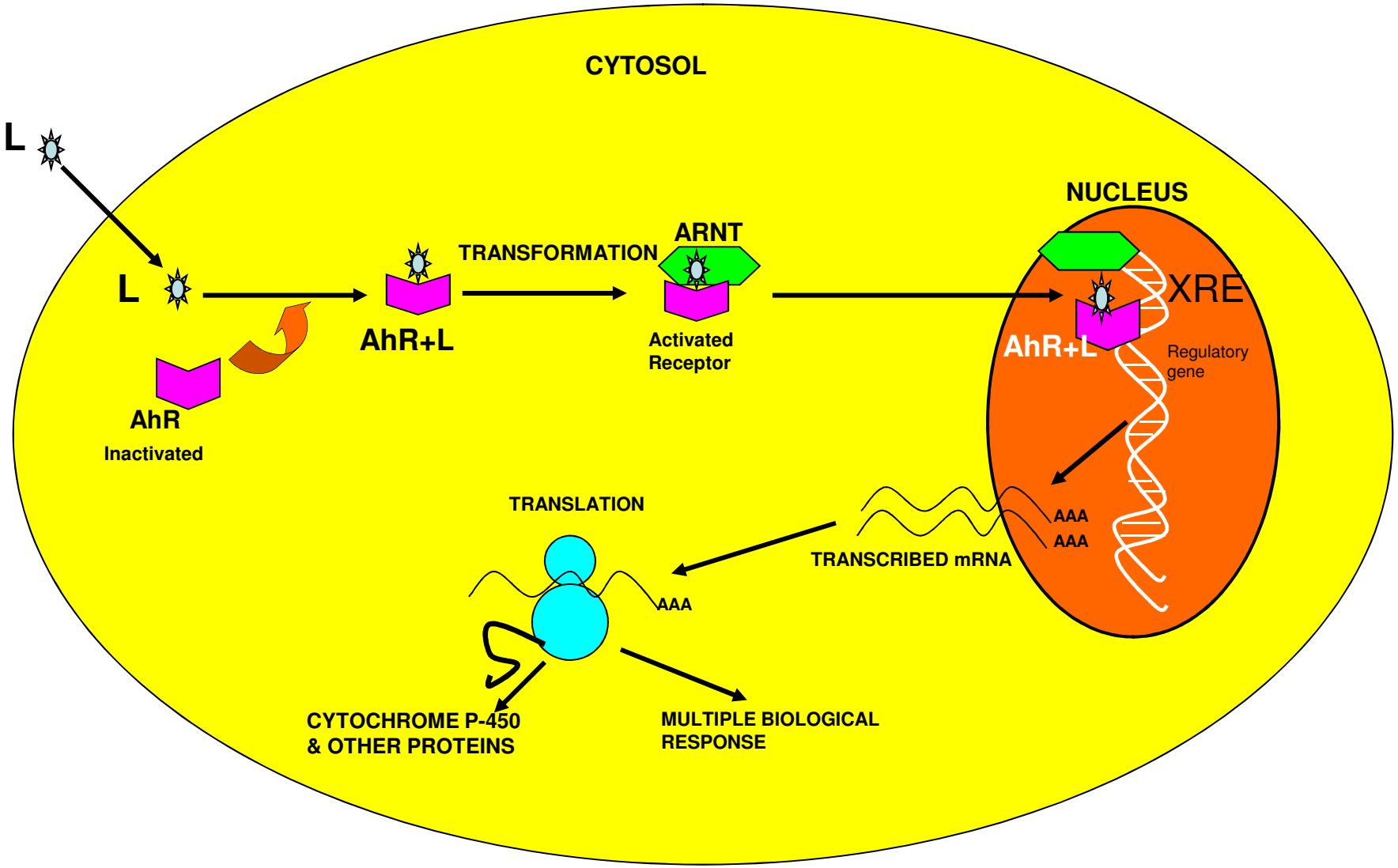
PCBs induced toxic manifestations are associated with the production of ROS (Tharappel *et al.*, 2002).

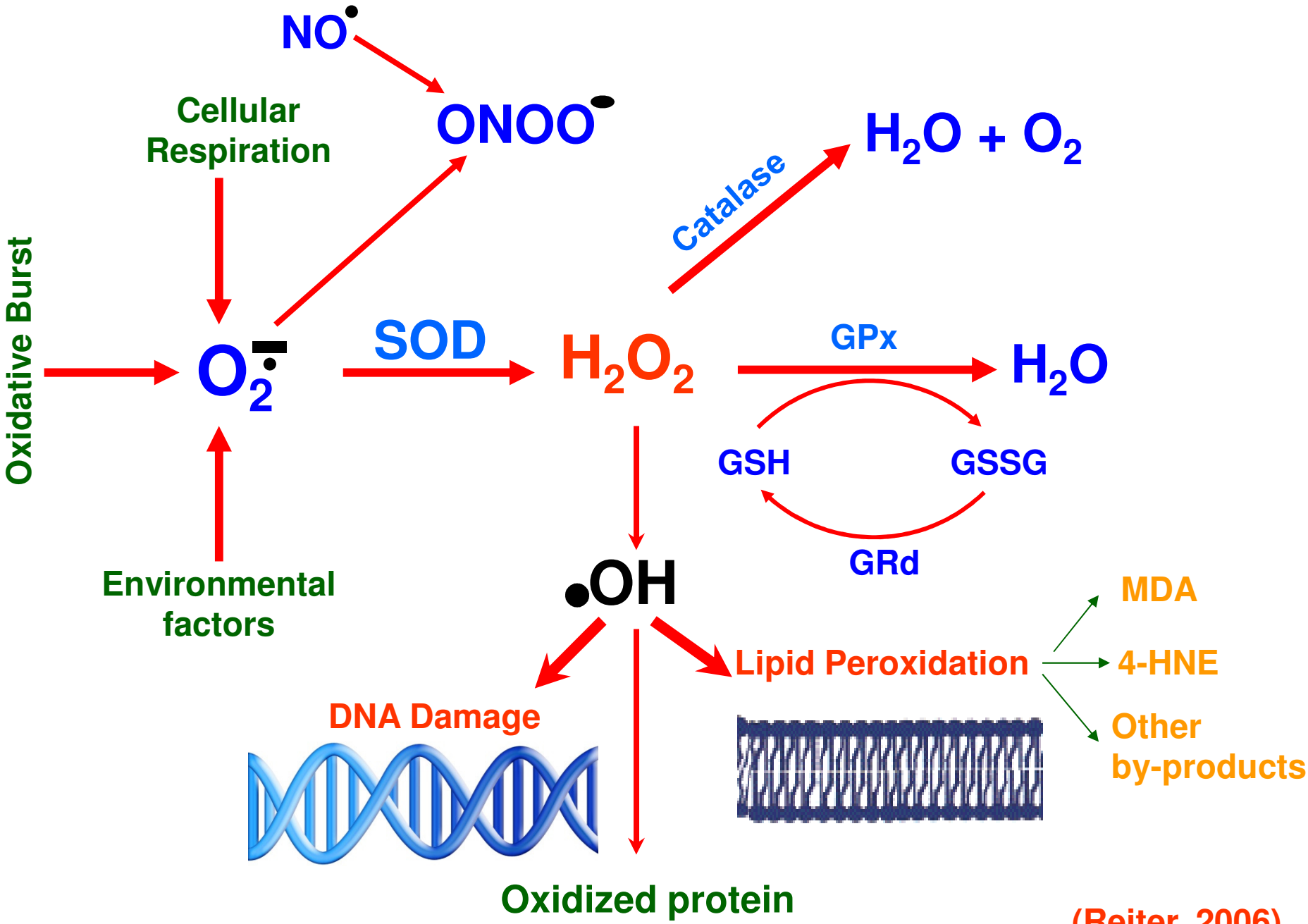
Aroclor 1254 induces oxidative stress in ventral prostate, testis, lung, kidney (Sridhar *et al.,* 2004; Murugesan *et al.,* 2005; Krishnamoorthy *et al.,* 2005; Banudevi *et al.,* 2006).

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




NO•
ONOO⁻
Cellular Respiration
Oxidative Burst
$O_2^{\bullet-}$
SOD
H_2O_2
Catalase
$H_2O + O_2$
GPx
H_2O
GSH
GSSG
GRd
Environmental factors
•OH
Lipid Peroxidation
MDA
4-HNE
Other by-products
DNA Damage
Oxidized protein

(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.

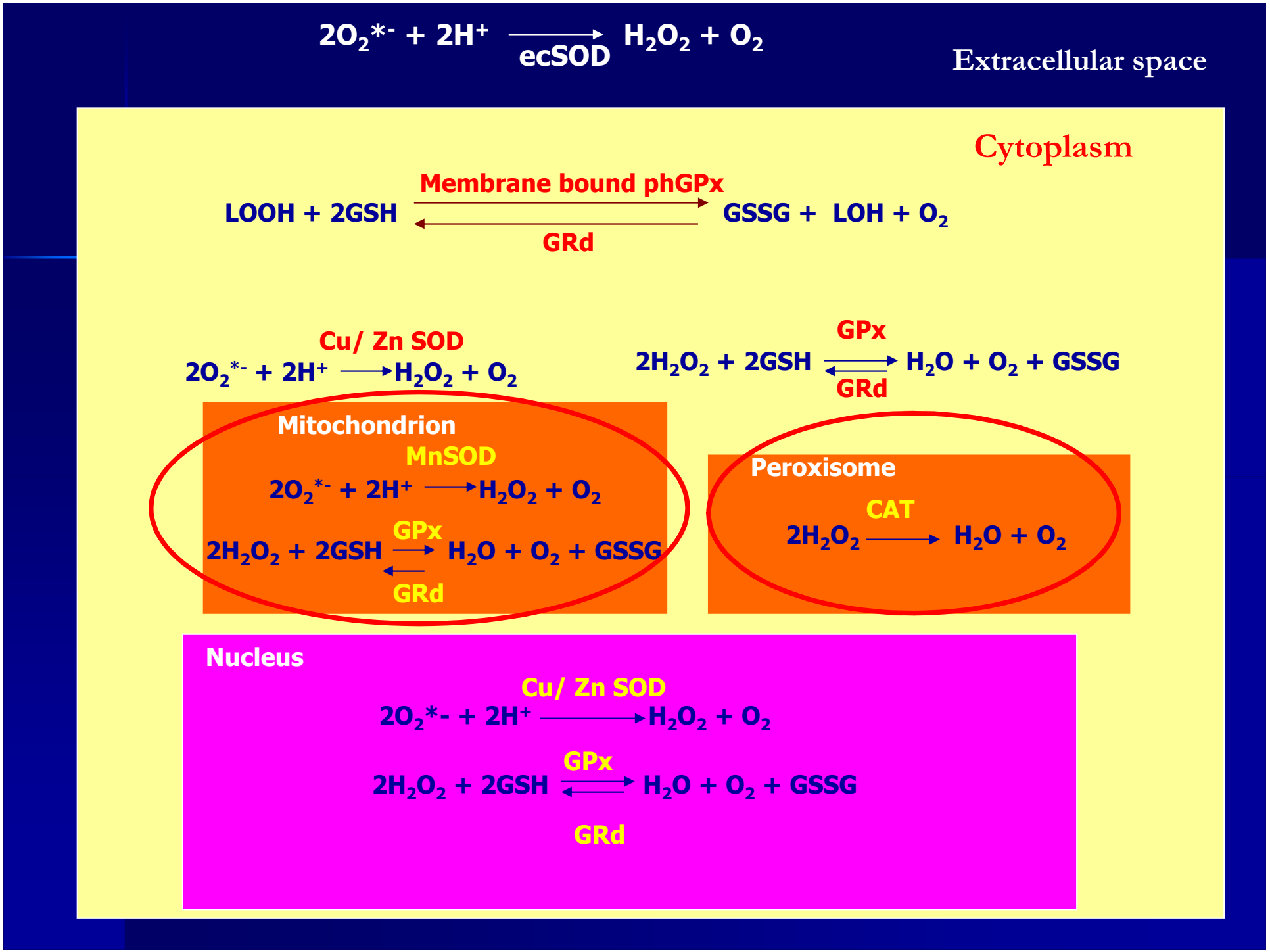
GPX4 is the only major antioxidant enzyme known to directly reduce phospholipid hydroperoxides within membranes and lipoproteins (Yant *et al.*, 2003).

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.

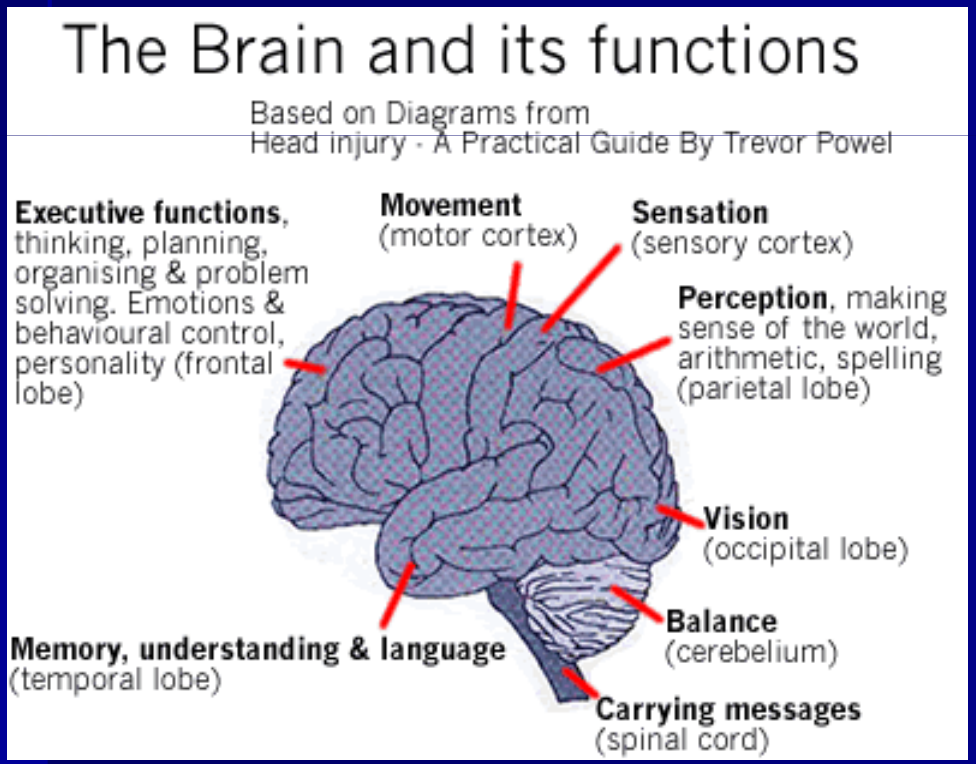
In fully mature adult brain, GPx-4 is expressed in all neuronal cell layers and most prominently in the hippocampus (Savaskan *et al.*, 2007).


$2O_2^{*-} + 2H^+ \xrightarrow{ecSOD} H_2O_2 + O_2$
Extracellular space
Cytoplasm
LOOH + 2GSH ⇄ GSSG + LOH + O_2
Membrane bound phGPx
GRd
$2O_2^{*-} + 2H^+ \xrightarrow{Cu/\ Zn\ SOD} H_2O_2 + O_2$
$2H_2O_2 + 2GSH \rightleftarrows H_2O + O_2 + GSSG$
GPx
GRd
Mitochondrion
MnSOD
$2O_2^{*-} + 2H^+ \longrightarrow H_2O_2 + O_2$
$2H_2O_2 + 2GSH \rightleftarrows H_2O + O_2 + GSSG$
GPx
GRd
Peroxisome
CAT
$2H_2O_2 \longrightarrow H_2O + O_2$
Nucleus
Cu/ Zn SOD
$2O_2^{*-} + 2H^+ \longrightarrow H_2O_2 + O_2$
$2H_2O_2 + 2GSH \rightleftarrows H_2O + O_2 + GSSG$
GPx
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 (Sqauire, 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).

Three isoforms: CK-BB, CK-MB, CK-MM (Dawson *et al.*, 1965).

(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

Aß1-42 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).

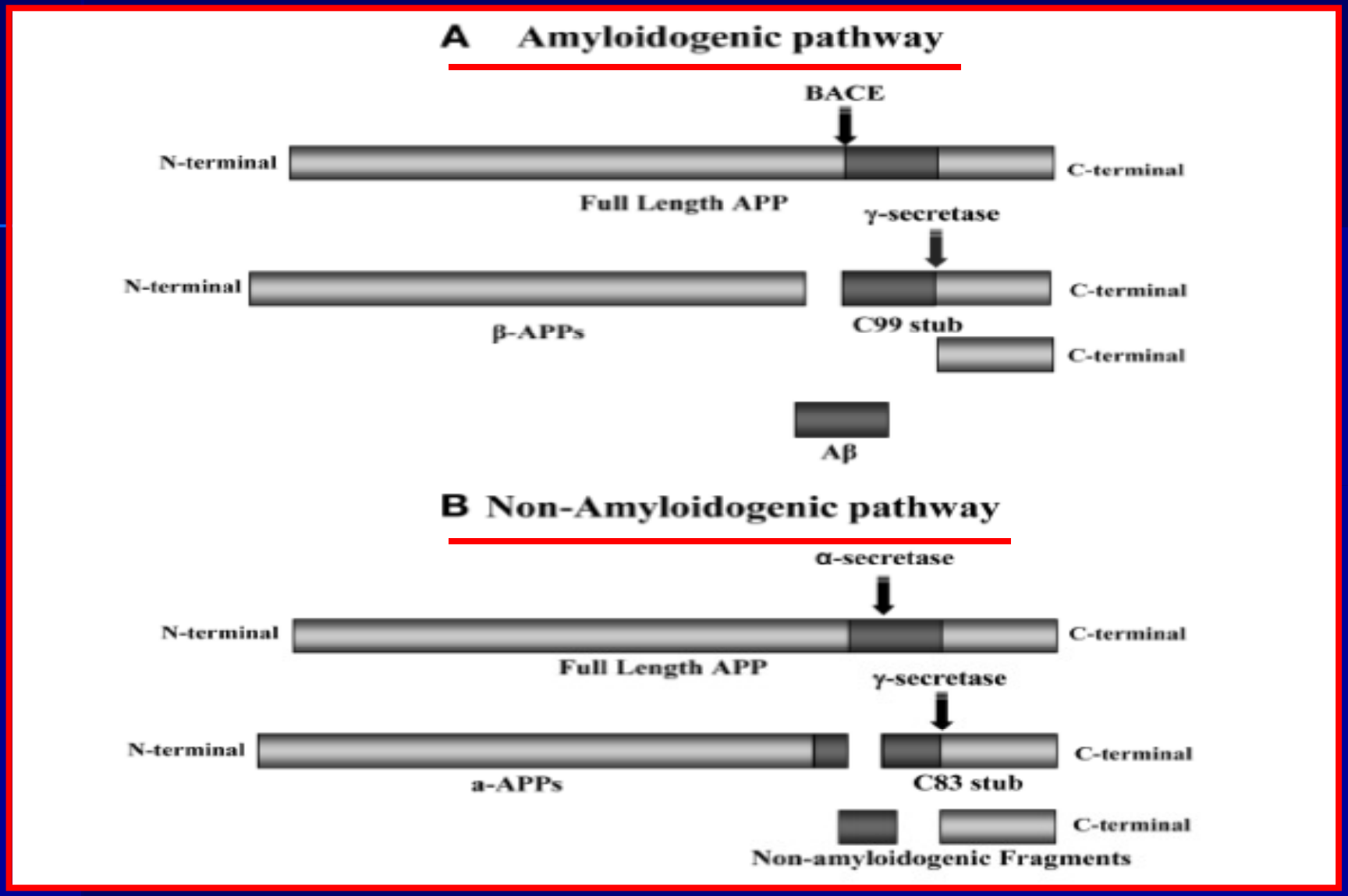
Oxidative stress promotes intra cellular accumulation of Aβ through enhancing the amyloidogenic pathway in SHSY5Y neuroblastoma cells (Misonou *et al.*, 2006).

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

Aβ(1-42):

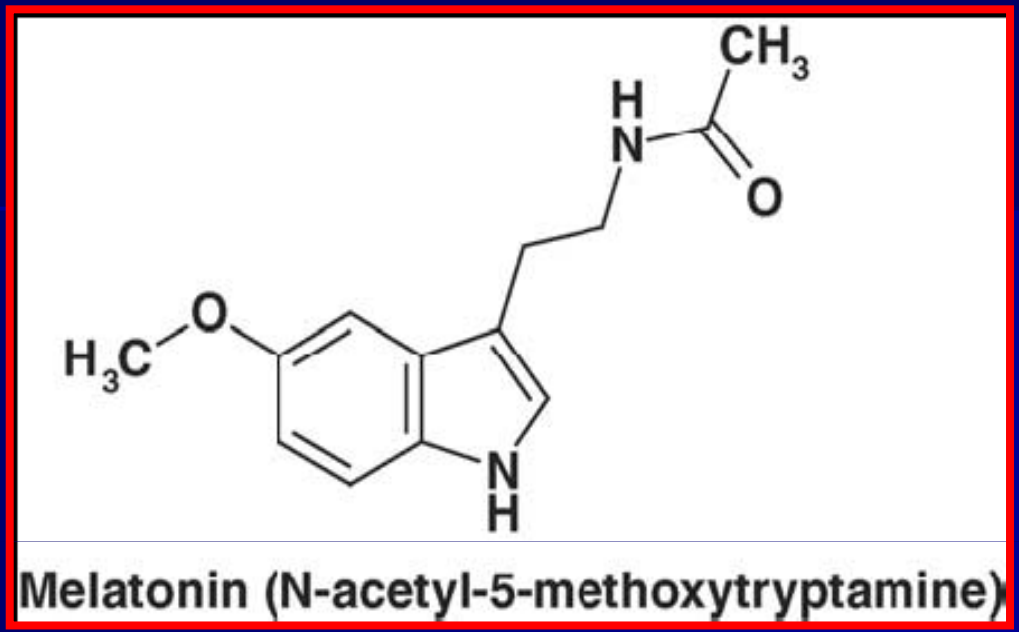
H_2N-Asp^{1}-Ala^{2}-Glu^{3}-Phe^{4}-Arg^{5}-His^{6}-Asp^{7}-Ser^{8}-Gly^{9}-Tyr^{10}-Glu^{11}-Val^{12}-His^{13}-His^{14}-Gln^{15}-Lys^{16}-Leu^{17}-Val^{18}-Phe^{19}-Phe^{20}-Ala^{21}-Glu^{22}-Asp23-Val^{24}-Gly^{25}-Ser^{26}-Asn^{27}-Lys^{28}-Gly^{29}-Ala^{30}-Ile^{31}-Ile^{32}-Gly^{33}-Leu^{34}-Met^{35}-Val^{36}-Gly^{37}-Gly^{38}-Val^{39}-Val^{40}-Ile^{41}-Ala^{42}-COOH

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



Barron *et al.*, 2006

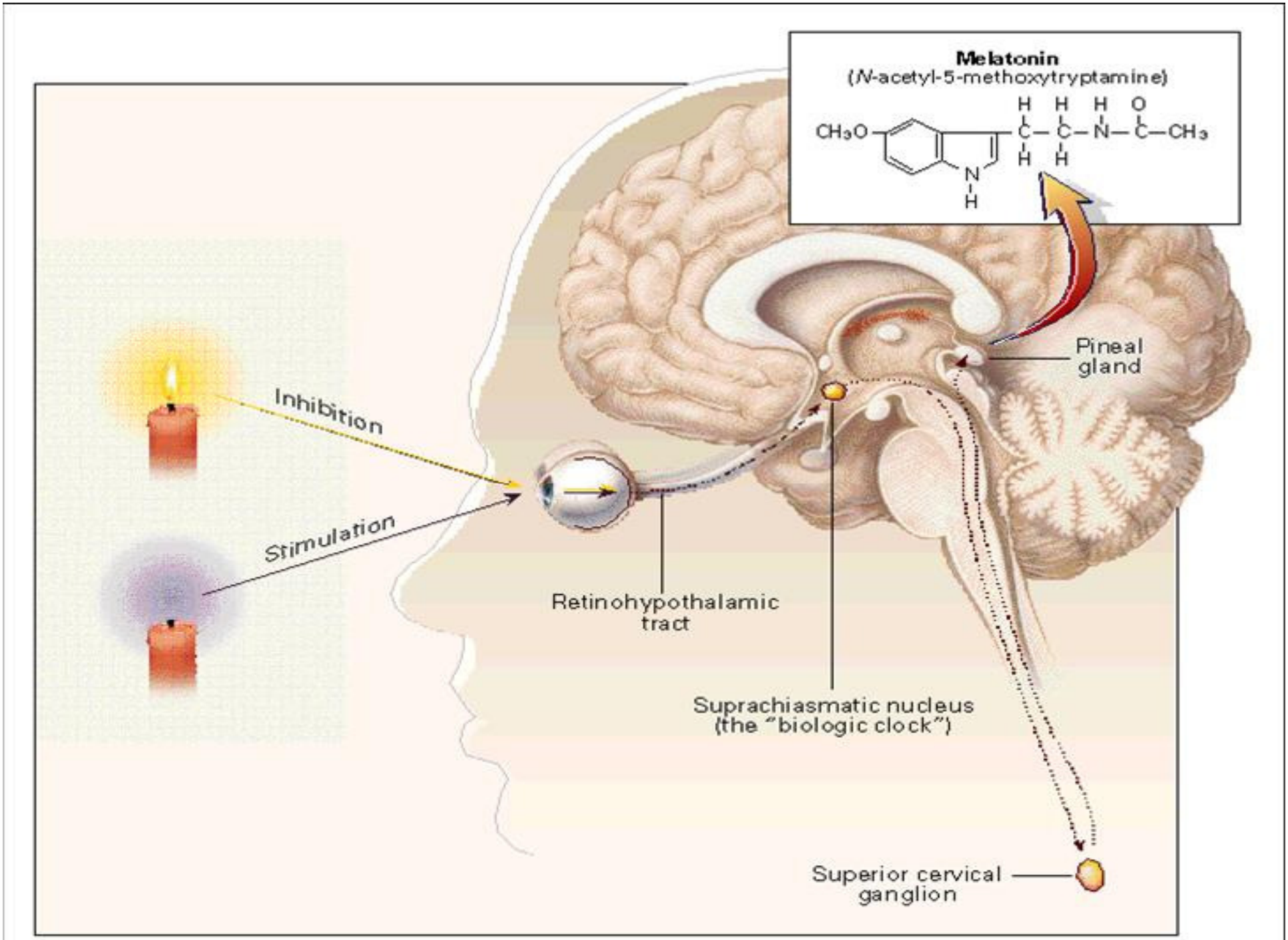
MELATONIN



Melatonin (N-acetyl-5-methoxytryptamine)

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).**


Melatonin
(N-acetyl-5-methoxytryptamine)
CH3O
H H H O
C C N C CH3
H H
N
H
Pineal gland
Inhibition
Stimulation
Retinohypothalamic tract
Suprachiasmatic nucleus (the "biologic clock")
Superior cervical ganglion

(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)

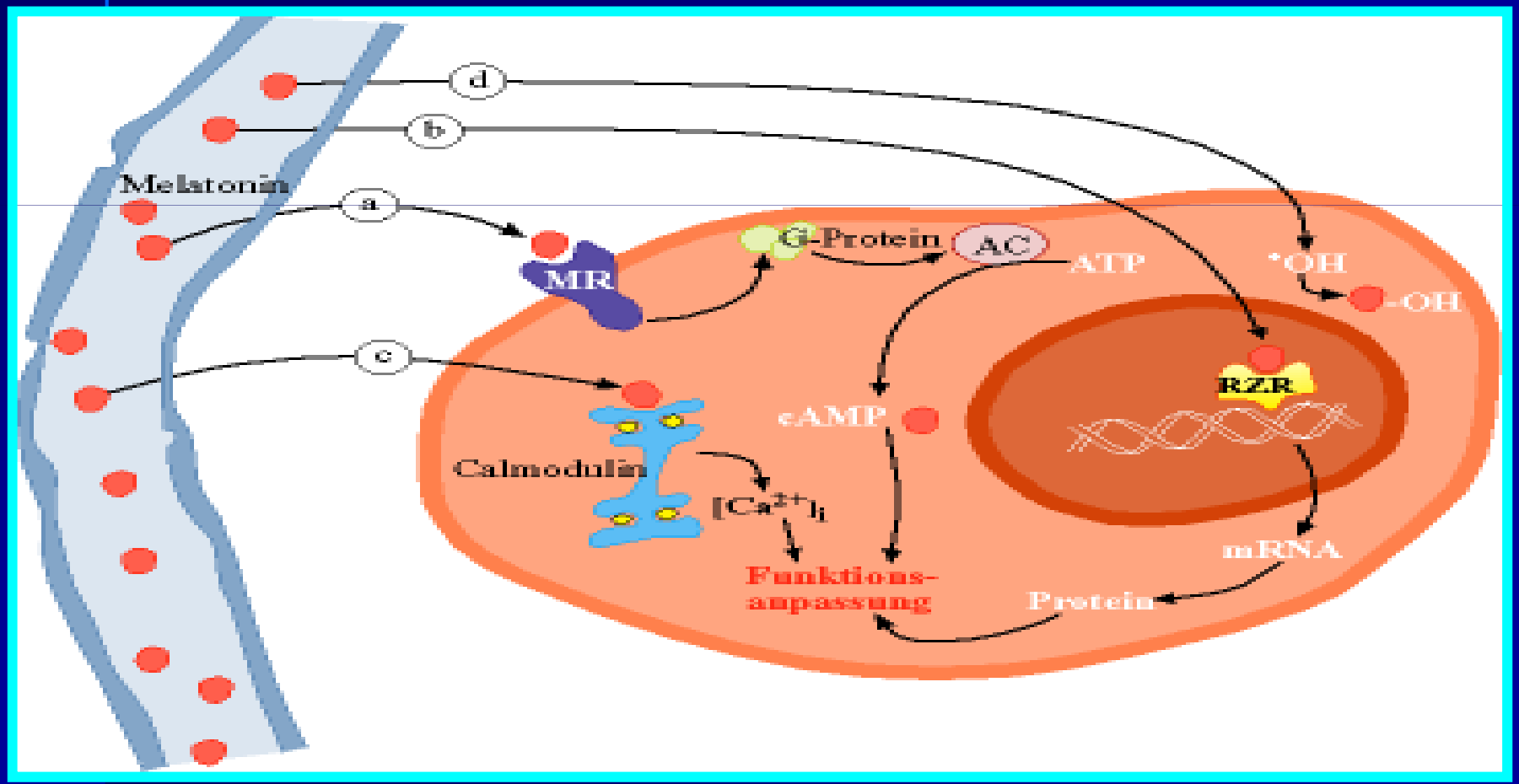
Upon oxidation, melatonin converts to a number of antioxidant compounds cyclic 3-hydroxymelatonin, N1-acetyl-N2-formyl-5-methoxykynuramine and N1-acetyl-5-methoxykynuramine (Hardeland *et al.*, 2006).

(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



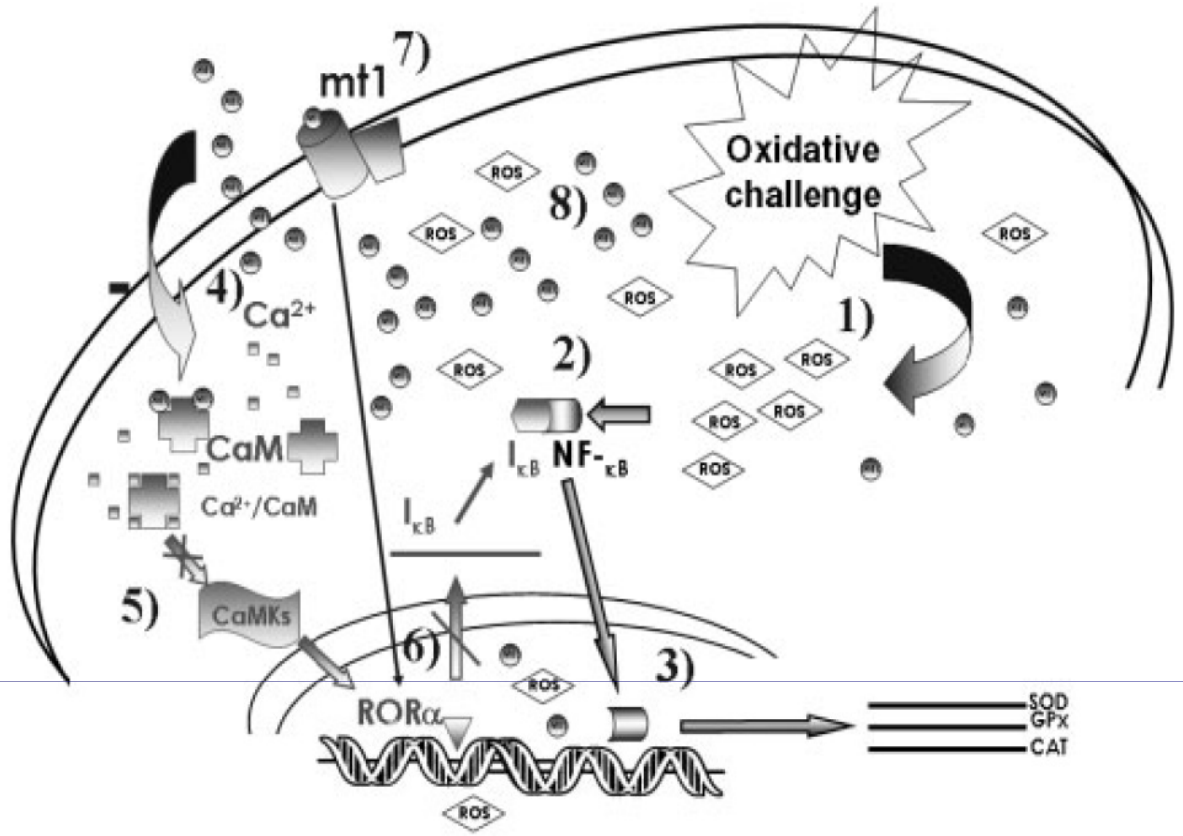


Fig. 1. Proposed melatonin mechanism of action against oxidative stress. When cells are subjected to an oxidative challenge, there is an increment of ROS production which alters the cellular redox state (1). I-κB is phosphorylated and NF-κB translocates into the nucleus (2) and binds to its κB response 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, mt1 (7). Likewise, melatonin is able to counteract oxidative stress, by means of its direct scavenging activity (8).

Cristina Tomas Zapico and Ana Coto-Montes, 2005, J. Pineal Research, 39: 99-104.

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)

Group I : Corn oil vehicle for 30 days.

Group II : Aroclor 1254 (2 mg/kg bw/ day i.p) for 30 days.

Group III : Aroclor 1254 (2 mg/kg bw/ day i.p) with simultaneous administration of melatonin (5mg/ kg bw/ day i.p) for 30 days.

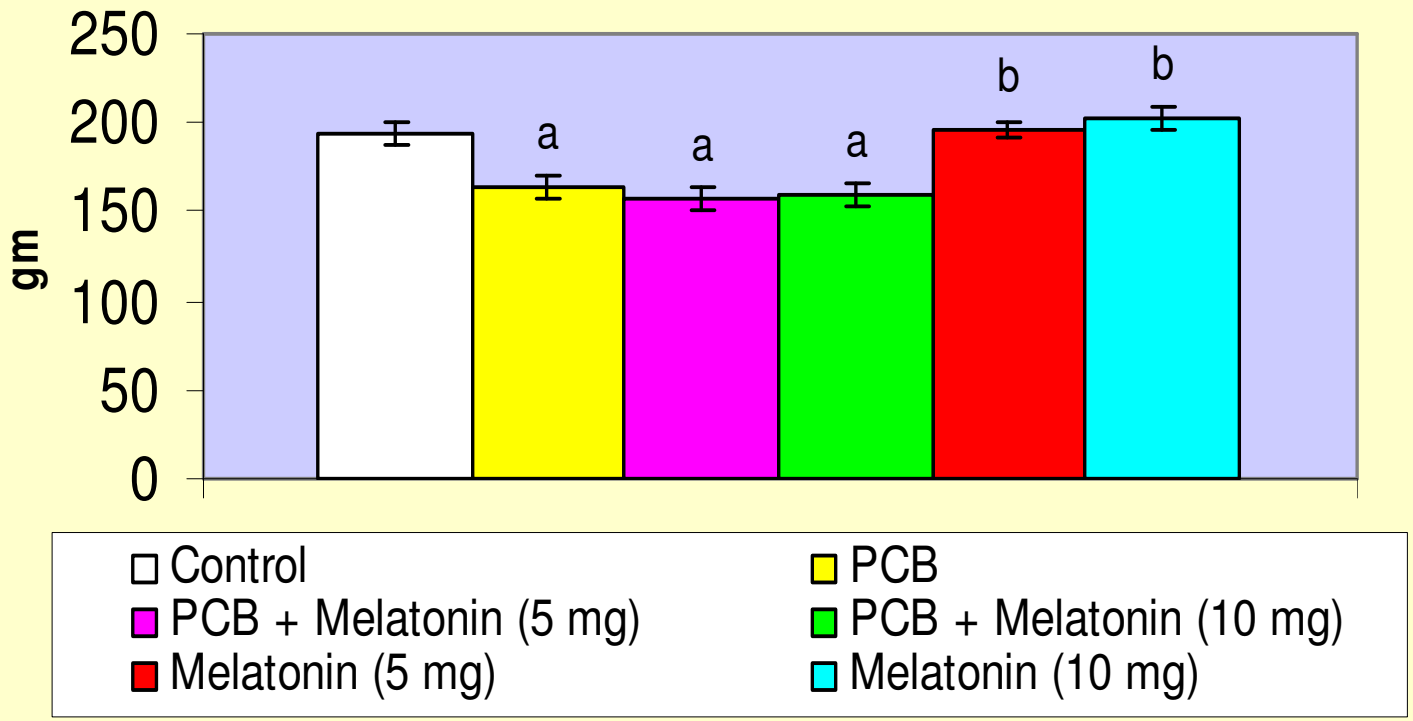
Group IV : **Aroclor 1254** (2 mg/kg bw/ day i.p) with simultaneous administration of **melatonin** (10 mg/ kg bw/ day i.p) for 30 days.

Group V : **Melatonin** (5 mg/kg bw/ day/ i.p) for 30 days.

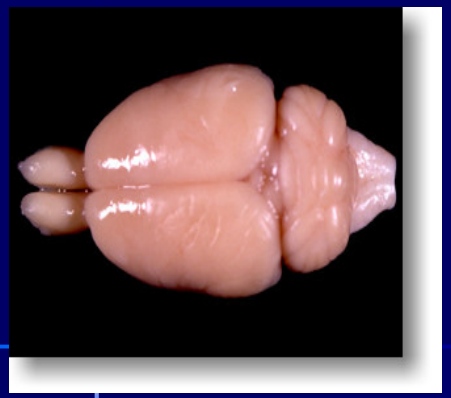
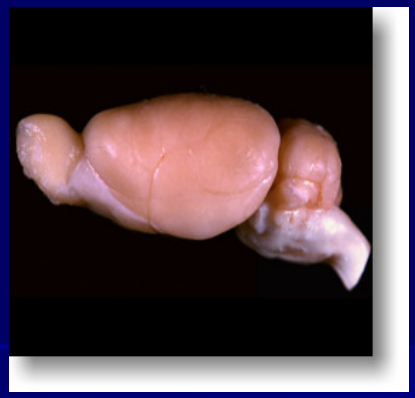
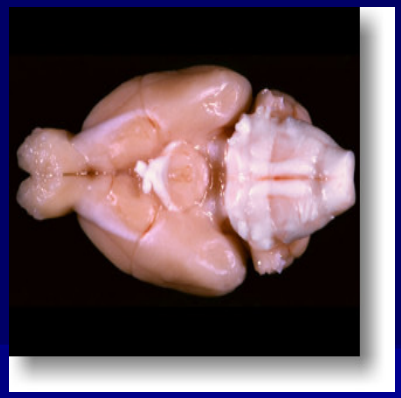
Group VI : **Melatonin** (10 mg/kg bw/ day/ i.p) for 30 days.

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.

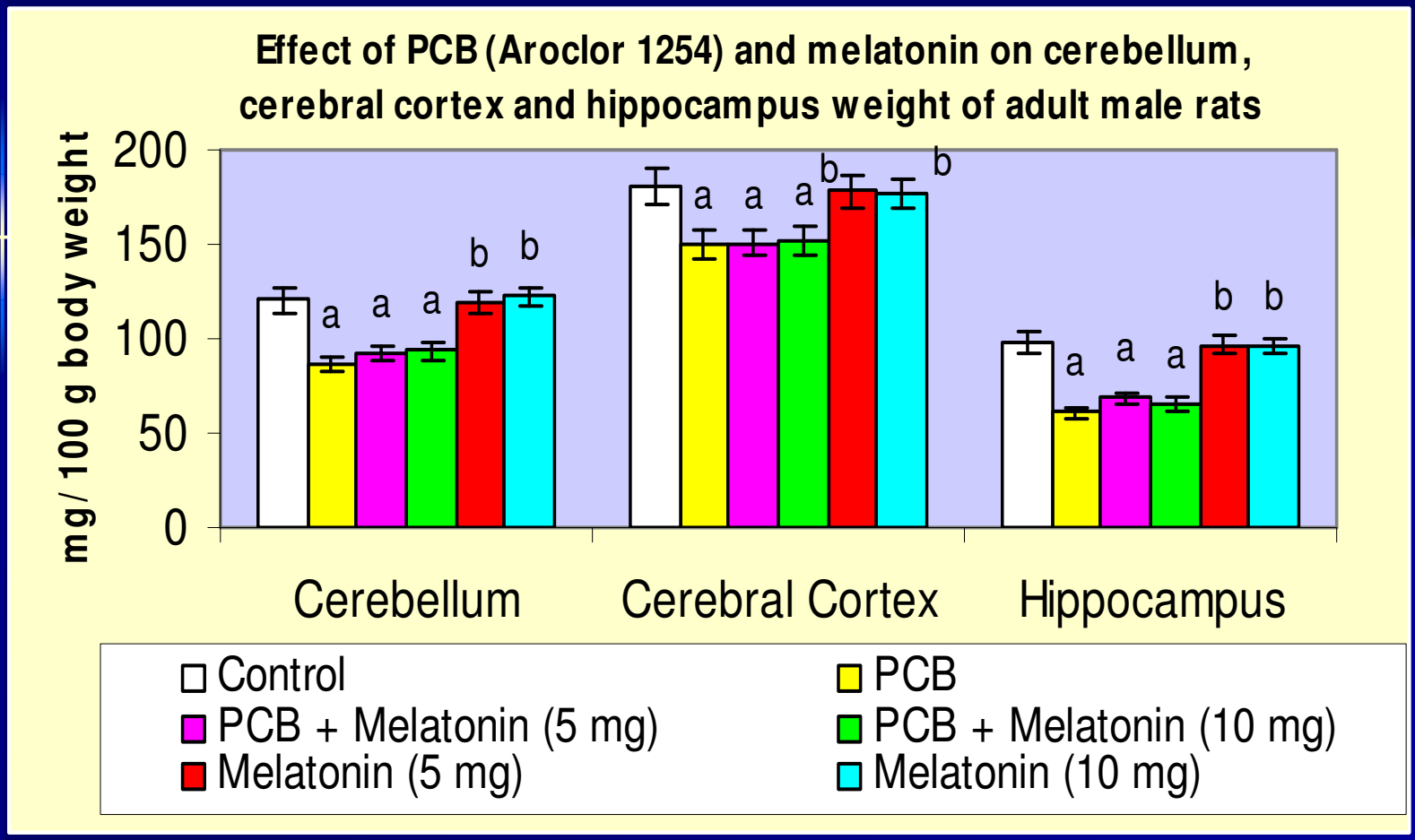





RAT BRAIN

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.



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.

Enzymatic Antioxidants

Total SOD, Cu/Zn SOD & Mn SOD (Del Maestro & Mc Donald, 1986)
Catalase (Sinha, 1972)
Glutathione peroxidase (Rotruck *et al.*, 1973)
Glutathione Reductase (Stall *et al.*, 1969)
Glutathione–s–transferase (Habig *et al.*, 1973)

Non Enzymatic Antioxidants

GSH (Moron *et al.*, 1979)
Vitamin C (Omaye *et al.*, 1979)
Vitamin E (Desai, 1984)

Reactive oxygen species

Lipid peroxidation (Devasagayam & Tarachand, 1987)
Hydrogen peroxide (Pick & Keisari, 1981)
Hydroxyl radical (Puntarulo & Cederbaum, 1988)

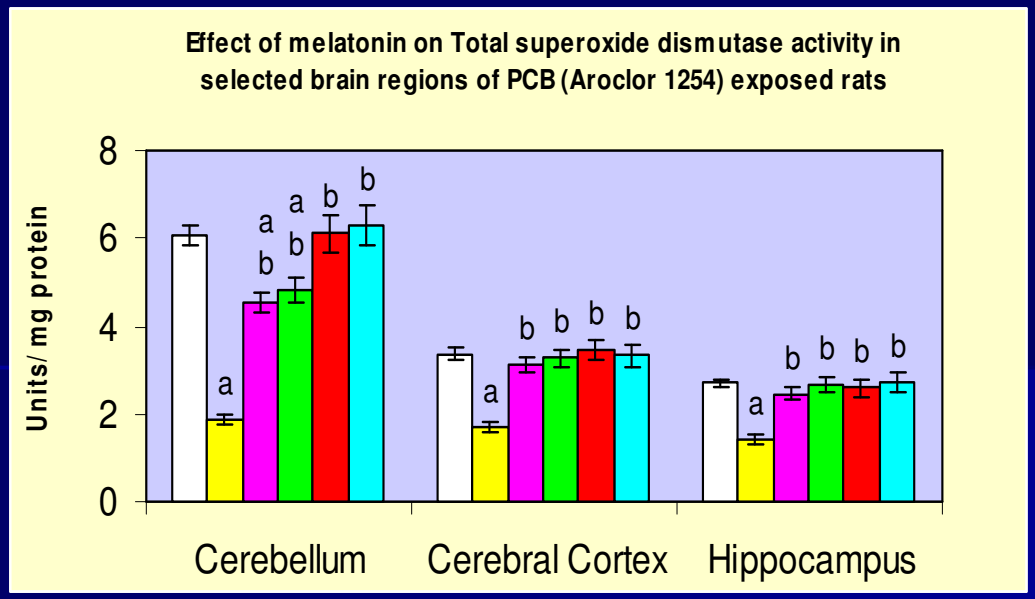
mRNA Expression

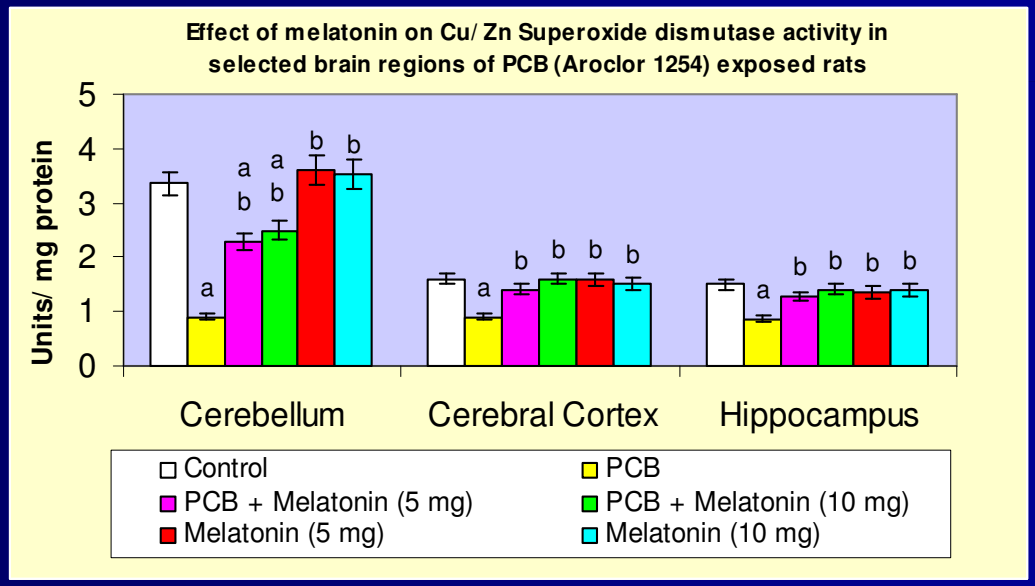
(i) Cu/ Zn Superoxide dismutase (SOD-1)

(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.





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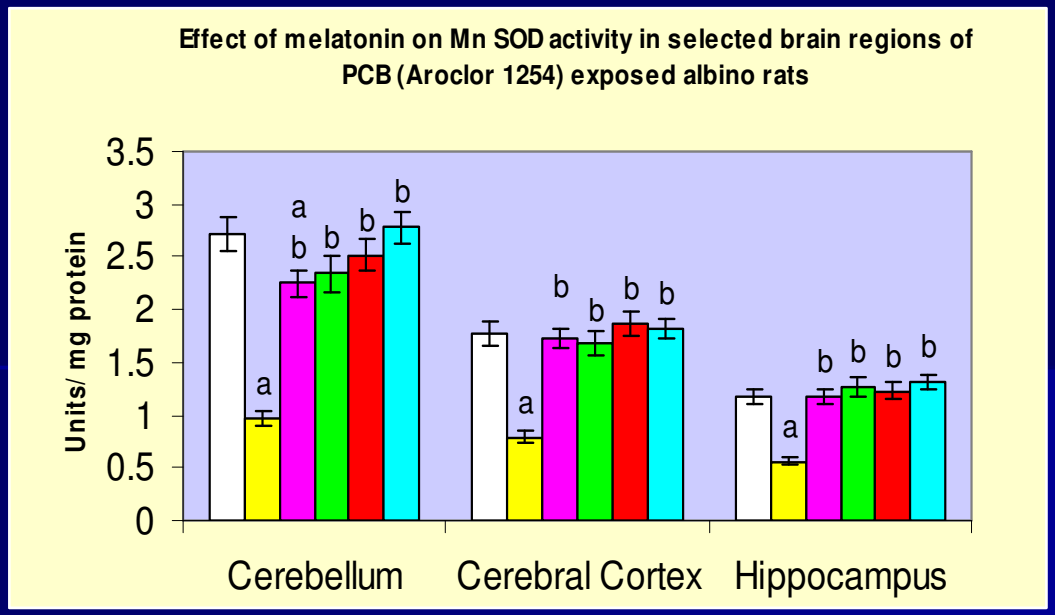

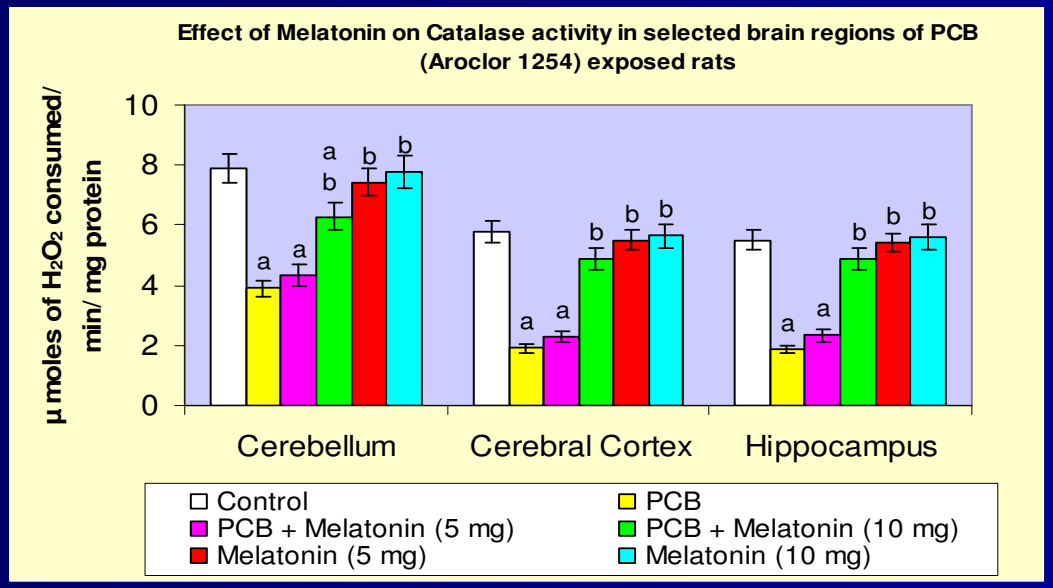

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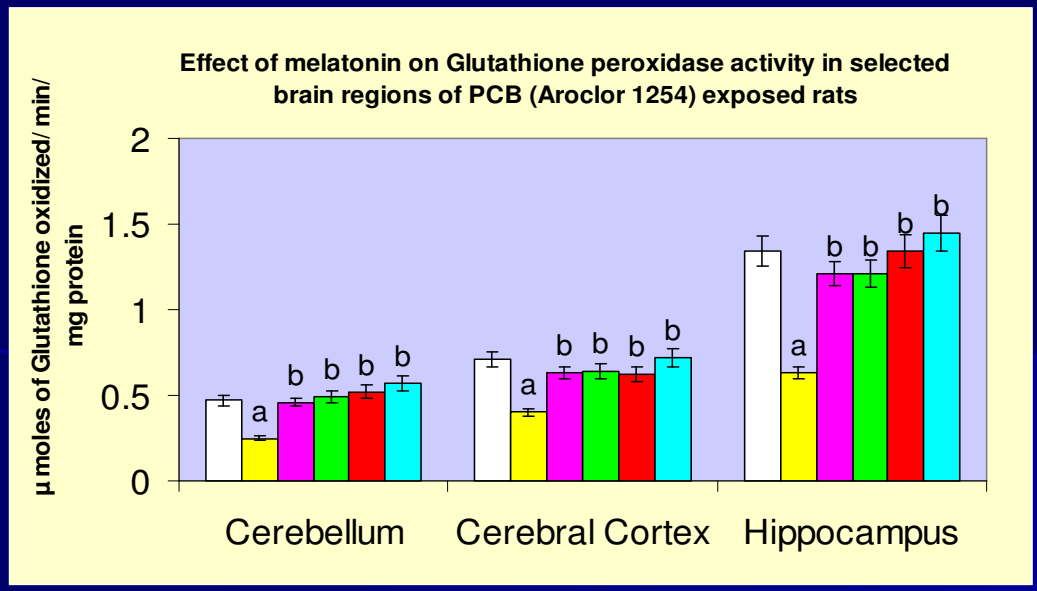

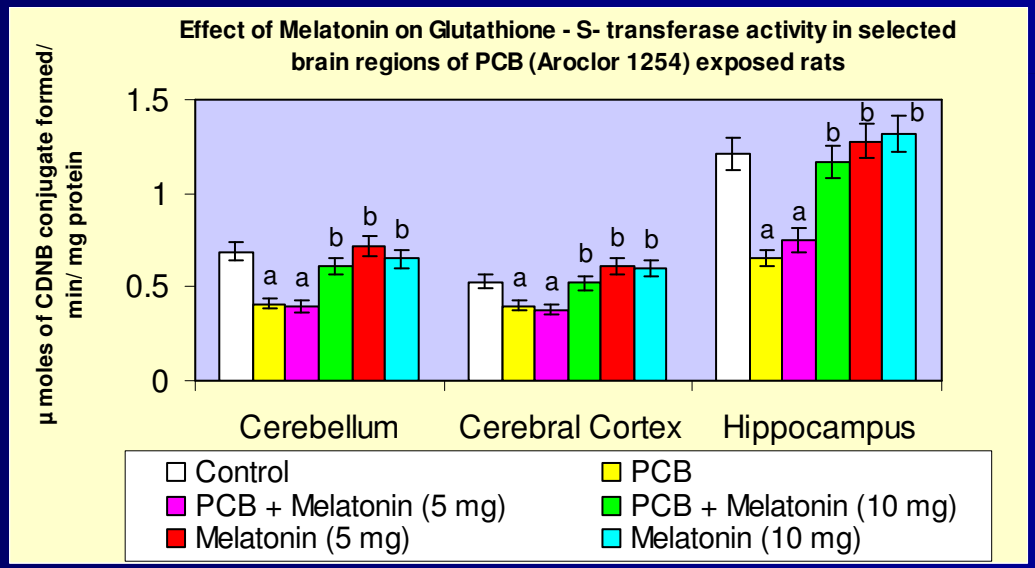

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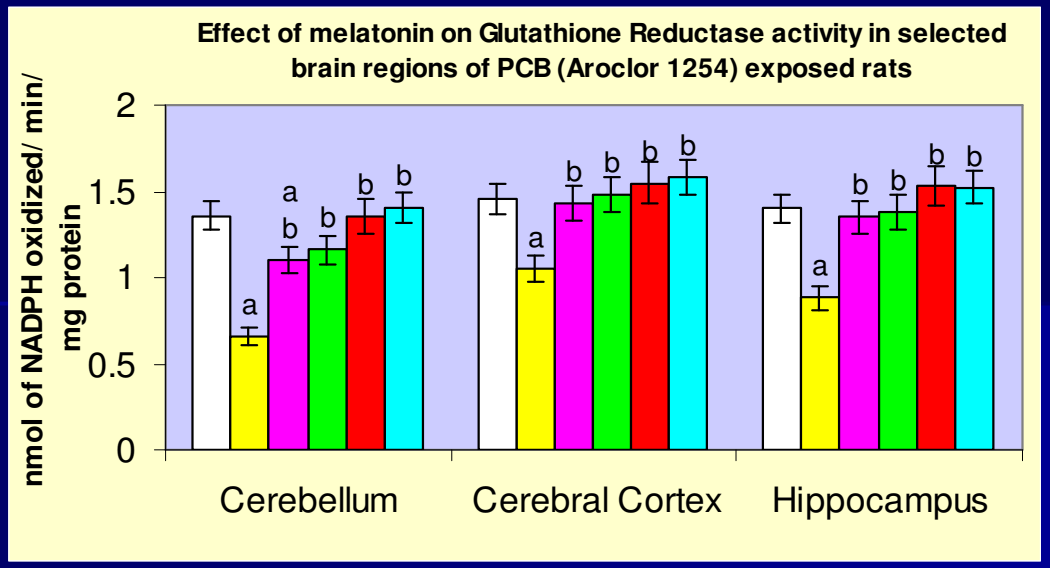

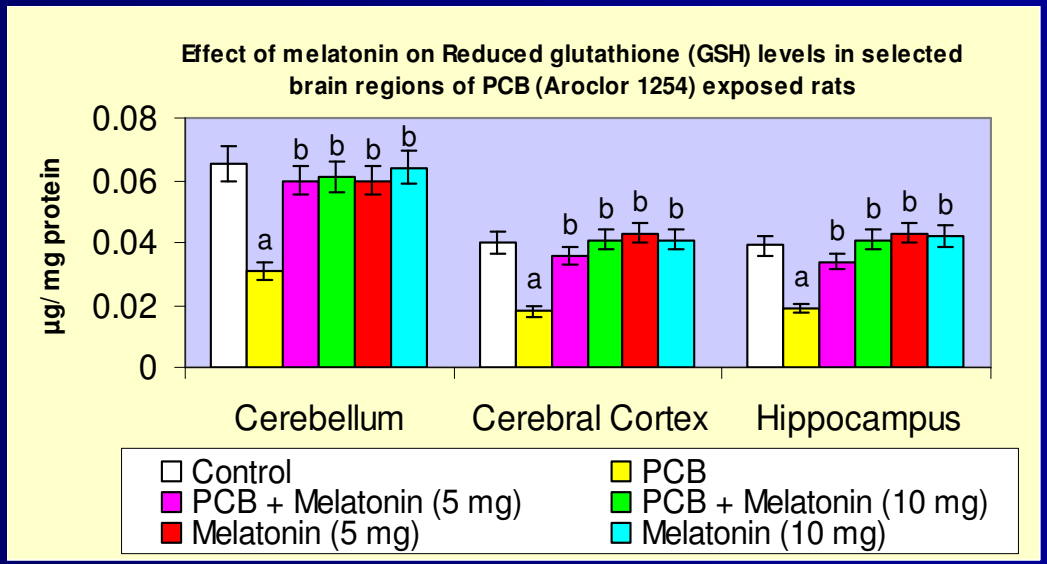

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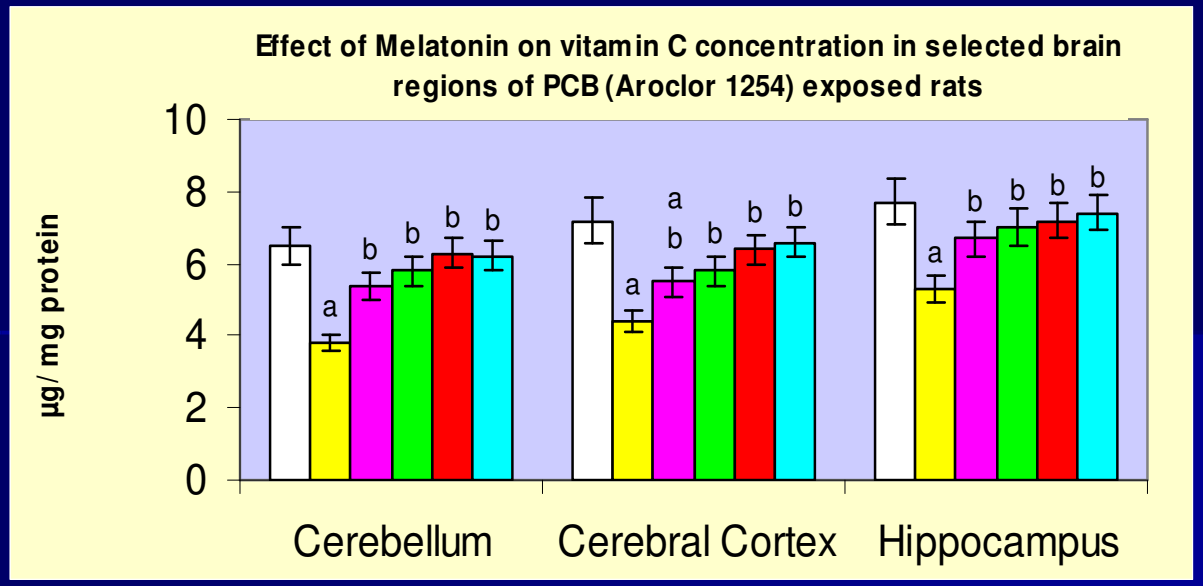

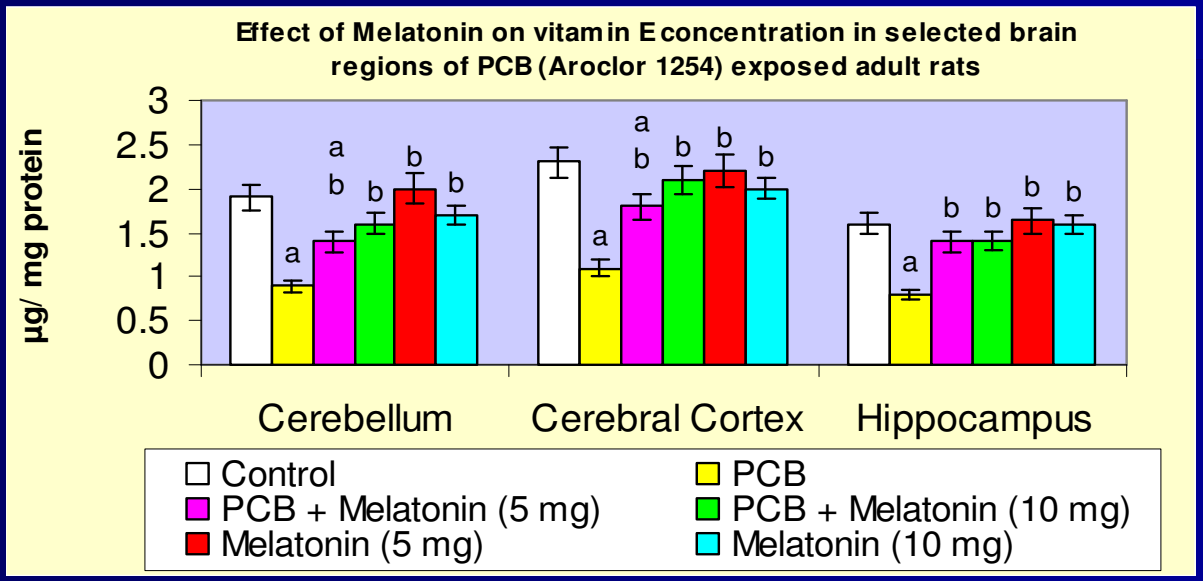

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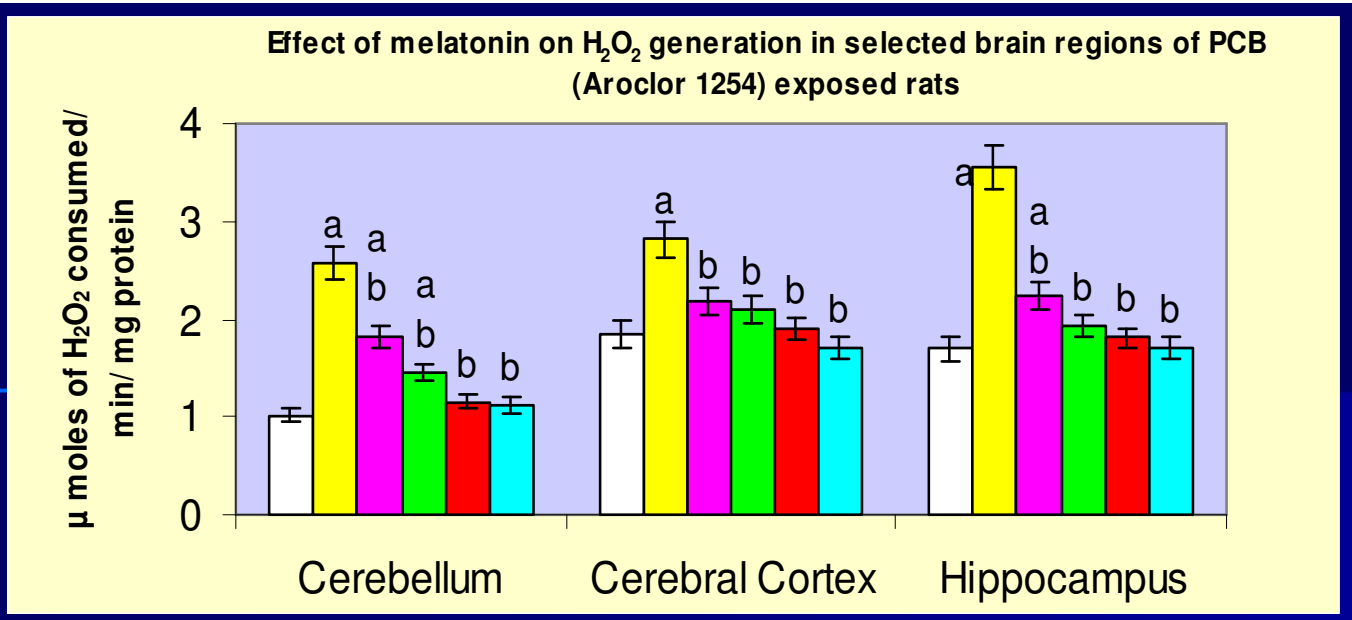

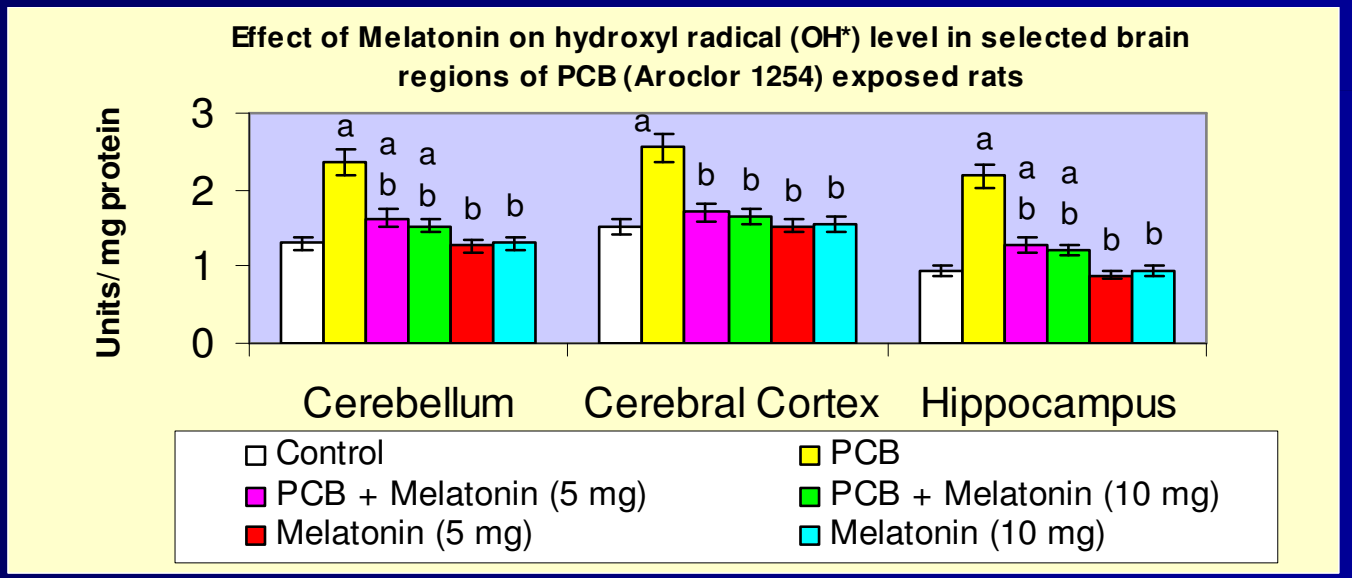

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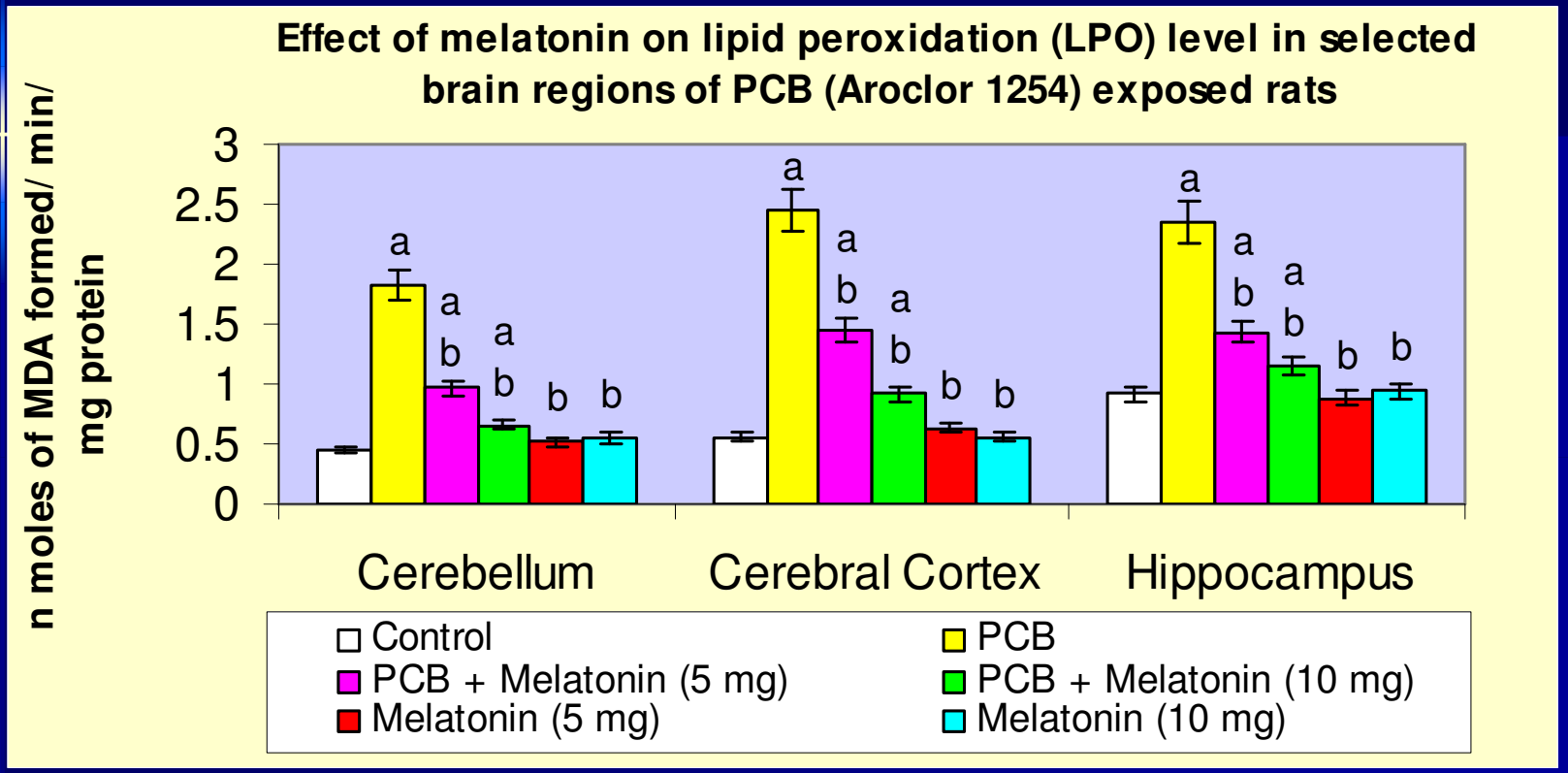

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Cu-Zn SOD (447 bp)

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)

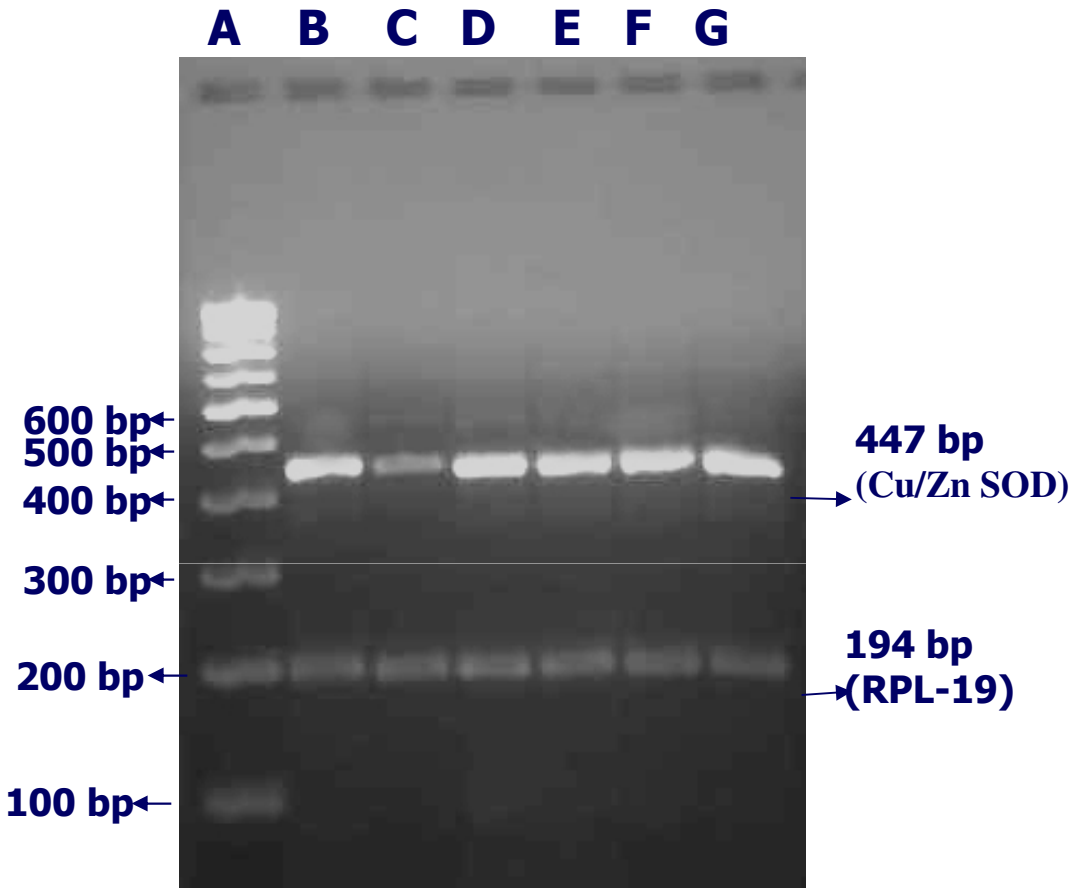
Gene bank accession no. for GPx4 is D87896

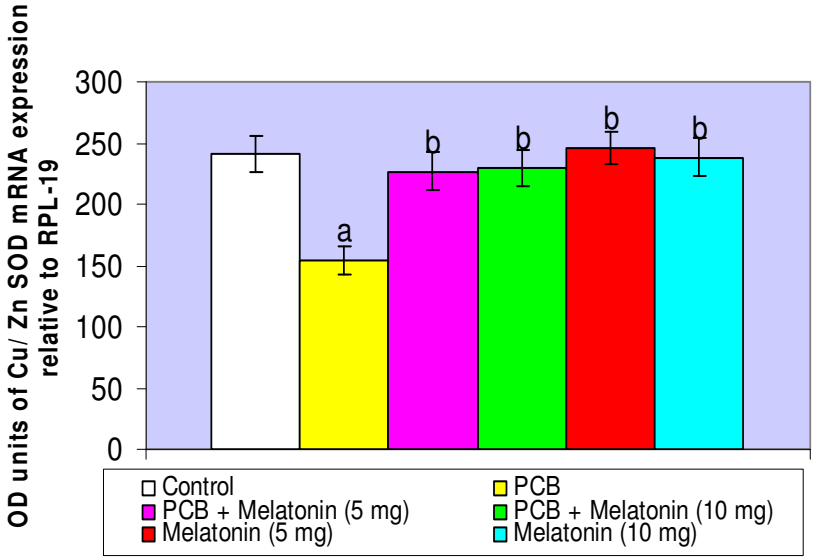
Sense : (nt.265-285) : 5’-ATGCACGAATTCTCAGCCAAG -3’

Antisense: (nt.725-709): 5’-GGCAGGTCCTTCTCTTCTAT -3’

Limaye *et al.*, 2003; Nam *et al.*, 2003

Cu/ Zn SOD mRNA Expression - Cerebellum

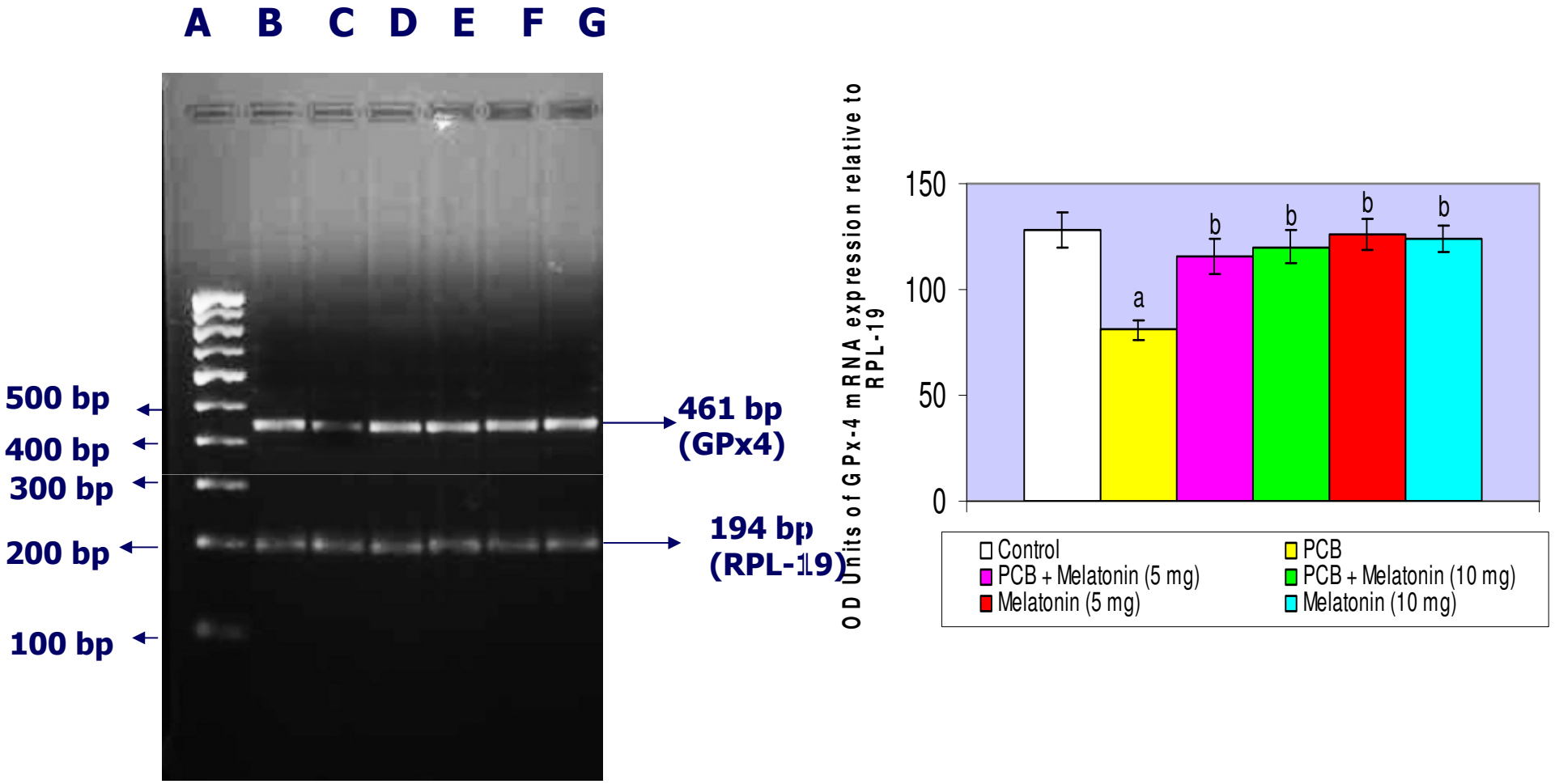




A -100 bp ladder; B - Control; C - PCB; D - PCB + Melatonin (5 mg);
E- PCB + Melatonin (10 mg); F-Melatonin (5 mg); G - Melatonin (10 mg)

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.

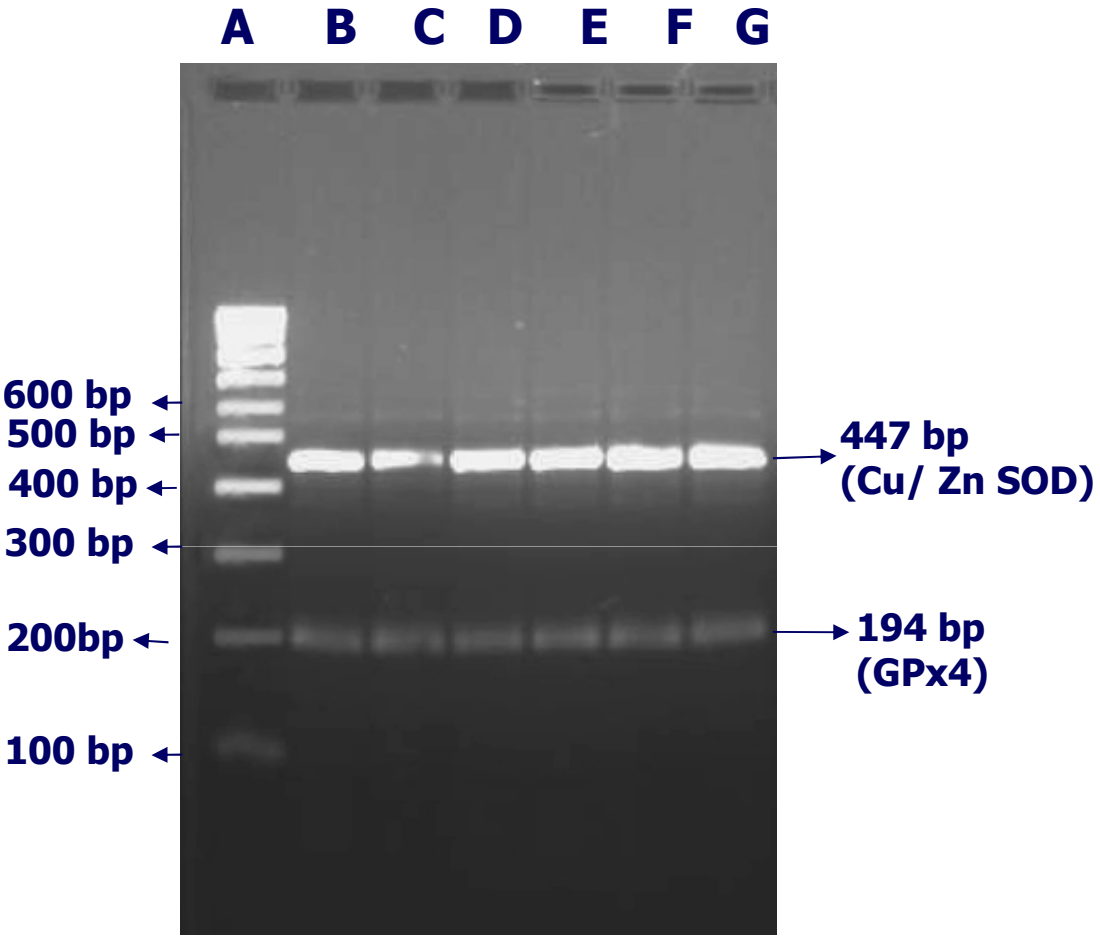
GPx-4 mRNA Expression - Cerebellum

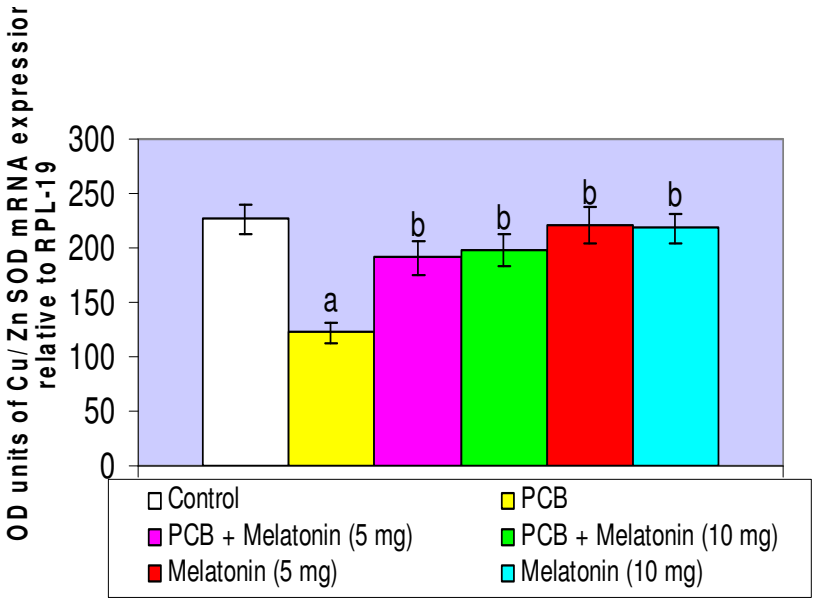


A – 100 bp ladder; B – Control; C – PCB; D – PCB + Melatonin (5 mg)
E - PCB + Melatonin (10 mg); F - Melatonin (5 mg); G – Melatonin (10 mg)

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

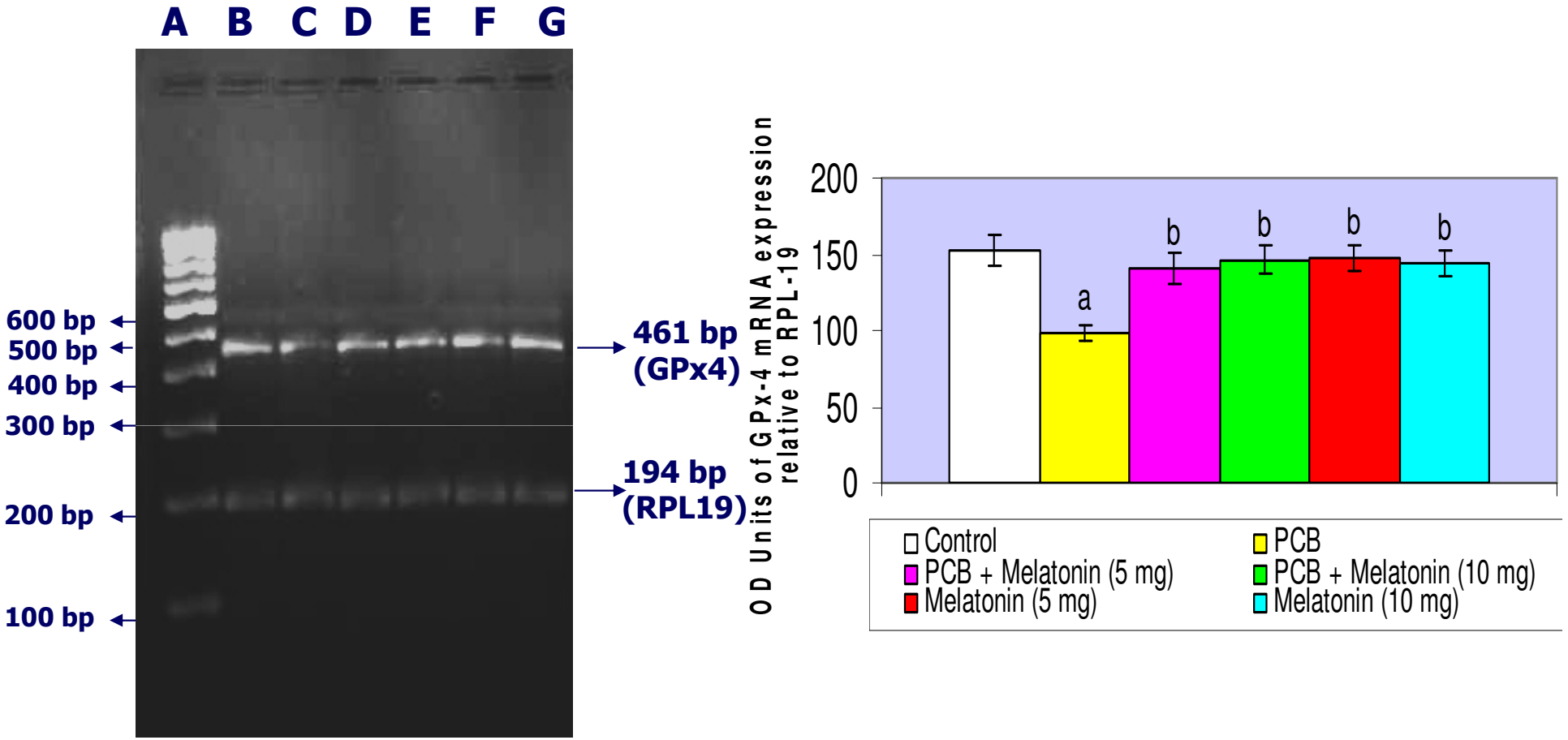




A – 100 bp ladder; B – Control; C – PCB; D – PCB + Melatonin (5 mg)
E - PCB + Melatonin (10 mg); F - Melatonin (5 mg); G – Melatonin (10 mg)

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.

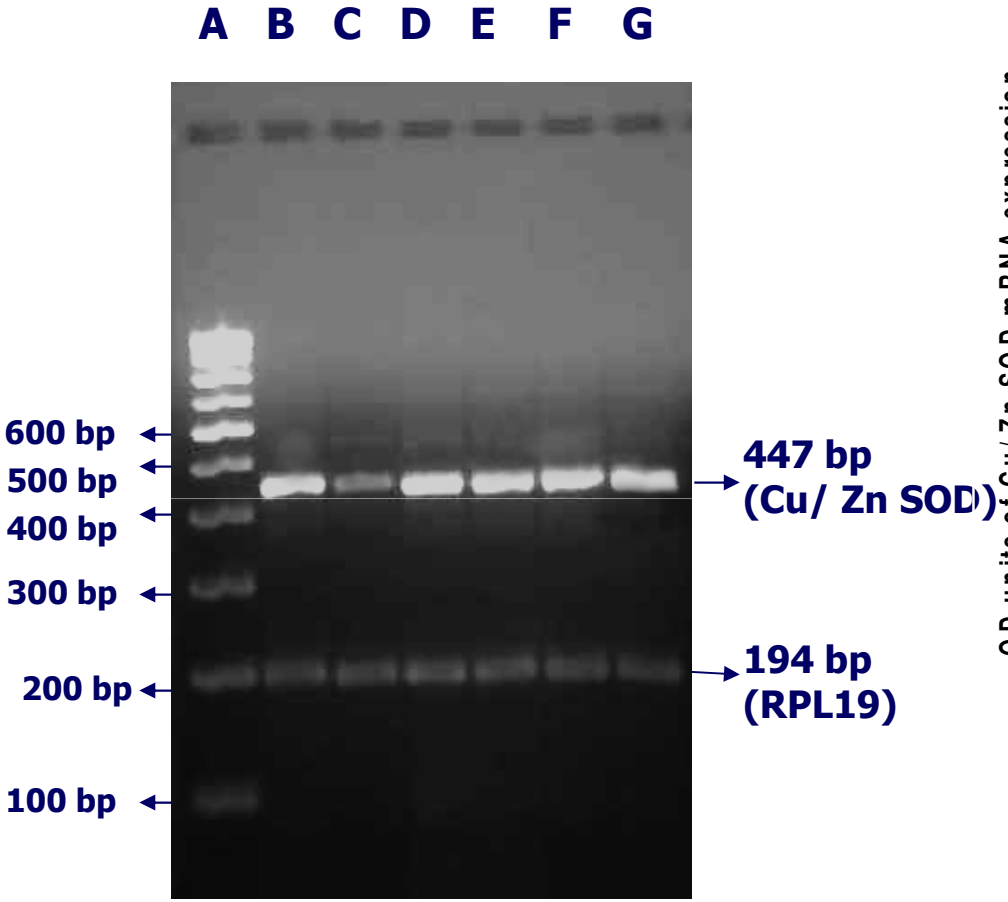
GPx-4 mRNA Expression – Cerebral cortex

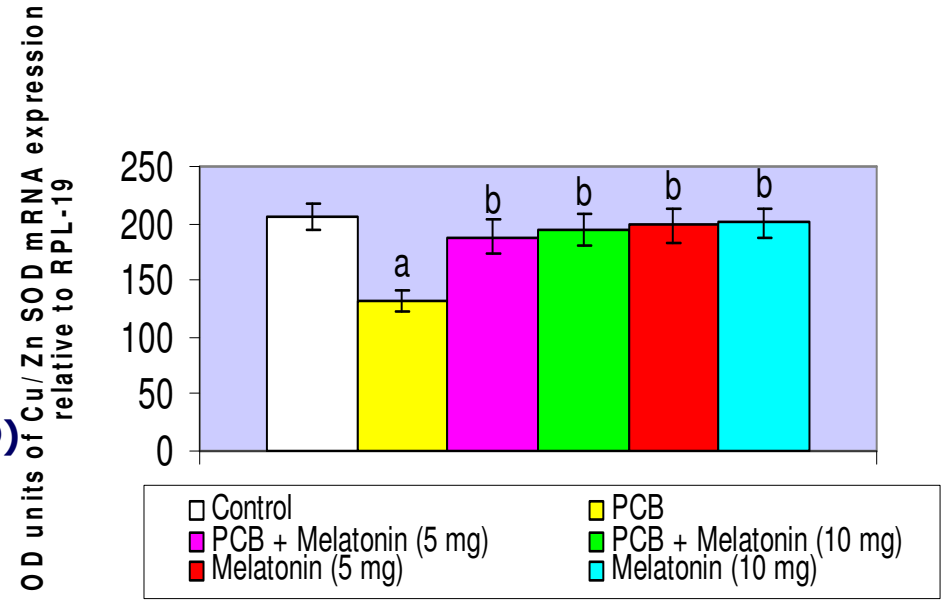


A – 100 bp ladder; B – Control; C – PCB; D – PCB + Melatonin (5 mg)
E - PCB + Melatonin (10 mg); F - Melatonin (5 mg); G – Melatonin (10 mg)

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,
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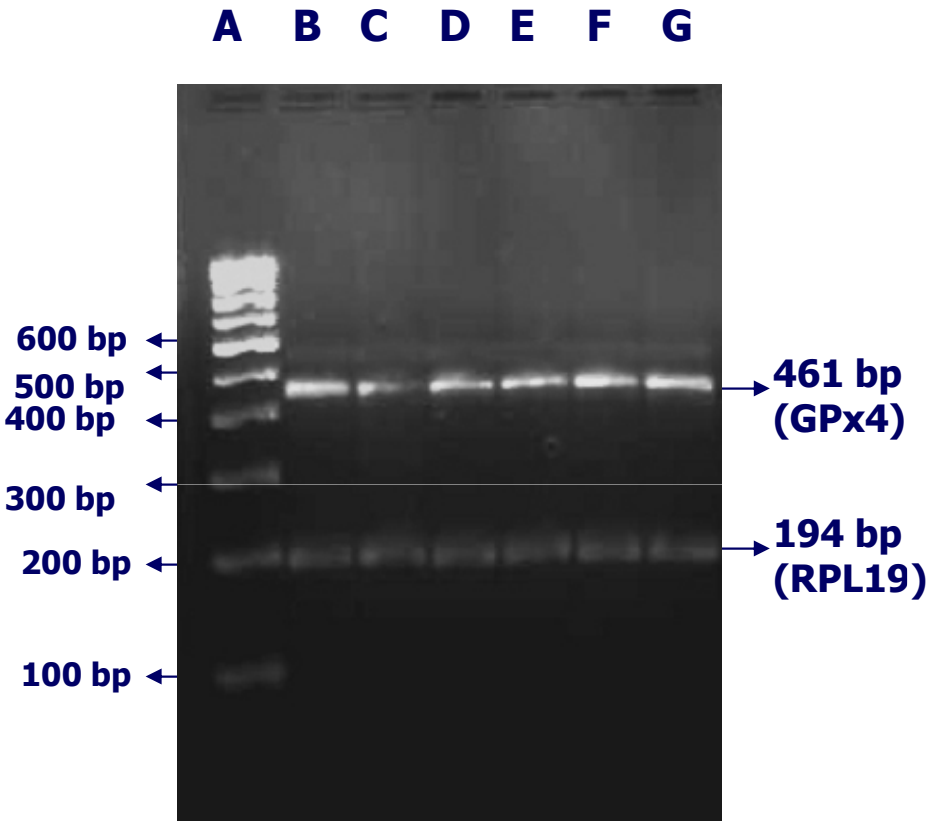
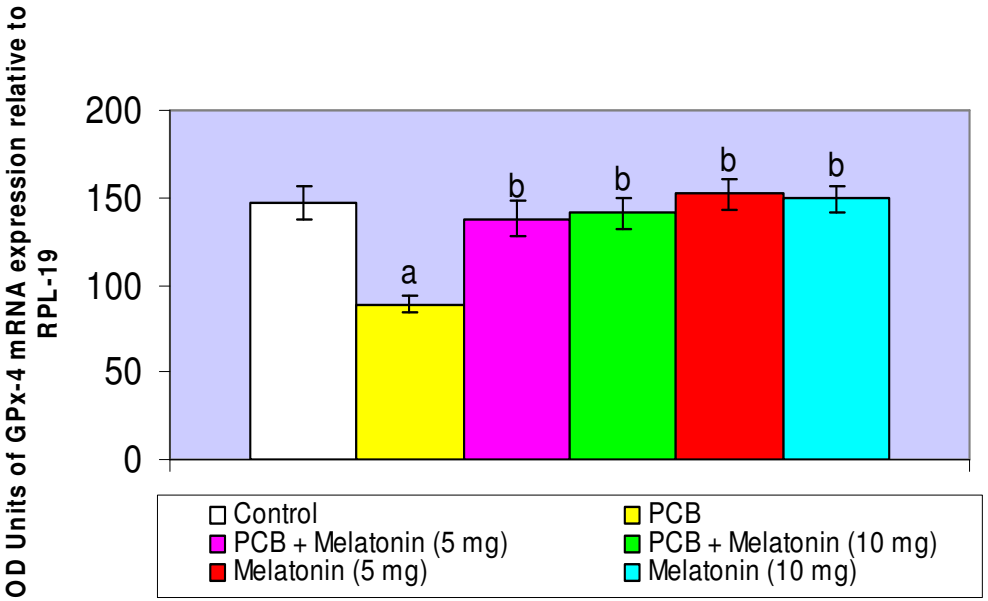
GPx-4 mRNA Expression – Hippocampus





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

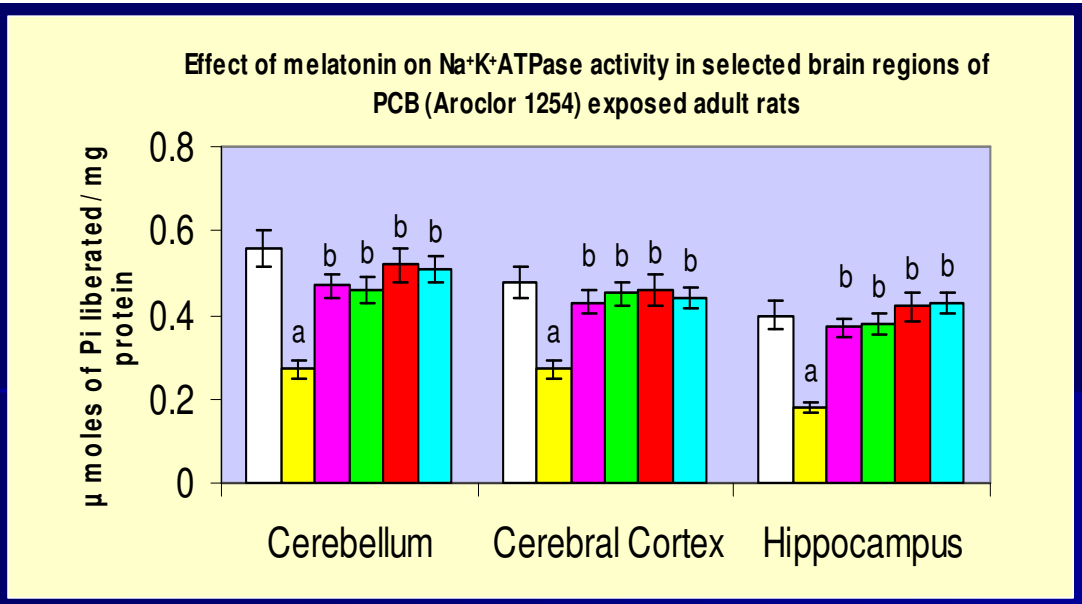
Membrane bound ATPases

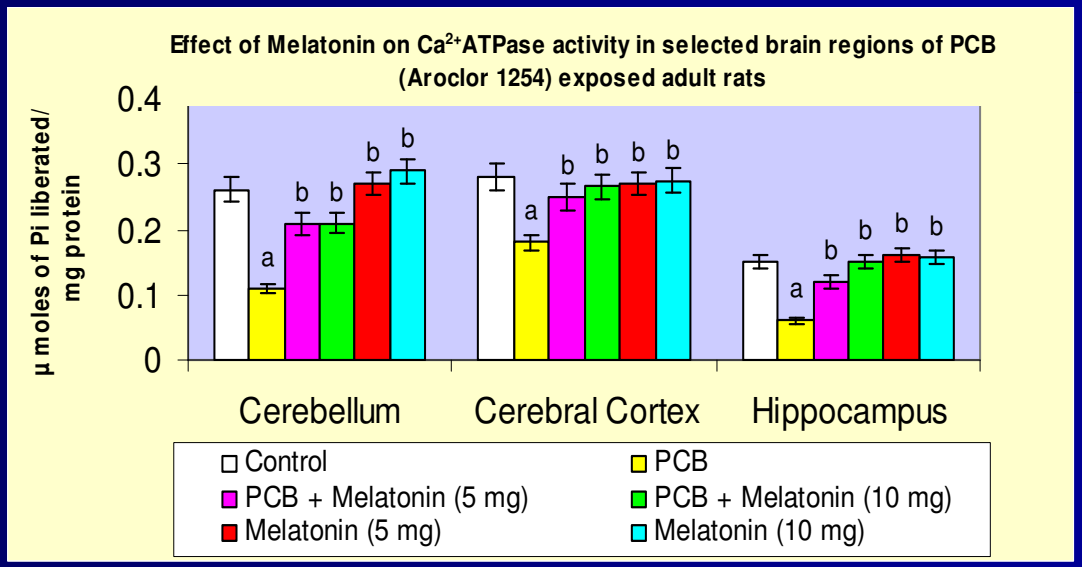
- $Na^{+}K^{+}$ ATPase (Bonting, 1970)
- Ca^{2+}ATPase (Hjerten and Pan, 1983)
- Mg^{2+} ATPase (Ohinishi *et al.,* 1982)
- Inorganic phosphorus (Fiske and Subbarao, 1925)

Creatine kinase (Okinaka *et al*., 1964).

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

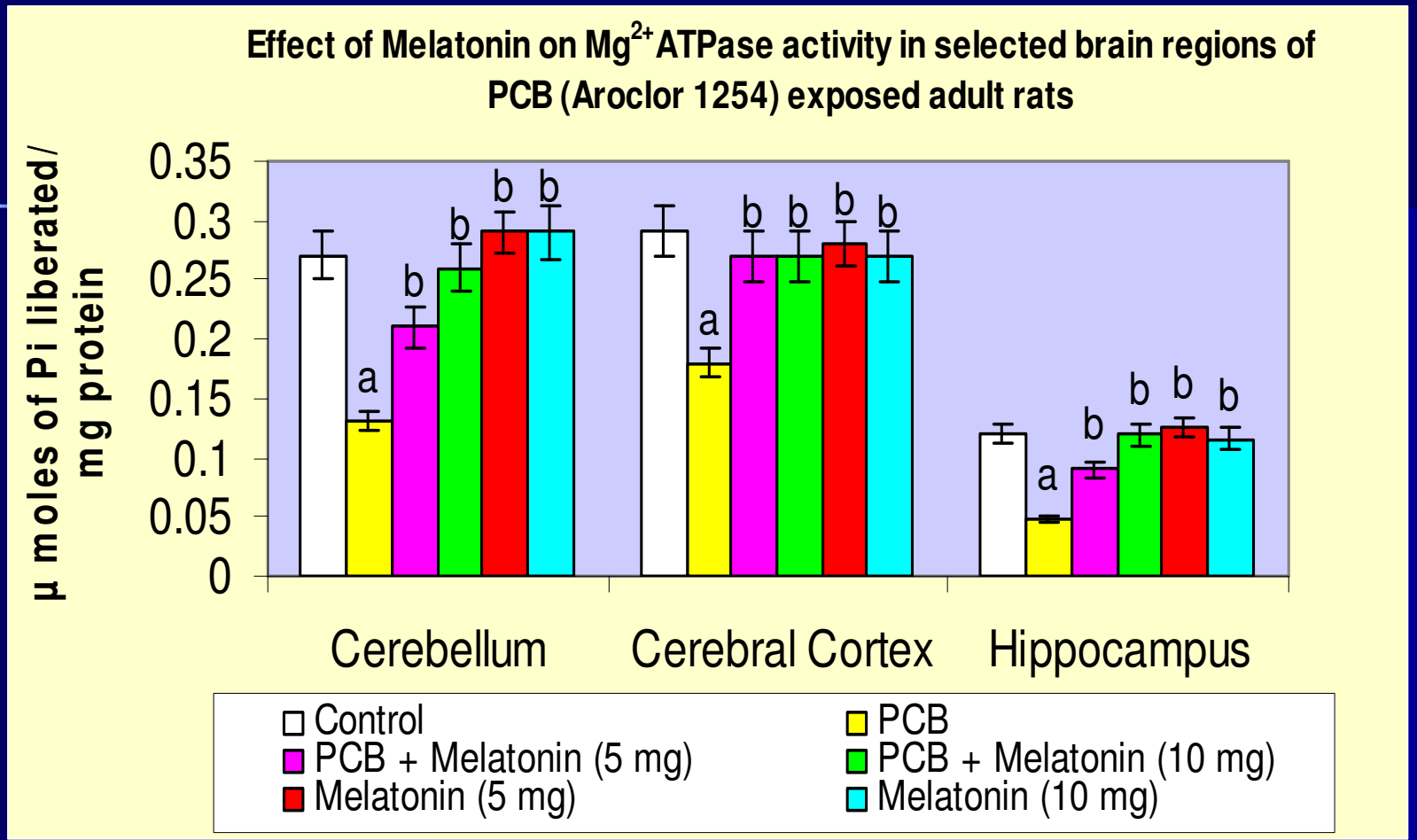
Acetylcholine esterase (Ellman *et al*., 1961).





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



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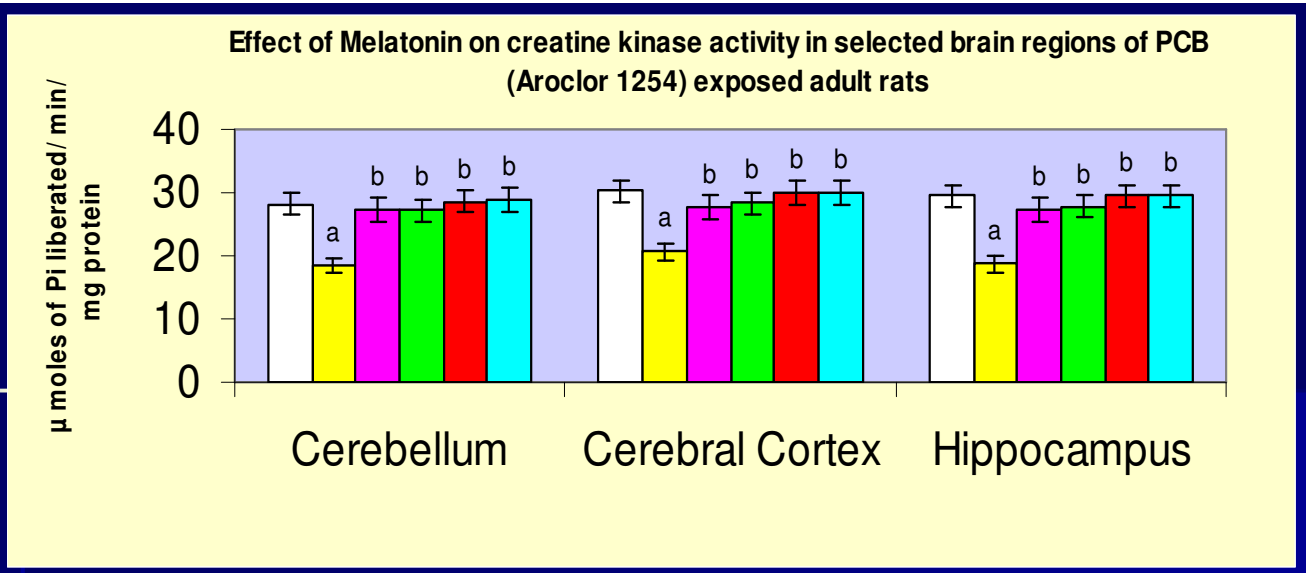

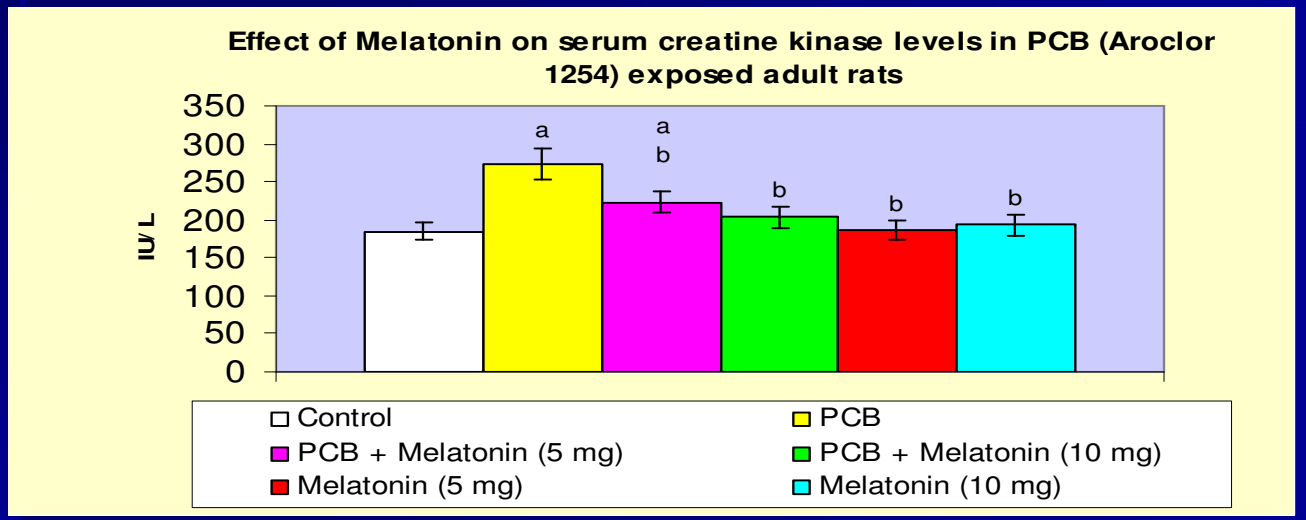

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Significance at P<0.05 followed by SNK,
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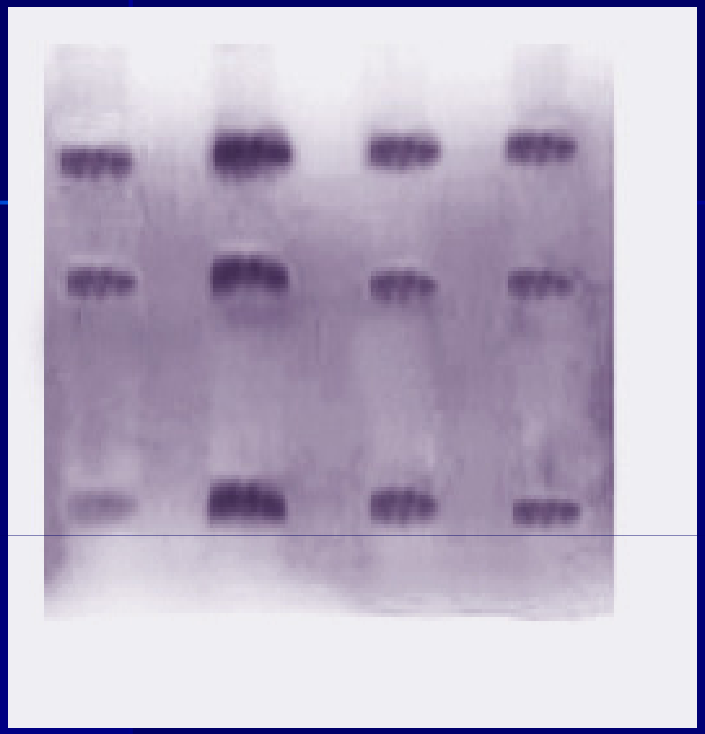
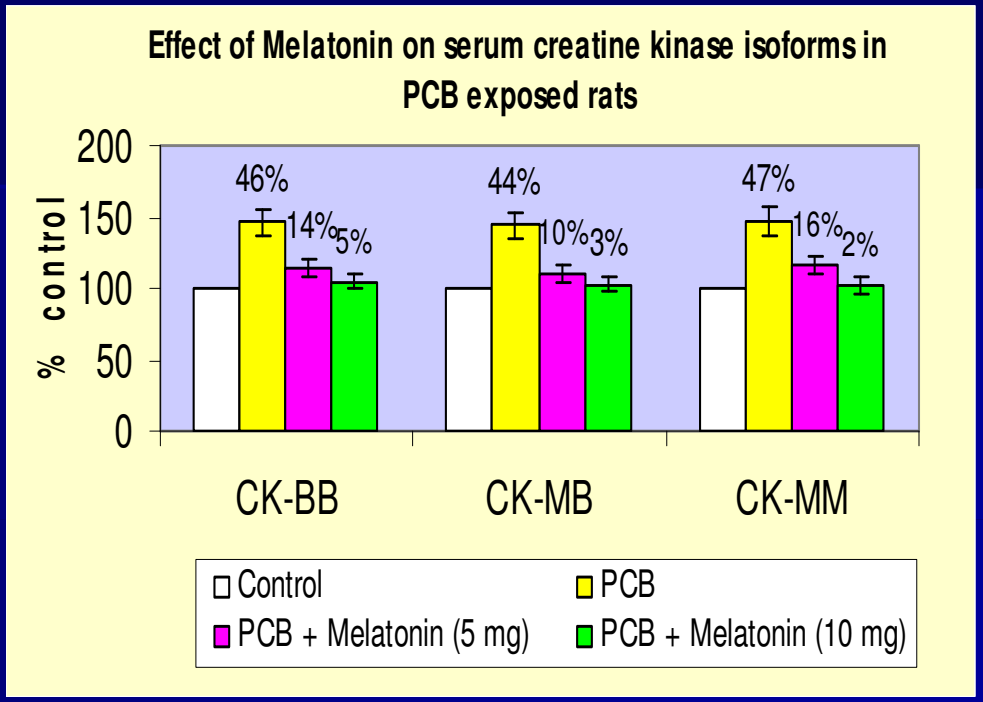
Basic & clinical Pharmacology & Toxicology, 2009, 105: 92-97

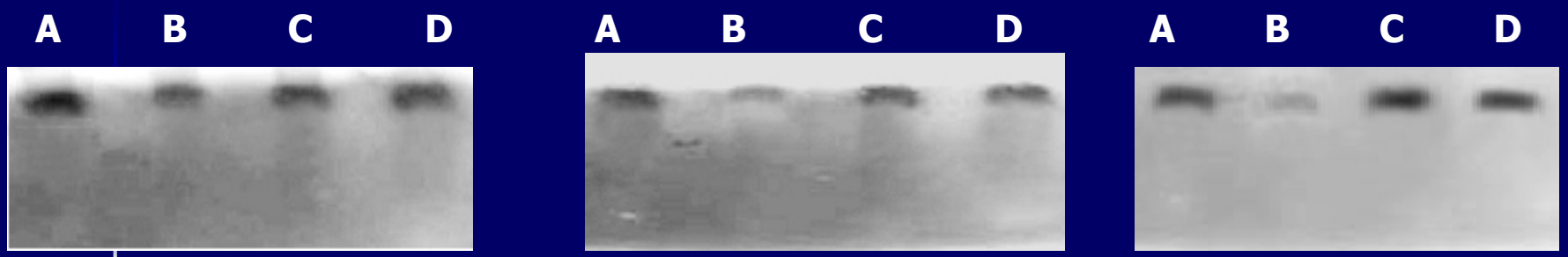




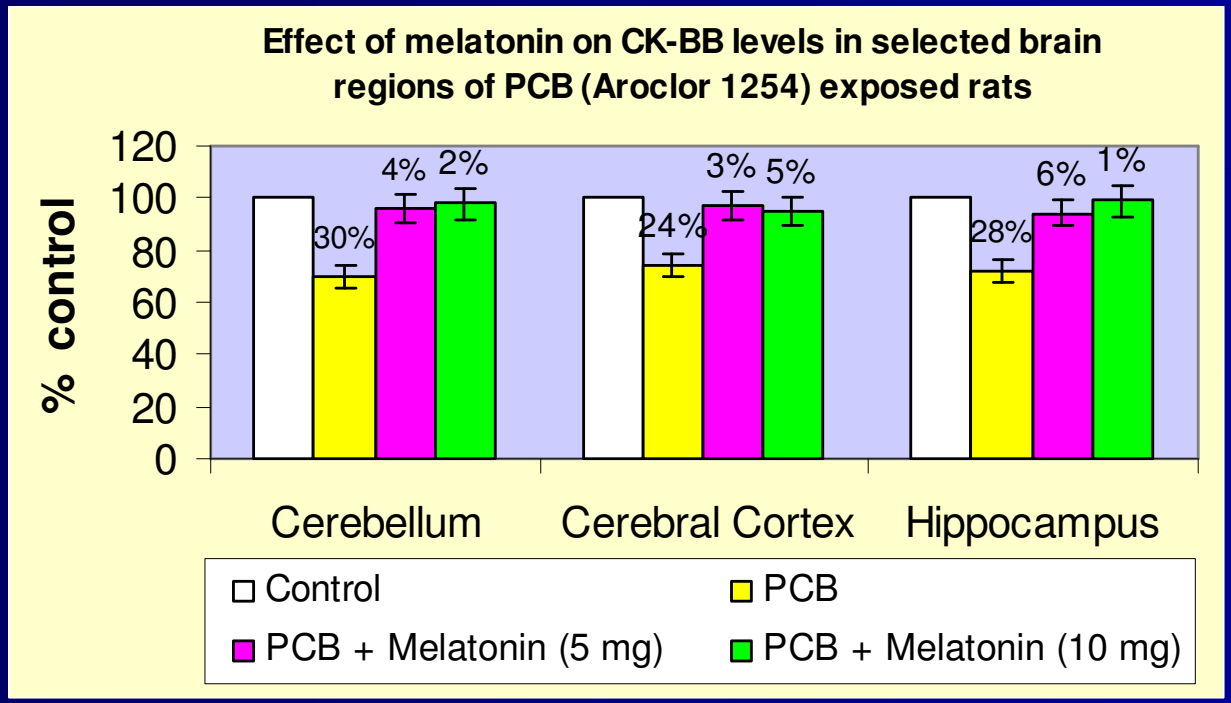
Each bar represents the mean ± SEM of 3 independent observations.

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



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



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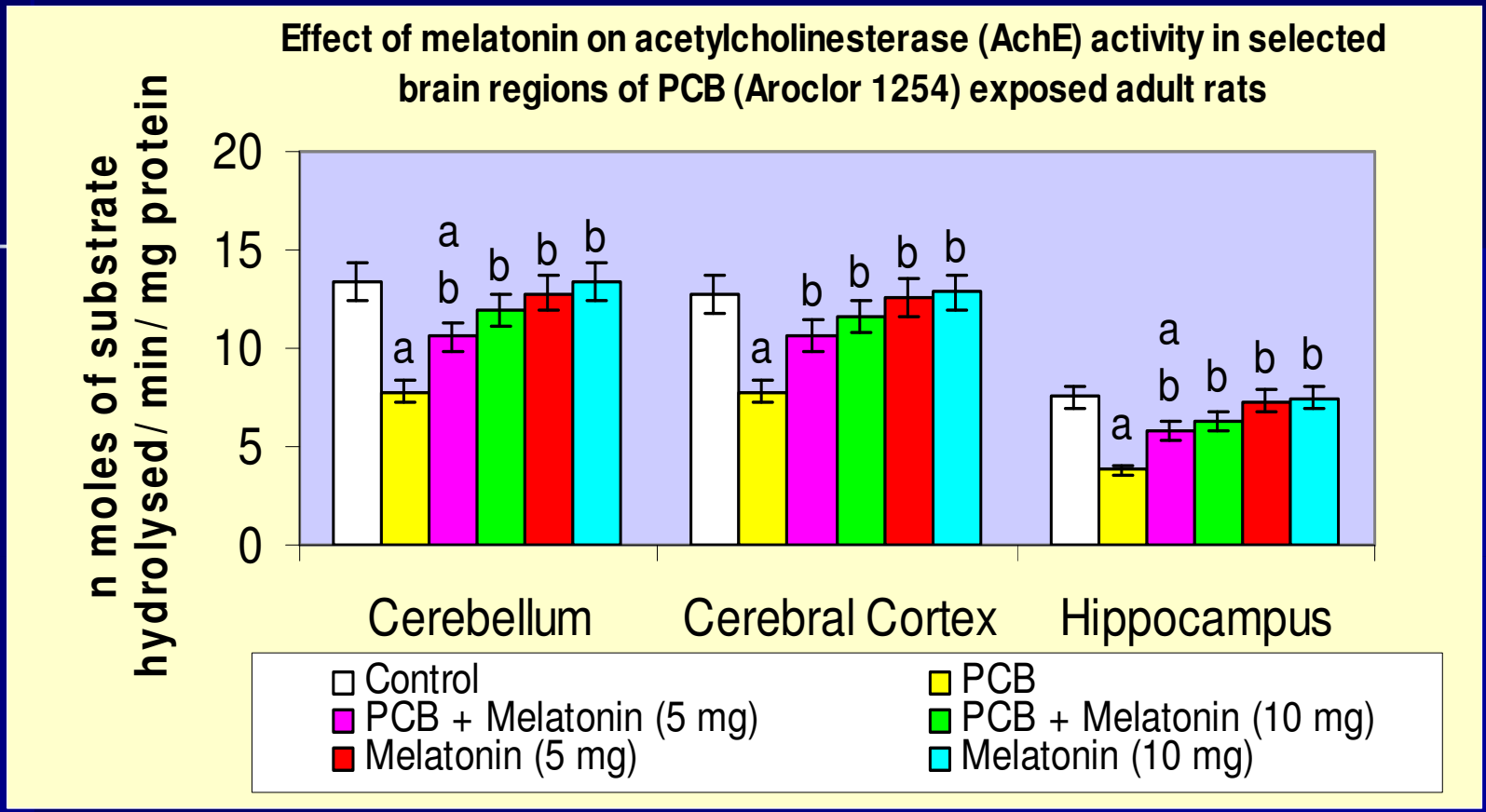


Each bar represents the mean ± SEM of 6 animals. Significance at P<0.05 followed by SNK, a: Control Vs others b: PCB Vs other groups.

Int J Dev Neurosci, 2008: 26: 585-591

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

Technique

Steps involved

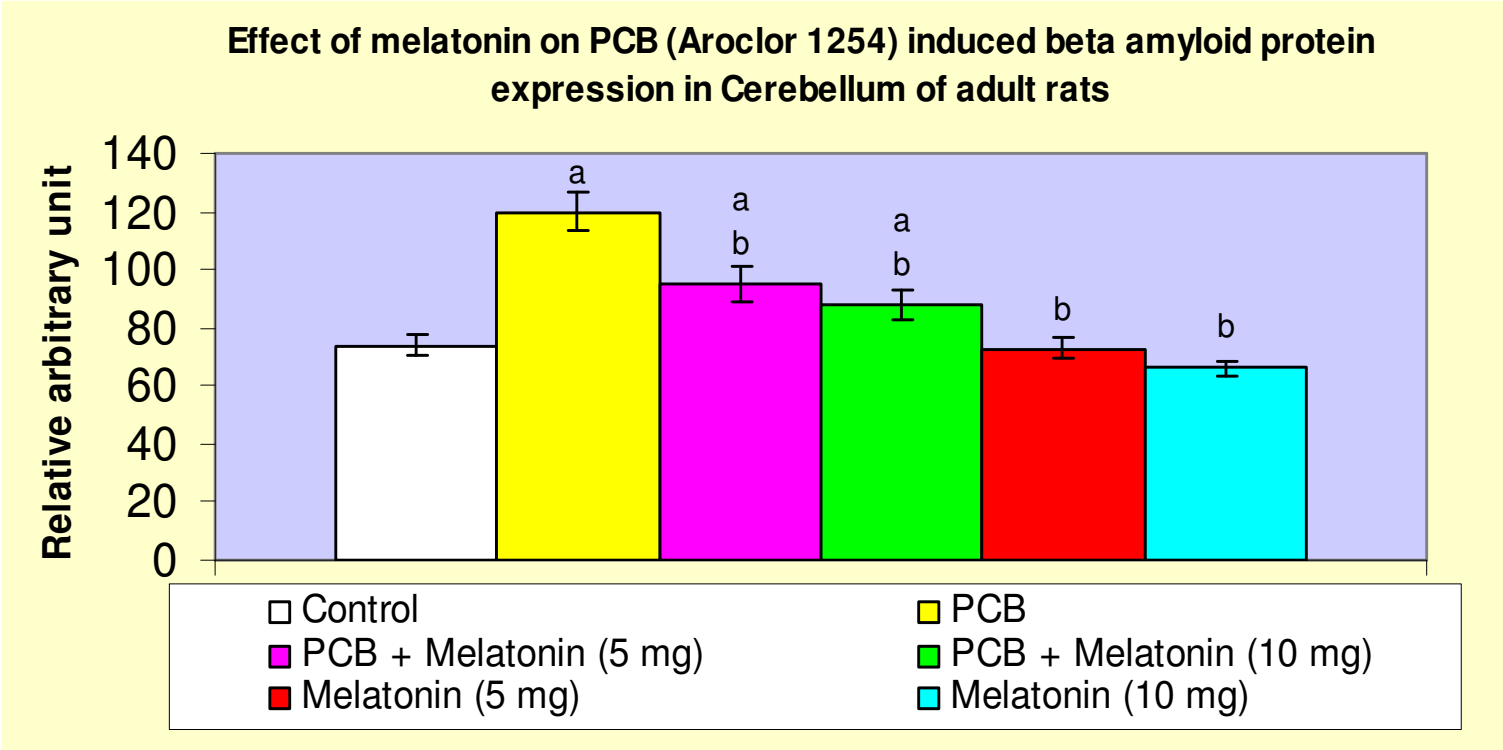
- Gel electrophoresis
- Transfer of proteins to the membrane
- Blocking non-specific binding
- Antibody incubation
- Protein detection

Primary : Beta Amyloid – Mouse monoclonal IgG2a (1: 1000)

Secondary : Rabbit antimouse IgG peroxidase conjugate (1:5000)

Primary : Beta Actin – Rabbit polyclonal (1: 1000)

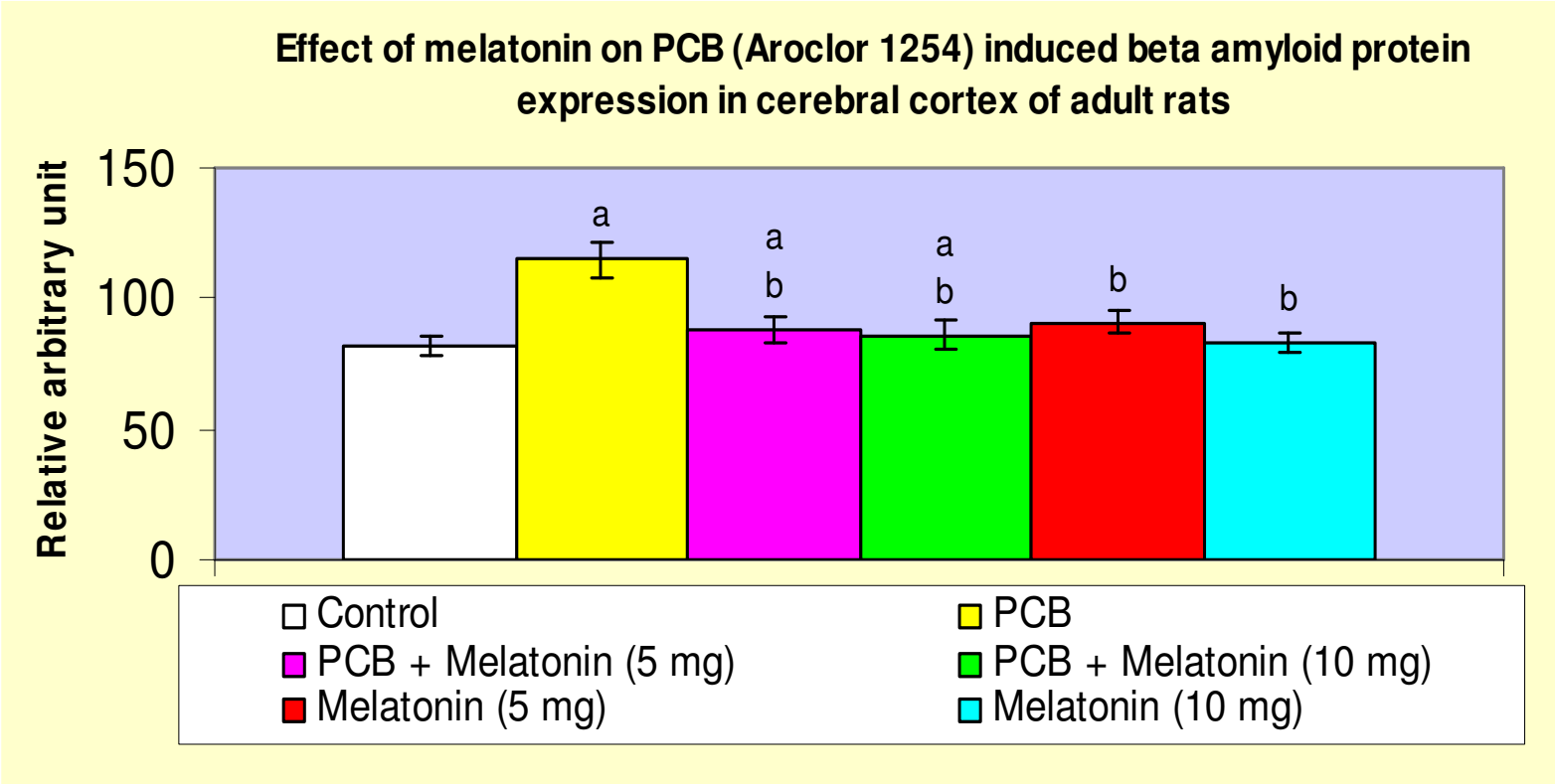
Secondary : Goat Anti rabbit (1: 5000)



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.

43 Kda Aβ

β actin



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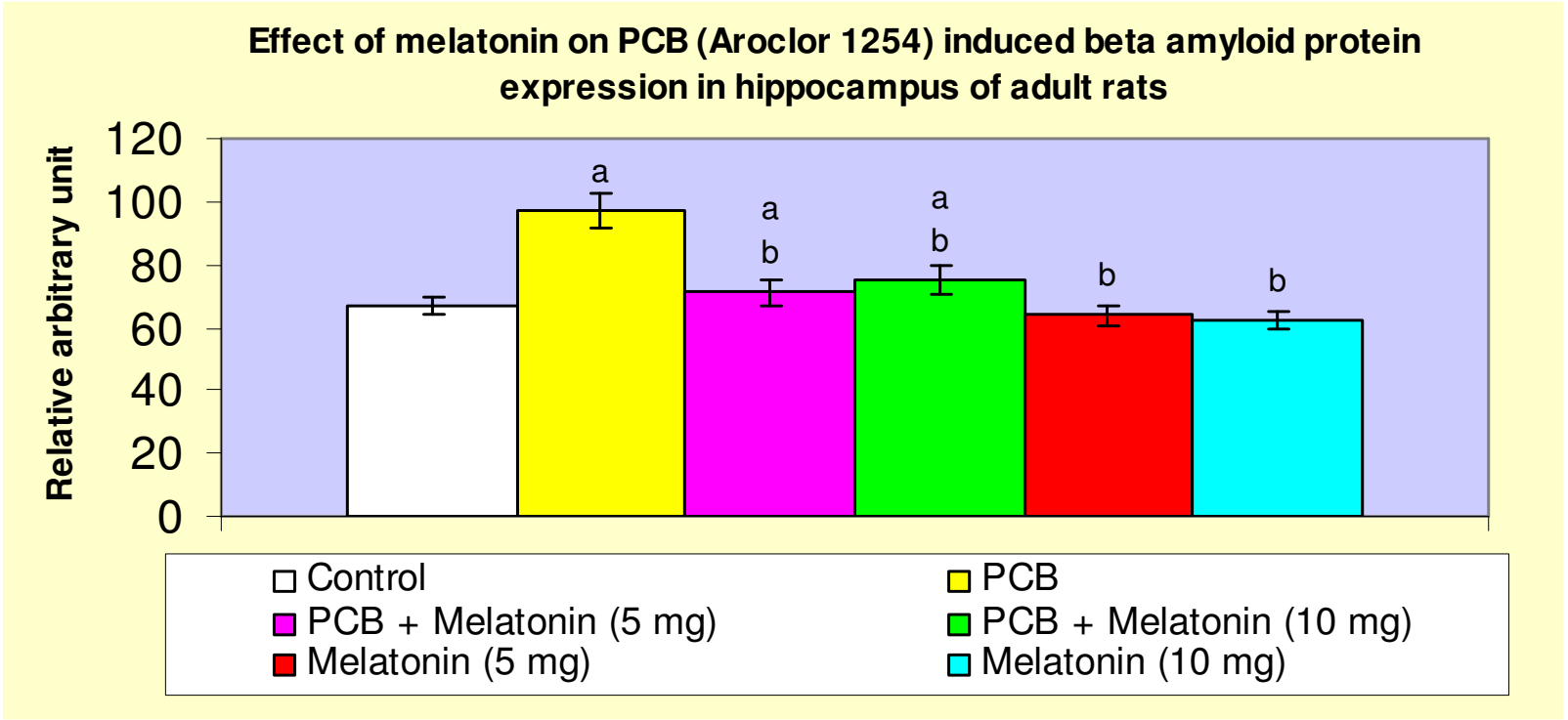


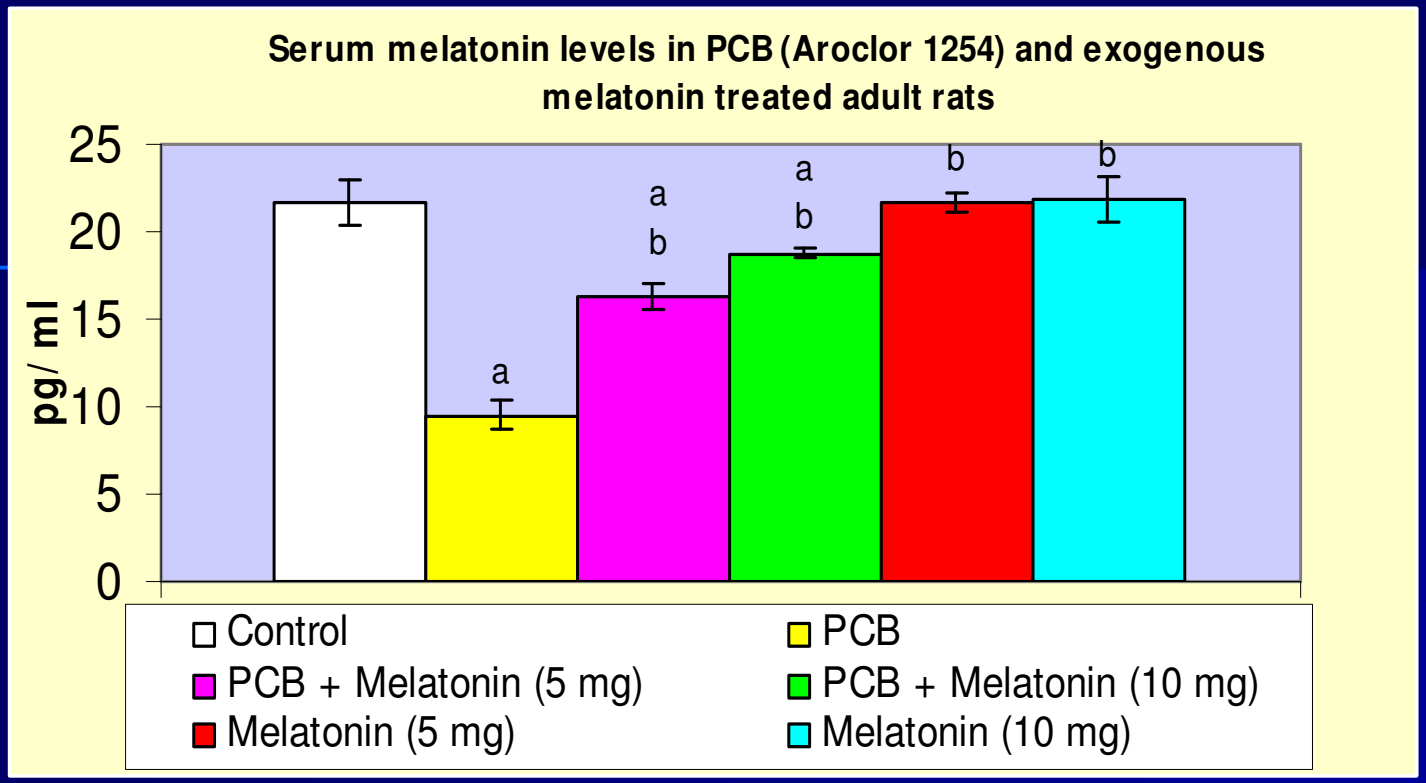
Each bar represents the mean + SEM of 3 observations. Significance at P<0.05 followed by SNK, a: Control Vs others, b: PCB Vs other groups.

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**



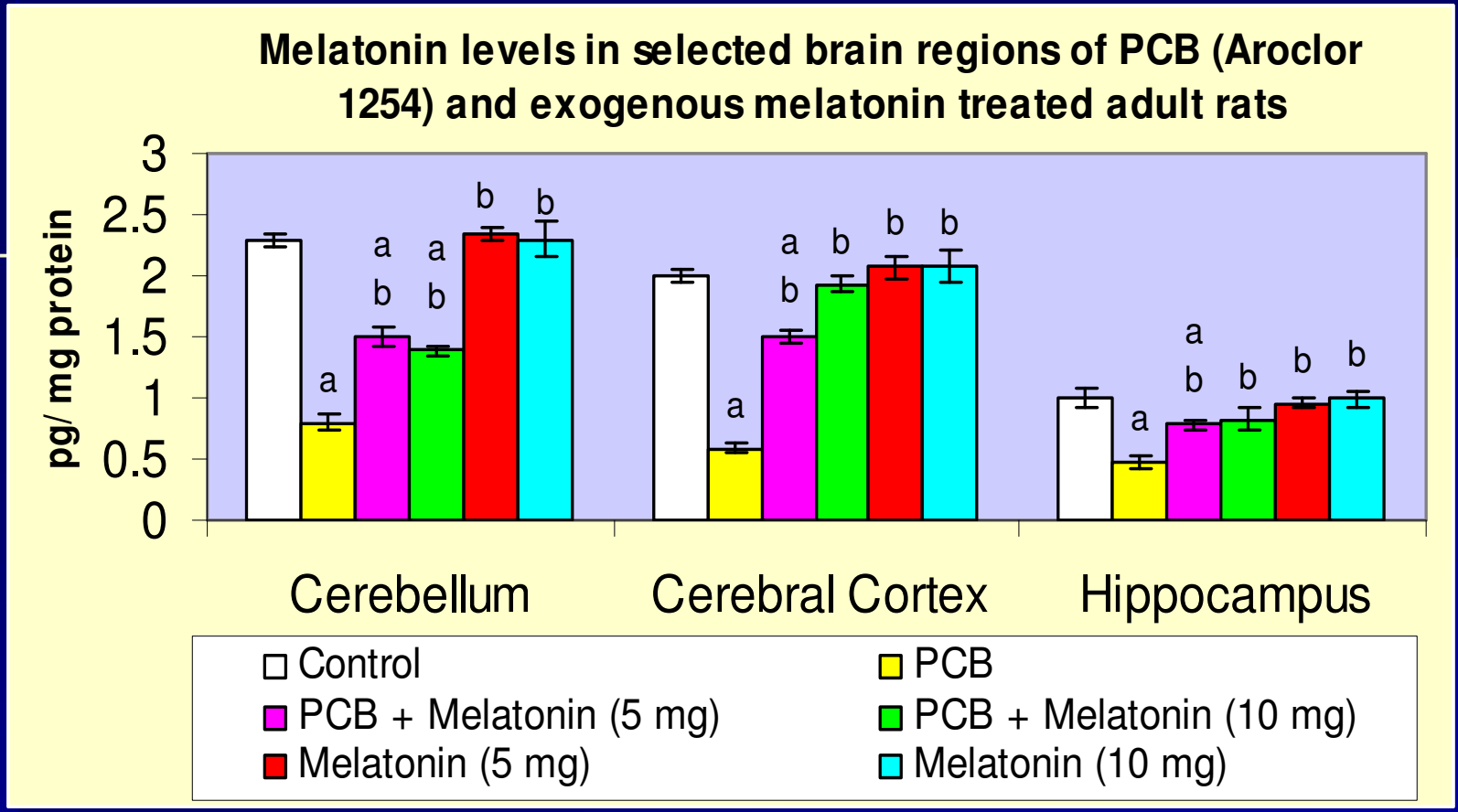
Each bar represents the mean ± SEM of 6 animals. Significance at P<0.05 followed by SNK, a: Control Vs others b: PCB Vs other groups.

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



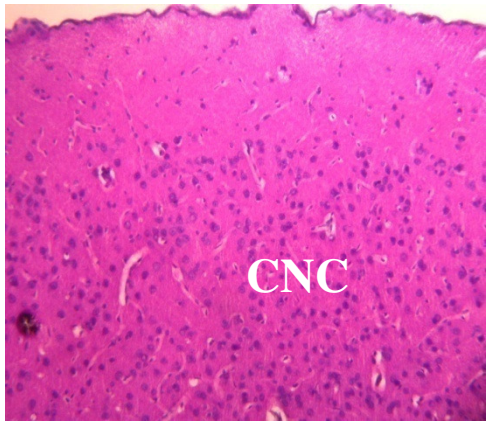
Each bar represents the mean ± SEM of 6 animals. Significance at P<0.05 followed by SNK, a: Control Vs others b: PCB Vs other groups.

CHAPTER – IV

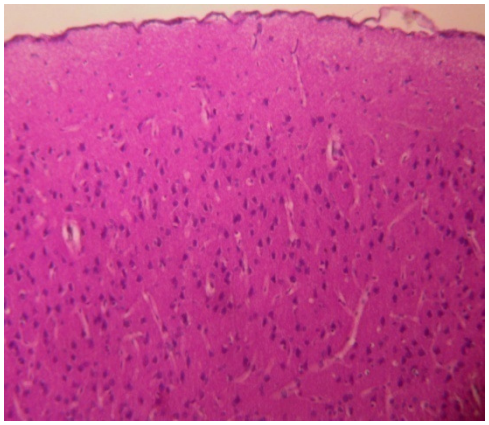
Histomorphological changes in cerebellum, cerebral cortex and hippocampus of PCB (Aroclor 1254) adult rats: Impact of melatonin

Neuroscience Research, 2010, 66: 189-197.

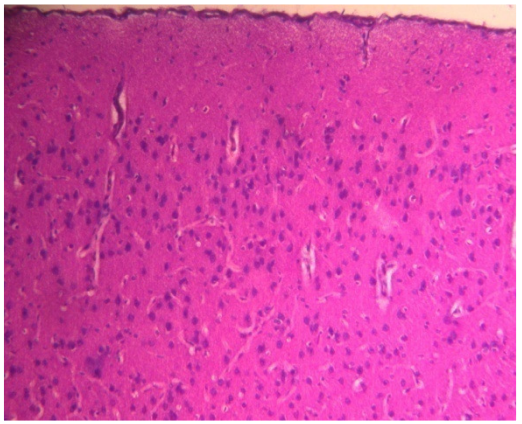
Cerebral cortex (10x)



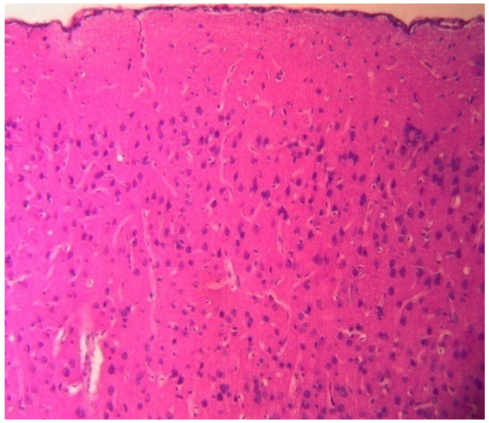
Control



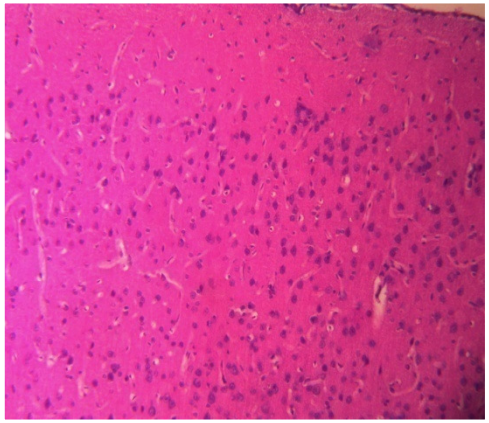
PCB



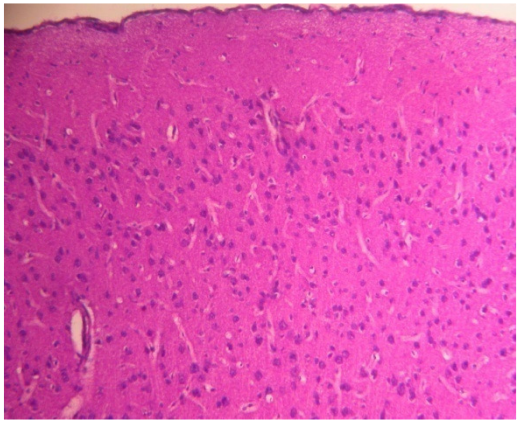
PCB + Melatonin (5 mg)



PCB + Melatonin (10 mg)



Melatonin (5 mg)

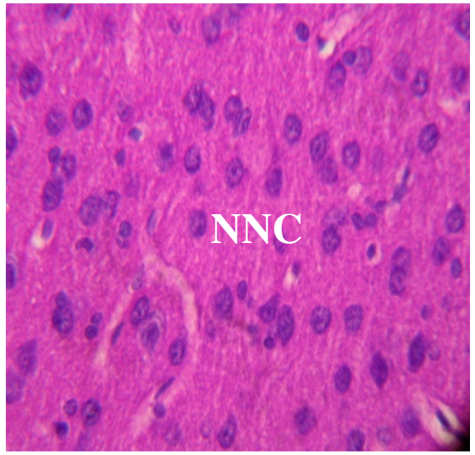


Melatonin (10 mg)

CNC- Cortical Neuronal cells

Haematoxylin and eosin staining

Cerebral Cortex – 40x



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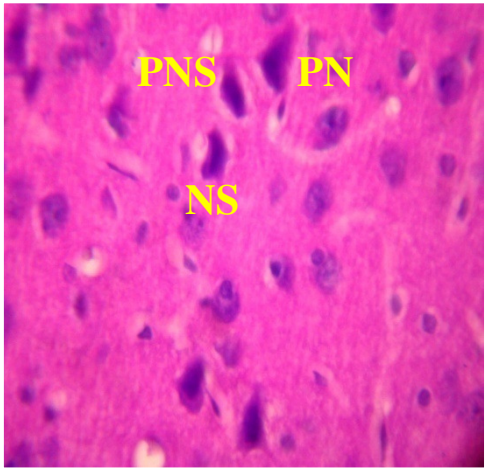
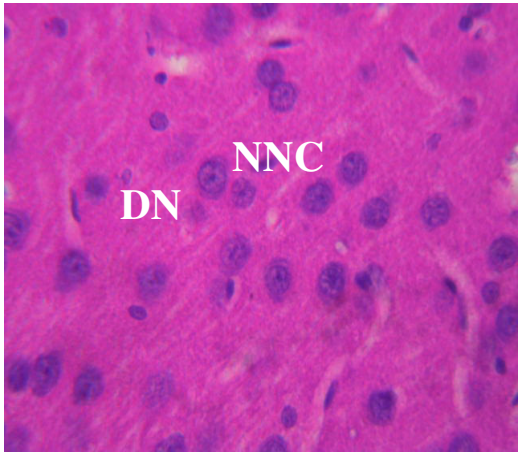
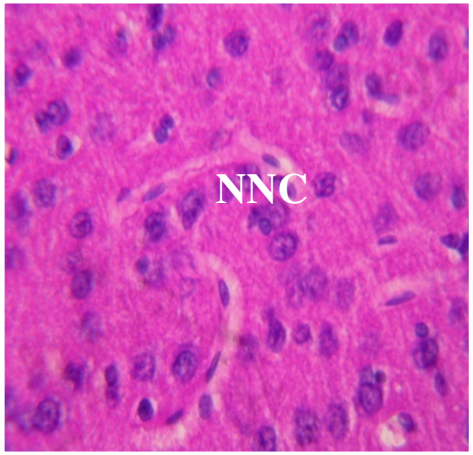


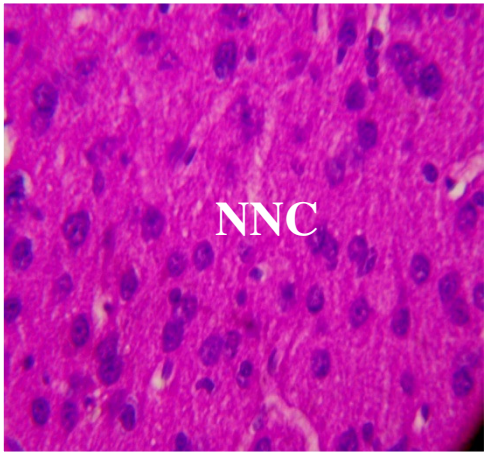
PCB



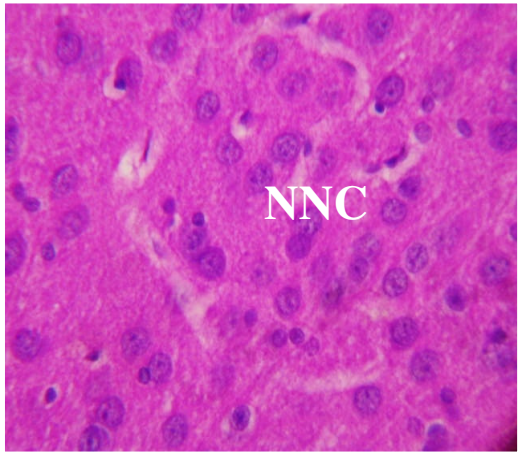
PCB + Melatonin (5 mg)



PCB + Melatonin (10 mg)



Melatonin (5 mg)



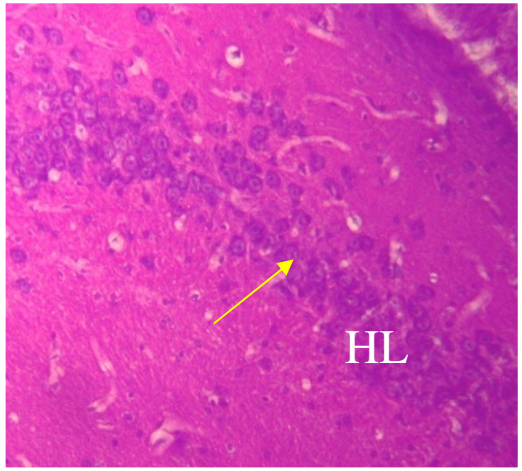
Melatonin (10 mg)

NS – Neuronal shrinkage
NNC – Normal Nerve Cells

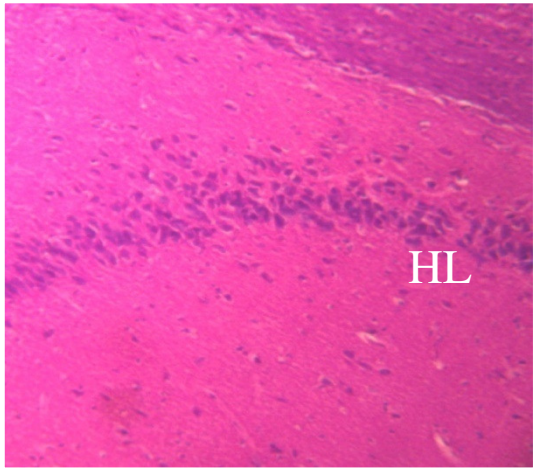
PNS – Peri Neuronal Spaces
DN – Degenerative neurons

PN – Pyknotic Nucleus

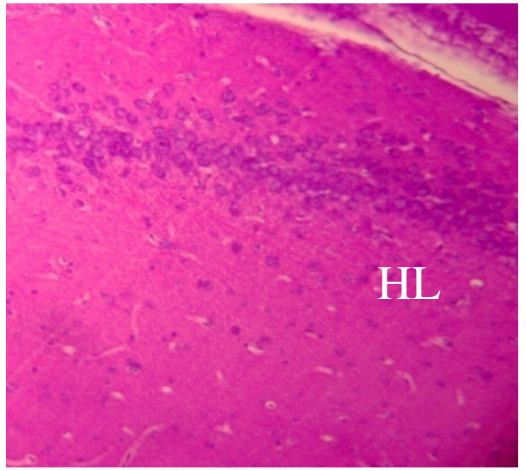
Hippocampus – 10x



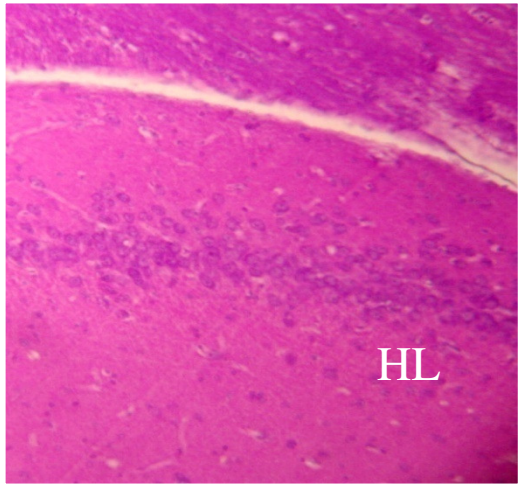
Control



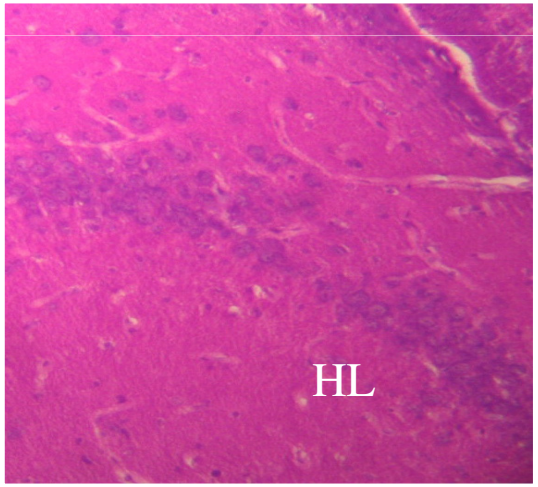
PCB



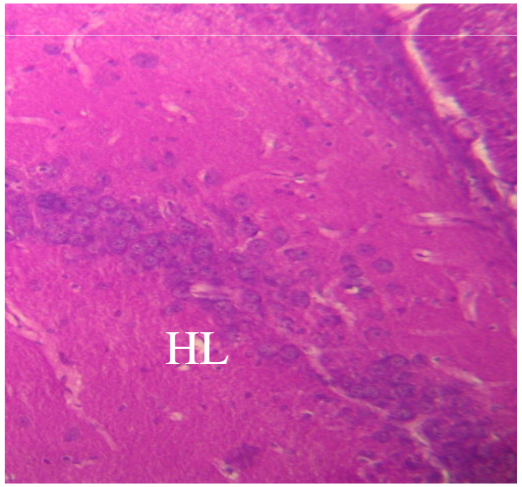
PCB + Melatonin (5 mg)



PCB + Melatonin (10 mg)



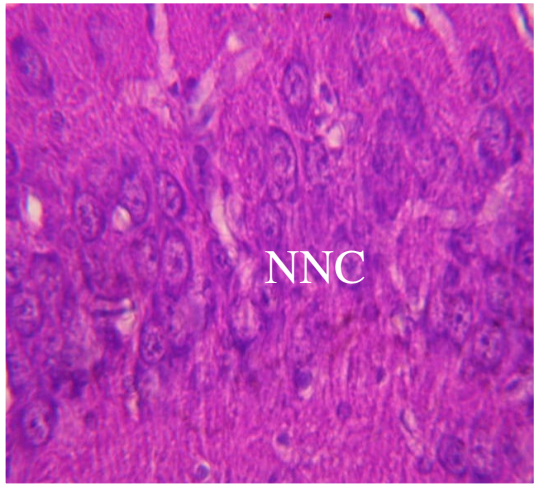
Melatonin (5 mg)



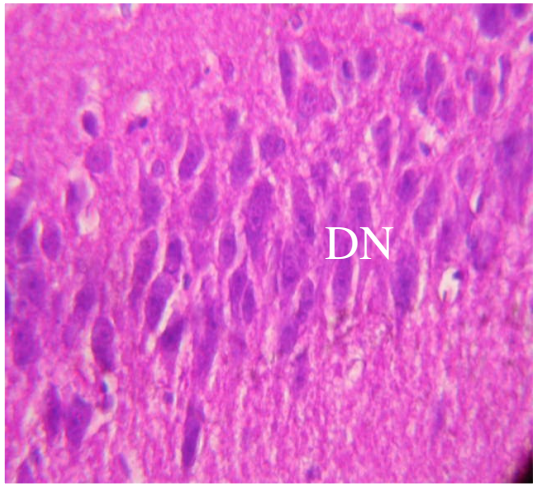
Melatonin (10 mg)

HL – Hippocampal layer

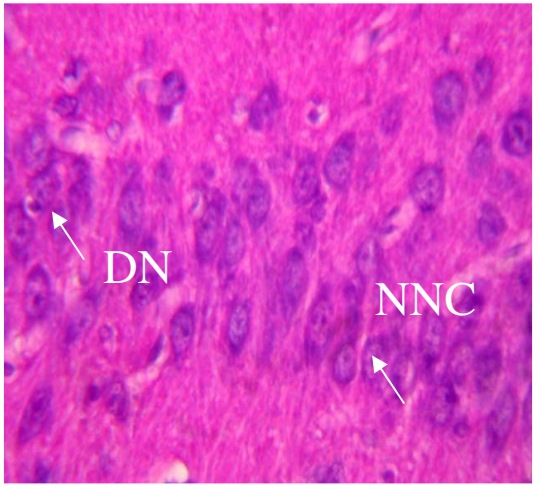
Hippocampus – 40x



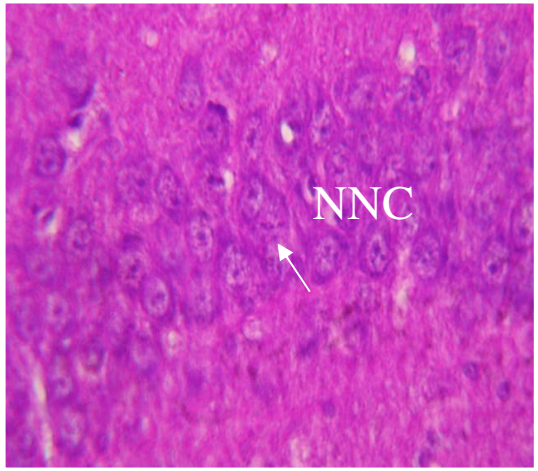
Control



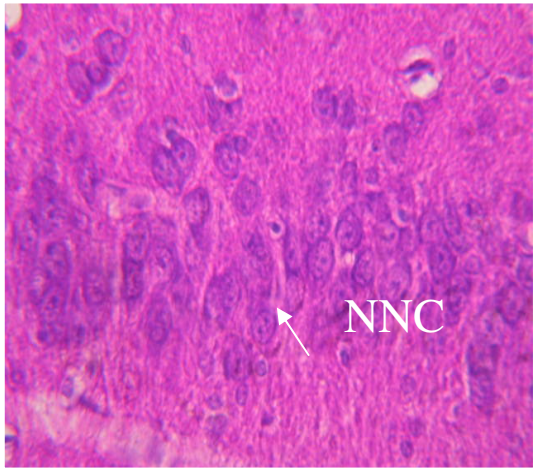
PCB



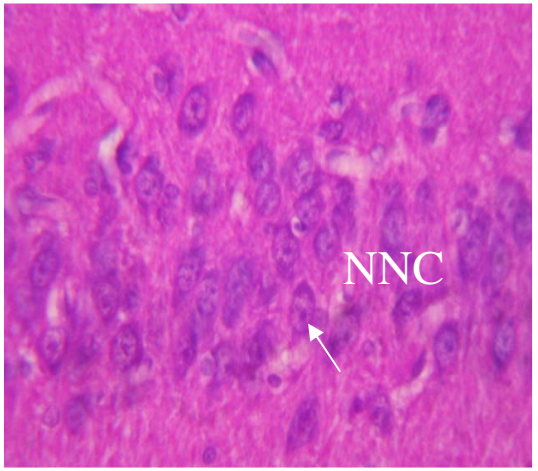
PCB + Melatonin (5 mg)



PCB + Melatonin (10 mg)



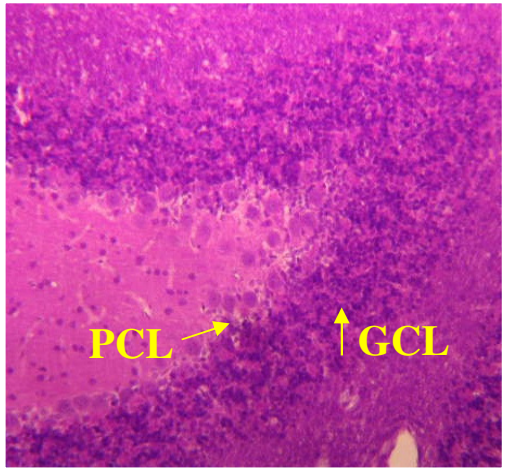
Melatonin (5 mg)



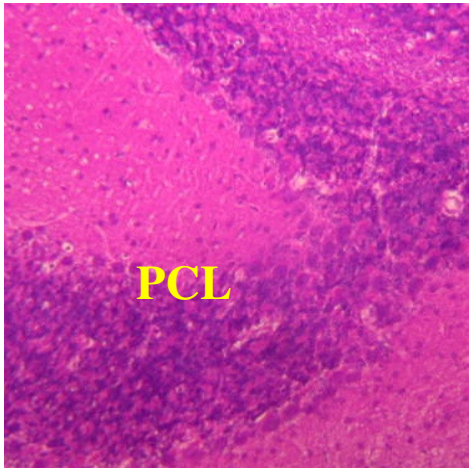
Melatonin (10 mg)

NNC – Normal nerve cells HL – Hippocampal layer DN – Degenerative neurons

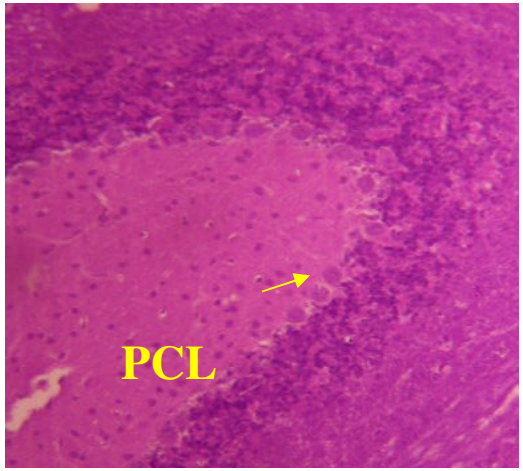
Cerebellum (10x)



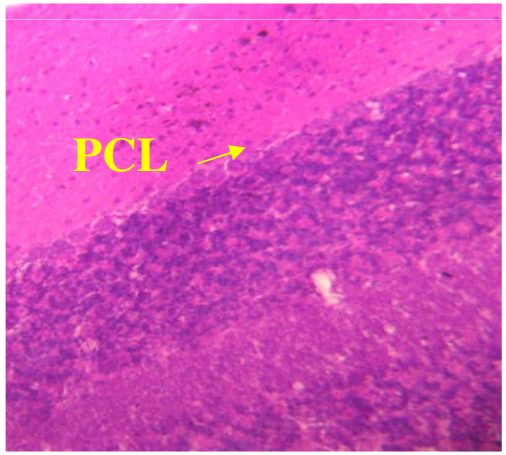
Control



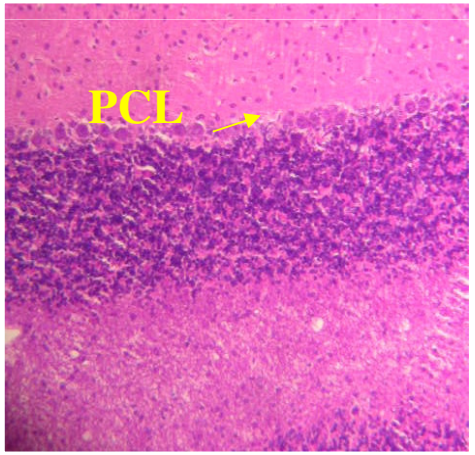
PCB



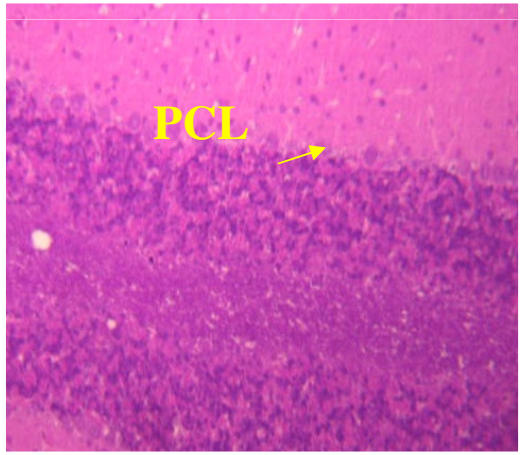
PCB + Melatonin (5 mg)



PCB + Melatonin (10 mg)



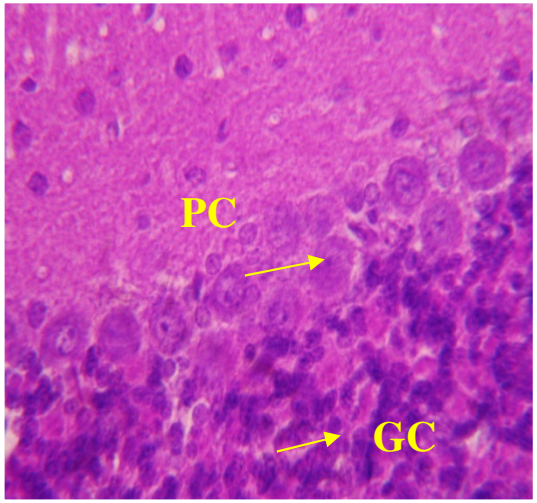
Melatonin (5 mg)

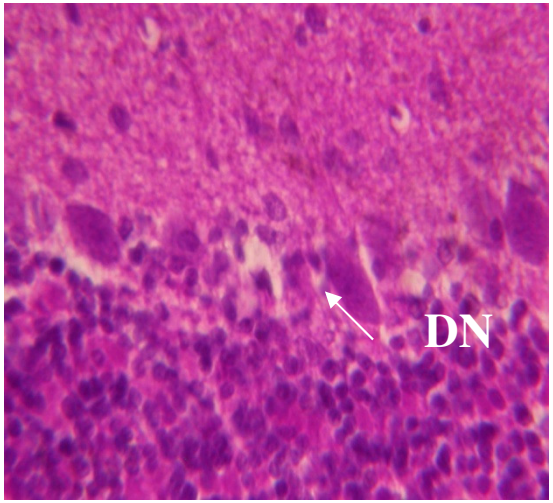


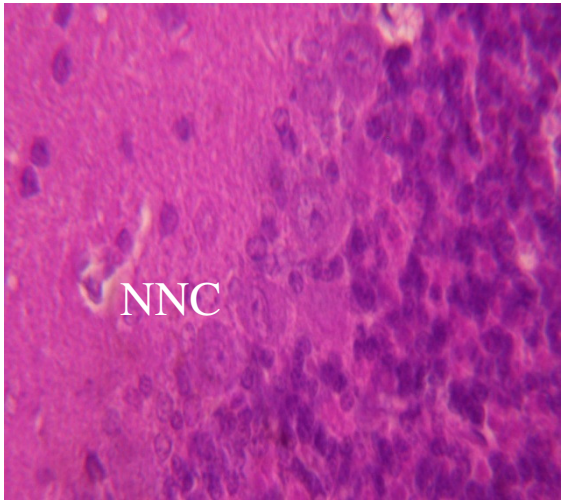
Melatonin (10 mg)

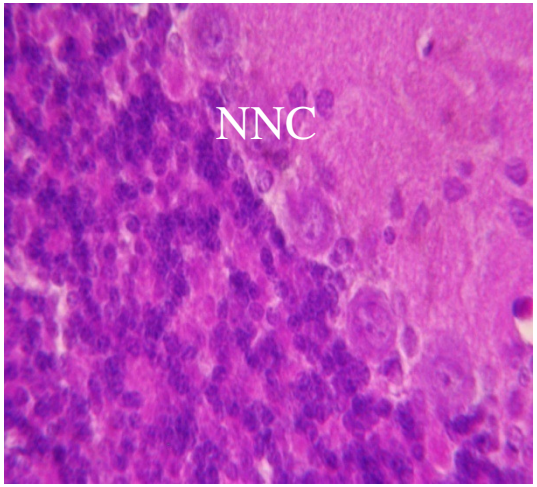
PCL - Purkinje cell Layer GCL – Granular cell layer

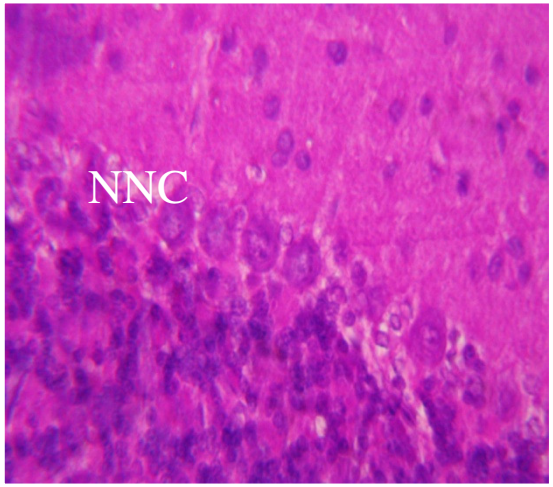
Cerebellum (40x)

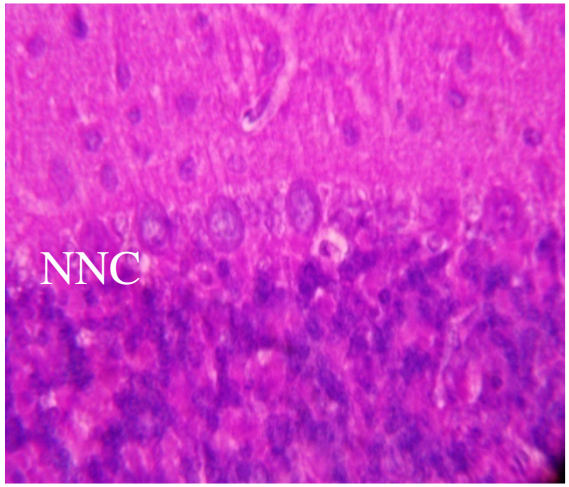






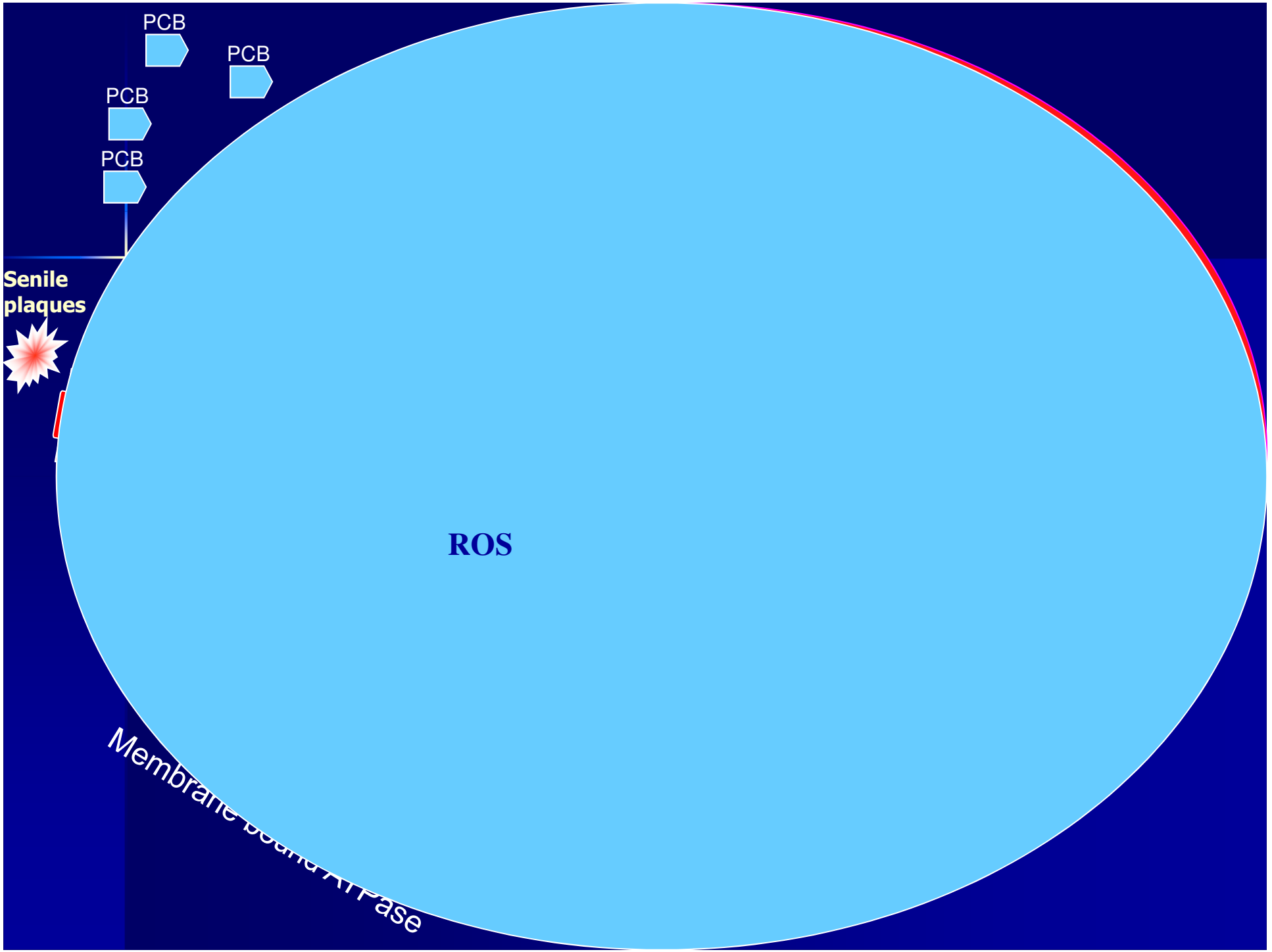


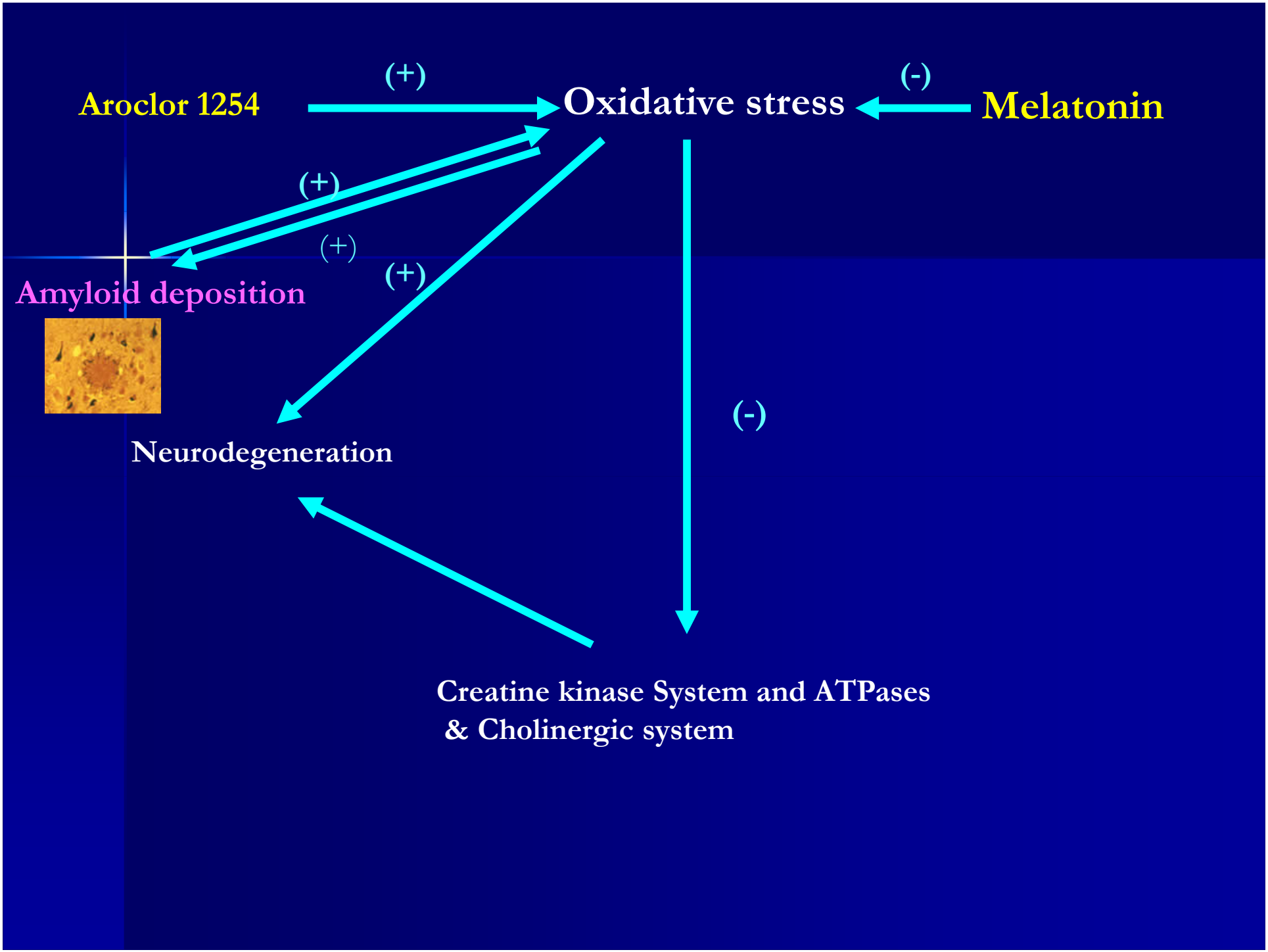




PC – Purkinje cells **GC – Granular cells**

NNC – Normal nerve cells **DN – Degenerative neurons**


PCB
PCB
PCB
PCB
Senile plaques
ROS
Membrane bound ATPase


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.

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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

Prabhu Venkataraman [a], Kandaswamy Selvakumar [a], Gunasekaran Krishnamoorthy [a], Sridhar Muthusami [a], Radhakrishnan Rameshkumar [b], Seepan Prakash [b], Jagadeesan Arunakaran [a,*]

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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

Prabhu Venkataraman, Gunasekaran Krishnamoorthy, Ganapathy Vengatesh, Narasimhan Srinivasan, Maria Michael Aruldhas, Jagadeesan Arunakaran *

Department of Endocrinology, Dr. ALM Post Graduate Institute of Basic Medical Sciences, University of Madras, Taramani Campus, Chennai 600113, India

 Basic & Clinical Pharmacology & Toxicology, **105**, 92–97

Doi: 10.1111/j.1742-7843.2009.00406.x

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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University of Madras, Taramani Campus, Chennai, India

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NeuroToxicology 28 (2007) 490–498

NeuroToxicology

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

Prabhu Venkataraman, Raju Muthuvel, Gunasekaran Krishnamoorthy, Arumugam Arunkumar, Muthusami Sridhar, Narasimhan Srinivasan, Karundevi Balasubramanian, Maria Michael Aruldhas, Jagadeesan Arunakaran *

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Neurotoxins: Free Radical Mechanisms and Melatonin Protection

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Department of Cellular and Structural Biology, University of Texas Health Science Center, San Antonio, Texas

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 H_2O_2 were elevated. Conversely, GSH concentrations as well as the activities of a variety of enzymes (Na^+K^+ATPase, Ca^{2+}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.

[255] Venkataraman, P., Krishnamoorthy, G., Vengatesh, G., Srinivasan, N., Aruldhas, M.M., Arunakaran, J. (2008) 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 the adult rat brain. *Int. J. Dev. Neurosci.*, **26**, 585-591.

[256] Venkataraman, P., Selvakumar, K., Krishnamoorthy, G., Muthusami, S., Rameshkumar, R., Prakash, S., Arunakaran, J. (2010) 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. *Neurosci. Res.*, **66**, 189-197.

Publications

Venkataraman P, Sridhar M, Siva Dhanammal, Vijayababu MR, Srinivasan N, Arunakaran J (2004b). Antioxidant role of Zinc in PCB (Aroclor 1254) exposed ventral prostate of albino rats. J Nutr Biochem 15 (10), 608-613.

Venkataraman P, Sridhar M, Sivadanammal, Vijaya Babu MR, Arunkaran A, Srinivasan N and Arunakaran J (2004a). Effect of vitamins supplementation on PCB (Aroclor 1254) induced changes in ventral prostatic androgen and estrogen receptors. *Endocr. Res.* 30: 469-480.

Sridevi N, **Venkataraman P**, Senthilkumar K, Krishnamoorthy G, Arunakaran J (2007). Oxidative stress modulates membrane bound ATPases in brain regions of PCB (Aroclor 1254) exposed rats: protective role of alpha-tocopherol. Biomed Pharmacotherab 61, 435-440.

Muthuvel R, **Venkataraman P**, Krishnamoorthy G, Gunadharini DN, Kanagaraj P, Jone Stanley A, Srinivasan N, Balasubramanian K, Aruldhas MM, Arunakaran J. (2006). Antioxidant effect of ascorbic acid on PCB (Aroclor 1254) induced oxidative stress in hypothalamus of albino rats. *Clin Chim Acta* 365: 297-303.

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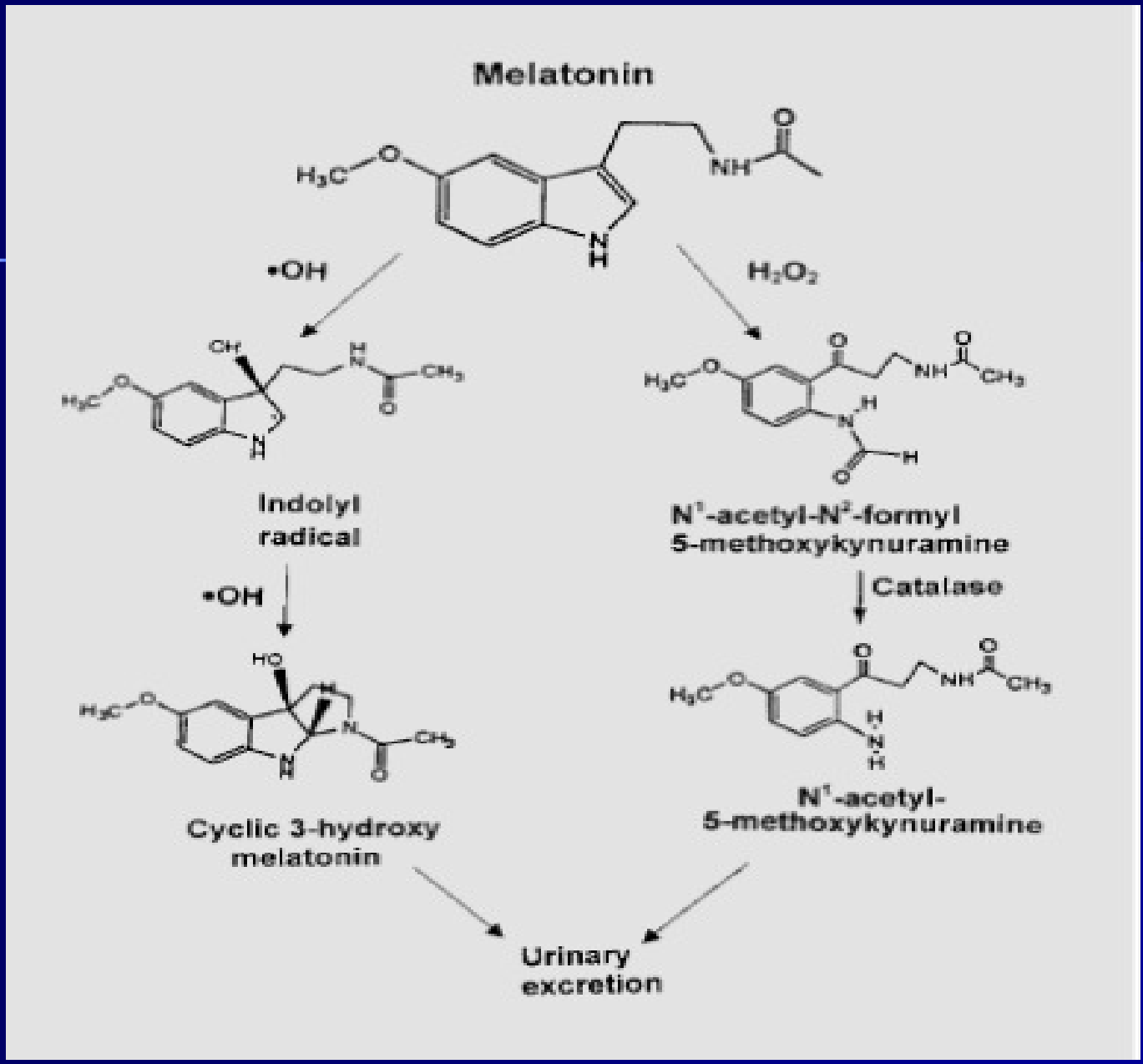
Dr. Sankar (Anatomy)

Dr. S. Prakash (Anatomy)

Dr. R. Ramesh kumar (Anatomy)

THANK YOU


Melatonin
•OH
H_2O_2
Indolyl radical
N^1-acetyl-N^2-formyl 5-methoxykynuramine
•OH
Catalase
Cyclic 3-hydroxy melatonin
N^1-acetyl-5-methoxykynuramine
Urinary excretion