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Effects of melatonin consumption on some oxidative stress biomarkers of stress-exposed pregnant rats.

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Abstract

Melatonin acts as a free radical scavenger and protects nuclear and mitochondrial DNA from free radicals-induced damage. This study was designed to test the hypothesis that melatonin may mitigate the effect of restraint stress in pregnant rats by mechanisms associated with stimulation of antioxidant enzymes and reduction in malondialdehyde (MDA) levels. Pregnant Wistar rats aged between 10-12 weeks and weighing 125±5.5g (mean ±SEM), were randomly divided into three groups (n= 8 each) that had the following treatments: non-stress with vehicle (NSV), stress with vehicle (SV) and stress with melatonin (SM). During the second week of pregnancy, the rats were individually restrained for 1 hour a day for one week. Melatonin (10mg/kg) was administered orally to the treatment group (SM) every day throughout the stress exposure period while the control groups (NSV and SV) received the vehicle (water). Three weeks after delivery, the rats were euthanized and the antioxidant enzymes [catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx)] and MDA levels were measured. The CAT values in SV rats were significantly ($P<0.001$) lower than in NSV and SM rats. The values for GPx and SOD followed the same pattern with CAT. The MDA value obtained from the SV rats was significantly ($P<0.0001$) higher than the values obtained from NSV and SM rats. The MDA values obtained from the SM rats were significantly ($P<0.05$) higher than in NSV rats. Results suggest that melatonin helps in countering the effects of restraint stress in pregnant Wistar rats by increasing the serum levels of CAT, GPx and SOD while reducing the serum level of MDA. It is concluded that stress-induced reduction in antioxidant enzymes and attendant increase in lipid peroxidation are mitigated by melatonin.

***Keywords*:** Stress, Melatonin, Pregnant rats, oxidative stress biomarkers

Introduction

Melatonin act as free radical scavenger and protect nuclear and mitochondrial DNA from free radicals-induced damage (Radogna *et al.,* 2010; Hardeland *et al.,* 2011; Chen *et al.,* 2013; Mauriz *et al.,* 2013; Olayaki *et al.,* 2015). This agent also stimulates the expression of antioxidant enzymes (Rodriguez *et al.,* 2004; Korkmaz *et al.,* 2012) and anti-inflammatory gene (Mauriz *et al.,* 2013) in neonatal and reverses oxidative stress during the prenatal period (Chen *et al.,* 2013;). Several studies have been done on effect of stress on pregnant mothers (Lee *et al.,* 2007; Fan *et al.*, 2009; Brunton & Russel, 2010; Argawal *et al.,* 2012; Chen *et al.,* 2013; Olayaki *et al.,* 2015). These studies have shown that oxidative stress occurs in these animals. However, there are few studies on effects of emerging antioxidants such as melatonin on stress-exposed pregnant rats.

Stress is a condition which threatens homeostatic equilibrium of body functions (Ashraf *et al.,* 2019). It arises when organisms observe that they cannot effectively cope with demands being made on them or with threats to their well-being (Malhotra, 2011; Amabebe and Anumba 2018; Palomba *et al.,* 2018;). Stress can be either acute

lasting for few minutes to hours, or chronic which persists for several hours, days, weeks or months (Chrousos, 2009; Ashraf *et al.,* 2019). The stress responses are necessary for survival but become harmful when they persist for a prolonged duration (Clarke *et al.,* 2016; Ashraf *et al.,* 2019). Acute stress is adaptive, and it involves multiple mediators, neurotransmitters, neuropeptide and hormones, while chronic stress is harmful and makes an individual vulnerable to various disease conditions (Amabebe and Anumba, 2018; Ashraf *et al.,* 2019). Long-term stress can induce changes in the multiple hormonal systems that can disrupt the stability of physiological systems like reproduction with negative impact on fertility.

Pregnancy is a physiological condition characterized by high oxygen requirements and increased metabolic demand making it susceptible to oxidative stress-induced organ damage (Chen *et al.,* 2013). In addition, because placenta is rich in polyunsaturated fatty acids it was recognized to be a main source of oxidative stress (Chen *et al.,* 2013). It is was shown that exposure to adverse environmental events such as stress during pregnancy can lead to long-lasting effects on normal individual's functions and risk of developing disease (Vaughan *et al.,* 2012).

Anti-stresses/antioxidants are molecules steady enough to donate an electron to a violent free radical and deactivate it, thus reducing its ability to damage. These agents hinder or inhibit cellular damage largely through their free radical scavenging properties (Halliwell, 1996; Lobo *et al.,* 2010). Antioxidants are used to deactivate free radicals by preserving the redox balance thereby safeguarding the body from damage by free radicals (Sen and Chakraborty, 2011). They also neutralize free radicals by acting as radical scavengers, enzyme inhibitors, hydrogen or electron donors, singlet oxygen quenchers, peroxide decomposers, synergist, and metal-chelating agents (Friel *et al.,* 2002; Lobo *et al.,* 2010)

Melatonin, identified chemically as N-acetyl-5-methoxytryptamine (Nassar *et al.,* 2007; Lobo *et al.,* 2010), is a hormone, found endogenously in animals' pineal gland. Exogenously, it occurs in some other living creatures, including dietary plants/food such as apple, barley, beans, cucumber, maize, potatoes, rice, tomato, feverfew and St John's wort (Manchester *et al.,* 2000; Reiter *et al.,* 2000; Caniato *et al.,* 2003; Arnao *et al.,* 2006; Arnao *et al.,* 2007; Tan *et al.,* 2007; Parades *et al.,* 2008; Posmyk and Janas, 2009; Arnao *et al.,* 2009; Rocha *et al*., 2010; Hardeland *et al.,* 2011; Chen *et al.,* 2012; Mauriz *et al.,* 2013; Zhang *et al.,* 2014; Parades *et al.,* 2014; Hernandez-Plata *et al.,* 2015; Ravishankar *et al.,* 2016; Salehi *et al.*, 2019).

Melatonin is a potent antioxidant that easily crosses cell membranes and the blood–brain barrier (Reiter *et al.,* 1997; Lobo *et al.,* 2010). In contrast to other antioxidants, melatonin does not go through redox cycling, which is the ability of a molecule to undergo reduction and oxidation. Once oxidized, melatonin cannot be reduced to its former state because upon reacting with free radicals, it forms several stable end-products. Thus, melatonin has been referred to as a terminal or suicidal antioxidant (Tan *et al.,* 2000; Lobo *et al.,* 2010). It is a powerful endogenous antioxidant and is exogenously taken safely (Rocha *et al.,* 2015). Amusingly, its antioxidant activities have been regularly reported to modify the reproductive dysfunctions associated with pathological conditions due to exposure to toxicants (Rocha *et al.,* 2015). Because melatonin freely crosses the physiological barriers, including the blood-testis barrier, and has a very little toxicity, it seems to be an excellent candidate in the prevention and/or treatment of the multiple male reproductive dysfunctions (Rocha *et al.,* 2015).

As a free radical scavenger, melatonin protects nuclear and mitochondrial DNA from free radical-induced damage (Reiter *et al.,* 2000). In addition, melatonin promotes the expression of antioxidant enzyme (Korkmaz *et al.,* 2012; Rodriguez *et al.,* 2013) and anti-inflammatory gene (Mauriz *et al.,* 2013). Although melatonin has been demonstrated to be an antioxidant scavenger that scavenges free radical species (Li *et al.,* 2016) and undoes oxidative stress during the prenatal period (Chen *et al.,* 2013). Anwar and Moustafa (2001) and Reiter and Tan (2002) reported that melatonin has strong antioxidant effects and reduces lipid

peroxidation. Melatonin itself is an oxyradical scavenger which stimulates the endogenous antioxidants systems [SOD, glutathione oxidase, glutathione-S-transferase (GST) and total thiol in blood and liver] (Reiter and Tan 2002; Martinez-Campa *et al.,* 2009).

Takasaki *et al*. (2003) investigated melatonin as a therapeutic agent that enhance oocyte quality in clients unable to conceive in earlier *in vitro* fertilization (IVF) cycles due to poor oocytes oocytes. A substantial decrease in the quantity of degenerated oocytes was described, and the quantity of inseminated germ cells improved. The higher levels of melatonin in the follicles decreased lipid peroxide level and may prevent damage to the DNA. Melatonin increases the immune system (Chen *et al.,* 2016), have anti-ageing (Oxenkrug *et al.,* 2001), anti-inflammatory (Li *et al.,* 2013) and anticancer activities (Anisimov *et al.,* 2006). It also shows neuroprotective effects (Pandi-Perumal *et al.,* 2013), accelerate the control of chronic diseases, including cardiovascular diseases (Pandi-Perumal *et al.,* 2005), diabetes and obesity (Agilli *et al.,* 2015). However, melatonin controls the mood (Chenevard *et al.,* 2008), sexual maturation (Esquifino *et al.,* 1987) and body temperature (Agilli *et al.,* 2015).

As a strong endogenous radical scavenger, melatonin can rapidly eliminate the excess free radicals. Moreover, melatonin at 10 mg/kg was discovered to rise the effectiveness of electron transport chain in mitochondria in old mice to decrease electron leakage and reduce free radical production (Okatani *et al.,* 2003). Thus, melatonin is necessary to keep a steady physiological status in human body. It could effectively play a vital role by modifying and acting synergistically with some non-enzyme reductants (Urata *et al.,* 1999), and other reducing molecules like reductases (Khaldy *et al.,* 2000) which are all involved in homeostasis. Furthermore, it was discovered that in rats, melatonin was many times more potent than vitamins C and E in safeguarding tissues from oxidative stress induced injuries (Hsu, *et al.,* 2000; Montilla *et al.,* 2001).

Materials and Methods

Melatonin was obtained from Sigma Chemical Company, St. Louis, MO, USA. While catalase (CAT), glutathione peroxidase (GPx), superoxide dismutase (SOD) and malondialdehyde (MDA) kits were obtained from MyBioscience Chemical Company, San Diego, California. USA.

Ethical clearance

Before the commencement of the study, ethical approval was obtained from the Animal ethical research committee of Usmanu Danfodiyo University Sokoto, Nigeria. The protocol number of the ethical committee approval was: PTAC/HS/(Ae)/OT/36-17.

Study Design

The study was carried out in three (3) phases:

Phase I Experimental rats and management (Breeding phase)

Ten in-bred virgin Wistar rats (7 females and 3 males), aged between 10-12 weeks and weighing 175±5.5 g (mean ± SEM), obtained from the Animal Research Unit, Department of Veterinary Pharmacology and Toxicology, Faculty of Veterinary Sciences, Usmanu Danfodiyo University Sokoto, Nigeria, were used. All rats were housed in cages in a facility with an ambient temperature of 25 -30^{o}C. The animals were given access a pelletized rat chow (Grow Maxx, Grand Cereals Ltd, Jos Plateau State, Nigeria) and tap water to drink *ad libitum*. Rats were housed in isosexual groups that is, males in one cage and females in another cage.

After acclimatization, the female rats were mated during puberty at 12 weeks of age with a male of the same genotype that is, a litter mate. Each female at proestrus phase was paired with one male at 9:00am and was checked for plugs the next morning. If a plug (sperm during cervical smearing) was present, this indicated copulation and the day of detection was designated as day 0 of gestation. The female rat was immediately moved to a new cage where it remained in isolation for the entire gestation period. If no plug was observed during the previous exposure, the animal was returned to its home cage for a new mating chance. After delivery, the pups (litters) were weaned at 21

days of age and housed in isosexual groups until 50 days of age. The female pups were used for the Phase II of the study

Phase II

There were two control groups: normal (vehicle/distilled water) control group without stress and a vehicle/distilled water control group with stress and one treatment group, stress with melatonin group (SM rats). After they were weaned at 21 days of age, 40 female pups from phase I were housed in isosexual groups and used for phase II of this study. At the onset of puberty (12 weeks), the female rats were inbred; that is, they were mated with their male litter mates as described above. Pregnant female rats were assigned randomly to three (3) groups as described above (n = 8 each).

Apparatus, Stress and Experimental Procedure

Starting from the second week of pregnancy (days 7 – 21) (Kovarik *et al.,* 2016), pregnant females in the study group were individually restrained for 1 hour a day. (8:00 - 9:00 am) from day 7 to day of delivery. The stress procedure described by Ward and Weisz (1984) with slight modifications was chosen because it has an indirect influence on the foetus via direct stress on the mother. Briefly, each pregnant rat was placed individually inside a transparent plastic tube (7 cm in diameter) with air holes for breathing and a closed end (Fig 1). Dams in the normal control group were left undisturbed in their home cages during the whole pregnancy. Treatment with melatonin began on day with stress exposure and was terminated after parturition.

Figure 1: The restraint stress procedure where a pregnant rat in the stress groups was placed individually into a transparent plastic tube (7 cm in diameter) with air holes for breathing and a closed end for 1 hour daily (8:00-9:00 am) from the second week of pregnancy, that is, from day 7 to delivery day.

Grouping procedure

Twenty-four dams originally obtained as pups from Phase I were randomly divided into three groups (n = 8 each) as follows:

Group I: Non-stressed with vehicle. This group was not stressed. It was administered 0.5 ml distilled water orally by gavage (vehicle; normal Control). They were tagged NSV rats.

Group II: Stressed with vehicle. This group was stressed and administered 0.5 ml distilled water orally by gavage (vehicle; vehicle Control). They were tagged SV rats.

Group III: This group was stressed and given 10 mg/kg melatonin orally by gavage (Okatani *et al.*, 2003). They were tagged SM rats.

Animals and treatments/interventions

Melatonin (10 mg/kg) were given orally daily from day 7 to delivery day to the treatment group that is, stress with melatonin (SM rats) while the control groups (NSV and SV rats) received 0.5 ml distilled water. After termination of stress exposure and treatment blood samples were taken three weeks after parturition for analyses.

Sample collection and **preparation of Serum**

Blood samples were collected via cardiac puncture into plain tubes. Each blood sample was allowed to clot and centrifuged at 4000 g for 10 minutes. The serum obtained was pipetted into a labelled tube for estimation of oxidative stress markers.

Sample collection** and **preparation of Serum
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Evaluation of oxidative stress biomarkers
The catalase (CAT), glutathione peroxidase (GPx), superoxide dismutase (SOD) activities and malondialdehyde (MDA) levels were determined in the serum and assayed using Cayman assay Catalase kit (MyBioscience Chemical Company, San Diego, California. USA.) according to the manufacturer's instructions.

Data Analyses
Data were subjected to analysis of variance (ANOVA) with Bonferroni's post-hoc test for multiple comparisons. Data were expressed and presented as Mean ± SEM. GraphPad Prism 5 software was used to carry out the statistical analyses. $P<0.05$ was considered significant.

Results
Catalase
Figure 2 shows the serum catalase (CAT) activities of stress exposed pregnant Wistar rats in the presence of vehicle and melatonin compared to the non-exposed group with vehicle. The catalase activity in SV rats was significantly ($P < 0.001$) lower than in NSV and SM rats.

Glutathione Peroxidase
The serum glutathione peroxidase (GPx) activities of stress exposed pregnant Wistar rats in the presence of vehicle and melatonin compared to the non-exposed group with vehicle are presented in Figure 3. The GPx activities obtained from SV rats were significantly ($P < 0.001$) lower than the activity obtained from NSV and SM. There was no significant difference between the GPx activities in SM rats compared to NSV rats.

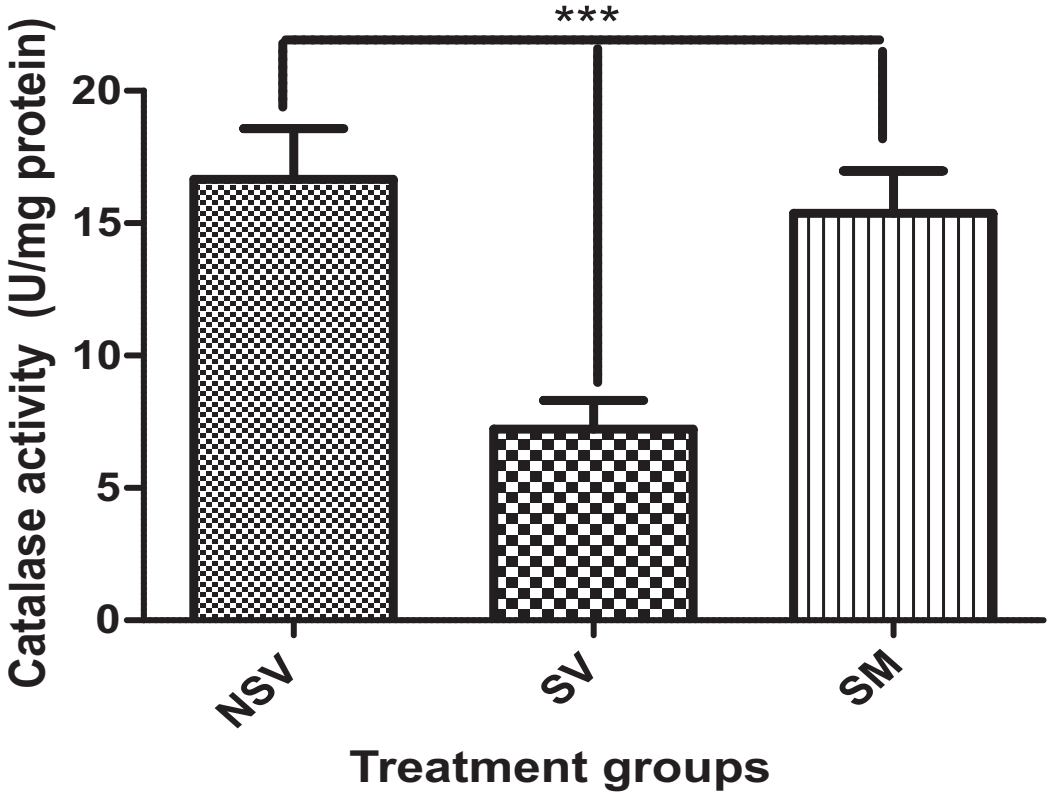

Figure 2: Serum catalase activities of stress exposed pregnant Wistar rats in the presence of vehicle and melatonin compared to non-exposed group given vehicle (n = 8). *** = $P < 0.001$ SV vs NSV and SM. Data was analysed using one way ANOVA and post-hoc test (Bonferroni) for multiple comparisons.

NSV = Non-Stress with vehicle (0.5ml distilled water), SV= Stress with vehicle (0.5 ml distilled water), SM = Stress with 10 mg/kg melatonin.

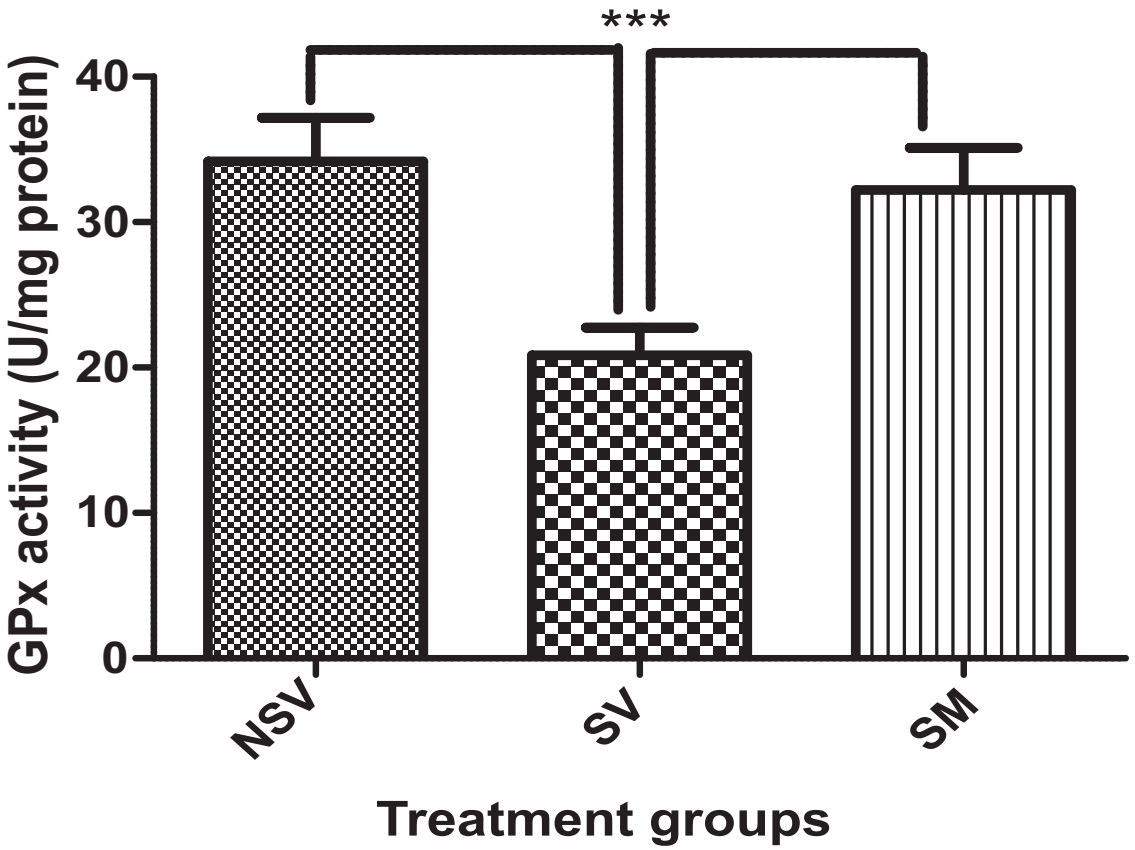

Figure 3: Serum GPx activities of stress exposed pregnant Wistar rats in the presence of vehicle and melatonin compared to non-exposed group with vehicle (n = 8 each). *** =p < 0.0001 SV vs NSV and SM. Data was analyzed using one- way ANOVA and post-hoc test (Bonferroni) for multiple comparisons.

Gpx = Glutathione peroxidase, NSV = Non-Stress with vehicle (0.5 ml distilled water), SV = Stress with vehicle (0.5 ml distilled water) SM = Stress with 10 mg/kg melatonin.

Superoxide dismutase (SOD)

Figure 4 shows the serum Superoxide dismutase activities of stress exposed pregnant Wistar rats in the presence of vehicle and melatonin compared to the non-exposed group given vehicle. The SOD activity obtained from the SV rats was significantly ($P < 0.001$) lower than the activities from the NSV and SM rats.

Malondialdehyde (MDA)

The serum Malondialdehyde levels of stress exposed pregnant Wistar rats in the presence of vehicle and melatonin compared to the non-exposed group given vehicle are presented in Figure 5. The MDA value obtained from the SV rats was significantly ($P < 0.0001$) higher than the values obtained from NSV and SM rats.

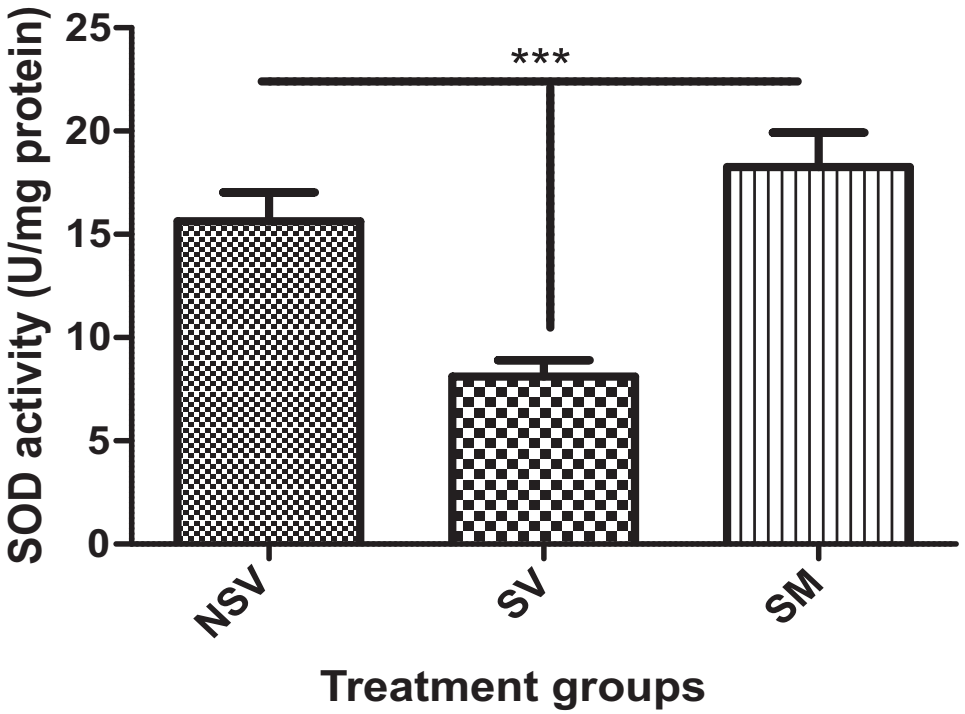

Figure 4: Serum SOD activities of stress exposed pregnant Wistar rats in the presence of vehicle and melatonin compared to the non-exposed group given vehicle (n = 8 each). ** = P < 0.001 SV vs NSV and SM.

Data was analyzed using one way ANOVA and post-hoc test (Bonferroni) for multiple comparisons. SOD = Superoxide dismutase, NSV = Non-Stress with vehicle (0.5 ml distilled water), SV = Stress with vehicle (0.5 ml distilled water) SM = Stress with 10 mg/kg melatonin.

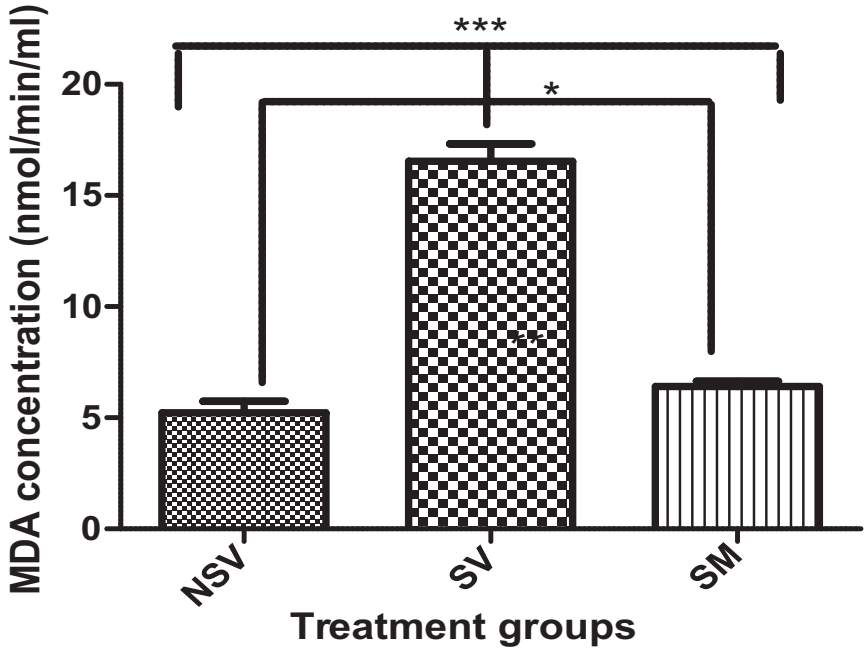

Figure 5: Serum MDA activities of stress exposed pregnant Wistar rats in the presence of vehicle and melatonin compared to the non-exposed group with vehicle (n = 8 each). *** = P < 0.0001 SV vs NSV and SM *= P < 0.05 NSV vs SM.

Data was analyzed using one- way ANOVA and post-hoc test (Bonferroni) for multiple comparisons. MDA = Malondialdehyde, NSV = Non-Stress with vehicle (0.5 ml distilled water), SV = Stress with vehicle (0.5 ml distilled water), SM = Stress with 10 mg/kg melatonin.

Discussion

The major finding of this study is that melatonin countered restraint stress in pregnant Wistar rats by significantly increasing the activities of catalase, glutathione peroxidase (GPx), superoxide dismutase (SOD) while reducing the serum level malondialdehyde (MDA). To the best of our knowledge, this study is the first to report the above findings in pregnant rats. In the current study, the activities of antioxidative enzymes (CAT, GPx and SOD) were decreased in stress with vehicle rats compared to non-stress with vehicle, stress with melatonin. The decrease in these parameters (CAT, GPx and SOD) was corrected by melatonin. This finding suggests that melatonin have anti-stress and antioxidant properties.

Catalase

The CAT concentration in the stress exposed pregnant rats that received vehicle (SV rats) was significantly reduced compared to the non-exposed rats that received vehicle (NSV rats). The results suggest that restraint stress caused a reduction in the catalase activity in the rats. This reduction was ameliorated by melatonin, compared to the non-exposed rats that received vehicle.

The decreased serum catalase activity in stress with vehicle rats might be clarified by numerous factors. It is likely that it was as a result of its oxidative inactivation or because of its kinetic properties (Awasthi *et al.,* 1975). It may be assumed that decreased catalase activity resulted in accumulation of H_2O_2 and other hydroperoxides in erythrocytes of the stress exposed rats. This fact may be responsible for additional generation of reactive oxygen species through the Fenton reaction (Murphy and Sies, 1991) within the cells/tissues in the body; and intensification of oxidation of vulnerable molecules, which might increase the damage of cells/tissues and consequently death (Murphy and Sies, 1991). The effect of stress on catalase activities appears to be controversial. Stress may reduce catalase activity (Belviranli *et al.,* 2013; Chang *et al.,* 2013). However, the current data are in accordance with the reports of Haider *et al.* (2015), who showed that increased oxidative stress produced inhibitory effects on catalase activity.

The present data suggest that melatonin have the efficacy to augment the activities of antioxidant enzymes to repair or prevent damage by reactive oxygen species in stress-exposed rats. The result of the current study is also similar to that of the previous study by Nku-Ekpang *et al.* (2016) which showed that stress in sickle anaemia patients caused a significant reduction in plasma catalase activity which was raised by Vitamins C and E supplementation and their combination.

From the present study, the low level of catalase activity in the stress exposed rats suggests that there was less scavenging activity on the continuously generated ROS, which is a steady state cellular event in respiring cells. This finding is in agreement with the previous study of Manfredini *et al.* (2008). The antioxidant enzyme defense system in stress exposed subjects is low and oxidative stress may develop because of the imbalance between enhanced generation of ROS and low cellular content of antioxidants (Cesquini *et al.,* 2003; Gutteridge and Halliwell, 2007; Nku-Ekpang *et al.,* 2016). The reduction in the levels of catalase activity observed in the stress exposed rats may hinder their normal cell functions of disposing organic peroxides and removal of H_2O_2. It may therefore, not be able to protect the membrane and haemoglobin from peroxidative damage (Antunes *et al.,* 1996; Nku-Ekpang *et al.,* 2016).

Diet rich in antioxidants may reduce oxidative damage or boost the antioxidant system (Choudhary and Kale, 2002; Aliahmat *et al.,* 2012). Aliahmat *et al.* (2012) showed that, complementation with the tocotrienol rich fraction of *Piper betle*, and *Chlorella vulgaris,* as exogenous antioxidants enhanced endogenous antioxidant function. The findings are in agreement with those of the present study which demonstrated that dietary supplements in the form of melatonin reversed the reduced activities of antioxidant enzymes observed in the stress exposed rats. The increased serum activity of catalase observed in the stress exposed rats in the presence of melatonin, may enhance its normal cell functions of disposing organic peroxides and removal of H_2O_2, thereby protecting the membrane from peroxidative damage.

Glutathione peroxidase

The glutathione peroxidase (GPx) activity in the stress exposed pregnant rats that received vehicle

(SV rats) was significantly reduced, compared to that of the non-exposed rats that received vehicle (NSV rats). The results suggest that restraint stress caused a reduction in the GPx activity in the rats. However, the reduction was ameliorated by melatonin compared to the non-exposed rats that received vehicle. In the current study, the serum GPx activity was reduced in the stress with vehicle rats. The finding is in agreement with those findings reported by other studies, which showed that stress reduced the serum GPx activity (Ranjekar *et al.,* 2003; Li *et al.,* 2006; Othmen *et al.,* 2008; Reyazuddin *et al.,* 2014).

The decreased serum GPx activity in stress with vehicle rats may be elucidated by many factors. It is probable that it was due to its oxidative inactivation (Reyazuddin *et al.,* 2014) or because of its kinetic properties (Awasthi *et al.,* 1975). During stressful condition, the affinity of selenium GPx for glutathione is low (Mukerjee *et al.,* 1996), therefore GPx is not saturated with glutathione even, at high concentrations of this substrate. The reduction in GPx activity observed in the stress exposed rats may prevent their normal cell functions of disposing organic peroxides and removal of H_2O_2, and may not, therefore, protect the membrane from peroxidative damage (Antunes *et al.,* 1996; Nku-Ekpang *et al.,* 2016). It is possible that the decreased GPx activity resulted in accumulation of H_2O_2 and other hydroperoxides in the erythrocytes of stress exposed rats. This finding may be responsible for further generation of ROS by the Fenton reaction (Murphy and Sies, 1991) in cells/tissues in the body, and magnification of oxidation of susceptible molecules, which may amplify the destruction of cells/tissues resulting in their death. The increased oxidative stress produces inhibitory effects on GPx activity, which is evident from decreased GPx activity. The results are in accordance with the reports of Haider *et al.* (2015), who showed that increased oxidative stress produced inhibitory effects on GPx activity.

Interestingly, in the current study, there were increases in serum GPx activities in stress with melatonin suggesting that melatonin have antistress and antioxidant properties. The present results suggest that melatonin augment the activities of antioxidant enzymes and enhance their ability to repair or prevent damage by ROS in stress-exposed rats. The findings of the present study are similar to those of Guti´errez-Salinas *et al,* (2013), although the authors showed that stress caused a decrease in the activities of the circulating antioxidant enzymes. The only difference between the study conducted by the authors and the present one was that the authors used sodium fluoride in induction of stress with vitamin C as an interventional agent, while the present study involved the use of restraint stress with HS and melatonin for intervention. Guti´errez-Salinas *et al.* (2013) showed that vitamin C increased the activities of antioxidant enzymes, while the present study showed that melatonin had a similar effect. The finding suggests that melatonin could induce the antioxidant defense system. The present result is also similar to findings in previous reports (Ranjekar *et al.,*2003; Li *et al.,*2006; Othmen *et al.,* 2008; Reyazuddin *et al.,* 2014) which indicated that stress reduced the serum activity of GPx. Similarly, other reports (Liu,1995; Özgüner *et al.,*1999; Belviranli *et al.,* 2013) reported that stress triggers oxidative stress with subsequent decrease in the serum antioxidant enzymes activities. The decrease obtained in the activity of GPx may be as a result of exhausted adaptive response to the oxidative stress (Reyazuddin *et al.,* 2014). However, the present results are inconsistent with the reports of Pajović *et al.* (2006) who showed that acute immobilization stress does not change the activities of GPx.

The decreased serum GPx activity in stress with vehicle rats may be explained in various ways. Possibly it was a result of its oxidative inactivation or because of its kinetic properties (Awasthi *et al.,* 1975). However, in the present study, there were increases in serum GPx activities in stress with melatonin, suggesting that melatonin have antistress and antioxidant properties. The results suggest that melatonin may augment the activities of antioxidant enzymes to repair or prevent damage by ROS in stress-exposed subjects. The increase in GPx activities in the treatment group following melatonin administration could boost redox reactions, thereby scavenging ROS, and terminating their harmful activity. The increase in the activities of antioxidant enzymes also reduces the damage to cellular macromolecules, such as DNA, proteins, and lipids (Stadtman and

Berlett, 1997; Dröge, 2002). The current study adds to the existing body of knowledge which show that stress lowers the serum activity of GPx and that supplementation with melatonin correct this adverse effect of stress.

Superoxide dismutase

The superoxide dismutase (SOD) action in the stress exposed pregnant rats that received vehicle (SV rats) was significantly reduced compared to the non-exposed rats that received vehicle (NSV rats). The finding suggests that restraint stress caused a reduction in the SOD activity in the rats. The reduction was corrected by melatonin compared to the non-exposed rats that received vehicle. In the presence of melatonin, the reduction was in addition led to significant increases in SOD activities compared to the non-exposed rats that received vehicle.

The decreased serum SOD activity in stress with vehicle rats may be due to an irretrievable inactivation of SOD by its product, H_2O_2, in a concentration-dependent manner (Salo *et al.,* 1988). It may also be because of its oxidative inactivation kinetic properties (Awasthi *et al.,* 1975). However, in the present study, there was increase in serum SOD activity in stress with melatonin indicating that melatonin has anti-stress properties, which appear to be consistent with their antioxidant actions. It may be presumed that reduced SOD activity resulted in accumulation of H_2O_2 and other hydroperoxides in erythrocytes of stress exposed rats. This might be accountable for supplementary generation of ROS through the Fenton reaction (Murphy and Sies, 1991) in cells/tissues in the body and amplification of oxidation of predisposed molecules, which could amplify the damage and consequently death of cells/tissues. The increased oxidative stress may have produced inhibitory effects on SOD activity, which was evident from decreased SOD activity. The results are in accordance with the reports of Haider *et al.* (2015) that increased oxidative stress produced inhibitory effects on SOD activity.

In the current study, the SOD activity was reduced in the stress with vehicle rats. The result is reliable with the findings described by other studies, which showed that stress reduced the serum SOD activity (Ranjekar *et al.,* 2003; Li *et al.,* 2006; Othmen *et al.,* 2008; Reyazuddin *et al.,* 2014). The decrease in this enzyme activity recorded in the present study may be as a result of exhausted adaptive response to oxidative stress (Reyazuddin *et al.,* 2014). The findings are similar to those of Chang *et al.* (2013) who showed that serum SOD activities in cataract stress were significantly decreased, compared to the control subjects. However, the present results are inconsistent with previous reports that acute immobilization stress had no effect on SOD activities (Pajović *et al*. 2006; Belviranli *et al.* 2013).

The results suggest that melatonin augment the activities of antioxidant enzymes to repair or prevent damage by ROS in stress-exposed subjects. The increase in the antioxidant enzymes activities may be involved in mitigating the damage of cellular macromolecules like DNA, protein, and lipids (Stadtman and Berlett, 1997; Dröge, 2002).

Lipid peroxidation

Malondialdehyde (MDA) levels were measured as an index of damage to polyunsaturated fatty acids. In the current study, the MDA concentration in the stress exposed pregnant rats that received vehicle (SV rats) was significantly higher compared to the non-exposed rats that received vehicle (NSV rats). The results suggest that restraint stress caused an increased lipid peroxidation and subsequent increase in MDA levels in the rats. However, the increase was corrected by melatonin compared to the non-exposed rats that received vehicle.

The correction of MDA by melatonin may be due to the fact that melatonin is vital antioxidant and reducing agent that decrease the production of lipid peroxidation products such as MDA. Indeed, its antioxidant activities might prevent cellular damage by inhibiting the peroxidation of polyunsaturated fatty acids contained in cellular and sub-cellular membrane phospholipids.

The present results suggest that stress increases serum MDA level in the pregnant Wistar rats was similar to the findings by Belviranli *et al.* (2013) who showed that the acute stress triggers oxidative stress with subsequent increase in serum MDA

level. However, in the presence of melatonin the serum MDA level was reduced. The finding suggests that melatonin enhanced the antioxidant defense system and so reduced the MDA level.

The increase in MDA in the serum of stress with vehicle rats is in agreement with the findings from other studies (Antunes *et al.,* 2000; Herken e*t al.,* 2001; Reyazuddin *et al.,* 2014). The current result is in accordance with previous studies, which showed that MDA levels increased in different forms of stress both in animals (Liu, 1995; Özgüner *et al.* 1999) and humans (Guti´errez-Salinas *et al.,* 2013; Chang *et al.* 2013; Haider *et al.,* 2015) studies.

The current results on the effects of melatonin are similar to earlier findings reported by Nku-Ekpang *et al.* (2016), who showed that basal level MDA was significantly lower in sickle cell anaemia patients than in normal non-sickle cell anaemia subjects, However, after vitamins C and E supplementation, the MDA levels in sickle cell anaemia became higher than the levels in non-sickle anaemia subjects (Nku-Ekpang *et al.,* 2016).

To summarise, the results of the current study showed that stress increased circulating MDA level which was associated with reduced activities of circulating CAT, GPx and SOD. The increased serum level of MDA in the treatment group is dampened in the presence of melatonin while the activities of CAT, GPx and SOD all rose in the presence of melatonin.

Conclusion

On the basis of the results obtained in this study, it was concluded that restraint stress caused a decrease in the activities of antioxidant enzymes, but increased the lipid peroxidation in pregnant rats. These stress induced changes were ameliorated by melatonin suggesting that it is an anti-stress agent. The results of the present study also suggest the possibility of using exogenous melatonin as dietary supplements in reducing/preventing stress-induced diseases including reproductive abnormalities such as infertility. It is concluded that, the stress induced reduction in the activities of antioxidant enzymes and the attendant increase in lipid peroxidation were mitigated by melatonin.

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