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Melatonin Improves Diabetes-Induced Foetal Growth Retardation in Rats

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Abstract

The aim of the present study was to investigate the effect of oral melatonin administration on foetal growth retardation, utero-placental antioxidant enzymes activities and lipid peroxidation in experimental diabetic rats. Twenty pregnant rats were divided into four groups of five rats each. Diabetes mellitus was induced by a single intraperitoneal administration of 120mg/kg body weight of alloxan. From gestational day 5 to 19, 5mg/kg and 10mg/kg of oral melatonin were administered to the rats with clearly manifested gestational diabetes. On the 19th day of gestation, the rats were sacrificed by cervical dislocation and placental, foetal and uterine tissues were harvested for estimation of tissue glutathione peroxidase (GPx) activity and malondialdehyde (MDA) levels. Foetal weight, foetal size, placental and plasma glucose were also determined. Results showed that, in diabetic rats, foetal growth retardation was associated with a significant reduction in placental and uterine antioxidant enzymes (GPx) activities ($P<0.001$) and increased lipid peroxidation as evidenced by raised MDA concentration ($P< 0.05$). Treatment with oral melatonin significantly improved the foetal weight, placental and uterine antioxidant enzymes activities as well as reduced lipid peroxidation, without affecting the degree of hyperglycaemia. Effects of melatonin on foetal growth are presumed to be dependent on its ability to improve uteroplacental antioxidant enzymes activities and reduce lipid peroxidation.

Key words: Gestational diabetes, uteroplacental, melatonin, glutathione peroxidise, malondialdehyde

Introduction

Despite the fact that perinatal mortality has dramatically declined over the past decades, the incidence of malformations has remained unchanged. Congenital malformations represent the single most important cause of perinatal mortality or severe morbidity in diabetic pregnancy (Cederberg *et al*., 2001). The pathogenesis of diabetic embryopathy is multifactorial (Kucera, 1971). In addition to maternal serum glucose levels, studies have shown that factors such as serum levels of triglycerides, β-hydroxybutyrate, branch-chain amino acids, creatinine, HbA1c, arachidonic acid, reactive oxygen species and somatomedin inhibitors are associated with embryonic dysmorphogenesis (Buchanan *et al*., 1994; Styrud *et al*., 1995). Despite recent advances in the management of gestational diabetes, the disease still remains a significant threat during pregnancy with an increased risk of malformation and still birth (Evans, 2009). Obesity and gestational diabetes are characterised by insulin resistance and metabolic derangements resulting from oxidative stress and protein glycation leading to impaired endothelial function, vascular inflammation and haemostasis. These have been reported to give rise to microcirculatory impairment and abnormal placental function leading to increased foetal morbidity and stillbirth (Mondestin *et al*., 2002). In addition to the increased incidence of congenital malformations associated with diabetic pregnancy, human and laboratory studies have shown the retarded growth in foetuses of diabetic mother (Mills, 1982; Pedersen and Molsted-Pedersen, 1981).

Previous studies showed that diabetes-induced congenital malformations could be prevented with treatment of antioxidants such as vitamins C, vitamin E

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and folic acid. (Wentzel *et al*., 1997 ; Cederberg *et al*., 2001). While the cellular mechanism of the diabetic embryopathy is not completely understood, a number of possible teratological processes have been suggested, based on the experimental studies. Pregnancy and diabetes have been reported to decrease antioxidant activities in rats (Szaleczky *et al*., 1999).

N-Acetyl-5-methoxytryptamine, commonly known as melatonin, is a hormone produced by a wide range of organisms including animals, plants and microbes. In vertebrates, melatonin is produced primarily by the pineal gland. While most of the biological effects of melatonin are exhibited through the activation of the melatonin receptors (Boutin *et al.*, 2005), studies have also reported its free radical scavenging (Tan *et al*., 2002) and indirect antioxidative activities (Reiter *et al*., 2000; Rodriquez *et al*., 2004). In this study, we investigated the effect of oral melatonin on foetal growth retardation, uteroplacental antioxidant enzymes activities and lipid peroxidation in rats with alloxan-induced diabetes.

Materials and Methods

Experimental animals: Foetuses were obtained from Wistar albino rats maintained in the Department of Biochemistry, Faculty of Life Sciences, University of Ilorin. Rats were fed a commercial pelleted diet (Bendel feeds, Edo State, Nigeria) and had free access to food and tap water. They were maintained at an ambient temperature of 25^{o}C with a 12hr light/dark cycle. To initiate mating, each female rat was placed in the same cage with a male and conception was verified by the presence of sperm in the vaginal smear. All experimental procedures were approved by the Animal Ethical Committee of the Faculty of Basic Medical Sciences, University of Ilorin, Nigeria.

Induction of diabetes and treatment with melatonin: Induction of diabetes was performed in a subset of female rats by intraperitoneal injection of 120mg/kg alloxan (Sigma St. Louis MO, USA) 1 week before mating commenced (3 weeks before conception). Diabetic status was confirmed 1 week after injection by serum glucose level ≥ 250 mg/dl using AccuCheck glucometer (Roche Diagnostics, Indianapolis, IN, USA). Non-diabetic control rats were injected with saline. Pregnant rats were administered saline (control) or graded doses of melatonin (Sigma, 5mg/kg and 10mg/kg) dissolved in absolute ethanol and dissolved in 0.9% NaCl (1.9w/v) with oral cannula from day 5 to day 19 of gestation.

Collection of terminal blood samples and organs: On day 19th day of gestation, rats were sacrificed by means of cervical dislocation. Blood samples were collected by cardiac puncture into EDTA bottles and centrifuged at 3,000 rpm for 20 min at 4°C, and plasma was stored below -20°C until used. Uteri of the animals were for the measurement of placental weight as well as foetal weight and size. Sections of excised uteri (100mg) were transferred to 2ml of ice-cold sucrose solution (0.3M) prior to homogenization at a speed of 3000g for 10 minutes at 4^{o}C. The supernatants were collected for the measurement of antioxidant enzymes activities.

Estimation of glutathione peroxidase (GPx) activity: The specific activity of glutathione peroxidase was determined using the glutathione peroxidase cellular activity assay kit from Automation Inc., Calabasas, CA, USA, following the manufacturer's instructions. This protocol, which is based on the report of Paglia and Valentine (1967), requires cumene hydroperoxide as a substrate and a five-fold dilution of homogenate. Absorbance was monitored at 340 nm for 3min and enzyme activity was reported in U/mg of protein.

Determination of lipid peroxidation: Tissue malonaldedyde (MDA) levels were estimated as described by Ohkawa *et al*, (1979). Part of iced homogenized tissues was placed in a total volume of cold 3ml/100mMkcl plus 0.003M EDTA and homogenised. Homogenates were centrifuged at 600g for 15 min. Then, 400 microliters of supernatant was added to 0.2 ml 8.1% SDS, 1.5 ml 20% acetic acid (PH 3.5), 1.5 ml 0.8% thiobarbituric acid and 0.6ml water. The solution was heated to 95^{0}C for 60 minutes. After addition of 1.0 ml water and 5.0 ml of n-butanol-pyridine mixture (15:1, vol/vol), the mixture was vigorously shaken and centrifuged at 2,000g for 15 minutes. The absorbance of the upper layer was read at 532 nm with Acurex Microplate Reader. MDA bis-dimethyl acetal was be used as the external standard. Results were expressed as nanomoles MDA per milligram protein. The intra-assay variability was determined in five sets of triplicate samples, and the coefficient of variation given.

Statistical analysis: All data are expressed as mean ± SEM. Statistical differences among the groups were calculated using analysis of variance (ANOVA) followed by Ducan's multiple range test (SPSS 20.0 version, Chicago, IL, USA). Differences with P<0.05 were considered significant.

Results and Discussion

Maternal plasma glucose levels, litter weight, litter size and plancenta weight in normal and melatonin-treated pregnant rats with alloxan-induced diabetes: A significant (P<0.05) increase in fasting maternal plasma glucose levels was observed in mice with alloxan-induced diabetes (260-270 mg/dl) compared with non-diabetic control rats (80-95 mg/dl) and treatment with melatonin produced no significant difference in plasma glucose levels (Figure 1A). Litter size of diabetic untreated rats reduced by 161% (P<0.001) compared with normal rats. However, administration of melatonin improved litter size by 3.7-fold (5mg/kg body weight, P<0.001) and 3.9-fold (10mg/kg body weight, P<0.001) (Figure 1B). Similarly, injection of alloxan produced a 49% (P<0.001) reduction in litter weight compared with normal rats while treatment with 5 or 10 mg/kg body weight of melatonin significantly (P<0.05) improved litter weight by 1.5- and 1.6-fold respectively (Figure 1C). Placental weight was slightly elevated (0.67±0.06) in diabetic untreated rats compared with normal rats (0.49±0.04) (Figure 1D). Placenta weight in melatonin-treated diabetic rats reduced by 13% (5mg/kg bw, P<0.05) and 16% (5mg/kg bw, P<0.05).

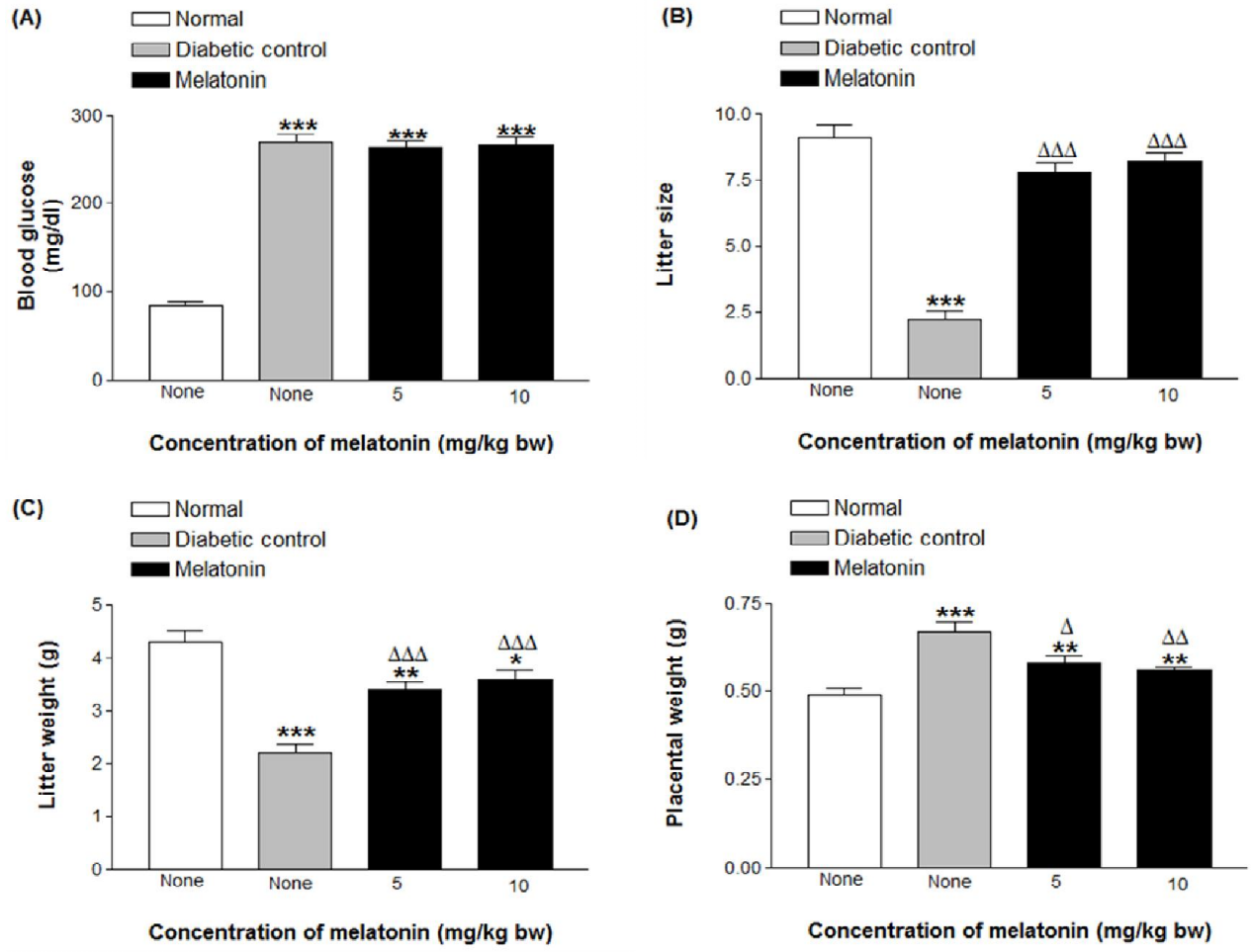

Figure 1. Plasma glucose (A), litter size (B), litter weight (C) and placental weight (D) in normal and diabetic rats treated with saline or graded doses of melatonin. Values are expressed as the mean ± SEM (n=5). *P<0.05, **P<0.01, ***P<0.001 compared with normal rats. $^{\Delta}$P<0.05, $^{\Delta\Delta}$P<0.01, $^{\Delta\Delta\Delta}$P<0.001 compared with diabetic control rats.

Glutathione peroxidase activity: Figure 2 showed a significant (P<0.001) reduction in uterine (38%) and placental (47%) glutathione peroxidase activities in untreated diabetic pregnant rats compared with normal rats. However, melatonin administration (5mg/kg body weight) improved uterine and placental glutathione activities by 20% (P<0.01) and 29% (P<0.05) respectively. Similarly, treatment with 10mg/kg body weight melatonin produced 25% (uterine, P<0.01) and 32% (placental, P<0.05) improvement in glutathione peroxidase activity compared with diabetic untreated rats.

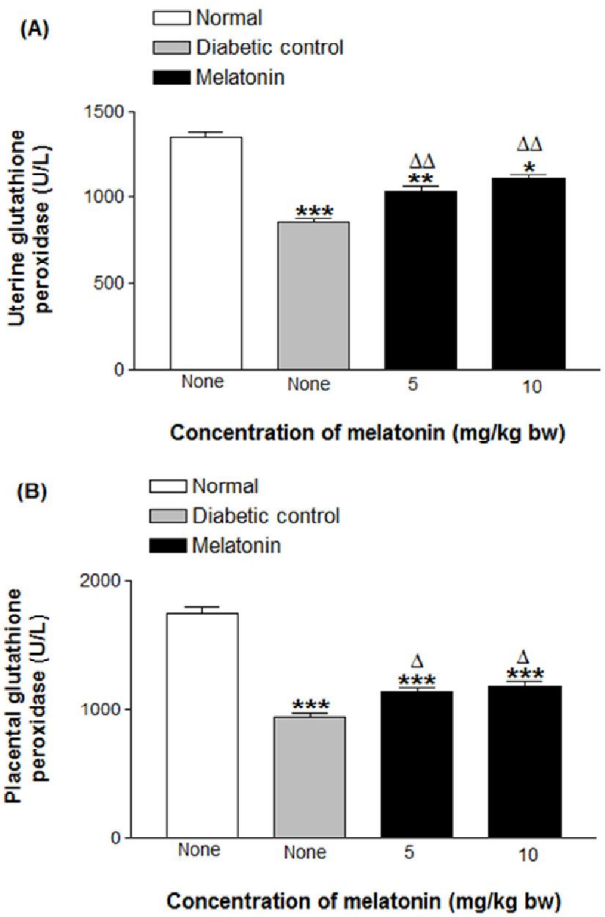

Figure 2. Effect of melatonin on uterine (A) and placental (B) glutathione peroxidase activity in normal and diabetic pregnant rats. Values are expressed as the mean ± SEM (n=5). *P<0.05, **P<0.01, ***P<0.001 compared with normal rats. $^{\Delta}$P<0.05, $^{\Delta\Delta}$P<0.01 compared with diabetic control rats.

Organ malondialdehyde (MDA) levels: Results presented in Figure 3 showed the effects of melatonin treatment on organ MDA levels in rats with alloxan-induced diabetes. Uterine and placental MDA levels were increased by 20% (P<0.05) and 39% (P<0.01) respectively in diabetic untreated rats. While treatment with graded doses of melatonin completely reversed elevated uterine MDA levels (Figure 3A), the increase observed in placenta MDA levels was reduced by significantly reversed 20% (P<0.05) and 25% (P<0.01) in rats treated with 5 and 10 mg/kg bw of melatonin respectively (Figure 3B).

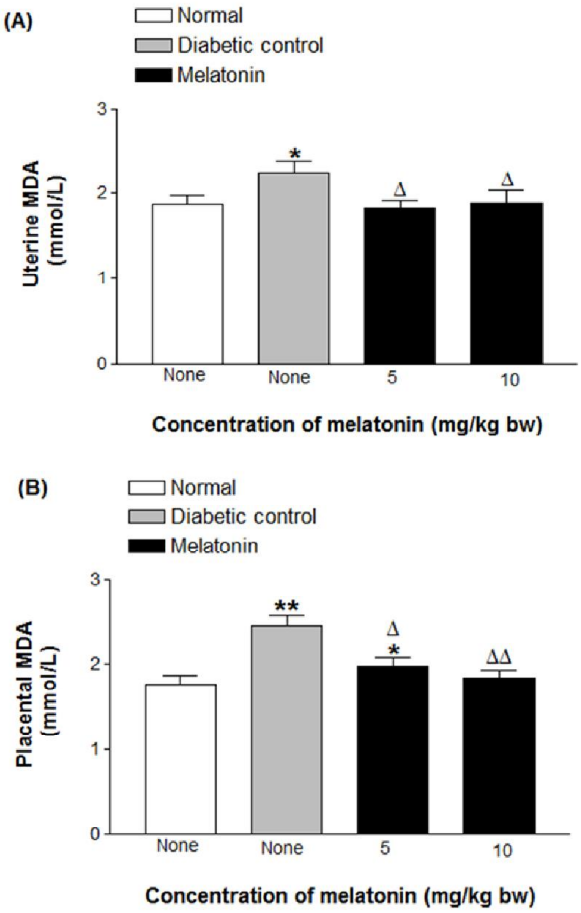

Figure 3. Effect of melatonin on uterine (A) and placental (B) malondialdehyde (MDA) levels in normal and diabetic pregnant rats. Values are expressed as the mean ± SEM (n=5). *P<0.05, **P<0.01, compared with normal rats. $^{\Delta}$P<0.05, $^{\Delta\Delta}$P<0.01 compared with diabetic control rats.

The antioxidant activity of melatonin was evaluated in this study using its effect on glutathione peroxidase (GPx) malondialdehyde (MDA) as a marker. Glutathione peroxidase promotes intracellular defence by catalysing decomposition of reactive oxygen species (Szaleczky *et*

al., 1999) and is a key indicator of oxidative stress (Goyal *et al.*, 2011). Reduced GPx activities observed in alloxan-induced rats in this study indicates higher levels of oxidative stress and is consistent with reports of previous human and animal studies with diabetes (Goyal *et al*., 2011). Protein glycation is a known consequence of hyperglycemia. Therefore, reduced GPx activities observed in this study may result from possible changes in structure and function of the enzyme in the presence of high concentration of glucose. Hyperglycemia could also affect the concentration of cofactors required for optimum functions of antioxidant enzymes. This observation is consistent with the reports of Wohaieb and Godin (1987) and Sukalski *et al*., (1993), which assessed glutathione peroxidase and superoxide dismutase activities in the liver tissue of diabetic rats. In the present study, administration of melatonin raised the level of antioxidants activity in both diabetic and non-diabetic rats. Several studies have previously reported relationships between antioxidant system and diabetic malformations (Wentzel *et al*., 1997; Sakamaki *et al*., 1999 and Cederberg *et al*., 2001). For instance, Sakamaki *et al*. (1999) implicated glutathione-dependent antioxidant system in the diabetic embryonic malformations while Wentzel *et al*. (1997) and Cederberg *et al*. (2001) reported that diabetes-induced congenital malformations could be prevented by treatment with antioxidant agents such as vitamin C, vitamin E and folic acid.

Oxidative stress is caused by free radicals or radical-generating agents at concentration that overwhelms natural radical-blocking or scavenging mechanisms (Block *et al*., 2002). Cigarette smoking and conditions such as diabetes mellitus and oxidative burst from activated macrophages have been known to induce oxidative stress. Endogenous effects of oxidative stress include damage to DNA, proteins and lipids and many other physiologically important chemicals; underscoring the importance of overwhelming the body's antioxidant defence system in the pathogenesis of many diseases (Halliwell and Guteridge, 1990). The present study showed that the concentrations of MDA, one of the biomarkers of oxidative damage, increased during gestational diabetes. However, administration of melatonin reduced MDA generation in the uterus and placental tissues. Diabetes has been associated with excess reactive oxygen species which is a cause of increased lipid peroxidation (Eriksson *et al*., 1996; Wentzel *et al*., 1997). The free radical scavenging ability of melatonin is due to activities of two of its metabolites, namely, N^1-acetyl-N^2-formyl-5-methoxy-kynuramine (AFMK) and N^1-acetyl-5-methoxy-kynuramine (AMK) (Tan *et al*., 2002; Ressmeyer *et al*., 2003). The superb antioxidative effects of melatonin have also been attributed to its ability to diffuse through biological membrane with ease and exert its actions in almost every cell in the body.

This study showed that melatonin had no effect on maternal blood glucose, indicating that though melatonin exhibited strong antioxidant and free radicals scavenging actions, its action is devoid of beneficial hypoglycaemic effects. This observation is consistent with the lack of hypoglycaemic effects reported for other antioxidants, such as vitamins C and E, in rats with gestational diabetes (Cederberg *et al*., 2001; Siman and Eriksson, 1997). However, improved litter size and weight observed in this study also indicate that beneficial effects of melatonin may include prevention of foetal malformations or morbidity in gestational diabetes.

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