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Assessment the Role of Melatonin in Iron Toxicity in Male Rats.

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Abstract Iron is a vital metal to normal cellular function. In spite of its requirement in the body, a misbalance in iron homeostasis can be devastating. The present study aimed to evaluate the possible protective effect of melatonin (MLT) on iron overload and its toxicity on brain and testes of male albino rats. The experimental animals were divided into four groups, the first group was left as control, the second group was treated with MLT at a dose of 15mg/kg B Wt., the third group was intoxicated with iron at a dose of 1g/kg B Wt. and the fourth group served as MLT and iron treated group. Administration of iron caused significant elevation in serum iron and ferritin contents, while a decrease in serum transferrin and in some hematological parameters, (WBCs & Ht %) were observed. These changes were associated with increased lipid peroxidation, decreased glutathione (GSH) levels, superoxide dismutase (SOD) and catalase (CAT) activities, in both of testis and the brain. In addition decreased dopamine, serotonin and nor epinephrine levels were observed. On the other hand, the administration of MLT showed an ameliorative effect in all of the investigated parameters. In conclusion, the present study showed that MLT is an effective agent in prevention and amelioration the toxic effects of iron-overload that can potentially cause oxidative stress. In addition, MLT counteracted the deleterious effects that disturbed by iron overload in both testis and brain.

Introduction

Iron is critical to normal cellular function and plays an important role in metabolic processes including O_2 transport, electron transport, oxidative phosphorylation and energy production, xenobiotic metabolism, DNA synthesis, cell growth, apoptosis, gene regulation and inflammation (Wang and Pantopoulos., 2011). It is also a necessary cofactor for the synthesis of neurotransmitters, dopamine, nor epinephrine and serotonin (He et al., 2007). In spite of its requirement in the body, a misbalance in iron homeostasis can be devastating; accumulation of iron has been associated with hemochromatosis and production of free radicals that potentially leads to neuronal damage (Altamura and Muckenthaler, 2009). Increased levels of iron in brain regions have been reported in neurodegenerative disorders (Rech et al., 2010). In addition, iron induces lipid peroxidation, protein carbonyl expression and depletion of lipid soluble antioxidants in testicular tissue results in disruption of spermatogenesis (Lucesoli et al., 1999; Doreswamy and Muralidhara., 2005), impairment in testicular activity and affects the androgenicity of male rats (El-Seweidy et al., 2010).

Melatonin(N-acetyl-5-methoxytryptamine), the main product of the pineal gland, which is

outside of the blood–brain barrier, acts as an endocrine hormone since it is released into the blood (Kaur and Ling, 2008). Melatonin has potent antioxidant activities (Korkmaz et al., 2009). Because of its highly lipophilic character, it can easily cross cell membranes (Pohanka., 2011) and the blood–brain barrier (Reiter et al., 2010). Several studies reported that melatonin and its metabolites are excellent scavengers of reactive oxygen and nitrogen species (Galano., 2011). Others demonstrated that melatonin prevents the brain oxidative damage induced by traumatic brain injury in the immature rats (Ozdemir et al., 2005). In addition, MLT displayed a marked role in the protection of testicular toxicity induced by radiation and different mutagenic and clastogenic agents (Anjum et al., 2011; Ji et al., 2012).

The aim of the present study was to evaluate the possible protective effect of melatonin on iron overload and toxicity on brain & testes of male albino rats.

Materials and Methods

Animals and animal's treatment

Adult male wistar albino rats, with an average body weight of 100-120g, were kept under good ventilation; adequate stable diet and water were allowed *ad libitum*. They were maintained on normal light/dark cycle. Rats were divided into four groups comprising of 6 rats in each. The first group served as a control group, where animals were given normal diet, and received nothing of treatment throughout the period of the study. Second group served as melatonin treated group, where animals were injected intraperitonealy (i.p) with freshly prepared melatonin at dose of 15 mg/kg body weight day after day for 8 weeks. Third group served as iron group, where animals were received ferrous sulphate dissolved in distilled water orally at dose of 1g/kg body weight day after day for 8 weeks. Forth group served as melatonin and iron treated group, where animals were injected i.p. with freshly prepared melatonin at dose of 15 mg/kg body weight and then received ferrous sulphate dissolved in distilled water orally at dose of 1g/kg body weight day after day for 8 weeks. All injections were administered in the volume of 0.5ml/ injection.

Sampling and biochemical analysis:

All experimental protocols were approved by the Animal Ethics Committee of Mansoura University. At the end of the experimental period, over night fasted rats were sacrificed. Blood samples were collected in clean non-heparinized centrifuge tubes and only few droplets were placed in clean heparinized tubes for hematological parameters then the tubes were let to stand for 15 min at 30◦C after which the non heparinized tubes were centrifuged at 3000 rpm for 15 min. Blood sera were carefully separated and were kept at - 20◦C for subsequent analysis.

Hematological parameters including the count of red blood corpuscles (RBCs), white blood cells (WBCs), hemoglobin (Hb), platelets count and hematocrit percent were conducted using hematological analyzer (Sysnex Ts-21) Japan (Dacie and Lewis, 1999). Serum ferritin was determined according to the method of Young, (1995). Serum transferrin was estimated according to the method of Starr, (1980). Iron concentration in serum, brain and testis were measured according to the method of Dreux (1977), zinc level according to the method of Hayakawa, (1961) and gamma-glutamyle transaminase (γ-GT) activity according to the method of Szasz, (1976).

Testis specimens were removed, weighed and then homogenized in phosphate buffer (50 mM, pH 7.0) to form about 10% (w/v) homogenate. The brain was carefully removed, weighed and then quickly frozen on dry ice until biochemical essay. There after, brain of each animal was divided longitudinally into two parts: One was weighed, homogenized in 75% high performance liquid chromatography (HPLC) methanol 10% (W/V) for HPLC determination of monoamines content. The other part was weighed and homogenized in phosphate buffer pH 7.0 to form 10% (w/v) homogenate for other biochemical assay. The homogenates were subjected to the following biochemical analysis: the lipid peroxidation product, malondialdehyde (MDA), was measured by the thiobarbituric acid assay, according to the method of Ohkawa et al.,

(1982). The reduced glutathione (GSH) content was measured according to the method of Prins and Loose (1969). Catalase (CAT) activity was assayed according to Bock et al., (1980). The superoxide dismutase (SOD) activity was assayed according to the procedure of Nishikimi et al., (1972). Brain neurotransmitter concentrations were determined by HPLC method Pagel et al., (2000).

Statistical analysis

The results were evaluated by One Way ANOVA (analysis of variance) test and post comparison was carried out with LSD test. Group results were then expressed as mean ± the standard error of the mean (S.E.M.). The values of $p \leq 0.05$ were considered statistically significant.

Results

In the current study, the administration of melatonin (15 mg/kg) did not change any of hematological parameters except hematocrit percent that observed a significant increase compared to the iron intoxicated rats. On the other hand, administration of ferrous sulphate (1g/kg body weight) caused significant decrease in white blood cells count and in hematocrit percent but no significant changes were observed in other hematological parameters comparing to control group (Table 1).

Table (1): Hematological parameters in control and different treated animal groups.

	Control	MLT	Iron	MLT+Iron.
RBCS(10^6/mm^3)	6.78±0.2	7± 0.1	6.7±0.3	7.0±0.1
WBCS (10^3/mm^3)	7.9±0.9	6.0± 0.7	4.5±0.2[a]	6.1±0.7
Hb (g/dl)	12.8±0.4	13.7±0.1	13.2±0.5	14.1± 0.2
Platelets (10^3/mm^3)	447±32.9	371.3±18.1	353.8±45.6	353.6±27.2
Haematocrit %	34.1±0.5	35.8±0.4	31.2±1[a]	35.1±0.3[b]

All values are expressed as mean ±SE of 6 animals.
[a] Significant (P< 0:05) when compared with the control group.
[ъ] Significant (P< 0:05) when compared with the iron group.

Table (2): Serum ferritin, transferrin, iron (Fe), and zinc (Zn) concentrations and γ-GT activities in control and different treated animal groups.

	Control	MLT	Iron	MLT+ Iron
Ferritin (ng/ml)	0.30±0.09	0.26± 0.05	0.76±0.1[a]	0.39±0.1[b]
Transferrin(mg/dl)	398±5.3	387± 5	348±1.3[a]	380±7[a,b]
Iron (μmol/L)	68.8±2.8	68.3±2	84.14±1[a]	76.42± 1.7[a,b]
Zinc (μg/dl)	43.04±4	35.08±1.1	28.6±0.4[a]	33.34±1.7[a]
γ-GT(U/L)	17.4±0.8	14.4±0.4	25.8±1.6[a]	20.2±1.1[b]

All values are expressed as mean ±SE of 6 animals.
[a] Significant (P< 0:05) when compared with the control group.
[b] Significant (P< 0:05) when compared with the iron group.

Table (3): Brain and testicular iron (Fe) in control and different treated animal groups.

	Control	MLT	Iron	MLT+ Iron.
Brain iron(µg/g)	15.49±0.4	15.41±0.2	20.24±0.4[a]	17.92±0.2[a,b]
Testicular iron(µg/g)	14.80± 0.5	14.76± 0.1	19.4± 0.5[a]	15.5±0.3[b]

All values are expressed as mean ±SE of 6 animals.
[a] Significant (P< 0:05) when compared with the control group.
[b] Significant (P< 0:05) when compared with the iron group.

Table (4): The activity of SOD, CAT, GSH and TBARS in brain and testis in control and different treated animal groups.

tissue		Control	MLT	Iron	MLT+Iron
Brain	TBARS (nmol/g)	270±2	207± 5[a]	374±6[a]	253±10[b]
	GSH (mg/g)	0.27±0.01	0.28±0.04	0.13±0.01[a]	0.24± 0.02[b]
	SOD (U/g)	186±0.8	192±0.8	166±2[a]	185±3[b]
	CAT (µmolH_2O_2/sec/g)	0.39±0.06	0.43±0.1	0.09±0.02[a]	0.36±0.08[b]
Testis	TBARS(nmol/g)	146±4	142± 3	274±9[a]	229±2[a,b]
	GSH(mg/g)	0.3±0.02	0.36±0.03	0.16±0.01[a]	0.26± 0.05$_b$
	SOD(U/g)	187±1.4	195±0.5[a]	183±1.6[a]	188±0.9[b]
	CAT(µmolH_2O_2/sec/g)	0.28±0.04	0.31±0.03	0.07±0.02[a]	0.24±0.02[b]

All values are expressed as mean ±SE of 6 animals.
[a] Significant (P< 0:05) when compared with the control group.
[b] Significant (P< 0:05) when compared with the iron group.

Table (5): Brain dopamine, serotonin, and norepinephrine levels in control and different treated animal groups.

	Control	MLT	Iron	MLT+Iron
Dopamine (µg/g)	8.23±0.4	9.61± 0.8[a]	5.89±0.3[a]	7.1±0.2[a,b]
Serotonin (µg/g)	3.9±0.3	3.8±0.08	2.8±0.1[a]	3.5± 0.1[b]
Norepinephrine (µg/g)	16.5±0.9	16.48±0.13	14.99±0.02[a]	15.22±0.04[a]

All values are expressed as mean ±SE of 6 animals.
[a] Significant (P< 0:05) when compared with the control group.

[b] Significant (P< 0:05) when compared with the iron group.

As shown in Table 2 and 3 administration of ferrous sulphate produced significant increase in the brain and testicular iron and in serum levels of ferritin, iron, and γ-GT but transferrin levels and zinc concentration were decreased as compared with control group. The administration of MLT against iron intoxication resulted in a significant reduction in the brain and testicular iron and in serum ferritin and iron levels as well as γ-GT activity, while significant increase was observed in serum transferrin level compared to iron intoxicated rats.

The data in Tables 4 shows the effect of various treatments on lipid peroxidation and antioxidant levels in the brain and the testis. In rats received iron alone, the values of TBARS were significantly higher in the brain and testicular tissues than in the control group. While the activity of GSH, SOD, and CAT were significantly decreased in these tissues when compared to the control group. The administration of MLT alone caused significant decreases in TBARS in the brain tissue, while produced significant increase in SOD activity in testicular tissue as compared with control group. On the other hand, the MLT administration as a protective agent to the rats intoxicated with iron had significantly increased in the measured antioxidant parameters including GSH, SOD, and CAT compared to iron intoxicated group.

Administration of MLT alone caused an increase in dopamine content. The iron intoxicated rats showed a significant decrease in the brain neurotransmitters as compared to the control group. On the other hand, MLT administration against iron intoxication caused a significant increase in dopamine and serotonin levels while no significant changes were observed in norepinephrine levels, all as compared with iron intoxicated animal group (Table 5).

Discussion

Iron is an essential metal involved in multiple physiological and biochemical processes throughout the body, including brain. In spite of its requirement in the body, accumulation of iron has been associated with hemochromatosis and production of free radicals that potentially leads to neuronal damage (Altamura and Muckenthaler., 2009). Excess iron is toxic due to oxidative metabolism, that free iron assists in the generation of the most devastatingly toxic hydroxyl radical (•OH) in what is referred to as the Haber-Weiss and Fenton reactions (Vidrio et al., 2008).

In the present study, the administration of ferrous sulphate to rats elevated serum iron levels, ferritin, and iron concentration in brain and testis homogenate but decreased serum transferrin, a situation permissive of iron overload. Increasing serum ferritin levels may be one of the first clinical signs of iron overload that within cells, iron is stored as ferritin. Supporting to these results, Abd El-Baky et al., (2009) demonstrated that the administration of 0.5 or 1.0 gm of ferrous sulfate/kg B wt caused significant increase in serum iron and ferritin levels.

Testicular iron content demonstrated significant increase, which in agreement with Lucesoli et al., (1999) and El-Seweidy et al., (2010) who considered testicular tissues as secondary target for accumulated iron. It considers that testes produce their own transferrin, and participates in iron shuttle system which fulfill the requirements of this metal for spermatogenesis (Sylvester and Griswold., 1993).

The current data showed no significant changes in blood Hb level, red blood corpuscles', and platelets' content in iron group which in line with the study of Barbosa et al., (2013). While there were significant decrease in white blood corpuscles' and Ht% in iron group as compared with the control group due to the accumulation of iron, which leads to suppression of bone marrow resulting in reduction of total and differential leucocytes counts. These findings in

accordance with the previous study of Tirgar et al., (2011).

The present results show a significant decrease in serum zinc concentration in iron group and in melatonin iron treated group as compared with the control group. Supporting these results, zinc levels in beta thalassemia major patients were significantly decreased in most of the studies as compared to the controls. This may be due to the release of zinc from hemolysed red cells (Shekhar et al., 2007). On the contrary, the present results disagree with those obtained by Elseweidy and Abd El-Baky, (2008) who demonstrated that serum zinc increased significantly after intake of iron-fortified diet.

The current study also demonstrated a significant increase in serum γ-GT activity after iron overload exposure. γ-GT is a key enzyme in the catabolism of GSH (Emdin et al., 2005). It has been shown that the extracellular cleavage of GSH by γ-GT induces the production of ROS, suggesting that γ-GT used as a marker of oxidative stress (Lee et al., 2004). Therefore, the present results suggested that serum γ-GT might be one of the enzymes related to oxidative stress induced by iron overload. This confirmed by decreased GSH level in iron overload animals. Meister (1994) reported that the GSH works as an antioxidant due to the presence of numerous –SH groups, which can react with the free radicals and products of lipid peroxidation such as lipid peroxides and aldehydes protecting in this way against development of oxidative stress (Tandon et al., 2002). Thus, it is supposed that the reduction in GSH might result from the utilization of –SH groups to scavenge free radicals formed (Jurczuk et al., 2006).

Malondialdehyde (MDA) is a secondary product of lipid peroxidation and is released because of the toxic effect of ROS (Guven et al., 2008). The major forms of cellular damage induced by iron overload is lipid peroxidation, The present study showed a significant increase TBARS concentration in rat brain and testis homogenates as compared to control levels, indicating high level of oxidative stress, which markedly enhanced with increasing iron toxicity. Despite the fact that brain is well protected by the blood brain barrier, it cannot provide full protection against circulating inflammatory agents that can generate radicals in the brain (Farkas et al., 2006). Supporting our results, Maharaj et al., (2006) stated that intrahippocampal injection of Fe (II) resulted in a significant increase in lipid peroxidation levels in comparison to control levels. In addition, El-Seweidy et al., (2010) reported that testicular contents of MDA in rats received biscuits enriched with iron (BEI) demonstrated significant increase while GSH showed significant decrease.

The current data showed significant decrease in SOD and CAT activities in brain and testicular tissues in iron group as compared with the control group. These results agreed with Lucesoli and Fraga, (1999) who indicated that the activities of SOD and CAT in testis were significantly decreased in iron overload animals. Jagetia et al., (2004) demonstrated that GSH depletion, reduced activities of antioxidant enzymes like SOD and CAT in testicular tissue after iron treatment which confirmed by the present data.

The present study showed that in iron treated group brain dopamine and serotonin decreased significantly as compared with the control group. These results were in accordance with the previous studies of Elseweidy and Abd El-Baky (2008) who demonstrated that intake of iron fortified diet significantly decreased brain serotonin and dopamine. Logroscino et al., (2008) also reported that Iron not only induces oxidative stress but damages proteins, membranes, and nucleic acids and eventually leads dopaminergic neuron death in the substantia nigra.

Supporting these data, Jimnez DeL Rio et al., (1993) have reported that serotonin-binding proteins (SBP), located in brain extract are involved in storage, protection and transport of serotonin as well as catecholamines. Fe (II) only increases such binding, it is approved that Fe (II) binds first to SH- group SBP. In addition, Monoamines form coordination bonds with trapped iron leading to potential change in SBP functions.

Other studies in animal models of Parkinson disease (PD) also suggested that a

high iron level in the brain is associated with loss of dopaminergic SNc neurons (Xie et al., 2003; Wang et al., 2004).

Effect of melatonin on iron toxicity

The current study demonstrated that melatonin has a protective action on brain and testis oxidative injury secondary to experimental iron overload. It is hypothesized that if oxidative stress is involved in the origin of a disease, then successful antioxidant treatment should delay or prevent the onset of that disease (Halliwell and Whiteman., 2004).

Melatonin, the chief product of the pineal gland, is an effective direct free radical scavenger and indirect antioxidant. It detoxifies both ROS and reactive nitrogen species (RNS), both groups of which are abundantly produced in the brain (Reiter et al., 2010). The present results suggest that melatonin might decrease the production of destructive free radical induced by iron toxicity. This is attributed, in apart, to the ability of melatonin to quench hydroxyl radicals and scavenge H_2O_2 generated from a Fenton reaction. Melatonin possesses an electron-rich aromatic indole ring and functions as an electron donor, thereby reducing and repairing electrophilic radicals **(**Martinez et al., 2005). Besides, melatonin as well as its metabolites reportedly possesses iron-binding properties (Limson et al., 1998; Maharaj et al., 2003) and have the ability to participate in maintaining iron pool at appropriate level resulting in control of iron haemostasis.

In other studies, Melatonin controlled iron levels after lead intoxication (Othman et al., 2004; El-Sokkary et al., 2005). In addition, melatonin controls oxidative stress and modulated the levels of iron and iron binding proteins during adriamycin treatment (Othman et al., 2008).

In the present study, the data showed that melatonin did not affect the levels of zinc in serum confirmed the findings by Othman et al., (2008). Moreover, it did bind several other heavy metals (Limson et al., 1998).Taken together, the chelation of iron ions by melatonin is an important factor in its anti-free radical effects subsequently, preventing or at least decreasing iron mediated tissue oxidative stress (Othman et al., 2008)

The current investigation demonstrated marked normalization of serum iron, ferritin and transferrin levels in melatonin iron treated group as compared with iron group. These findings are consistence with Othman et al., (2008) who suggested that melatonin could prevent oxidation of iron binding proteins. Because of small size of melatonin enough to have direct access to the iron core of ferritin through the pores of the protein shell, inhibits redox cycling and generation of toxic ROS.

In the present data, treatment with melatonin resulted in a significant increase in haematocrit percent, which in agreement with the study of Ozmerdivenli et al., (2011) but no significance changes were observed in other hematological parameters. On the other hand, γ-GT is inversely associated with antioxidants (Kilanczyk and Bryszewska, 2003), while melatonin has the ability to protect macromolecules against oxidative stress and increase the activity of antioxidant enzymes (Hardeland et al., 2009 and Korkmaz et al., 2009). Accordingly, melatonin might inhibit serum γ-GT activity to exert its antioxidant effect. This might be in agreement with Ogeturk et al., (2004) who stated that the elevations in γ-GT and total iron levels induced by CCl4 injections were significantly reduced by melatonin. El-Missiry et al., (2007) found that the treatment with melatonin before irradiation displayed significant amelioration in the elevation of serum γ-GT activity.

The present result demonstrates that melatonin has a protective action against oxidative stress in brain and testis induced by iron overload, evidenced by a significant decrease in MDA levels in brain and testis homogenate of melatonin iron treated group as compared to iron group. These results were in accordance with Maharaj et al., (2006) who reported that the injection of melatonin or 6-OHM in combination with Fe2+ significantly inhibited lipid damage in the hippocampus induced by the neurotoxin. In addition, Chen et al., (2003) reported that melatonin able to protect against Fe^{2+} induced lipid peroxidation in vivo. Furthermore, melatonin reportedly

suppressed lipid peroxidation induced by iron (Kabuto., 1998).

Melatonin not only does not consume cellular GSH, it also preserves or even increases the content of GSH in tissues (Tan et al., 2002). According to this view, melatonin cause significant increase in brain and testis GSH levels in melatonin treated group as compared with iron group, and hence to inhibit formation of extracellular and intracellular ROS *(Reiter et al., 2004)*. This result is in agreement with those obtained by El-Sawi et al., (2007) that when melatonin was given to animals injected with Delta-aminolevulinic acid, the decrease in GSH levels in rat brain was significantly improved. In addition, Feng et al., (2006) demonstrated that early melatonin treatment in Alzheimer's disease is able to reduce thiobarbituric acid-reactive substances (TBARS) levels, increase GSH content, SOD activity and prevent those processes that lead to cell death in a amyloid precursor protein (APP) transgenic mouse model.

It is commonly accepted that SOD is considered as a major antioxidative enzyme, because it dismutases the superoxide anion ($O_2 \cdot^-$) radical to hydrogen peroxide (H_2O_2) and reduces the formation of peroxynitrite. Antolin et al., (1996) indicated that melatonin increased the tissue mRNA levels for both manganese and copper SOD levels in animals treated with melatonin.

CAT is an inducible enzyme, decomposes H_2O_2, and is involved in the antioxidant defense mechanisms of mammalian cells. Thus, it is an index of increased H_2O_2 production (Meneghini., 1997). Melatonin was found also to stimulate the activity of CAT, which is also involved in reducing the H_2O_2 and thus reduce the generation of hydroxyl radicals (Montilla et al., 1997).

In the current study, both CAT and SOD activity improved by melatonin treatment , melatonin administration was able to neutralize a number of toxic reactants including ROS and free radicals (Reiter et al., 2008) such combination of actions indicating in reduced accumulation of lipid peroxidation hence prevent cascade generation of other harmful species. This might due to the important role of melatonin in activating antioxidant defenses such as SOD and CAT (Hardeland., 2009). This in agreement with Okatani et al., (2000) who demonstrated that administration of melatonin to pregnant rats, increased the activities of SOD in fetal brain tissues.

In the current result, significant increase in both neurotransmitter, dopamine and serotonin activity were observed in melatonin iron treatment group as compared with iron group. Cabrera et al., (2000) found that 6-OHM, like melatonin, is able to protect the hippocampus against lipid peroxidation and neuronal damage caused by the neurotoxin Fe2+. Furthermore, Melatonin as well as its metabolites are multi-faceted free radical scavengers and are effective against both oxygen and nitrogen-based reactants and may clearly attenuate neural damage resulting from craniocerebral trauma (Peyrot and Ducrocq., 2008). These results also agreed with Dabbeni-sala et al., (2001) who stated that the administration of melatonin ameliorates a hemi-Parkinson condition in rats caused by intranigral application of the catecholaminergic neurotoxin 6-OHDA.

In conclusion, MLT is effective in the prevention and amelioration of the toxic effects of iron-overload that can potentially cause oxidative stress in addition to counteracting the deleterious effects of iron overload in both testis and brain.

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تقييم دور الميلاتونين في التسمم بالحديد في ذكور الجرذان

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يعتبرالحديد عنصرضروري للوظائف الخلوية الطبيعية . على الرغم من احتياجاته للجسم؛ التوازن الخاطئ للحديد يمكن ان يكون مدمر. صممت هذه الدراسة لدراسة امكانية التأثير الوقائي للميلاتونين على زيادة تراكم الحديد وسميته على المخ والخصيتين في ذكور الجرذان. وقسمت حيوانات التجارب الي اربع مجموعات:- مجموعة ضابطة ،مجموعة معالجة بالميلاتونين بجرعة (١٥ ملجم/ كجم)، مجموعة مستحثه بزيادة الحديد بجرعة (١جرام/ كجم) من الحديد ومجموعة معالجه بالميلاتونين (١٥ ملجم/ كجم) ثم حقنت ب(١جرام/كجم) من الحديد. وفي نهاية فترة التجربة تم ذبح الفئران وتجميع عينات الدم والمخ والخصية لاجراء قياسات الدم ،تركيز الحديد والزنك ،الضغط التأكسدي للدهون ومضادات الاكسده في كل من المخ والخصية والموصلات العصبية في المخ.

ويمكن تلخيص النتائج كالتالي:-

تسبب زيادة تراكم الحديد في حدوث خلل واضح في كل القياسات ويتضح هذا الخلل في زيادة معنوية في تركيز الحديد وفي مستوى الفريتين في الدم مع نقص واضح في عدد خلايا الدم البيضاء ونسبة الهيماتوكريت.وارتبطت هذه التغيرات بزيادة الضغط التأكسدي للدهون (MDA) والتي كانت مصحوبة بنقص واضح في مضادات الأكسدة في المخ والخصية مثل الجلوتاثيونGSH، ونشاط انزيم SOD وCAT. بالاضافة الى نقص واضح في مستوى الدوبامين ،السيروتينين والنورادرينالين في المخ.

عند المعالجة بالميلاتونين للفئران المستحثه تجريبيا بزيادة الحديد أوضحت النتائج تحسن معنوي في معظم القياسات والخلاصة يمكن القول بأن استخدام الميلاتونين بجرعة ١٥ملجم/ كجم لمعالجة حيوانات التجارب المستحثة بزيادة الحديد خفف من حدة الاكسدة واستطاع التصدي للآثار الضارة الناتجة عن الحديد الزائد في كل من المخ والخصية.