Int J Diabetes & Metabolism (2009) 17:87-92

Melatonin effects on macrophage in diabetic rats and the maternal hyperglycemic implications for newborn rats

Adenilda Cristina Honório França[1,4], Karina Aparecida Silva[2], Nagilla Daliane Feliciano[3], Iracema Mattos Paranhos Calderon[4], Marilza Vieira Cunha Rudge[4], Eduardo Luzía França[1].

University of Araguaia, Federal University of Mato Grosso, Pontal do Araguaia, Brazil [1] Institute of Health Sciences, University Center of Planalto de Araxá, Araxá, Brazil [2], Institute of Biomedical Sciences, Federal University of Uberlândia, Uberlândia, Brazil[3] Post Graduate Program in Gynecology, Obstetrics and Mastology, Botucatu Medical School, Unesp, São Paulo, Brazil[4]

Abstract

The modulatory effects of melatonin (MLT) on maternal and fetal macrophages in diabetic rats and the repercussion of maternal hyperglycemia on fetus-placenta parameters were studied. This was achieved by determining maternal and fetal blood glucose, weight and superoxide release by macrophages. Placental weight, protein, DNA and RNA concentration were also verified. Superoxide levels in macrophages isolated from pregnant healthy rats were higher than those obtained from diabetic animals. Melatonin increased significantly in the macrophages of control animals (18.7 ± 2.8 with MLT compared to 14.2 ± 1.6 without MLT) but decreased with melatonin stimulation in diabetic rats (8.8 ± 1.4 with MLT compared to 12.9 ± 2.1 without MLT). Melatonin significantly decreased superoxide levels in newborns of diabetic mothers (7.3 ± 3.4) compared to those of healthy (14.6 ± 3.5) mothers. Blood glucose levels were significantly higher ($p<0.05$) in newborn rats of diabetic mothers (108.3 ± 7.8) compared to blood glucose levels in newborn control rats (81.2 ± 10.7). Body weight was significantly higher ($p <0.05$) in the offspring of rats with alloxan-induced diabetes. No statistical difference ($p> 0.05$) was observed in the placenta weight, total protein concentration and DNA of rats. The RNA concentration was significantly lower ($p <0.05$) in the placentas of rats with alloxan-induced diabetes (156.1 ± 71.8), when compared to the concentration of RNA in the placentas of control rats (239.5 ± 77.3). In conclusion, maternal hyperglycemia modified the fetus-placental parameters and melatonin modulated the macrophages activation in maternal and fetal diabetic rats.

Keywords: *diabetes, hormone, macrophages, melatonin, pregnancy, superoxide.*

Introduction

Diabetes mellitus consists of a group of chronic disorders characterized by hyperglycemia or diminished insulin secretion, or both, and has been associated to certain infections.[1] Phagocytic disorders and changes in superoxide release are major factors for infectious diseases.[2] Diabetic patients have reduced phagocytic activity, low microbicidal activity in lysosomal enzyme release and production of reactive oxygen species, owing to changes in antioxidant systems.[2] The reduction in phagocytic and microbicidal activity of leukocytes is likely related to an increase in blood glucose levels.[3] Phagocyte functional activity can be examined through indirect mechanisms to determine oxygen consumption[4] or derivatives of cell oxidative metabolism, such as superoxide anion.[5,6]

Rats with diabetes induced by chemicals agents (alloxan or streptozotocin) have neutrophil quiomitaxia,[7] low phagocytic activity,[8] low hydrogen peroxide production[9] and increased glucose levels. Diabetic pregnant rats have low antibody levels and decreased T-lymphocyte activity. These alterations are also found in their newborns.[10]

Diabetes may change pregnancy conditions, thus affecting the mother and even more so the fetus. Gestational diabetes is the most frequent metabolic disorder of pregnancy and occurs in 1% to 10% of gestations.[11] The main pathophysiological consequences for the fetus are macrosomia and oxidative stress.[12] Oxidative stress plays an important role in the pathogenesis and complications of diabetes. Some studies have shown that melatonin (N-acetyl-5-methoxy tryptamine) reduces oxidative stress induced in diabetic rats.[13] Melatonin is an endogenous neurohormone produced by the pineal gland in mammals, and its actions are related to its capability to scavenge free radicals, increase the antioxidant activity of enzymes and beneficial effects for controlling diabetes complications,[15] mainly due to its scavenging capacity against reactive oxygen species in diabetic rats.[14] Furthermore, melatonin at physiological concentrations can stimulate the phagocytic activity of macrophages in animals.[16]

Received on: 6/5/2009
Accepted on: 3/11/2009

Correspondence to: Adenilda Cristina Honorio França, Campus Universitário do Araguaia ICBS/UFMT-Pontal do Araguaia – MT, Rodovia MT100, Km 3,5 s/n^{o} , Pontal do Araguaia - MT. CEP: 78698-000, Brasil. E-mail: denifran@terra.com.br

Phagocytes are considered the first line of the body's immune defense and the reduction in their functional activity contributes to increased susceptibility and severity in diabetes infections.[17] Several immunological functions are affected by diabetes,[1] but little is known about its influence of hormones on maternal and fetal macrophages, and the possible implications of maternal hyperglycemia for physiopathological disorders in newborns.

This study investigated immunomodulatory effects of melatonin (MLT) on macrophage activity in maternal and fetal rats with alloxan-induced diabetes and the repercussion of maternal hyperglycemia on fetus-placenta parameters.

Materials and methods

Animals and operation procedures

Adult female Wistar rats, weighing between 200g and 250g (10-12 weeks of age) and still in their reproductive phase, were housed under constant conditions of light (photoperiod L:D 12:12) and temperature (25^{o}C) with *ad libitum* access to water and food. The animals were separated into two groups: control pregnancy (n=10) and alloxan-induced diabetic pregnancy (n=10). For diabetic induction, 12-h fasted animals received a single intravenous injection of alloxan (42 mg/kg body weight) in the medial portion of the tail. Control animals were given an equivalent volume of 0.9% saline.

For diabetes confirmation, blood glucose levels were determined by an Accu-Check Advantage (Roche, Sao Paulo, Brasil) glucometer 15 d after alloxan injection (mean glucose blood control group = 74 ± 6.5 and alloxan-induced diabetic =146 ± 30.3). Animals of both groups were paired for mating. The fertilization day was assessed by the vaginal presence of sperm.

In the morning at days 1, 7, 14 and 21 of pregnancy, maternal blood glucose levels were determined by puncture of the tail vein of rat. The mean maternal blood glucose level was calculated using all glycaemic values obtained during pregnancy.

On the morning of day 21 of pregnancy the animals were anesthetized with sodium phentobarbital (50 mg/kg body weight, intraperitonealy) and submitted to a surgical procedure for newborn removal. The spleen was removed and placed in phosphate buffered saline (PBS) solution. The newborn rats were weighed and decapitated and blood samples were collected and pooled all puppies for determination of glucose.

The spleens were also removed, pooled and stored in PBS for further superoxide determination. The placentas were removed, weighed and conserved at -20^{o}C for further analysis of total protein, RNA and DNA. The research project was evaluated institutionally and approved in July, 2005.

Isolation, Purification and Identification of Macrophages

Maternal and newborn spleens were macerated in PBS. The cells were fractioned by centrifugation (160 x g, 30 min.) through gradient density (density 1.077 g/l) using Ficoll-Paque (Pharmacia, Uppsala, Sweden). Purified macrophages were resuspended independently in serum-free medium 199 (Gibco, MD, USA) and washed twice. Cell count (using Newbauer hemocytometer) and macrophage suspension were adjusted to a final concentration of $1x10^{6}$cells/ml.[6] Before undergoing superoxide release measurements, the macrophages were identified under light microscope and their viability was measured by the trypan blue exclusion test.

Release of Superoxide Anion

To measure superoxide release, cytochrome C (Sigma) reduction was determined as previously described.[5,6] The macrophages were centrifuged twice at 160 x g for 10 min at 4^{o}C, and 500 mL of the phagocytes was resuspended in PBS containing 2.6 mM $CaCl_2$ and 2.0mM $MgCl_2$ and cytochrome C (Sigma-2 mg/ml) for verified the spontaneous superoxide release. Phorbol myristate acetate (PMA-Sigma) stimulation was performed as positive control at final concentration of 10^{-7}M. To evaluated the melatonin effect the macrophages were stimulated with melatonin hormone (Sigma, final concentration of 10^{-7}M).[16] The suspensions (100 μL) were incubated for 60 min at 37^{o}C in culture plates. The reaction rates were measured by absorbance at 450 nm and the results were expressed as nmol/O_2^-. All the experiments were performed in duplicate or triplicate.

Total protein, ribonucleic acid (RNA) and desoxiribonucleic acid (DNA) in the placenta

The placentas were preserved in liquid nitrogen, macerated in PBS and filtered to determine total protein concentration, DNA and RNA.[18,19,20,21] The results were expressed in mg%.

Statistical analysis

Analysis of variance (ANOVA) with F statistics was used to evaluate the quantitative data on maternal and newborn blood glucose, weight, superoxide release, placental weight, protein concentration, DNA and RNA; significant differences were evaluated by Tukey's test.[22] All the results were expressed as mean ± SD. Statistically significant differences were considered for alpha error below 0.05 ($p < 0.05$).

Results

Confirmation of diabetes

Mean glucose level, determined four times over 21 days of pregnancy, was significantly higher ($p < 0.05$) in diabetic rats treated with alloxan than those of untreated rats (Table 1). There was no difference ($p > 0.05$) in body weight between diabetic and control animals.

The effect of diabetes on maternal macrophages

Diabetes not influenced significantly ($p > 0.05$) the several parameters of isolated macrophages. The number of maternal macrophages retrieved as well as their viability. The functional activity also were not affected by diabetes ($p > 0.05$), according to analysis of spontaneous superoxide release (control group =14.2 ± 1.6 – diabetic group 12.9 ± 2.1; Table 1).

Table 1: General characteristics (mean ± sd) of control and alloxan-induced diabetic rats during pregnancy.

Parameter	Control rats (n=10)	Diabetic rats (n=10)
Glucose level (mg/dl)	87.7 ± 10.4	148.2 ± 12.8*
Body weight	329,2 ± 59.2	288,5 ± 14.1
Number of macrophages (x106 cells/ml)	4.2 ± 0.5	3.9 ± 0.4
Viability of macrophages (%)	89 ± 4.7	87.9 ± 5.6
Superoxide release (nmol) (unstimulated cells)	14.2 ± 1.6	12.9 ± 2.1

*p<0.05 (ANOVA) comparing control rats to alloxan-induced diabetic rats.

Table 2: General Characteristics (mean ± sd) of newborn rats of alloxan-induced diabetic and control mothers. *p < 0.05 (ANOVA) comparing the control group to the alloxan-induced diabetic group

Parameter	Newborn of Control rats (n=10)	Newborn of Alloxan-diabetic rats (n=10)
Glucose level (mg/dl)	81.2 ± 10.7	108.3 ± 7.8*
Body weight (g)	3.1 ± 0.9	4.3 ± 1.0*
Placental weight (g)	0.56 ± 0.4	0.55 ± 0.2
Placental total protein (mg %)	60.8 ± 9.6	64.5 ± 7.7
Placental DNA Concentration (mg %)	266,9 ± 72.3	226.1 ± 62.9
Placental RNA Concentration (mg %)	239.5 ± 77.3	156.1 ± 71.8*
Number of macrophages (x10^6 cells/ml)	2.7 ± 0.35	2.1 ± 0.45
Viability of macrophages (%)	81.9 ± 2.5	80.8 ± 3.6
Superoxide release (nmol) (unstimulated cells)	13.3 ± 2.9	8.3 ± 1.9*

*p<0.05 (ANOVA) comparing control group to alloxan-induced diabetic group

Influence of melatonin on the superoxide release from macrophages of pregnancy rats

In control pregnant rats, melatonin at 10^{-7} M significantly ($p < 0.05$) stimulated superoxide release from macrophages compared to non-stimulated macrophages (18.7 ± 2.8 versus 14.2 ± 1.6) (Fig. 1). There was no significant difference ($p > 0.05$) between macrophage stimulation by melatonin and PMA (21.1 ± 1.8).

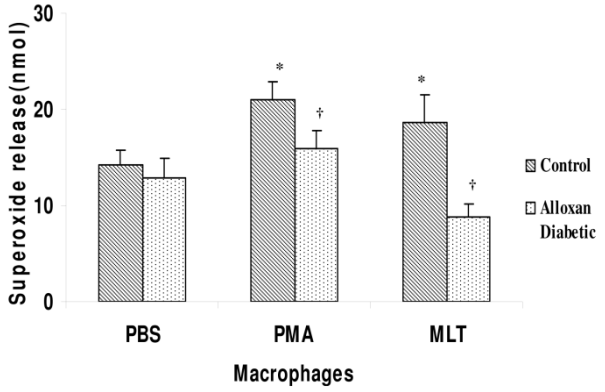

Figure 1: Effects of melatonin (MTL, 10^{-7} M), PBS (control) and PMA (10^{-7}M- positive control) on macrophage superoxide release (mean ± sd) in control or alloxan-induced diabetic rats during pregnancy (n=10 for each treatment). *p < 0.05 superoxide release in cells stimulated with PMA or melatonin versus PBS; †p < 0.05 control group versus alloxan-induced diabetic group.

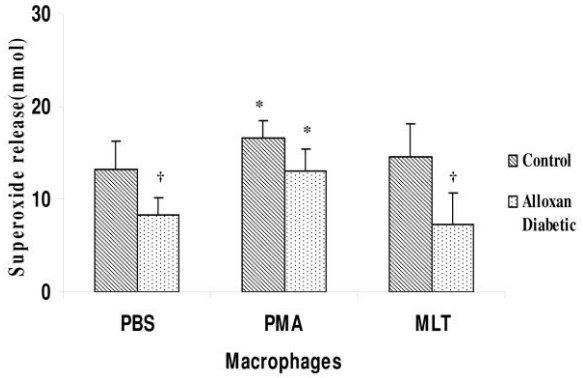

Figure 2: Effects of melatonin (MTL, 10^{-7} M), PBS (control) and PMA (10^{-7} M, positive control) on macrophage superoxide release in newborn rats of control and alloxan-induced diabetic mothers (n=10 for each treatment). *p < 0.05 superoxide release in cells stimulated with PMA or MLT versus PBS, within the same experimental group; †p < 0.05 control group versus the alloxan-induced diabetic group.

In diabetic pregnant rats, melatonin stimulation failed to induce superoxide release (8.8 ± 1.4 compared to 12.9 ± 2.1 of control group). Melatonin decreased macrophage superoxide release in diabetic pregnant rats (8.8 ± 1.4; ($p < 0.005$) compared to control pregnant rats (18.7 ± 2.8; Fig. 1).

Influence of maternal hyperglycemia on fetus-placenta parameters

Blood glucose levels were significantly higher ($p < 0.05$) in the newborn rats of diabetic mothers (108.3 ± 7.8)

compared to blood glucose levels in newborn control rats (81.2 ± 10.7). Body weight was significantly higher ($p < 0.05$) in the offspring of rats with alloxan-induced diabetes compared to control (Table 2). No statistical difference ($p > 0.05$) was observed in placental weight, total protein concentration and DNA of rats with alloxan-induced diabetes, when compared with the placentas of control rats (Table 2). The RNA concentration was significantly lower ($p < 0.05$) in the placentas of rats with alloxan-induced diabetes (156.1 ± 71.8), compared to the concentration of RNA in the placentas of control rats (239.5 ± 77.3) (Table 2).

The effect of diabetes on the number and function of newborn macrophages

Diabetes influenced ($p > 0.05$) neither the number nor the viability of spleen macrophages in newborn rats. In contrast, the functional activity of the offspring of rats with alloxan-induced diabetes decreased significantly ($p < 0.05$), as shown by the lower superoxide release by non-stimulated macrophages in the group with alloxan-induced diabetes (Table 2).

Influence of melatonin on the superoxide release of macrophages in newborn rats.

Melatonin at a concentration of 10^{-7} M, did not stimulate ($p > 0.05$) superoxide release from macrophages of offspring of control rat (14.6 ± 3.5 in MLT-stimulated versus 13.3 ± 2.9 innon-stimulated macrophages) (Fig. 2). Spleen macrophages from newborns of both diabetic and control mothers had higher superoxide release when stimulated by PMA (16.6 ± 1.8), more than that of non-stimulated macrophages (13.3 ± 2.9) and melatonin-stimulated macrophages (14.6 ± 3.5). Melatonin failed to stimulate spleen macrophages of newborns of diabetic mothers (7.3 ± 3.4 compared to 8.3 ± 1.9 without melatonin). Melatonin significantly decreased ($p < 0.005$) superoxide release by newborn macrophages of diabetic rats (7.3 ± 3.4) compared to newborn macrophages of control rats (14.6 ± 3.5) (Fig.2).

Discussion

The present study evaluated, in vitro, the effect of melatonin on the release of superoxide anion by macrophages isolated from the spleen of alloxan-induced diabetic pregnant rats and their pups. This work also studies the repercussion of maternal hyperglycemia fetus-placental parameters.

The pregnant rats with alloxan-induced diabetes had moderate hyperglycemia accompanied by low body weight (although not significant) (Table 1). On the other hand, there was no change in the count or viability of spleen macrophages in both diabetic and control groups. The activation ability of these cells, indicated by superoxide release, was also similar in the two groups, suggesting that diabetes does not affect these cells during pregnancy in rats. Similar results regarding the functional activity of leukocytes[23]and phagocytic activity of macrophages[16] were observed in patients with diabetes and in experimental studies on diabetic animals.

Other studies indicate that neurohormones pocess immunomodulatotory effects.[24] Melatonin is an endogenous neurohormone produced by the pineal gland in mammals, and its beneficial action has been linked to its ability to scavenge different free radicals and increase the antioxidant activity of enzymes.[14,15,25] Many studies have postulated that melatonin has a stimulatory action on the immune cells.[16,26] In the present study, melatonin stimulated the cellular oxidative metabolism of control pregnant rats. This was evidenced by the increased superoxide release by macrophages, which reached values similar to those obtained in PMA-stimulated macrophages (positive control). Moreover, the cells of diabetic pregnant rats were not stimulated by melatonin. Similar results were found by Pawlak et al,[16] who observed that melatonin stimulates phagocytosis in normal rats, but failed to stimulate this activity in cells isolated from diabetic rats.

During oxidative stress, cells generate high contents of superoxide radicals.[28] Free radical generation has been reported as an important mechanism for body protection in infectious processes, mainly intestinal infections.[6,29] In the present study, these radicals were not released in diabetic pregnant animals. In contrast to control rats, superoxide release by macrophages from diabetic rats was not modified by melatonin and was only weakly stimulated by PMA, which indicates that diabetes may partially affect the function of these cells. Diabetes mellitus is known to affect the immune system and some studies have demonstrated that blood neutrophils and B and T lymphocytes from diabetic patients show impaired immune function [17,23] Similar results were reported in the leukocytes of diabetic patients[23] and macrophages from diabetic rats.[16]

The association between diabetes and pregnancy may cause severe immunological changes because pregnancy alone makes diabetes control difficult, accentuating maternal hyperglycemia levels. Some studies demonstrate that immune alterations due to maternal hyperglycemia are reflected in alterations of immunological and biochemical parameters in the fetus, increased frequency of embryonic death as well as malformations and changes in placental parameters.[10, 11, 30,31]

Diabetic pregnancy represents an important risk factor for overnutrition and the development of obesity in the offspring.[32] Some literature reports have associated fetal growth disturbances with maternal blood glucose and weight with the placenta. In the present study, the offspring of diabetic rats had increased blood glucose and body weight. A physiological state of insulin resistance is required to better direct maternal nutrients toward the fetus-placenta unit, allowing adequate fetal growth. Women with gestational diabetes mellitus have more a severe insulin resistance, which disrupts the intrauterine milieu, resulting in accelerated fetal development with increased risk of macrosomia. In the present paper, maternal blood glucose was characterized by mild hyperglycemia, which probably interfered in the weight of the newborn rats. As a natural interface between mother and fetus, the placenta is the obligatory target of such environmental changes.[33] The

maternal metabolic alterations associated with profound diabetes in the uterine environment leads to an abnormal pattern of fetal growth.[34]
Studies that investigated the impact of placental alterations on fetal development revealed that rats with alloxan-induced diabetes had lower placental RNA and cell count. Abnormal placental structure and function in diabetic pregnancies are also related to increased oxidative stress and reduced antioxidant capacity in diabetic rats.[35] Oxidative stress occurs in diabetic pregnancy, likely compromising antioxidant defense mechanisms and increasing free radical production.[36]

In contrast, no alterations were observed in the count and viability of the spleen macrophages of offspring from both control and diabetic groups. Offspring macrophages, however, had compromised functional activity. The decrease in spontaneous superoxide release by newborn macrophages shows the effects of maternal hyperglycemia on the activity of these cells. Melatonin also failed to stimulate offspring macrophages to release superoxide, indicating that maternal hyperglycemia may affect macrophage function in newborn rats. The role that melatonin plays as an immunomodulator is explained in the literature. Many studies have reported melatonin effects in the scavenging of free radicals[14] and diabetes control,[15] whereas others found that melatonin does not scavenge superoxide radicals.[37] Phagocytic activity and superoxide alterations have been considered major factors of infectious diseases, mainly the bacterial infections,[2,6] and are likely associated to higher infection rates in children of diabetic mothers.

The maternal hyperglycemia modified the fetus-placental parameters and the melatonin modulated the macrophages activation in control and diabetics maternal rats and the newborn of the diabetic mothers. Melatonin can stimulate macrophage superoxide release in healthy pregnant rats, but not in the macrophages of newborn rats. When diabetes is induced in these animals, spontaneous superoxide release remains unaffected, but melatonin treatment decreases superoxide release in both mother and offspring.

References

1. Rocha JLL, Baggio H CC, da Cunha CA, et al. Relevant issues in the interaction between diabetes mellitus and infection. Arq Bras Endocrinol Metab 2002; 46: 221-229.
2. Larijani B, Shooshtarizadeh P, Mosaffa N, Heshmat R. Polymorphonuclear leucocyte respiratory burst activity correlates with serum zinc level in type 2 diabetic patients with foot ulcers. Br J Biomed Sci 2007; 64: 13-17.
3. Jakelic J, Kokic S, Hozo I, Maras J, Fabijanic D. Nonspecific immunity in diabetes: hyperglycemia decreases phagocytic activity of leukocytes in diabetic patients. Med Arh 1995; 49:9-12.
4. Nakamura M, Baba AS, Tamura N. Polarographic micromethods for the rapid assay of phagocytosis connected oxygen consumption by leukocytes in diluted peripheral blood. J Lab Clin Med 1981; 97: 31-38.
5. Pick E, Mizel D. Rapid microassays for the measurement of superoxide and hydrogen peroxide production by macrophages in culture using an automatic enzyme immunoassay reader. J Immunol Methods 1982; 46:211-226.
6. Honorio-França AC, Carvalho MPSM, Isaac L, et al. Colostral mononuclear phagocytes are able to kill Enteropathogenic Escherichia coli (EPEC) opsonized by colostral IgA. Scand J Immunol 1997; 46: 59-66.
7. Fortes ZB, Farsky SP, Oliveira MA, Garcia-Leme J. Direct vital microspcopic study. Br J Pharmacol 1991; 40: 1267-1273.
8. Panneerselvam S, Govindasamy S. Sodium molybdate improves the phagocytic function in alloxan-induced diabetic rats. Chem Biol Interact 2003; 145:159-163.
9. Alba-Loureiro TC, Hirabara SM, Mendonca JR, et al. Diabetes causes market changes in fuction and metabolism of rat neutrophils. J Endocrinol 2006; 188:295-303.
10. Honorio AC, Rudge MVC, Oliveira SL, et al. Maternal and Fetal Immune Response in Diabetic Rats. Rev. Bras Ginecol Obstet 1996; 18: 233-238.
11. Gabbe S. Gestational diabetes mellitus. N Engl J Med 1986; 315: 1025-1026.
12. Grissa O, Atègbo JM, Yessoufou A, et al. Antioxidant status and circulating lipids are altered in human gestacional diabetes and macrossomia. Transl Res 2007; 150:164-1671.
13. Donder E, Bayadas G, Sokmen S. Investigation of antioxidant and glucometabolic efects of melatonin in experimental diabetes mellitus. Biomed Res 1999; 10:537-546.
14. Klepac N, Rudes Z,Klepac R. Effects of melatonin on plasma oxidative stress in rats with streptozotocin induced diabetes. Biomed Pharmacoter 2005; 60: 32-35.
15. Sudnikovich EJ, Maksimchik YZ, Zabrodskaya SV, et al. Melatonin attenuates metabolic disorders due to streptoxotocin-induced diabetes rats. Eur J Pharmacol 2007; 569:180-187.
16. Pawlak J, Singh J, Lea RW, Skwarlo-Sonta K. Effect of melatonin on phagocytic activity and intracellular free calcium concentration in testicular macrophages from normal and streptozotocin-induced diabetic rats. Mol Cell Biochem 2005; 275:207-213.
17. Alba-Loureiro TC, Munhoz CD, Martins, JO, et al. Neutrophil function and metabolism in individuals with diabetes mellitus. Braz J Med Biol Res 2007; 40:1037-1044.
18. Lowry O, Rosenbrough N, Farr A. Protein measurement with the folin phenol reagent. J Biol Chem 1951; 193: 265-275.
19. Munro HN, Fleck A. The determination of nuclear acids. Methods Biochem Anal 1996; 14:114-116.
20. Burton K. Study of the conditions and mechanism of the diphenylamine reaction for the colorimetric

stimation of deoxyribonucleic acid. Biochemistry 1956; 62:315-323.
21. Giles KW, Myers A. An improved diphenylamine method for the stimation of dioxiribonucleic acid. Nature 1965; 206:93-94.
22. Zar JH. Biostatical analysis. Englewood Cliffs, Prentice-Hall International Editions, New Jersey, 1984.
23. Seyrek N, MarcinkowskiW, Smogorzewski M, et al. Amlodipine prevents and reverses the elevation in (Ca2+)*i* and the impaired phagocytosis of PMNL of diabetic rats. Nephrol DialysisTransplant 1997; 12: 265–272.
24. Besedovsky HO, del Rey A. Immune-neuro-endocrine interactions: Facts and hypotheses. Endocr Rev 1996; 17: 64–102.
25. Pandi-Perumal SR, Trakht I, Srinivasan V, et al. Physiological effects of melatonin: Role of melatonin receptors and signal transduction pathways. Prog Neurobiol 2008; 85: 335-353
26. Skwarlo-Sonta K. Bi-directional communication between pineal gland and immune system. J Physiol 2002; 543: 6S-7S.
27. Robertson RP. Chronic oxidative stress: a central mechamism for glucose toxicity in pancreatic islet beta cell in diabetes. J Biol Chem 2004; 279:4351-4354.
28. Rodriguez C, Mayo JC, Sainz RM, et al. Regulation of antioxidant enzymes: a significant role for melatonin. J Pineal Res 2004; 36:1-9.
29. França-Botelho AC, França EL, Honório-França AC, et al. Phagocytosis of Giardia lamblia trophozoites by human colostral leucocytes. Acta Paediat 2006; 95: 438-443.
30. Calderon IMP, Rudge MVC, Ramos MD. Biochemical and histochemical longitudinal analysis of placentas of diabetic rats: Relationship with macrosomatia and intrauterine growth retardation. Rev Bras Ginecol Obstet 1999; 21:91-98.
31. Padmanabhan R, Shafiullah M. Effect of maternal diabetes and ethanol interactions on embryo development in the mouse. Mol Cell Bioch 2004; 22:15-36
32. Merzouk S, Madani S, Hichami A, et al. Age related changes in fatty acids in obese offspring of streptozotocin-induced diabetic rats. Obes Res 2002; 10:703-7014.
33. Radaelli T, Varastehpour A, Catalana P, et al. Gestational diabetes induces placental genes for chronic stress and inflammatory pathways. Diabetes 2003; 52:2051-2058.
34. Catalano P, Huston L, Anini S, Kalhan SC. Longitudinal changes in glucose metabolism during pregnancy in obese women with normal glucose tolerance and GDM. Am J Obstet Gynecol 1999; 180:903-916.
35. White V, Jawerbaum A, Sinner D, et al. Oxidative stress and altered prostanoid production in the placenta of streptozotocin-induced diabetic rats. Reprod Fertil Dev 2002; 14: 117-123.
36. Biri A, Onan A, Devrim E, et al. Oxidant status in maternal and cord plasma and placental tissue in gestational diabetes. Placenta 2006; 27: 327-332.
37. Marshall KA, Reiter RJ, Poeggeler B, Aruoma OI, Halliwell B. Evaluation of the antioxidant activity of melatonin in vitro. Free Rad Biol Med 1996; 21:307-315.