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RESEARCH ARTICLE

IMMUNE REGULATION BY PLANT MELATONIN: A REVIEW

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ABSTRACT

The basic structure of melatonin is evolutionarily conserved. Hence, it was speculated that melatonin may be present in different animals (from unicellular to multi-cellular) and even in plants. Melatonin in plans is generally regarded as phyto-melatonin. Like the role of melatonin in animals, phyto-melatonin can perform a number of functions like attenuation of apoptosis, prevention of free radical generation, protection against UV irradiation etc. But, unlike phyto-estrogen, the role of phyto-melatonin in animals is totally an unexplored area. Hence, aim of the present study was to note the role of phyto-melatonin in maintenance of general health and immunity of goats. To fulfil the aim, we supplemented the goats with phyto-melatonin rich diet i.e. corn (*Zea mays*) which is having 1.4 ng/gm of dry weight of tissue and they are also edible to goats. We noted significantly high level of body weight, haematological (AST, ALT level, total RBC count and %Hb), immunological (TLC, %LC, %SR of PBMCs), metabolic (plasma glucose, cholesterol, HDL, LDL, protein levels and HDL: LDL ration), free radical (SOD, catalase, GPx levels), hormonal (estrogen, melatonin), cytokine (IL-6 and TNF-α) levels and significantly low level of MDA. However, plasma testosterone was unaffected upon phyto-melatonin treatment. Thus, for the first time role of phyto-melatonin as a protective molecule with improving effect on the health and immunity of Indian goat *Capra hircus*is being proposed, as the effect of phyto-melatonin supplementation can be brought back to normal and this dietary supplement might be utilizing the similar pathway as commercial melatonin. There are so many less expensive and readily available sources of phyto-melatonin that requires the proper knowledge of exploitation of these sources for extreme benefit for animals as well as for the human beings in near or far future.

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INTRODUCTION

The basic structure of melatonin is evolutionarily conserved (Reiter *et al*., 2007). Hence, it was speculated that melatonin may be present in different animals (from unicellular to multi-cellular) and even in plants. Around nineteenth century the presence of melatonin in plants were evidenced by scientists (Dubbels *et al*., 1995). Recent studies have suggested that not only the higher plants but also different macro and micro alga (Balzer *et al*., 1998), red algae (Lorenz and Lu¨ning, 1998), metazoans (Hardeland and Poeggeler, 2003) are also having substantial amount of phyto-melatonin. But, the presence of phyto-melatonin in other plant groups like bryophyta, pteridophyta and most gymno-sperms is under controversy (Reiter *et al*., 2007). The amount of phyto-melatonin present in plants is also variable due to the extraction and quantification techniques in different tissues of plants (Badria, 2002).

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It is also evidenced that plants can directly absorb melatonin from soil which has come to the soil upon degradation of different micro-organisms and fungi (Tan *et al*., 2007; Muller and Hardeland, 1999). However, it is also evidenced by several studies that plants can also synthesize melatonin (Tan *et al*., 2007) and melatonin can be stored in different body parts of edible plants like fruits (Reiter *et al*., 2007), dry seed (Badria, 2002) etc. The physiological function of phyto-melatonin in plants is almost similar to those of animals. Phyto-melatonin is responsible for circadian time management (Kola´r and Macha´ckova´, 2001), protection against harsh environment (Posmyk *et al*., 2008), promotion of vegetative growth (Herna´ndez-Ruiz *et al*., 2004), attenuation of apoptosis (Lei *et al*., 2004), scavenging of free radicals (Tan *et al*., 2007) under normal or physiologically stressed conditions like UV irradiation (Tettamanti *et al*., 2000). In some countries of Europe, the common maize (*Zea mays*) is offered to cattle for milk enhancement (www.fao.org) and the corn seed is most popular to farmers, breeders and raisers due to their palatability to the cattle. However, in corn the amount of phyto-melatonin is less (1.4 ng/gm of dry weight of tissue)

than other phyto-melatonin rich seeds like white mustard seed (189 ng/gm of dry weight of tissue). The role of phyto-melatonin in regulation of different physiological functions in animals is totally lacking. We identified the lacunae of previous studies and therefore, the aim of the present study was to note the role of phyto-melatonin rich diet (i.e. *Zea mays*) in regulation of immunity and physiology of Indian goat *Capra hircus.*

MATERIALS AND METHODS

Animals and maintenance: Goats of approximately same age (~1 year) and weight (~20 ± 2 kg) were procured from commercial goat raiser and then were housed in goat shelter under natural conditions of Varanasi ($25^0$18’ N, 83^0 01’ E, India) in order to maintain a consistency in food and hygiene throughout the year. At the time of procurement, the goats were weighed (Calf Weighing Sling, Munk’s Livestock, Kansas, USA) and the age was determined by dentition as described by Fandos *et al*. (1993). The male and female goats were kept separately to avoid mating or pheromonal effects. The detection of heat period was purely based on the visual observations i.e. more vocalization, reddening of vulva and mucorrhea. Goats were fed with usual ration of roughages (dry and green) and concentrate as suggested by Central Institute for Research on Goats, (CIRG), Mathura, Uttar-Pradesh, India. Single goat generally requires 4-5 kg of fodder/day and was fed with usual ration made up of roughages (dry and green) and concentrate. Dry roughages contained crushed barley (*Hordeum vulgare*, 1 part), crushed maize (*Zea mays*, 2 parts), linseed (*Linumusitatissimum*) or mustard seed cake (*Brassica juncea*, 2.25 parts), rice bran (*Oryza sativa*, 2 parts) along with small amount of molasses or a pinch of salt when required. Green roughages contained maize (*Zea mays*), elephant grass (*Pennisetum purpureum), pearl millet (Pennisetum glaucum*), sorghum (*Sorghum* sp.) and oat (*Avena sativa*). The concentrate contained oilseed cakes and soaked gram (*Cicer arietinum*) and water *ad libitum*. They were exposed to 8 hours outdoor for free grazing and 16 hours indoor (during night) conditions. Health of the goats was monitored by noting down the body temperature (normal rectal temperature, 102.5^0F–103^0F) and rumen movement by authorized veterinary doctors. Goats were treated with helminthicide twice per year and 0.5% solution of malathion (acaricidal baths) as described by Chowdhury *et al*. (2002).

The slaughtering of the goats was performed according in the city abattoir to the Slaughter of Animal Act under “Central Provinces Gazette” 1915 and modified in 2002. All the experiments were conducted in accordance with the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines and Institutional practice within the framework of revised Animal (Specific Procedure) Act of 2007 of Government of India on animal welfare. The study was carried out during three major seasons of a year i. e. summer, monsoon and winter. Thus, the climatic condition during summer months was (April–June, temperature $43.87^0 \pm 1.02^0$ C, percent relative humidity [%RH] 36.74 ± 4.28%, day length, light–dark cycle-13.42 hours:10.18 hours), monsoon months (July–September, temperature $28.68^0 \pm 2.76^0$ C, %RH 87.04 ± 3.50%, day length, light–dark cycle-12 hours:12 hours), and winter months (November–January, temperature $10.76^0 \pm 3.63^0$ C, %RH 64.12 ± 3.05%, day length, light–dark cycle 10.35 hours: 13.25 hours). All of the results were validated with the samples collected from CIRG in a seasonal manner.

Experimental design: A total number of 12 goats (six males and six females) were selected from the flock for every month of winter season (i.e. n = 6/sex/every month of winter) and were numbered on ears. Thus, for winter, the total numbers of male goats were 18 and the total numbers of female goats were also 18. Hence, for winter the total number of males and females were 36 (18 males + 18 females). The goats were were fed with 250 gm (~350ng melatonin) of maize/animal/day as dietary supplement during the months of winter season. The study was continued for 40 days. After completion of entire experimental period blood of both male and female goats were collected (as described in materials and methods section) and processed for different hematological, biochemical, hormonal, immunological and free radical parameters.

Measurement of body weight: The goats were weighed using Calf Weighing Sling of Munk’s Livestock, Kansas, USA.

Hematological parameters

Estimations of AST and ALT activities in plasma: Aspartate aminotransferase (AST) also known as glutamate oxaloacetate transaminase (GOT) is a transaminase. The principle of AST estimation is as follows:

Kinetic determination of the aspartate aminotransferase (AST) activity: L-Aspartate + α - Ketoglutarate ------------> Oxaloacetate + L-Glutamate Oxaloacetate + NADH +H^+-->L-Malate + NAD^+. These levels may be estimated as plasma levels and can be regarded as an assay of hepatotoxicity upon hormonal/drug treatment.

Alanine aminotransferase (ALT) also known as glutamate pyruvate transaminase (GPT) is a transaminase. The principle behind the ALT test is as follows:

ALT
L-Alanine + α- Ketoglutarate --------> Pyruvate + LGlutamate
LDH
Pyruvate + NADH + H^+ --------> L-Lactate + NAD^+.

Assessment of Red Blood Cell (RBC) count in blood: The total blood (as collected from peripheral circulation) was taken in RBC counting tube with RBC fluid i.e. Ringer’s solution, mixed well and the entire mixture is placed on Neubaeur Chamber and RBCs were counted.

Assessment of % haemoglobin (%Hb) blood: % Hb in blood was estimated with the help of Sahli’s hemoglobin meter (Systonic Instruments, India). In principle, hemoglobin is converted to acid haematin by the action of HCL (0.1N). The acid haematin solution is further diluted with HCl until its color matches exactly with that of the permanent standard of the comparator block. The hemoglobin concentration is read directly from the calibration tube. The % hemoglobin was found to be calculated from the standard value as grafted on the standard tube.

Immunological parameters

Total Leukocyte Count (TLC): Blood was taken in a WBC

pipette and diluted 20 times in Natt-Herrick diluents and white blood cells counted in Neubauer's counting chamber (Spencer USA) under the microscope. For DLC, a thin blood film was prepared and stained with Leishman's stain and leukocyte subpopulations were counted under oil immersion lens of Nikon microscope (Nikon, E200, Japan; Haldar *et al*., 2004). Lymphocyte counts (no./mm^3) was determined from total and differential leukocyte count by using the following formula:

$$\text{Lymphocyte count} = \frac{\text{TLC X Lymphocyte percent}}{100}$$

% Lymphocyte Count

TLC X Lymphocyte percent

% Lymphocyte count was performed following the protocol of Haldar *et al*., (2004) as published elsewhere.

%SR of Peripheral Blood Mononuclear Cells (PBMCs)

Separation of Peripheral Blood Mononuclear Cells (PBMCs): Blood was diluted with PBS (RT) in 1:1 ratio. Three mL of Ficoll (HiSep, Cat. No. LSM 1084) was transferred to a 15 mL sterile centrifuge tube. Ficoll was carefully overlaid with 6 mL of diluted blood and mixing of both was avoided. The centrifuge tube was centrifuged (without brake) at 400 × g for 30 minutes at room temperature. Centrifugation at lower temperature was not performed to avoid the cell clumping and poor recovery. After centrifugation, the sedimentation of erythrocytes, polynuclear leukocytes and band mononuclear lymphocytes above was obtained. Supernatant containing plasma and most of the platelets were aspirated carefully without disturbing the interface band. The opaque interface containing the mononuclear cell band was aspirated with the help of a glass Pasteur pipette and transferred in a sterile 15 mL tube. Ten mL of PBS/appropriate cell culture medium was mixed gently with mononuclear cells. The tube was gently inverted several times only to ensure a proper mixing. The mixture was centrifuged for 10 minutes at 250 × g. The supernatant was discarded. This step was repeated for thrice and finally the cell number was counted and viability of the cells (≥95%) was determined with the help of trypan blue exclusion method.

Cell harvesting and MTT assay: Cell harvesting and MTT assay was done following the protocol of Pauly *et al*., (1973) with few modifications as suggested by Ghosh *et al*., (2013). Plates were incubated at 37^0C with 5% CO_2 in incubator (Heracell, Germany) for 48 h and blastogenic response of thymocytes and splenocytes were measured by using a colorimetric assay based on the reduction of tetrazolium salt (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium

bromide (MTT, SRL, Mumbai, India) following the protocol of Mosmann, (1983). At 48 h, 200 µL of acidified propanol (0.04M HCl in isopropanol) was added to each well and the optical density (OD) of each well was determined with a micro-plate reader (ELx-800, Biotek Instruments, Winooski VT, USA) equipped with a 570 nm wavelength filter. Mean OD values for each set of triplicate were used in subsequent statistical analysis. Response was calculated as percent stimulation ratio (%SR) representing the ratio of absorbance of mitogen stimulated (challenged with Con A) cultures to basal cultures (without Con-A) for each groups.

$$\text{\% Stimulation ratio (\%SR)} = \frac{\text{Optical density of Challenged (Con A)} \times 100}{\text{Optical density of Basal}}$$

Metabolic parameters

Estimation of plasma glucose: Glucose was estimated by commercially available glucose estimation kit (Beacon India Pvt. Ltd., Mumbai) following manufacturer's protocol. The plasma was directly used for the assay.

Estimation of plasma cholesterol: The cholesterol estimation was done by method of Sackett, (1969). The stock solution of cholesterol was made of 1 mg/mL. Then serial dilutions were made from 0-200µg/mL in chloroform for standard curve. A mixture of acetic anhydride and sulphuric acid (20:1) was added to it and incubated in dark for 30 minutes and the O.D. was measured at 640 nm. For experimental plasma samples the cholesterol was extracted in a mixture of ether: ethanol (3:1). Then it was centrifuged at 3000 rpm for 10 minutes. The supernatant was taken out and evaporated to dryness in boiling water bath. Finally it was reconstituted in 5 mL of chloroform and 1 mL of Acetic Anhydride and sulphuric acid (20:1) mixture was added to it and incubated in dark for 30 minutes and the O.D. was measured at 640 nm (ELx-800, Biotek Instruments, Winooski VT, USA).

Estimation of plasma protein: Protein was estimated using commercially available Bradford's reagent following the protocol of Bradford (1976). Plasma was directly used for the protein estimation.

Estimation of plasma HDL, LDL levels and HDL: LDL ration: Plasma level of HDL and LDL were measured using a commercial kit (Sigma Aldrich, USA, Cat. No. MAK045) following manufacturer's protocol. The lower and upper limits of detections are 2mg/dL to 300mg/dL.

Free Radical parameters

SOD activity in plasma: Superoxide dismutase (SOD; EC 1.15.1.1) activity was assayed following the method of Das *et al*. (2000). 0.5 mL of plasma was added to 1.4 mL of reaction mixture comprised of 50 mM phosphate buffer (pH 7.4), 20 mM L-methionine, 1% (v/v) Triton X- 100, 10 mM hydroxylamine hydrochloride, 50 mM ethylene diaminetetraacetic acid (EDTA) followed by a brief pre-incubation at 37 °C for 5 min. Next, 0.8 mL of riboflavin was added to all samples along with a control containing buffer instead of sample and then exposed to two 20W fluorescent lamps fitted parallel to each other in an aluminium foil coated wooden box. After 10 min of exposure, 1 mL of Greiss reagent was added and absorbance of the colour formed was measured at 543 nm on a spectrophotometer (ELx-800, Biotek Instruments, Winooski VT, USA). One unit of enzyme activity is defined as the amount of SOD inhibiting 50% of nitrite formation under assay conditions.

Catalase activity in plasma: Catalase (CAT; EC 1.11.1.6) activity was measured following the procedure of Sinha (1972). This method is based on the fact that dichromate in

acetic acid is reduced to chromic acetate when heated in the presence of H_2O_2 with the formation of perchromic acid as an unstable intermediate. The chromic acetate thus produced is measured calorimetrically. The catalase preparation is allowed to split H_2O_2 for different periods of time. The reaction is stopped at a particular time by the addition of dichromate/acetic acid mixture and the remaining H_2O_2 is determined by measuring chromic acetate calorimetrically after heating the reaction mixture. There is production of green color at the end of the process. 10% homogenate of tissues were prepared in PBS (10 mM; pH 7.0) and then centrifuged at 12,000 × g for 20 min at 4^0 C. Supernatant was taken for enzyme estimation. 5 mL of PBS was added to 4 mL of H_2O_2 (200 mM) and then 1 mL of plasma was added. After 1 min 1 mL of this solution was taken in a tube and 2 mL of $K_2Cr_2O_7$ (5%) solution was added. Then it was boiled for 10 min and absorbance was measured at 570 nm (ELx-800, Biotek Instruments, Winooski VT, USA). The activity of CAT was expressed as amount of H_2O_2 degraded per minute.

GPx activity in plasma: Glutathione peroxidase (GPx; EC 1.11.1.9) activity was assayed as described by Mantha *et al*. (1993). The reaction mixture (1 mL) contained 50 µL plasma, 398 µL of 50 mM phosphate buffer (pH 7.0), 2 µL of 1 mM EDTA, 10 µL of 1 mM sodium azide, 500 µL of 0.5 mM NADPH, 40 µL of 0.2 mM GSH and 1 U glutathione reductase. The reaction mixture was allowed to equilibrate for 1 min at room temperature. After this, the reaction was initiated by addition of 100 mM H_2O_2. The absorbance measured kinetically at 340 nm (ELx-800, Biotek Instruments, Winooski VT, USA) for 3 min. The GPx activity was expressed as nmol of NADPH oxidized to $NADP^+$ per min per mg of protein using an extinction coefficient (6.22 mM/cm) for NADPH.

MDA level in plasma: The supernatant was subjected to thiobarbituric acid (TBA) assay by mixing with 8.1% sodium dodecyl sulphate (SDS), 20% acetic acid, 0.8% TBA and then digested it for 1 h at 95 °C (Sharma *et al*., 2008). The reaction mixture was immediately cooled in running water, vigorously shaken with 2.5 mL of n-butanol and pyridine reagent (15:1) and centrifuged for 10 min at 1500 × g (Ohkawa *et al*., 1978). The absorbance of the upper phase was measured at 534 nm (ELx-800, Biotek Instruments, Winooski VT, USA). Total thiobarbituric acid reactive substances (TBARS) were expressed as malondialdehyde (MDA; nmol/g tissue weight) taking 1,1,1,1-tetraethoxy propane (TEP) as standard. The standard curve was calibrated using different dilutions of 10 nM TEP. For the activity assay in plasma instead of tissue homogenate, equal volume of plasma was used.

Hormonal parameters

Plasma level of testosterone: An ELISA kit for peripheral testosterone assay was purchased from DiaMetra (Lot No; DKO 002), Italy and was measured according to the manufacturer's protocol. The coefficient of intra and inter assay variation was less than 9% and 15% respectively. The assay was carried out in triplicate.

Plasma level of estradiol: The ELISA kit for peripheral estradiol assay was purchased from Biotron Diagnostics Inc., Palm Ave Hemet, CA, USA and was measured according to the manufacturer's protocol. Intra and inter assay variation was less than 5% and 14% respectively. The analytic sensitivity was 10 pg/mL. The assay was carried out in triplicate.

Plasma level of melatonin: Peripheral melatonin level was measured in the blood collected at night with the help of a commercial kit (Biosource, Nivelles, Belgium; Cat. No. KIPL3300) according to the manufacturer's protocol. Analytic sensitivity (limit of detection) for melatonin serum was 2 pg/mL. Inter and intra-assay variations were between 9.0% and 15%, respectively. The assay was carried out in triplicate.

Cytokine parameters

Plasma level of IL-6: Sandwich ELISA was performed to quantify the level of IL-6 in plasma collected from the goats according to the manufacturer's instruction (KomaBiotech, Seoul, Korea; Cat. No. K0331230). Lower and upper limits of analytic sensitivities were 16 pg/mL and 1000 pg/mL. All the assays were carried out in triplicate.

Plasma level of TNF-α: Sandwich ELISA was performed to quantify the level of TNF-α in plasma collected from the goats according to the manufacturer's instruction (Koma Biotech; Cat. No. K0331186). Lower and upper limits of analytic sensitivities were 16 pg/mL and 2000 pg/mL. All the assays were carried out in triplicate.

Statistical analyses: The data were presented as the mean ± standard error of the mean (SEM). All the hematological, biochemical, hormonal, immunological and free radical parameters were analyzed by one way ANOVA followed by Student's unpaired t- test. The mean difference was considered to be statistically significant at the 0.05 level ($p < 0.05$). Statistical analyses were done with Statistical Package of Social Sciences (SPSS), IBM, software version 17.0 in accordance with Bruning and Knitz (1977).

RESULTS

Since the studies are underway (unpublished data); the glimpses of results alongwith the p-value will be provided where ever necessary.

Effect on body weight: Upon phyto-melatonin supplementation we noted significant increase in body weight ($p < 0.05$) in both the sexes.

Effect on haematological parameters: Upon phyto-melatonin supplementation there was no significant effect on plasma AST and ALT levels which are markers for hepato-toxicity (Fig. 2A and 2B). However, RBC count and %Hb were significantly high only in female goats upon phyto-melatonin supplementation than males ($p < 0.05$).

Effect on immunological parameters: %SR of PBMCs were significantly high ($p < 0.05$) in both the sexes upon phyto-melatonin supplementation. Total Leukocyte Count (TLC) in both the sexes of goats was significantly high upon phyto-melatonin supplementation ($p < 0.01$) but the males presented significantly higher level than females ($p < 0.05$). % Lymphocyte count (%LC) was significantly high in both the sexes upon phyto-melatonin supplementation ($p < 0.05$).

Effect on metabolic parameters: Plasma glucose level was

significantly high in both the sexes of goats upon phyto-melatonin supplementation ($p < 0.01$) which is significantly higher in females ($p < 0.05$) than males. Further, plasma cholesterol level was significantly high in both the sexes in phyto-melatonin supplemented groups ($p < 0.05$), however plasma protein level did not show any significant variation. Plasma HDL concentration was significantly high in both the sexes upon phyto-melatonin supplementation ($p < 0.05$) which is higher in females ($p < 0.5$) than males. Plasma LDL concentration was significantly low ($p < 0.05$ in males and $p < 0.01$ in females) upon phyto-melatonin supplementation, which further resulted in significantly higher levels of plasma HDL and LDL ration ($p < 0.01$) particularly in females than males ($p < 0.01$).

Effect on free radical parameters: SOD level was significantly high in both the sexes upon phyto-melatonin supplementation ($p < 0.05$). Similarly catalase and GPx levels were significantly high in both of the sexes ($p < 0.05$ in male goats for catalase and $p < 0.01$ in males for GPx; $p < 0.01$ in females for both catalase and GPx) upon phyto-melatonin supplementation. The reverse relationship was observed in case of MDA level in plasma which was significantly low ($p < 0.01$) in both the sexes upon phyto-melatonin supplementation.

Effect on hormonal parameters: Upon phyto-melatonin supplementation the plasma level of melatonin was significantly high only in males ($p < 0.01$) but the level is unaffected in females. Plasma level of estradiol was significantly high upon phyto-melatonin supplementation ($p < 0.05$) but plasma level of testosterone was unaffected.

Effect on cytokine parameters: Upon phyto-melatonin supplementation plasma level of IL-6 was significantly high in both the sexes ($p < 0.05$ in males and $p < 0.01$ in females) while females presented a significantly higher level ($p < 0.05$) than males. Plasma level of TNF-α was significantly high in both the sexes ($p < 0.01$) upon phyto-melatonin supplementation.

DISCUSSION

The effect of phyto-melatonin on regulation of goat health management and immunity is the first attempt in the field of research in ruminant and goat physiology in particular. Corn is not the normal/common food of goats in India and hence, we used the maize as a dietary supplement with the normal food for goats. Further, it was necessary to check if the supplementation had any side effect(s) on the digestive system of goats as this is the major system which maintains the body homeostasis. Therefore, we noted AST and ALT as the markers for liver function test (LFT). This is because, AST catalyses the transfer of the amino group of L-aspartate to a keto-glutarate to give L-glutamate. AST is widely distributed in the body, but the highest levels are found in heart, liver, skeletal muscles and kidneys. ALT catalyzes the transfer of the amino group of L-alanine to a keto-glutarate to produce L-glutamate. The highest levels are found in the liver and kidneys, and in smaller amounts in heart and skeletal muscle. ALT concentration is increased when hepatic cells are damaged (liver cell necrosis or injury of any cause). Our results suggested that there was no significant variation in the AST, ALT in experimental groups. Further, there were no sex dependent variations in AST and ALT levels suggesting that neither the corns (as a source of phyto-melatonin) nor their metabolites were affecting the body homeostasis in a detrimental manner or having any negative impact on goat health. Upon supplementation we found there was a significant increase in body mass in the phyto-melatonin treated groups. This might be due to the reason that it provoked basal metabolism (anabolism) of the body and as a result body mass increased. To confirm the same we further studied the different circulatory metabolic parameters. We checked the circulatory level of glucose (a ready source of energy) that was significantly high in phyto-melatonin supplemented groups particularly in females. Cholesterol, the sustainable source of energy was found to be significantly higher in both the sexes of supplemented groups. However, the circulatory level of protein did not show any sex dependent variation in goats. Thus, we may conclude that there might have been an increase in body metabolic processes upon treatment and for that the higher requirement of energy was needed. To balance the same circulatory level of glucose was increased. However, protein, which is only being used as a source of energy under severe pathological or starving condition, was found to be unaffected. Simultaneously and most interestingly, the cholesterol level was also high in the phyto-melatonin treated groups and this higher level of cholesterol might have then deposited to increase body mass. Further, HDL the “Good Cholesterol” was significantly high in both males and females of phyto-melatonin treatment.LDL, the “Bad Cholesterol” was significantly low in both the sexes upon treatment. The ratio of HDL and LDL was high in supplemented groups suggesting a beneficial physiological aspect of phyto-melatonin.

Higher cholesterol in circulation proved to be physiologically beneficial for goats. The higher circulatory level of glucose suggests that body metabolism is high. Increase in peripheral cholesterol level suggests that this high cholesterol might have been used as source of energy for near or far future. This stored energy is channelized to modulate energy demanding process of goat, as during winter two most energy demanding events (reproduction and immune modulation) occur simultaneously. To explore the issue, we studied the haematological, immunological and hormonal parameters. The haematological parameters including total RBC count and %Hb content were significantly high in phyto-melatonin supplemented groups being higher in females. Thus high level of metabolic parameters might be providing higher fitness level to females than males during stressful months of winter.

In the cell mediated immune parameters the Total Leukocyte Count (TLC), % Lymphocyte Count (%LC) and % Stimulation Ratio of Peripheral Blood Mononuclear Cells (%SR of PBMCs) were significantly high in the supplemented groups. Explanation may be that, phyto-melatonin supplementation might have increased the peripheral melatonin level and the increased melatonin level then might have increased the peripheral cell mediated immune parameters which is in agreement with earlier reports of Carrillo-Vico *et al*., (2013). A similar trend was observed in case of circulatory level of cytokines. We noted significantly high TNF-α and IL-6 levels in both the sexes upon supplementation, thus, like cell mediated immune system, cytokine levels were in parallel with high level of circulatory melatonin and inflammatory status of the body. We noted a significant increase in circulatory melatonin level particularly in males, upon supplementation of phyto-melatonin but, testosterone level was unaffected while the estrogen level in females was significantly high.

Thus, we may conclude that in males the higher level of melatonin might have increased the immune parameters and to cope up with this higher energy demand metabolic parameters were also increased. But, for females, melatonin and the elevated estrogen level increased inflammatory factors or cytokines being in agreement of previous reports in other animals that estrogen might have up regulated inflammatory cytokines (Calippe *et al*., 2008).Thus, under the influence of both the hormones, the higher immune status of the females were maintained with higher levels of metabolic and haematological parameters than male goats. In male goats melatonin alone was sufficient enough to well manage the immune functions as testosterone is an immune suppressor (Ahmad and Haldar, 2010).

The elevated metabolism of body resulted in generation of high level of Reactive Oxygen Species (ROS). The level of ROS generation can be estimated by the activities of their scavenger enzymes. The main free radical scavenging enzymes in the system are Super oxide Dismutase (SOD), Catalase (CAT) and Glutathione Peroxidases (GPx). MDA level, a marker for lipid peroxidation was lower in both the sexes of phyto-melatonin supplementation being low in females. We noted the SOD, CAT and GPx in blood of goats upon phyto-melatonin supplementation and found there was a significant increase in SOD, CAT and GPx activities in phyto-melatonin supplementation than control group however sex dependent variation was statistically non-significant.

Thus, in both the sexes of phyto-melatonin supplementation group, increased melatonin level maintained its parallelism with increased levels of free radical scavenging enzymes as metabolic activity increased. But, lipid per-oxidation, which generally depicts the level of cellular disintegration (as MDA level is a universal marker of lipid per-oxidation caused due to cell-membrane disruption; Wong-ekkabut, 2007) was low. Thus, the free radical generation could be the only causal effect of increased metabolism.

Conclusion

For the first time role of phyto-melatonin as a protective molecule with improving effect on the health and immunity of Indian goat *Capra hircus* is being proposed, as the effect of phyto-melatonin supplementation can be brought back to normal and this dietary supplement might be utilizing the similar pathway as commercial melatonin. There are so many less expensive and readily available sources of phyto-melatonin that requires the proper knowledge of exploitation of these sources for extreme benefit for animals as well as for the human beings in near or far future.

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REFERENCES

Ahmad, R and Haldar, C, Melatonin and androgen receptor expression interplay modulates cell-mediated immunity in tropical rodent *Funambulus pennanti*: an *in-vivo* and *in-vitro* study, Scand J Immunol, 71, 420-430, 2010.

Badria, FA, Melatonin, serotonin, and tryptamine in some Egyptian food and medicinal plants, J Med Food, 5, 153–157, 2002.

Balzer, I, Bartolomaeus, B and Ho¨cker, B, Circadian rhythm of melatonin content in Chlorophyceae, Proc Conference News Plant Chronobiol Res Markgrafenheide, 55–56, 1998.

Bruning, JL and Knitz, BL, Computational handbook of statistics, Eds. Scott, F and Company, Illinois, 1977.

Calippe, B, Douin-Echinard, V and Laffargue, M, Chronic estradiol administration *in vivo*promotes the proinûammatory response of macrophages to TLR4 activation: involvement of the phosphatidylinositol 3-kinase pathway, J Immunol, 180, 7980-7988, 2008.

Carrillo-Vico, A, Patricia, JL, Álvarez-Sánchez, N, Rodríguez-Rodríguez, A, and Guerrero JM, Melatonin: Buffering the Immune System, Int J Mol Sci, 14, 8638-8683, 2013.

Chowdhury, SA, Bhuiyan, MSA and Faruk, S, Rearing Black Bengal goat under semi-intensive management 1. Physiological and reproductive performances, Asian-Aust J Anim Sci, 15, 477-484, 2002.

Das, K, Samanta, L and Chainy, GBN, A modified spectrophotometric assay of superoxide dismutase using nitrite formation by superoxide radicals, Ind J Biochem Biophys, 37, 201–204, 2000.

Dubbels, R, Reiter, RJ, Klenke, E, Goebel, A, Schnakenberg, E and Ehlers, C, Melatonin in edible plants identified by radioimmunoassay and by high performance liquid chromatography-mass spectrometry, J Pineal Res, 18, 28–31, 1995.

Fandos P, Orueta JF and Olanda A, Tooth wear and its relation to kind of food: the repercussion on age criteria in *Capra pyrenaica,* Acta Theriol, 38, 93-102, 1993.

Ghosh, S, Singh, AK, Haldar, C, Seasonal modulation of immunity by melatonin and gonadal steroids in a short day breeder goat *Capra hircus*, Theriogenol, 82, 1121-1130.

Haldar, C, Rai, S and Singh, R,Melatonin blocks dexamethasone-induced immunosuppression in a seasonally breeding rodent Indian palm squirrel, *Funambulus pennanti*, Steroids, 69, 367-377, 2004.

Hardeland, R and Poeggeler, B, Non-vertebrate melatonin, J Pineal Res, 34, 233–324, 2003.

Herna´ndez-Ruiz, J, Cano, A and Arnao, MB, Melatonin: a growth-stimulating compound present in lupin tissues, Planta, 220, 140–144, 2004.

Kola´r, J and Macha´ckova´ I, Occurrence and possible function of melatonin in plants, A review, Endocytobio Cell Res, 14, 75–84, 2001.

Lei, XY, Zhu, RY, Zhang, GY and Dai, YR, Attenuation of cold induced apoptosis by exogenous melatonin in carrot suspension cells: the possible involvement of polyamines, J Pineal Res, 36, 126–131, 2004.

Lorenz, M and Lu¨ning, K, Detection of endogenous melatonin in the marine red macroalgae *Porphyra umbilicalis* and *Palmaria palmata* by enzyme-linked immunoassay (ELISA) and effects of melatonin administration on algal growth, Proc Conf Plant Chronobiol Res, Markgrafenheide, 42–43, 1998.

Mantha, SV, Prasad, M, Kalra, J and Prasad, K, Anti oxidant enzymes in hypercholesteromia and effect of vitamin E in rabbits, Atherosclerosis, 101, 135–144, 1993.

Mueller, U and Hardeland, R, Transient accumulations of exogenous melatonin indicate binding sites in the dinoflagellate *Gonyaulax polyedra*, In: Studies on antioxidants and their metabolites, ed. R. Hardeland, 140–147, Gottingen: Cuvilier, 1999.

Ohkawa, H, Ohishi, N and Yagi, K, Reaction of linoleic acid hydroperoxide with thiobarbituric acid, J Lipid Res, 19, 1053–1057, 1978.

Pauly, JL, Sokal, JE and Han, T, Whole-blood culture technique for functional studies of lymphocyte reactivity to mitogens, antigens, and homologous lymphocytes, J Lab Clin Med, 82, 500–512, 1973.

Posmyk, MM, Kuran, H, Marciniak, K and Janas, KM, Pre-sowing seed treatment with melatonin protects red cabbage seedlings against toxic copper ion concentrations, J Pineal Res, 45, 24–31, 2008.

Reiter, RJ, Tan, DX, Manchester, LC, Simopoulos, AP, Maldonado, MD and Flores, LJ, Melatonin in edible plants (phyto-melatonin): identification, concentrations, bioavailability and proposed functions, World Rev Nutr Diet, 97, 211–230, 2007.

Sackett, DL, Clinical epidemiology, Am J Epidemiol, 89, 125-128, 1969.

Sharma, S, Haldar, C and Chaube SK, Effect of exogenous melatonin on X-ray induced cellular toxicity in lymphatic tissue of Indian tropical male squirrel, *Funambulus pennanti*, Int J Radiat Biol, 84, 363-374, 2008.

Sinha, AK, Colorimetric assay of catalase, Anal Biochem, 47, 389–394, 1972.

Tan, DX, Manchester, LC, Di Mascio, P, Martinez, GR, Prado, FM and Reiter, RJ, Novel rhythms of N1-acetyl-N2-formyl-5-methoxykynuramine and its precursor melatonin in water hyacinth: importance for phyto-remediation, The FASEB J, 21, 1724–1729, 2007.

Tettamanti, C, Cerabolini, B, Gerola, P and Conti, A, Melatonin identification in medicinal plants, Acta Phytotherap, 3, 137–144, 2000.

Wong-ekkabut, J, Xu,Z, Triampo, W, Tang, I-M, Tieleman, DP and Monticelli, L, Effect of Lipid Peroxidation on the Properties of Lipid Bilayers: A Molecular Dynamics Study, Biophy J,93,4225–4236, 2007.

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