Effect Of Melatonin On C>2 -Free Radical Production And Some Metabolic Changes Induced By Stress

Ahmed I. Agamy and Awad M. El-Abd
Physiology and Biochemistry Departments
Benha Faculty Of Medicine - Zagazig University

ABSTRACT

Stress is known to produce many effects on the body as 62 -free radical production, hyperglycemia, hypercholesterolemia and hypertriglyceridemia. Melatonin is known to be a highly efficient free radical scavenger, it is also known to produce some metabolic changes. Little is known about the role of melatonin in stress. The aim of the present work was to study the effect of melatonin on stress induced C>2 -free radical production, hyperglycemia, hypercholesterolemia and hypertriglyceridemia. The results obtained showed that immobilization stress caused a significant increase in the plasma level of ()2 -free radical, as indicated by the plasma level of malondialdehyde (MDA), glucose, cholesterol and triglycerides ($P < 0.05$). Melatonin administration prior to stress caused a significant decrease in the stress-induced 62 free radical production, ($P < 0.01$) while it has no significant effect on the stress induced hyperglycemia, hypercholesterolemia and hypertriglyceridemia. Thus, the present study might give a new meaning for the emerging role of melatonin as anti-stress hormone.

INTRODUCTION AND AIM OF THE WORK

Stress is known to produce multiple effects in the body as hyperglycemia[1], hypercholesterolemia and hypertriglyceridemia[2]. Recently stress was found to be one of the processes that generate free radicals[3]. These free radicals have received a great deal of investigative interest because they exert extensive damage to cells, tissues and organs.

Melatonin is known to be highly efficient free radical scavenger in different situations as ischemia reperfusion injury[4] and ionizing radiation[5]. Melatonin also, is known to affect some metabolic parameters as plasma glucose[6] **cholesterol*** and triglyccridcs[8].

Little is known about the effect of melatonin on the stress-induced 02 -free radical production and metabolic changes.

The aim of the present work is to study the effect of melatonin administration on the stress-induced 62 free radical production (as j indicated by the level of plasma MDA) and stress induced hyperglycemia, hypercholesterolemia and hypertriglyceridemia.

MATERIAL AND METHODS

Experimental animals:

Adult male albino rats weighing 150-250 grams without previous preparation. **Sample Collection:**

Trunk blood was obtained by decapitating the rats after a blow on the head without anaesthesia. The blood was collected in heparinized tubes, then centrifugated. Plasma was separated and used for estimation of malondialdehyde (a marker of lipid peroxidation) by the method of Draper and Hadly[9], glucose by the method of Trinder[} , cholesterol by the method of Stein[11] and triglycerides by the method of Wahlefeld[12].

Design of the study:

Three groups were tested. All groups had free access to food and water. Before decapitation to get the blood sample, rats were fasted for 12-18 hours with free access to water

Group I (control group): consisted of 7 rats that were saline injected.

Group II (Stress group): in which the effect of immobilization stress on plasma O_2 -free radical production (indicated by MDA concentration), plasma glucose, cholesterol and triglycerides were studied. The rats were subjected to immobilization stress by tying them to a wooden board with four limbs fully extended.

It was classified into 3 subgroups according to period of stress:

Group II a: in which 7 rats were stressed for !/- hour.

Group II b: in which 7 rats were stressed for 1 hour.

Group II c: in which 7 rats were stressed for 2 hours.

Group III: in which the effect of melalonin on stress induced O_2 - free radical production, hyperglycemia and hyperlipidemia were studied, Melatonin was injected intraperitonealy (I.P) at two doses; the first dose was injected 1.0 hour before stress and the second dose was injected immediately before stress.

Depending on the dose of melatonin, this group was classified into 2 subgroups:

Group III a: in which 7 rats were injected melatonin at a dose 5 mg /kgm for 2 doses I.P, 1 hour before and immediately before stress, then subjected to immobilization stress for 1 hour.

Group III b: in which 7 rats were injected melatonin at a dose 10 mg /kgm for 2 doses I.P, 1 hour before and immediately before stress, then subjected to immobilization stress for 1 hour.

Statistical analysis:

Student's t-test was used to compare the mean values. Differences with P values < 0.05 were considered statistically significant.

RESULTS

* RITcct of stress on plasma MDA, glucose, cholesterol and triglyccrides: (Table l)and(l'ig. 1,2,3,4).

Immobilization stress for 1/2 hour caused a significant increase in plasma MDA ($P < 0.05$), glucose (p O.001), cholesterol ($P < 0.01$) and triglycerides($P<0.01$).

Increasing the period of stress for 1 hour caused a more significant increase in plasma MDA ($P < 0.001$), glucose ($P < 0.001$), cholesterol ($P < 0.001$) and triglycerides ($P < 0.01$).

When the duration of stress was increased to 2 hours, the increase was more and more in plasma MDA ($P < 0.001$) glucose ($p < 0.001$) cholesterol ($P < 0.001$) and triglycerides ($P < 0.001$).

* Effect of melatonin on stress induced increase in plasma MDA, glucose, cholesterol and triglycerides:

injecting rats intraperitonealy with melatonin at a dose of 5 mg /kgm body weight for 2 doses 1 hour before and immediately before stress caused a significant decrease in the stress-induced increase in plasma MDA ($P < 0.01$) as compared with rat exposed to stress without melatonin injection, but it caused no significant change in stress-induced increase in plasma glucose, cholesterol and triglycerides (Table 2) and (fig. 5).

Increasing the dose of melatonin to 10 mg/kgm body weight caused a more significant decrease in stress-induced increase in plasma MDA ($P < 0.001$) as compared with rats subjected to stress without melatonin

prclrcalment but with no significant change in stress induced increase in plasma glucose, cholesterol and triglycerides (Table 3) and (fig. 6)

Table (1): Mean Values, ± SD of the effect of stress for 1/2 hour, one hour and 2 hours on plasma MDA, Glucose, Cholesterol and Trigylcerides compared with the control group.

Parameters / Studied Group	Plasma • MDA (nmol/ml)	Plasma Glucose (mg/di)	Plasma cholesterol (mg/dl)	Plasma Triglycerides (mg/dl)
Control (Non, stressed) group n = 7	50.313.2	86.1 ±8.6	91.9±9.1	62.9 ± 7.0
Stress for !/2 hour n=7	54.9 ± 3.9 P<0.05	11418.7 P< 0.001	115±10 P<0.01	75.7 ±7.3 P<0.01
Stress for 1.0 hour n=7	65.4 ± 4.2 P< 0.001	145 ± 10 P< 0.001	138 ±8.3 P< 0.001	83.6 ±8.3 P<0.01 192.9' ±0.001 P< 0.001
Stress for 2.0 hours n=7	80 ±7. 3 P<0.001	172.6 ±7.9 P<0.001	165. 7 ±6.2 P< 0.001	

P: Probability versus Control group.

Table (2): Mean values, ± SD of the effect of melatonin at dose 5 mg/kg body weight on the stress-induced increase in plasma MDA, glucose, cholesterol and triglycerides compared with rats under stress for one hour without melatonin pretreatment.

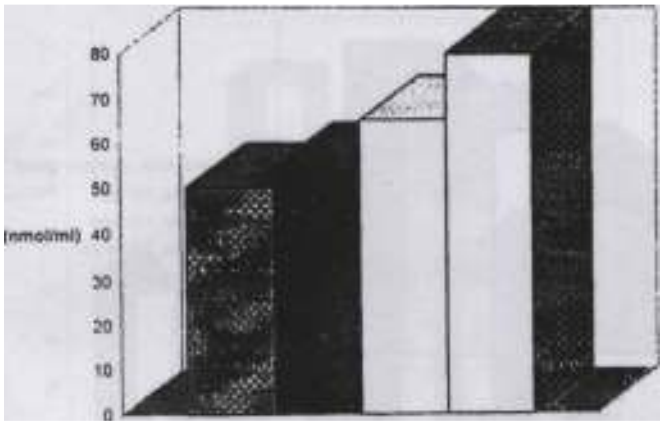
"^ —-Siiodied groups	Stress for 1 hour (group II, b)	Melatonin 5 mg/kg before stress (Group III a)	P
Plasma MDA (nmol/ml)	65.4 ±4.2	58.1 ±3.3	<0.01
Plasma glucose Jmg/dl.)	145 ±10	143 ±9.2	RS
Plasma cholesterol (mg/dl)	138.6 ±8.3	135.7 ±7.9	N.S
Plasma triglycerides (mg/dl.)	83.6 ±8.3	8 1.9 ±8.0	N.S

N.S.: Non Significant

Table (3): Mean Values, ± SD of the effect of Melatonin at dose 10 mg/kg body weight on the stress-induced increase in plasma MDA, glucose, cholesterole and triglycerides compared with rats under stress for 1 hour without melatonin pretreatment.

Studied groups / Parameters	Stress for 1 hour (group II, b)	Melatonin 10 mg/kg before stress (Group 111 b)	P
Plasma MDA (nmol/ml)	65.4 ±4.2	53.2 ±2.9	< 0.001
Plasma glucosejnig/dl.l	145 ±10	141 ±9.1	N.S
Plasma cholesterol (mg/dl)	138.6 ±8.3	133.2 ±8.5	N.S
Plasma triglycerides (mg/dl.)	83.6 ±8.3	80.1 ±8.1	N.S

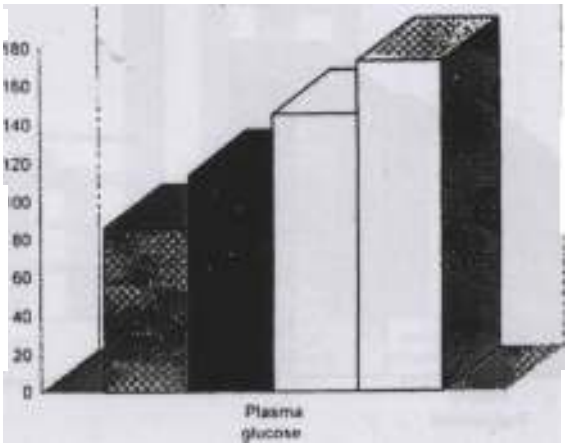
Flg.(1): **Mean values of the effect of stress for 1/2 hour, one hour and two hours on plasma MDA compared wiht the control group**



a Control Non, stressed group • Stress for 1/2 hour D Stress for 1.0 hour D Stress for 20 hours

Fig.(2): Mean values of the effect of stress for 1/2 hour.one hour and two hours on plasma glucose compared with the control group

(mg/dl)



| O Control Non, stressed group]: • Stress for 1/2 hour D Stress for 1 0 hour D Stress for 2 0 hours

Fig.(3): **Mean values of the effect of stress for 1/2 hour, one hour .and two hours on plasma cholesterol compared with the control group**

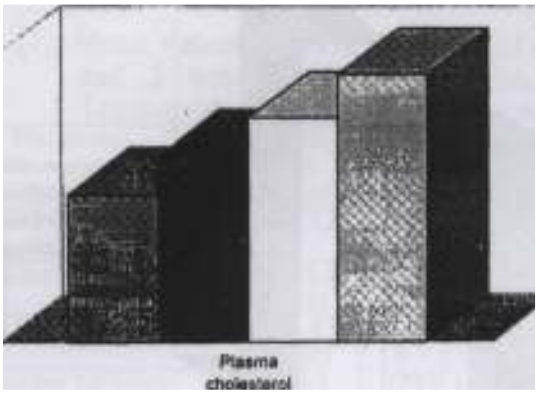


Fig.(4): **Mean values of the effect of stress 1/2 hour, one hour and two hours on plasma triglycerides compared with the control group**

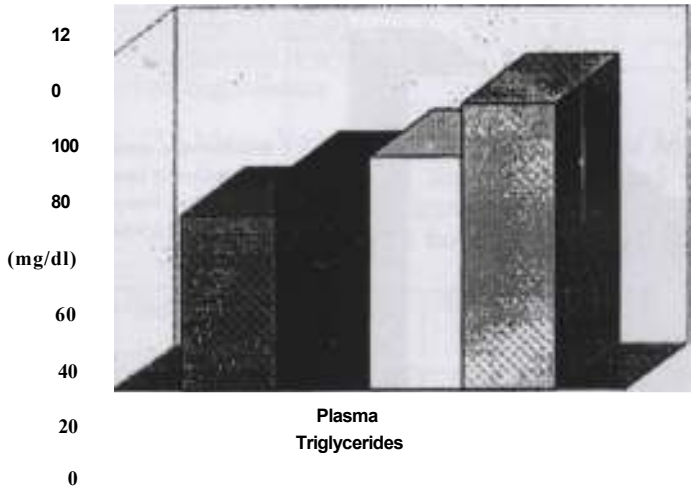
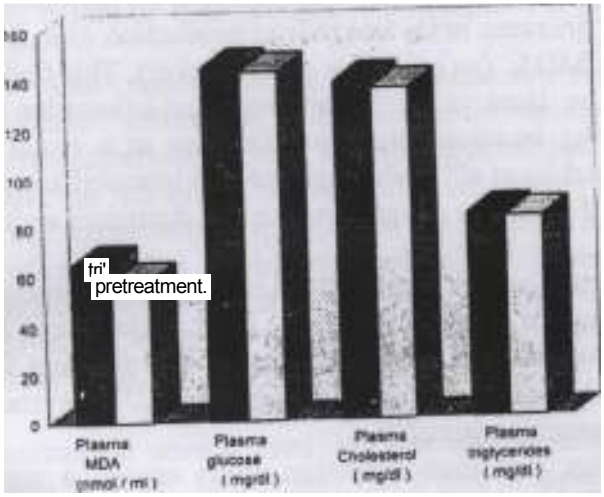


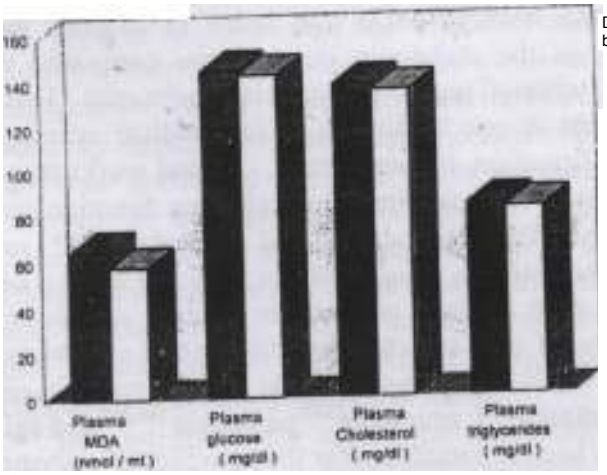
Fig. (5): Mean values of the effect of melatonin at 5 .0 mg/kg body weight on the stress-induced increase in plasma MDA . glucose cholesterol and triglycerides compared with rats under stress for one hour without melatonin pretreatment



• stress for 1.0 hour i group U b

DMeltonin 5 0 mg/kg before stress group III

Fig.(6): Mean values of the effect of melatonin at 10 mg/kg body weight on the stress - induced increase in plasma MDA . glucose, cholesterol and iglycendes compared with rats under stress for one hour without melatonin



• stress for 1.0 hour group II b

DMeltomn 10 mg/kg before stress group III

DISCUSSION

Our results, showed that immobilization stress for different periods caused a significant increase in 02 free radical production as indicated by the plasma level of MDA (an oxidative stress marker). This finding was reported previously by Hara et al.,[3] who found that exhaustive stress in the form of swimming increased lipid pcroxidation as a result of free radical production and liu et al[13] who reported that immobilization stress induces generation of reactive oxygen species and decreases endogenous antioxidant defenses.

Also, the present results showed that immobilization stress caused a significant increase in plasma glucose level. This stress induced hyperglycemia was reported by many authors in the different forms of stress such as immobilization stress[14,15] ether stress[1} psychological stress[17] and water immersion stress[18].

We found also, that immobilization stress caused a significant increase in plasma cholesterol and triglycerides, this was confirmed by many authors in different forms of stress e.g. immobilization stress[X] and psychological stress[2].

The results of this work showed that melatonin reduced the immobilization stress-induced 02 free radical production as indicated by the decreased plasma level of MDA (an index of induced membrane oxidative damage) in the melatonin injected rats compared with that subjected to stress without pre-treatment with melatonin. This finding proves that melatonin is one of the chief free radical scavengers and antioxidants. In confirmation to our results, several works reported that melatonin detoxify free radicals directly via electron donation such as the highly toxic hydroxyl (OH) radical[19, 0] and peroxyl (Roo**) radical [21] Additionally, other studies clone both in vitro and in vivo found that melatonin protected cells, tissues and organs against oxidative damage induced by a variety of free radical generating agents and processes e.g. carcinogen safrole [23>24] lipopolysaccharide * "[7], ischemia-reperfusin injury (^, ionizing radiation [5', excessive[3] paraquate [28,29] and aging[20,30], Also, melatonin has been reported to alter the activities of enzymes which improve the total antioxidative defense capacity of the organism e.g. stimulate glutathione peroxidase enzyme which metabolizes

hydroperoxides including hydrogen peroxides (H2O2), thereby reducing the formation of the highly toxic (OH) radical[31] and inhibition of nitric oxide synthase thus reduces the formation of the free radical nitric oxide [32]

Our results showed that melatonin pre-treatment produced no significant ciTect on immobilization stress-induced hypcrglyccmia, hypercholesterolemia and hypertriglyceridemia. Regarding the previous reports on the effect of melatonin on plasma glucose level, it showed marked controversy, some, like our results found that melatonin had no significant effect on plasma glucose [6<33]. Others, reported that melatonin decreases plasma glucose level[34] and in contrast to this John et al.,[35] and Sandyk, [} reported that melatonin caused significant increase in plasma glucose level.

This controversy could be explained by the difference in the experimental animal used as those who found melatonin had no effect on plasma glucose used frogs [33] or a type of Japanese birds[6] and those who found nn increase in plasma glucose as a result of melatonin administration used pigeon[0] but those who found that melatonin administration decreases plasma glucose used mice or rats[34]. This might raise the possibly that the effect of melatonin on plasma glucose is species dependant. It might be also explained by the difference in the experimental model used as some studied the effect of melatonin on normal blood glucose[(l)]. Others, studied the effects of melatonin on alloxan-indueed hyperglycemia[13'] and others studied its effect on the hyperglycemia-induced by intracerebroventricular injection of 2 deoxy-D glucose and our work was conducted on the stress - induced hyperglyemia. The mechanism of hyperglycemia in each of these conditions is different. Another explanation for this controversy is the difference in gonadal function and environmental temperature during the time of the experiment. Alonso- Bedate et al./[3]-' reported that inhibitory effect of melatonin on plasma glucose could be observed only when gonadal function and melatonin are stimulated by temperature.

In contrast to our results on the effect of melatonin on plasma cholesterol, many authors found that melatonin lowers plasma cholesterol level in hereditary hypercholesterolemia[37] in hypercholesterolemia

resulting form fatty diet[38] and in drug-induced hypercholesterolemia[7]. This contradiction might be due to the difference in the experimental model of hypercholesterolemia as we used the stress - induced model.

In agreement with our results on the effect of melatonin on plasma triglycerides, Sandyk and Awerbuch[39] found that serum triglycerides are not affected 6y melatonin.

In conclusion: stress caused a significant increase in plasma MDA, glucose, cholesterol and triglycerides. Plasma ML)A was significantly decreased in rats injected by melatonin and the effect was dose-dependant. Melatonin has no significant effect on plasma glucose, cholesterol and triglycerides. So, melatonin is a good antioxidant.

REFRENCES:

1- Curi C.M., Ribeiro E.B., Zaia C.T and Dolnikoff M.S. (1990):
Glycemic response to stress stimulation by ether exposure in adrenalectomized rats. Pharmacol. Biochem. Behave., 37: 399.

2- Spence J.D., Manucks S.B., Munoz C., Cheng H., Huff. M., Dennis Band Borkwski K. (1990):
Hemodynamic and endocrine effects of mental stress in untreated borderline hypertension. Am. J. Hypertens., 3 : 859.

3- Hara M., Abe M., Suzuki T. and Reiter R.J. (1996):
Tissue changes in glutathione metabolism and lipid peroxidation induced by swimming are partially prevented by melatonin. Pharmacol. Toxicol., 78: 308.

4- Bertuglia S., Marchiafava P.L. and Colantuoni A (1996):
Melatonin prevents ischemia reperfusion injury in hamster cheek pouch. Microcirculation Cardiovasc. Res., 31: 947.

5- Blinkenstaff R.T., Brandstadter S.M, Reddy S. and Witt R. (1994):
Potential radioprotective agents. 1. Homologous of melatonin J. Pharm.Sci., 83:216.

6- Zeman M., Vyboh P., Jurani M., Lamosova D., Kostal L., Bilcik B., Blazicek P, and Juraniova E, (1993):
Effects of exogenous melatonin on some endocrine, behavioral and metabolic parameters in Japanese quail conturnix japonica Comp. Biochem. Physiol Comp. Physiol., 105: 323.

7- Mori W., Aoyama H. and Mori N. (1984):
Melatonin protects rats from injurious effects of glucocorticoid, dexamethasone. Japanese J. of Experm. Med., 54; 255.
8- Ruiz de Gordao J.C, Santafe J., Segarra Domenech J. and Santisteban A. (1994):
Modifications of rat Plasma lipoproteins induced by acute immobilization stress. Psychosom. Med., 56: 486.
9- Draper 11.11., and lladlcy M. (1990):
Malondialdehyde determination as index of lipid peroxidation. Methods Enzymol., 186: 421.
10- Trinder, P(1969):
Enzymatic Determination of Glucose. Ann. Clin. Biochem., 6: 24.
11- Stein, EA:(1986):
Quantitative Enzymatic Colorimetcric Determination of total & HDL Cholesterol in serum or plasma In: Textbook of clinical chemistry, NW Tietz, (Ed). W.B Saunders, Philadelphia, 879- 886, 1818, 1829.
12- Wahlefeld,AW(1974):
Quantitative Enzymatic Determination of Triglycerides in serum or plasma. In: Methods of enzymatic analysis. Vol. (5), Bergmeyer I I U (Ed.), Academic press, N.Y, p: 1831
13- Liu J. Wang X. and Mori A. (1994):
Immobilization stress-induced antioxidant defense changes in rat plasma: Effect of treatment with reduced glutathione. Int. J. Biochem., 26:511.
14- Yamada F., Inoue S., Saitoh T., Tanaka K., Satoh S., and Takamura Y. (1993):
Gluco-rcgulatory hormones in the immobilization stress -- induced increase of plasma glucose in fasted and fed rats. Endocrinology, 132:2199.
15- Tajima T, Edo H., Suzuki Y., Ikari H., Gotok M., & Iguchi A (1996)
Immobilization stress-induced increase of hippocampal acetylcholine and of plasma epinephrine, norepinephrine and glucose in rats. Brain Res., 720: 155.
16- Reis P.M., Santos M.A., Reis A.M. and Coimbra C.C. (1994):
Effects of hyperprolactinemia on plasma glucose and prolactin in rats exposed to ether stress. Physiol. Behav., 56: 495.

17- Armario A., Marti 0., Molina T., De Pablo J. and Valdes M. (1996): Acute stress markers in human: response of plasma glucose, cortisol and prolactin to two examination differing in (he anxiety they provoke. Psychoneuro endocrinology, 21: 17.

18- De Boer S.F., Kopmans. J., Slangen L., & Van der Guenj J. (1990): plasma catecholamine, corticosterone and glucose responses to repeated stress in rats, effect of inter stressor interval length. Physiol. Bchav.,47: 117.

19- Tan D.X., Chen D, Poegeller B., Manchester I,; and Reiter R. (1993): Melatonin a potent endogenous hydroxyl radical scavenger, Endocrine J., 1:57.

20- Reiter, R.J., (1995a): oxidative processes and antioxidative defense mechanisms in the aging brain. FABES J., 9: 526.

21- Cao G., Allesio K.M., and Cuther R.G.(1993): Oxygen -radical absorbance capacity assay for antioxidants. Free Radical Biol. Med. 14: 303.

22- Pieri C, Marra M., Recchioni R & Marcheselli F. (1994): Melatonin: A peroxyl radical scavenger more effective than vitamin E. LifeSci., 15:271.

23- Reiter R.J. (1995b): The role of the neurohormone melatonin as a buffer against macromolecular oxidative damage. Neurochem. Int., 27: 453.

24- Tan D.X., Reiter R.J., Chen L.D., Poeggeller B., Manchester L.R. and Barlow-Walden L.R. (1994): Both physiological and pharmacological levels of melatonin reduce DNA adduct formation induced by carcinogen safrole. Corcinogenesis, 15:215.

25- 25- Sweeryneck E, Melchiorri D., Reiter R, Ortiz G, Leinsk A(1995a) Lipopolysaccharide- Induced hepatoloxicity is inhibited by the antioxidant melatonin. Eur. J. Pharmacol., 293: 327.

26- Seweryneck E., Abe M., Reiter R.J., Lope/, B. (1995b): melutonin administration prevents lipopolysueehuride- induced oxidative damage in phenobarbital- treated animals. J. Cell Biochem., 58:436.

27- Sewerynceck E., Ortiz G.G., Reiter R.j., Pablos M.L., Melchiorri D., Daniels W.M. (1996):
lipopolysaccharide- induced DNA damage is greatly reduced in rats treated with the pineal hormone melatonin. Mol. Cell Endocrinol., 117: 183.

28- Ogata T. and Manobe S. (1990):
Correlation between lipid peroxidation and morphological **m;imfcskilinn of parmjtiatCHtKUtcctl** lung in rats. Arch. Txicol., 64: 7.

29- Melchiorri D, Reiter R.J., Sweerynek E., Hara M., Chen L. and Nistico G. (1996):
paraquat toxicity and oxidative damage reduction by melatonin. Biochem. Pharmacol., 51: 1095.

30- Reiter R.J (lW5e) :
Oxygen radical detoxification process during aging: The functional importance of melatonin. Aging, 7 : 340.

31- Pablos M., Chuang J., Reiter R.J., Ortiz G.G. Daniels W.M. Seweryneck E., Nelchiorri D. and Poeggeler B. (1995):
Time course of the melatonin-induced increase in glutathione **peroxidase** activity in chick tissues. Biol. Signals, 4: 325.

32- Pozo D., Reiter R.J., Calvo J. P. and Guerreo J.M. (1994): Physiological concentrations of melatonin inhibit nitric oxide synthase in rat cerebellum. Life Sci., 55 : 455.

33- Alonso - Bedate. M., Carballada R. and Delgado M.J. (1990): Effects of melatonin on gonadal steroids and glucose plasma levels in frogs. J. Pineal Res., 8 : 79.

34- Pierrefiche G.Jopall G., Courboin G., Henriet I & LaboritH. (1993) : Antioxidant activity of melatonin in mice. Res. Commun. Chem. Path. Pharmacol., 80:211.

35- John T.M., Viswanathem M., George J.C. and Scahes C. G. (1990): Influence of chronic melatonin implantation on circulating levels of **catecholamines,** growth hormone, thyroid hormones, glucose and F.F.A. in the pigeon. Gen. Comp. Endocrinol., 79: 226.

36- Sandyk R. (1993):
Weak magnetic fields antagonize the effects of melatonin on blood glucose in parkinson's disease. Int. j. Neurosci., 68: 85.

37- Aoyama H., Mori N., and Mori W. (1988):
Effects of melatonin on genetic hypercholesterolemia in rats. Atherosclerosis, 69: 269.

38- Mori N., Aoyama H. and Mori W. (1989) :
Anti - hypercholesterolemic effect of melatonin in rats. Japanese Soc. Of Path., 39: 613.

39- Sandyk R. and Awerbuch G.I. (1994) :
Relationship between melatonin secretion and serum cholesterol in patients with multiple sclerosis. Int. J. Neurosci 76 : 81.