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Effect of exogenous melatonin on endocrinological profiles, biochemical and antioxidant and oxidative stress profiles in post-partum anestrus Andaman local buffaloes (*Bubalus bubalis*) in tropical island ecosystem

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ABSTRACT

Andaman local buffalo (ALB) is distributed in Andaman and Nicobar Islands. ALB is non-descriptive and represents admixture of different Indian breeds of buffaloes. ALB is suffering infertility due to anestrus in dry (summer) season. Therefore, this study was conducted to assess the effect of slow release subcutaneous exogenous melatonin (MT) implant on hematological profiles, endocrinological profiles (cortisol and prolactin), biochemical profile (total protein, albumin, globulin, glucose and total cholesterol), antioxidant profiles [total antioxidant capacity (TAC), catalase (CAT), glutathione (GSH) and superoxide dismutase (SOD)] and oxidative stress profile (malondialdehyde; MDA) in anestrus buffalo cows during summer to improve its reproductive efficiency. Experimental buffaloes (5–7 years of age) were selected and divided into two groups, Gr I: Control (n=6) and Gr II: Treatment (n=6; melatonin implant @ 18 mg/50 kg b.wt). Statistical analysis revealed that these experimental profiles differed significantly between treatment and control groups. Blood profiles revealed that anestrus buffalo suffered severe macrocytic hypochromic anemia with increased leukocytosis and MT has improved the health status of reproductive system and whole body systems. Concentration of cortisol and prolactin were lower in MT treated than in control group. Similarly, antioxidant profiles were higher and oxidative stress profile was lower in MT treated group than in control group. Biochemical profiles were increased in MT treated than in untreated buffalos. Study concludes that melatonin had significant beneficial effects in improvement of the antioxidant profiles, minimization of oxidative stress with cascading beneficial effects on hormone, biochemical and hematological profiles, which will improve the cyclicity and fertility rate in anestrus buffalo during summers in humid tropical island ecosystem.

Keywords: Antioxidant, Biochemical profiles, Buffalo, Endocrinological profiles, Melatonin, Summer anestrus

Andaman local buffaloes are distributed in Andaman group of Islands and few numbers in Campbell Bay of Nicobar group of Islands (De *et al.* 2019). ALB is an admixture of different Indian breeds, closely related to north Indian buffalo breeds (De *et al.* 2019). It is well adapted to special type of tropical humid island climatic environmental condition and thrives well in minimal management and low input. Herd size varies from 4–21 and age at puberty is 3–5 years and dry period is 6–12 months (Pal and Gupta 1987). ALB has length and yield of lactation of 232.5 days and 331.8 kg, respectively and average milk yield is 1.4 kg (Jayakumar *et al.* 2012). The buffalo population has declined from 16,000 in 2003 to 7860 in 2012 (19th Livestock Census 2012). The population of this buffalo germplasm is declining rapidly due to the unavailability of proper breeding strategy and making the species very vulnerable to extinction.

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Anestrus is a kind of infertility broadly defined as considerable absence of estrus expression at the expected time frame. It is more appropriately defined as functionless, quiescent ovaries and reproductive tract. Thermal humid stress in buffaloes induces antigonadotrophic and antisteroidogenic functions (Shimamura *et al.* 1995) which in turn alter the estrous cycle and uterine environment leading to adverse effect in the uterine embryo development causing reproductive failure in buffaloes (Khan and Das 2012), causes longer the post-partum anoestrus (Megahed *et al.* 2006). Increased temperature humidity index (THI) and longer photo period in summer cause reduction of MT secretion (i.e. short day breeder); decreased MT secretion induces increased prolactin and cortisol secretion, which in turn inhibits secretion of GnRH and gonadotropins leads to anestrus (Ramadan *et al.* 2017). Anestrus is caused by various factors including lack of sufficient nutrients, hormonal imbalance, lack of antioxidants, anaemia, etc (Barile 2005). Further, percentage of anestrus was higher in adult than in heifers (Markandeya and Bharkad 2003).

Hematological profiles (total red blood cell count: TRBC, haemoglobin: Hb) are decreased significantly in anestrous than in normal cycling buffaloes (Dhoble and Gupta 1981) whereas total white blood cell count (TWBC) is increased significantly in anestrus acyclic cows (Kumar and Sharma 1991).

Melatonin, secreted by the pineal gland in the brain (Reiter 1991), acts as a neurotransmitter and regulates the circadian rhythm (Reiter 1993) and seasonal reproduction in mammals (Revel *et al.* 2009). In mammals, melatonin considerably modulates the fertility as its low concentration results into infertility (Klein *et al.* 1991). Animal studies clearly indicated that MT could regulate the firing frequency of the hypothalamic GnRH pulse generator, thereby alteration in the releasing pattern of gonadotropins, which in turn could stimulate the gonadal hormone secretion (Bittman *et al.* 1985) and bring subsequent improvement in development of gonads and reproductive system.

Moreover, MT and its metabolites act as antioxidants and powerful direct scavengers of free radicals (Tomás-Zapico and Coto-Montes 2005). Melatonin stimulates the enzymes responsible for metabolising the reactive oxygen species, thereby; helps in protection of the cell membrane fluidity and integrity (Tan *et al.* 1993). During the post-partum period of dairy cows, antioxidants play significant role in changes in reproductive tract and ovary in cyclic animals, which is lacking in anestrus animals (Derar *et al.* 2011). Increased concentration of free radicals with reduced level of antioxidant profiles in follicular fluid of anestrus buffaloes indicated that the oxidative stress has significant adverse effect on estrous cycle of buffaloes (Jan *et al.* 2014). Similarly, in buffalo heifers, decreased ovarian function due to significant increment of free radicals with simultaneous decrement of antioxidants was reported (Ahmed *et al.* 2010). Melatonin has higher potency than Vit-E (Pieri *et al.* 1994) and GSH/mannitol (Hardeland *et al.* 1993) in removing the peroxyl radicals and in scavenging of hydroxyl radicals, respectively. Though, earlier studies pointed out that exogenous MT significantly improved the endocrinological profiles, fertility profiles in different species (sheep: Buffoni *et al.* 2015, cattle: Aggarwal *et al.* 2005, goat: Jiménez *et al.* 2014, buffalo: Ramadan *et al.* 2019, Kumar *et al.* 2015, mithun: Perumal *et al.* 2018); perusal of available literature revealed no information on hematological, biochemical, endocrinological, antioxidant and oxidative profiles in post partum anestrus Andaman local buffaloes during summer season in island ecosystem of Andaman and Nicobar Islands. Therefore, the present study was designed to assess the effect of exogenous slow release MT implantation in hematological, biochemical, endocrinological profiles, antioxidant and oxidative stress profiles in post-partum anestrous Andaman local buffalo cows during summer season in tropical humid island ecosystem.

MATERIALS AND METHODS

Area of the study: The present study was conducted at Buffalo Breeding Farm, ICAR-CIARI, Port Blair, Andaman and Nicobar Islands, India and is placed in between 6°45′ to 13°44′ North Latitude and in between 92°12′ to 93°57′ East Longitude. Average maximum and minimum temperature were 33.5 and 23°C, respectively. Average width of Andaman group of Islands is 24 km (maximum 52 km). Relative humidity was in range of 70–92% and annual rainfall is >3100 mm spread over 7 months. These buffaloes were maintained in the semi-intensive system where they were allowed for grazing from 0700 to 1200 h. The seasons were classified into rainy/wet (May to November) and dry/summer (December to April) as per the monsoon availability for five whole calendar years in Andaman and Nicobar Islands. Average sunlight hours per day differed significantly between rainy (4.28±0.89) and dry summer (9.20±0.74) seasons. From December to April, the sun regularly shines, while from June to September, the sky is often cloudy. Average relative humidity (%) differed between rainy (84.21±1.93) and dry summer (75.80±2.06) seasons. Average temperature (°C) differed between rainy (29.71±0.62) and dry summer (31.42±0.80) seasons. Average rainfall (mm) differed significantly between rainy (444.92±13.62) and dry summer (89.04±8.84) seasons. Average THI differed between rainy (84.92±1.59) and dry summer (85.59±1.15) seasons. THI was calculated with the following formula:

$$THI = (0.81 \times Ta) + \{(RH \div 100) \times (Ta - 14.4)\} + 46.6$$

where Ta, average ambient temperature (°C) and RH, average relative humidity (Lakhani *et al*. 2018).

Experimental animals: Twelve apparently healthy (body condition score: 5–6 out of 10, classified as good) buffalo cows of 5–7 years of age were selected for the present study. ALBs were maintained in the semi-intensive system where they were allowed for grazing from 0700 to 1400 h. Experimental animals were maintained under uniform feeding, lighting, housing and other standard managemental practices as per the farm schedule. Buffalo cows that had shown anestrous for more than 120 days of post-partum, lack of overt sign of oestrus, corpus luteum with smooth ovaries and less than 1 ng/mL of blood progesterone were considered as anoestrus (Kumar *et al.* 2015). The experiment was conducted in dry/summer season. Each experimental animal was fed with 30 kg mixed jungle forages (17.30% and 10.50% dry matter and crude protein, respectively) and 4.5 kg concentrates (88.00% and 13.20% dry matter and crude protein, respectively) fortified with mineral mixture and salt and offered *ad lib.* potable drinking water.

Experimental groups: Experimental animals (n =12) were divided into two groups, Gr I: Control (n=6) and Gr II: Treatment (n=6; melatonin implant @ 18 mg/50 kg b.wt). Due to unavailability of melatonin implants in India, crystalline melatonin powder (HiMedia Laboratories Pvt Ltd, Mumbai, India) was dissolved in refined corn oil at room temperature in quantities sufficient to make a final concentration of 18 mg/mL. Once dissolved, the suspension was used immediately on the same day (Perumal *et al.* 2018). Control animals were administered with corn oil as placebo in subcutaneous route. In the earlier studies in buffalo (Kumar *et al.* 2015) and mithun (Perumal *et al.* 2018), MT concentration reached highest level (9–12 folds) on day 8 of administration and followed by gradual decrease to the level of control on day 40, although the availability of MT in treated animal was observed up to day 36 (Perumal *et al.* 2018). Based on this, MT supplementation was done once in a month during the experimental period. Blood samples were collected and analysed the hematological, biochemical, endocrinological, antioxidant and oxidative profiles.

Collection of blood and estimation of experimental profiles: Blood samples were collected from the experimental buffalo cows by venipuncture of jugular vein into heparin tubes (20 IU of heparin per mL of blood) at 0400 h interval throughout the day (one day only; 6 collections) on day 37–40 of MT implant initiation (6^{th} week). These blood samples were used for haematology profiles analysis. Remaining blood samples were centrifuged at 1200 × g for 15 min at 4°C. The plasma samples were separated rapidly, labelled and preserved at –20°C for further analysis of biochemical, antioxidant and oxidative and endocrinological profiles.

Hematological profiles: Blood analysis was carried out using Veterinary fully automatic hematological analyzer (Prokan, PE-6800) directly after the samples were received by the research laboratory and within 30 min after samples were collected. Heamatological profiles such as total red blood cells (TRBC), haemoglobin (Hb), erythrocyte sedimentation rate (ESR), packed cell volume (PCV), total white blood cell (TWBC), differential leucocyte count (DLC: lymphocytes, monocytes, neutrophils, eosinophils) and platelets were estimated.

Biochemical profiles: The biochemical indices such as total protein, albumin, globulin, glucose and total cholesterol were estimated with commercially available diagnostic kits (ERBA Mannheim, Germany) using biochemistry analyser (Automated Clinical Chemistry Analyser EM200, ERBA Diagnostics Mannheim GmbH, Germany).

Hormone analysis: Prolactin (analytical sensitivity: 0.12 ng/mL; intra- and inter-assay coefficients of variation: 4.76 and 7.56%, respectively) and cortisol (analytical sensitivity: 35 pg/mL; intra- and inter-assay coefficients of variation: 6.78 and 10.12%, respectively) were estimated using commercially available ELISA kits (500730, 500360, Cayman Chemical Company, Ann Arbor, MI, USA, respectively) in 96-well clear polypropylene microplate using Alere Microplate Reader (Alere Medical Pvt Ltd, India, AM 2100).

Antioxidant profiles: Total antioxidant capacity (709001), Superoxide dismutase (706002), Glutathione (703002) and Catalase (707002) in the blood plasma were estimated by using Cayman's Catalase assay kit (Cayman Chemical Company, Ann Arbor, MI, USA) as per the manufacturer's guidelines in 96-well clear polypropylene microplate using Alere Microplate Reader.

Oxidative profile: Malondialdehyde in plasma was separated and determined as conjugate with TBA. Plasma proteins were precipitated by TCA and then removed by centrifugation. MDA–TBA complex was measured at 534 nm (Shah and Walker 1989) in 96-well clear polypropylene microplate using Alere Microplate Reader.

Statistical analysis: The statistical analysis of the data was performed as per standard procedures. Analysis of variance (ANOVA) was performed using a generalized liner model (SPSS 16, SPSS, Chicago, IL, USA). Figures present the non-transformed data. The data used in the study were tested for normality before analysis using Shapiro-Wilk statistics. Means were analyzed by student t-test to determine the significant differences between the treatment and control groups using the SPSS software/PC computer program. The mean values with a significance of $P<0.05$ were considered to be statistically significant.

RESULTS AND DISCUSSION

MT treated buffalo cows had significantly higher TRBC, Hb, ESR, and PCV and significantly lower TWBC at the rate of 23.68, 25.05, 27.88, 18.67 and 21.94%, respectively than in untreated control cows (Fig.1). Similarly neutrophil, lymphocyte, monocyte, eosinophil and platelet were significantly lower in MT treated than in untreated cows at the rate of 28.73, 23.63, 36.48, 44.73 and 16.78%, respectively (Fig.2.). Endocrinological profiles revealed that cortisol and prolactin were significantly ($P<0.05$) lower in MT treated than in untreated anestrus buffaloes at the rate of 31.66 and 35.46% respectively (Fig. 3). Biochemical profiles revealed that total protein, albumin, globulin, glucose and total cholesterol were significantly ($P<0.05$) higher in MT treated than in untreated anestrus buffalo cows at the rate of 24.80, 28.76, 21.37, 15.89 and 14.19% respectively (Fig. 4.). Similarly antioxidant profiles such as TAC, CAT, GSH and SOD were significantly higher and

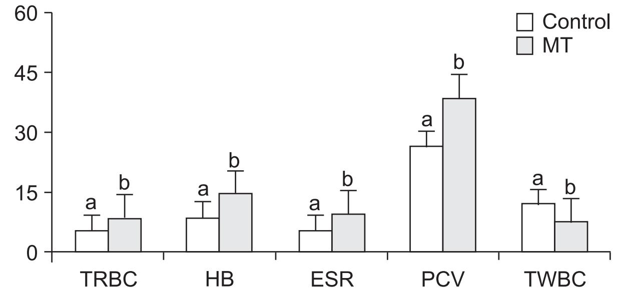

Fig. 1. Heamatological profiles in melatonin (MT) treated anestrus buffalo cows(mean ± SEM). Vertical bar on each point represents standard error of mean. Vertical bar with small letters (a, b) indicates significant ($P<0.05$) difference between the control and MT treated cows. TRBC, Total Red Blood Cell ($\times10^6$/mm^3); HB, Haemoglobin (g/dl); ESR, Erythrocyte Sedimentation Rate (mm/h); PCV, Packed Cell Volume (%) and TWBC, total white blood cell ($\times10^3$/mm^3). n= 6 cows for control and MT treated buffalo cows.

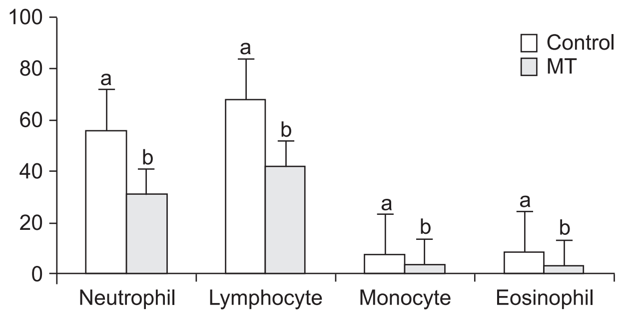

Fig. 2. Differential leukocyte count in melatonin (MT) treated anestrus buffalo cows (mean ± SEM). Vertical bar on each point represents standard error of mean. Vertical bar with small letters (a, b) indicates significant (P<0.05) difference between the control and MT treated buffalo cows.

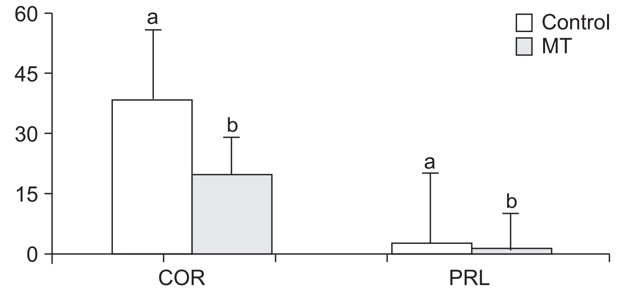

Fig. 3. Endocrinological profiles in melatonin (MT) treated anestrus buffalo cows (mean ± SEM). Vertical bar on each point represents standard error of mean. Vertical bar with small letters (a, b) indicates significant (P<0.05) difference between the control and MT treated buffalo cows. COR, Cortisol (pg/mL) and PRL, Prolactin (ng/mL). n= 6 cows for control and MT treated buffalo cows.

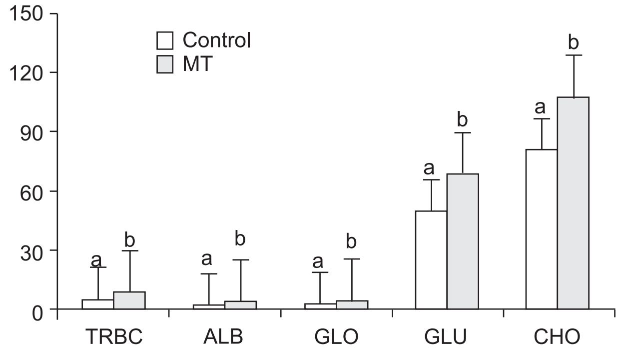

Fig. 4. Biochemical profiles in melatonin (MT) treated anestrus buffalo cows (mean ± SEM). Vertical bar on each point represents standard error of mean. Vertical bar with small letters (a, b) indicates significant (P<0.05) difference between the control and MT treated buffalo cows. TP, Total protein (g/dL); ALB, Albumin (g/dL); GLO, Globulin (g/dL); GLU, Glucose (mg/dL); and CHO, Total Cholesterol (mg/dL). n= 6 cows for control and MT treated buffalo cows.

MDA concentration was lower significantly (P<0.05) in MT treated than in untreated acyclic buffalo cows at the rate of 14.08, 27.06, 19.80, 40.81 and 23.84%, respectively (Fig. 5.). These heamatological parameters has shown significant (P<0.05) positive correlation with antioxidant profiles whereas significant (P<0.05) negative correlation

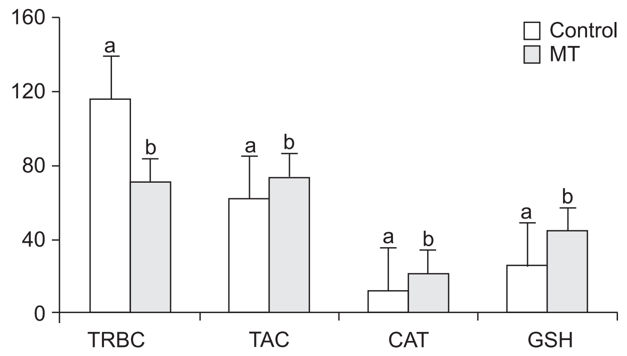

Fig. 5. Biochemical profiles in melatonin (MT) treated anestrus buffalo cows (mean ± SEM). Vertical bar on each point represents standard error of mean. Vertical bar with small letters (a, b) indicates significant (P<0.05) difference between the control and MT treated buffalo cows. MDA, Malondialdehyde (nmol/L); TAC, Total antioxidant capacity (nmol/μL); CAT, Catalase (nmol/min/L); GSH, Glutathione (nmol/min/L). n= 6 cows for control and MT treated buffalo cows.

observed with TWBC, DLC, MDA, cortisol and prolactin.

Although beneficial effects of exogenous MT on endocrinological profiles and antioxidant profiles were reported in different livestock species (sheep: Buffoni *et al.* 2015, cattle: Aggarwal *et al.* 2005, goat: Jiménez *et al.* 2014, buffalo: Ramadan *et al.* 2019, Kumar *et al.* 2015, mithun: Perumal *et al.* 2018), similar line of studies were lacking in anestrus buffalo cows in tropical island ecosystem during summer season in Andaman and Nicobar Islands. MT release from the implant is continuous and does not follow the diurnal rhythm of endogenous MT secretion (Perumal *et al.* 2018). MT treatment in buffalo induced cyclicity led to increased metabolic rate which in turn increased the RBC production which causes increased the values of other blood profiles (Patil *et al.* 1992). Again Coles (1986) reported that increase of RBC in cyclic estrus cows is due to higher excitement and hyperactivity under favour of oestrogen indicating that the MT treated cows may have significantly higher estrogen level. Therefore, these blood profiles such as TRBC, Hb, PCV and ESR were significantly higher in MT treated buffalo cows. Total WBC was significantly increased in anestrus than in MT treated animals as MT has pain attenuation effect, immune development effect and cytotoxic effects (Gilad *et al.* 1998). Leukocytosis was observed in anestrus cows indicating these animals suffered infection (Benjamin 1978). Anestrus cows had significantly higher total WBC and DLC counts as compared to treated ones which might be due to bacterial or any other infection in the anestrus cows (Connell *et al.* 2008). Anestrus group had significantly (P<0.05) higher total DLC which was clearly indicated by marked neutrophilia than in treatment group. Similarly, eosinophil and lymphocyte were also significantly (P<0.05) increased in anestrus than in treatment group. Ahmed (2011) reported melatonin administration in chicks improve the health and immune status of the chicks by increasing RBCs count, PCV and Hb concentration. The increase in RBCs count, PCV and Hb concentration obtained may be attributed to its direct stimulatory effect on bone marrow. Anwar *et al.* (1998) found that melatonin treatment in rats numerically increased RBC, Hb and PCV. Melatonin has anti-inflammatory effects as it reduced the TNF-α and IL-1β concentrations (Bahrami *et al.* 2018). Besides, MT modulates the inflammatory genes expression in immune cells and regulates the immune/ inflammatory responses. As a potential antioxidant, MT minimises the generation of ROS leads to reduction of pro-inflammatory cytokine production through inhibition of the activation and nuclear translocation of NF-κB (Mehrzadi *et al.* 2018).

Cortisol and prolactin were significantly lower in MT treated than in untreated control group. Heat stress in hot summer months causes increased blood concentrations of prolactin (Perera 2011), leading to decreased progesterone secretion and increased calving to conception intervals which in turn results in repeated breeding and reduced reproductive performance (Zicarelli 2007). MT regulates the prolactin secretion by multiple complex mechanisms which depend *o*n the physiological status of animals (Villauna *et al.* 2007). MT alters lactotrophs sensitivity through its direct actions at pituitary level and also has direct effect on the hypothalamic site (Villauna *et al.* 2007) to modify the prolactin release. Similarly, Lincoln and Clarke (1995) reported that MT primarily influenced the pituitary gland to regulate the prolactin secretion and development of partial refractoriness to control the prolactin secretion. Moreover, prolactin stimulates the adrenal corticoid's hyper secretion and inhibits the GnRH secretion through its receptors on dopaminergic neurons which in turn cause hyperprolactinemia, which is one of the major key causes of infertility in female. In our study, prolactin concentration was significantly reduced in MT treated group and this result was expected due to the well-known suppressive effect of MT on prolactin secretion and its concentration. Further, higher concentration of MT acts directly on pars tuberalis (Santiago-Moreno *et al.* 2000) to decrease the prolactin secretion. Similarly, in the present study, the MT treated buffalo cows had significantly lower level of prolactin. Cortisol is a stress hormone secreted in response to stress reactions. Slow release MT has significantly reduced the concentration of cortisol in anestrus buffalo cows as it inhibits adrenocortico tropic hormone (ACTH) stimulated cortisol production (Frank *et al.* 2004). MT has been reported to decrease the aggressive behaviour in dogs due to cortisol and improved their quality of life (Rosado *et al.* 2010). Similarly, supplementation of MT decreased cortisol and its side effects before, during and after surgery in canine species (Desborough 2000).

In the present study, the biochemical profiles significantly increased in MT treated than in untreated anestrus buffalo cows. Optimum total serum proteins are essential for the expression of estrus (Tandle *et al.* 1998) whereas in anestrus cows, the protein level is very low. Similar type of result was reported in MT treated anestrus buffalo heifers (Ramadan *et al.* 2015) whereas Singh *et al*. (2010) reported decreased plasma albumin in MT treated than in untreated lactating buffaloes. Albumin is the most abundant plasma protein which could play a major role as an antioxidant in plasma, mediating thiol oxidation and carbonyl formation (Himmelfarb and Mcmonagle 2001). Similarly, plasma glucose concentration were also increased by MT treatment (Ramadan *et al*. 2015). Glucose is the primary energy source for the ovary and it is possibly metabolized in the ovary through anaerobic pathways. It also stimulates the ovarian follicular growth (Rabiee *et al*. 1991). Furthermore, plasma glucose is a positive metabolic signal for the central control of GnRH release (Foster and Nagatani 1999) and subsequent reproductive cycle. High plasma glucose was previously reported in cycling dairy cattle compared to anestrus (Singh and Singh 2006). Plasma cholesterol concentration was increased by melatonin treatment than in untreated control buffalo cows (Ramadan *et al*. 2015). Cholesterol has a significant role in the ovarian function as it is the pre-cursor molecule for steroid biosynthesis (Arshad *et al*. 2005). Also, expression of estrus is more likely in dairy animals with higher plasma cholesterol (Westwood *et al*. 2002).

MT is a universal, multifunctional potent antioxidant and reduces the level of ROS and oxidative stress in mammalian species (Vinogradova *et al.* 2010). In the present study, slow release MT stimulated the TAC, enhanced the activities of antioxidant enzymes and reduced the MDA level in buffalo cows. Similar observation was reported in summer anestrus buffaloes (Kumar *et al.* 2015). After interaction with ROS, MT is converted into its metabolites and these metabolites have free radical scavenging activities (Tan *et al.* 2007). Moreover, MT induces the expression of cellular antioxidant [GSH] and enzymatic antioxidants [SOD, GSHRx, GSHPx, γ-glutamyl cysteine synthase and CAT] (Mehrzadi *et al.* 2016). MT also improves the activity and expression of the antioxidant vitamins (α-tocopherol and ascorbic acid) (Bahrami *et al.* 2018). Therefore, it is reasonable to say that MT has indirectly minimized the number of ROS and free radicals through enhanced production of the molecules that are protecting the cells against oxidative stress. Indeed, MT has double effect as potent as Vit-E in eliminating the peroxyl radicals (Pieri *et al.* 1994) and has higher potency than GSH or mannitol in scavenging the hydroxyl radicals (Hardeland *et al.* 1993). Because of its multiple facets and universal actions, especially at the level of mitochondria, MT strongly suppresses the oxidative stress. In our study, activity of TAC, CAT, SOD and GSH has been increased in MT treated than in untreated control buffalo cows. Similar results were reported as MT treated buffalo anestrus cows improved their antioxidant potency and decreased the MDA formation (Kumar *et al*. 2015, Singh *et al*. 2016). This may also be due to stimulatory effects *of* MT on enzymes involved in antioxidant defence mechanism (Ashrafi *et al.* 2013). MT reduces Bisphenol A-induced toxicity by increasing the level of GSH and activation of antioxidant enzymes including SOD, GSHPx and GSHRx as well as inhibition of lipid peroxidation (Anjum *et al.* 2011). In *in-vivo* study in rat, treatment of MT for endometrial lesions resulted in decreased MDA concentration and significantly increased antioxidant profiles (Reiter *et al.* 1999). Earlier reports indicated that anestrus buffaloes have suffered lower TAC and higher MDA concentration than in normal cyclic buffaloes (Ahmed *et al.* 2010, El-Moghazy *et al.* 2011) and similar result was observed in the present study. The result of the present work indicated that prolonged slow release melatonin protected the post-partum anoestrous buffaloes in summer season as summer stress alleviating agent.

The study concluded that exogenously administered slow release MT had increased the oxygen carrying capacity of blood to the gonad and reproductive tract, protected the reproductive tract and gonads from lipid peroxide as a potential antioxidant and regulate the hormone level (prolactin and cortisol) as an endocrine regulator. The results of present study can be utilized in improving the reproductive efficiency of buffaloes suffering anestrus in summer or dry season within the country or in other countries have similar environment or climatological condition of humid tropical island ecosystem of Andaman and Nicobar Islands. However, further investigation is needed to assess the correlation patterns between endocrinological profiles and antioxidant profiles with fertility rate in MT treated buffaloes during summer seasons to confirm the present findings.

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