

Effect of Melatonin and Caffeine Interaction on Caffeine Induced Oxidative Stress and Sleep Disorders

Obochi G. O., Amali O.O.E., and Ochalefu D.O

Department of Biochemistry, Benue State University, Makurdi, Nigeria.

Summary: Effect of interaction of melatonin and caffeine on caffeine induced oxidative stress and sleep disorders was studied. Fifteen wistar rats were randomly assigned into three study groups. The animals in group 1 (the control) received a placebo of 10.0 ml distilled water via gastric intubation. The hosts in groups 2 and 3 were treated with 100 mg caffeine/ kg, or melatonin/ kg, respectively, in a total volume of 10.0 ml vehicle. The experiment lasted for 30 days. One day after the final exposure, the animals were euthanized by inhalation of overdose of chloroform. Blood was collected by cardiac puncture. Serum was obtained by centrifugation (6000 Xg, 30 mins), and used for serum total protein and serum blood urea nitrogen levels. The brain of each rat was also harvested and processed into whole homogenate, frozen in liquid nitrogen (N₂), and maintained at -80°C until used for total brain cholesterol and tryptophan levels. The results showed that interaction of melatonin and caffeine enhanced protein synthesis; stimulated gonadotrophin release, and could be used as oral contraceptive for women, and may be beneficial in the treatment of impotence (androgen depression), leading to improved reproductive and sex life; stimulated tryptophan metabolism, which prevents vitamin B₆ deficiency, anemia, negative nitrogen balance, tissue wasting and accumulation of xanthurenic acid, which promotes sleep; and could be beneficial in the treatment of hyper cholesterolemia, thereby preventing coronary heart disease, and post menopausal osteoporosis.

Key words: *melatonin, caffeine, oxidative stress, sleeps disorders and free radicals.*

**Address for correspondence:*

P.M.B. 102119 Makurdi, Nigeria. Phone: 07096045538, 08089506087, 08052707200 E-mail: gobochi@yahoo.com

Manuscript Accepted: April, 2010

INTRODUCTION

Caffeine, 1, 3, 7 – trimethylxanthine is consumed worldwide by people of all races and social class through processed cocoa, coffee, tea or kolanut based foods and beverages, stimulants, drugs and cosmetics (Eteng et al, 1997; Obochi, 2006). Caffeine, theophyllines and theobromines are related compounds and are classified as alkaloids. An alkaloid is a compound that contains nitrogen, and is physiologically active. Caffeine is metabolized in the liver and is excreted in kidneys, faces, saliva, semen and breast milk (Julien, 1996). Liver diseases, pregnancy, and use of oral contraceptives slow the metabolism and excretion while nicotine and other therapeutic drugs interact with caffeine to increase or decrease its metabolism and excretion (Julien, 1996). Caffeine is equally distributed in total body water and freely crosses the placenta to the fetus, leading to

spontaneous abortion or miscarriage (Julien, 1996). The metabolism and toxicity of caffeine are viewed to be dose dependent, resulting in non-linear accumulation of metabolites (Methylxanthines – dimethyluric acid, 7-methyl uric acid, 3-methyluric acid), which may lead to cardiovascular diseases such as heart failure, high blood pressure and neuronal disorder as schizophrenia (Julien, 1996; Eteng et al, 1997; Obochi, 2006).

The central nervous system stimulatory action of caffeine elevates mood. This accounts for much of the popularity that coffee and tea have as morning wake-up beverages. Also, people who are afflicted with significant depression medicate themselves by using caffeine products. Caffeine acts to block adenosine's inhibition of cells in the brain, and this underlies alertness and up beat mood (Julien, 1996). Adenosine is an inhibitory neurotransmitter, synthesized from AMP by the action of s-nucleotidase, which acts to result in behavioural sedation, regulation of oxygen delivery to cells, dilation of cerebral and coronary

blood vessels (Eteng et al, 1997). Caffeine and other methylxanthines occupy adenosine receptors and then block the action of the neurotransmitter. Adenosine receptors are linked with interplay of release, reuptake, metabolism and excretion of neurotransmitters (Julien, 1996; Eteng et al, 1997; Obochi, 2006). Thus, blockade of adenosine receptors by caffeine results in alteration in behavioural pattern by delaying neuronal tube closure (Eteng et al, 1997); activates phosphorylase and lipase, thereby enhancing glycogenolysis and lipolysis, resulting in loss of weight (Julien, 1996). The blockade also inhibits glutamate release, resulting in low protein synthesis, poor growth, low protein efficiency ratio, poor nitrogen retention (Eteng et al, 1997), and decreases bone mineral density, leading to osteoporosis, palpitations, tremor, headache, anxiety, peptic ulcer and insomnia (Julien, 1996). The blockade of adenosine receptors by caffeine inhibits androgen binding protein, resulting in decreased cauda epididymis sperm reserve, seminiferous tubular fluid volume, low sperm production and infertility (Eteng et al, 1997; Obochi, 2006). Thus, the toxicity of caffeine is viewed as a phenomenon of rapid absorption but slow excretion (Obochi, 2006).

Therapeutic properties of caffeine include relief of pain, headache and cold (through inhibition of the inflammatory action of prostaglandins), depression and in appetite-suppressant medications due to its diuretic effects (Obochi, 2006). Melatonin (N-acetyl-5-methoxytryptamine) is a hormone synthesized from tryptophan in the pineal gland of the brain in humans and animals (Reiter, 1995); stimulated by the dark and inhibited by light (Reiter, 1995). It also occurs naturally in plants such as oats, tomatoes, banana and barley (Webb and Pulg-Domingo, 1995). The principal role of melatonin involves regulation of neuro-endocrine reproductive functions (Reiter et al, 1995; Webb and Pulg-Domingo, 1995). It also plays a role in signaling changes in reproductive hormone levels (Maestroni, 1993). Melatonin is metabolized to 6-hydroxy-melatonin in the liver, and the main metabolite, 6-sulphatoxy-melatonin is excreted (Maestroni, 1993; Cavallo, 1993); lowers body temperature and blood flow to the brain, (Lamberg, 1996; Brzezinski, 1997).

Melatonin is an effective antioxidant that protects DNA, cells and tissues from free radical assault (Sharma et al, 1989; Stokkan et al, 1991; Pierpaoli et al, 1993; Tan et al, 1993; Lesnikov et al, 1994; Pierpaoli et al, 1994; Reiter, 1995). It stimulates the main antioxidant enzyme of the brain, glutathione peroxidase (Reiter, 1995) and inhibits proliferation and growth of cancer cells (Blask et al, 1991; Lissoni et al, 1992; Cos et al, 1994; Neri et al, 1994; Aldeghi

et al, 1994; Lissoni et al, 1994; Sandyk, 1994; Chan et al, 1995; Lissoni et al, 1995). It also lowers total cholesterol and LDL levels in rats (Chan et al, 1995); acts as a powerful chronobiotic maintaining synchronicity and preventing desynchrony of circadian rhythms (Armstrong, 1989; Attenburrow et al, 1996); improves sleep (Garfinkel et al, 1995) and sex life (Laudon et al, 1988).

Melatonin stimulates gonadotrophin release hormone, estrogen levels and androgen responses (Terzollo et al, 1993; Laudon et al, 1996), and may inhibit ovarian function in women and may be beneficial as oral contraceptive (Voordouw et al, 1992). It is involved in the regulation of calcium and phosphorous metabolism by stimulating the parathyroid gland and by inhibiting calcitonin release and prostaglandins synthesis (Sandyk et al, 1992). Melatonin stimulates immunoenhancing effects (Maestroni, 1993), improves leucopenia and winter depression (Neri et al, 1994; Webb and Pulg-Domingo, 1995). Because of these therapeutic properties of melatonin, it is often bought over the counter in health food shops and is consumed for improved sleep, sex life, longevity and lowering of blood pressure, leading to self medication. The current study is therefore focused on assessment of how melatonin could impact upon caffeine induced oxidative stress and sleep disorders in animals exposed daily to caffeine and melatonin.

MATERIALS AND METHODS

Fifteen Wistar albino rats weighing between 150g to 250g obtained from the disease free stock of the animal house, Department of Biochemistry, College of Medical Sciences, Benue State University, Makurdi, Nigeria, were used for the study. The animals were randomly assigned into three study groups of five animals per group. Each rat in a study group was individually housed in a stainless steel cage with plastic bottom grid and a wire screen top. The animal room was adequately ventilated, and kept at a room temperature and relative humidity of $29 \pm 2^\circ\text{C}$ and 40 – 70% respectively with 12 hour natural light-dark cycle. The animals were fed ad libitum with water and rat chow (Livestock Feeds Ltd, Makurdi, Nigeria). Good hygiene was maintained by constant cleaning and removal of faeces and spilled feeds from cages daily. All animal experiments were approved by the Animal Care and Use Committee of the Medical College, Benue State University, Makurdi, Nigeria.

Treatment regimen

All rats received daily treatment with their test solutions for a period of 30 days. All treatments were conducted between the hours of 6.00 – 7.00 pm because melatonin is most active in the evening. The rats in Group 1 (the control) received a placebo of 4.0ml distilled water by gastric intubation. The rats in Groups 2 and 3 were treated with 100mg caffeine/kg body weight or a combination of 100mg caffeine/kg body weight + 100mg melatonin/kg body weight, respectively, in a total volume of 4.0ml vehicle.

Preparation of caffeine

Synthetic caffeine was obtained from May and Baker (M & B) Limited (Enfield, Middlesex, England), and used for the study. A stock solution of caffeine was prepared by dissolving 20g of powdered caffeine in 500ml of hot distilled water. The solution was allowed to cool to room temperature, and from this, and based on the animal's weight that morning, the 100mg/kg body weight dosages were administered to the animals in Groups 2 and 3 as part of the 10.0ml volume used for gastric intubation.

Preparation of Melatonin

Synthetic melatonin was obtained from May and Baker Limited (Enfield, Middlesex, England), and used for the study. A stock solution of melatonin was prepared by powdered melatonin in 500ml of distilled water. From this, and based on the animal's weight that morning, the 100mg/kg body weight dosages were administered to the animals in Groups 3 as part of the 10.0ml used for gastric intubation.

Sample preparation

One day after the final exposure, the animals were euthanized by inhalation of overdose of chloroform. Blood was collected by cardiac puncture. Serum was obtained by centrifugation (6000 Xg, 30min.), and used for serum total protein, blood urea nitrogen levels. The brain of each rat was also harvested and processed into whole homogenate. Briefly, the brain tissues were ground using mortar and pestle, and buffered with Tris-HCl (pH 7.4). a whole homogenate (WH) of the tissue was then prepared by centrifugation at 6000 Xg for 30min; the supernatant generated was recovered and subjected to a second centrifugation at 8000 Xg for 20min. The whole homogenate (WH) was then frozen in liquid nitrogen (N₂) and maintained at -80°C until used for total brain cholesterol and tryptophan levels.

Determination of Total Serum Protein Level

Total serum protein was determined by the Biuret method described by Gornall et al (1949). To 0.5ml

of the sample solution was added 1.0ml distilled water to bring the volume to 1.5ml in each tube. Tube 1, the blank received 1.5ml distilled water. The suspension was mixed, and 0.2ml of 5% sodium deoxycholate (DOC) in 0.01N KOH was added and mixed to make the suspension more soluble. Then, 1.5ml of biuret reagent (1.50g CuSO₄ · 5H₂O, 6.0g Sodium potassium tartrate, and 300ml of 10% NaOH per litre) was added (including the blank). The contents in each tube were mixed in a vortex mixer, and incubated at 37°C for 15 min, and the absorbances read at 50nm in a 6400/6405 spectrophotometer (Jenway, Essex, England) against the reaction blank. The concentration of the standard bovine serum albumin (BSA) was 2mg/ml (1.0g BSA was dissolved in 500ml H₂O). 10% NaOH was prepared by dissolving 100g NaOH in 1 litre of distilled water.

Determination Of Blood Urea Nitrogen Levels

Serum blood Urea nitrogen was determined with the method of Taylor (1989). In this assay, Urea, the end product of nitrogen metabolism in humans is hydrolysed to ammonia (NH₃) and carbon dioxide (CO₂) by an enzyme, urease. The ammonia is then converted to indophenol blue in the presence of sodium nitroferrocyanide, phenol and alkaline hypochlorite reagents. Briefly, 10ml of the EDTA (dissolved 7.5g EDTA- free acid in 400ml H₂O, pH adjusted to 6.5 with 10% NaOH, and diluted to 750ml) buffered serum were pipetted into 13 X 100mm test tubes, and 10ml distilled water added to bring the volume to 20ml. The blank tube received 20ml distilled water. Then, 20ml of urease solution (5ml urease-glycerol extracts in 100ml volumetric flask, and diluted to 100ml with EDTA buffer) was added to all tubes including the blank. The tubes were incubated at 37°C for 30min. Then, 0.5ml of phenol reagent [5ml of liquefied Phenol to 100ml volumetric flask, added 80ml H₂O and 1.0ml of 2.5% sodium nitroferrocyanide stock solution (prepared by dissolving 2.5g Na nitroferrocyanide in 100ml H₂O) and brought to volume with H₂O] was added to all tubes. Then, 0.5ml of alkaline hypochlorite reagent [mix 25ml of 2.5N NaOH (10%) and 4.0ml Clorox, and diluted to 100ml with H₂O] was added to all tubes. The tubes were mixed, and incubated at room temperature for 40 min. then, 4.0ml distilled water added to all tubes, mixed and the absorbencies read at 625 nm in a 6400/6405 spectrophotometer (Jenway, Essex, England). 0.2mg/ml urea working standard was prepared by adding 2.0ml stock solution to 98ml H₂O. 10.0mg/ml urea stock standard was prepared by dissolving 2.14g Urea in 100ml H₂O, and freeze in 2.5ml batches.

Determination Of Total Brain Cholesterol Levels

Total brain cholesterol was determined with the method of Kates (1986). Briefly, 1.0ml of the whole homogenate (WH) were pipetted, and evaporated to dryness in small test tubes under nitrogen (N₂). The dried samples were redissolved in absolute ethanol. Then, 0.1ml of the WH was delivered using an automatic micropipettor into tubes and sufficient absolute ethanol added to bring the volume to 2.0ml. 2.0ml absolute ethanol only was used for the blank. Then, 1.0ml of the cholesterol reagent [add 40ml iron stock reagent (prepared by dissolving 5.0g FeCl₃ · 6H₂O in 200ml conc. H₃PO₄) to 460ml conc. H₂SO₄] was added to all tubes. Then, 2.0ml of ferric chloride reagent (consists of 80ml of a solution of 2.5% FeCl₃ · 6H₂O in conc. H₃PO₄ and 920ml of conc. H₂SO₄ per litre) was added, and mixed carefully at the lowest setting of the vortex mixer. The tubes were incubated for 30 min. at room temperature, and the absorbencies read at 550nm in 6400/6405 spectrophotometer (Jenway, Essex, England). The concentration of the cholesterol standard solution was 0.1mg/ml (dilute 5.0ml of cholesterol stock solution in 50ml absolute ethanol). Cholesterol stock standard was prepared by dissolving 0.1g cholesterol in 100ml absolute ethanol.

Determination of total brain tryptophan Levels

Total brain tryptophan was isolated and determined according to the standard method described by Davidson and Henry (1979). Briefly, in the isolation assay, 1.0ml of the brain whole homogenate (WH) were pipetted into 20ml ampule, 9.0ml of 6.0N NaOH added, mixed, and the ampule sealed by pulling the glass tip over an intense flame. The suspension was incubated at 110°C for 24 hours. The extracts were transferred to 50ml beakers; each titrated with 6N HCl to pH 7.0 while mixing on a magnetic stirrer. The titration was then changed to 1N HCl at a pH 8.5, and centrifuged at 10,000 Xg for 20min. the supernatant was decanted into 25ml graduated cylinders, and the volumes brought to 20ml with 0.1M Tris-HCl buffer, pH 7.4, and mixed well. The hydrolysate (whole homogenate-WH) was used for the analysis of total tryptophan levels.

In the determination assay, briefly, whole sample homogenates were pipetted into test tubes. The blank received 1.0ml distilled water. Then, 5.0ml of tryptophan free growth medium (10.5g K₂HPO₄, 1.0g (NH₄)₂SO₄, 1.0g Na citrate · 2H₂O, 0.65g MgSO₄, 2.0g glucose per litre) was added to all tubes including the blank. Then, 5.0ml of 4% glucose solution was added. The tubes were autoclaved for 15min (120°C, 15-1b pressure). All the tubes were

allowed to cool to room temperature, and incubated in a shaking water bath at 37°C for 48 hours. The tubes were mixed vigorously in a vortex mixer, and the absorbances read at 450nm in 6400/6405 spectrophotometer (Jenway, Essex, England) against the blank.

Statistical Analysis

Data collected were expressed as mean ± standard deviation (SD), and the Student's t- test was used for analysis. Values of P < 0.05 were regarded as significant.

RESULTS

Table 1 presents the results of treatments on total serum protein and blood serum urea nitrogen levels in the rats. The results of the total serum protein levels showed that there was significant (P < 0.05) increase (12.66%) in the values of the caffeine treated hosts, and a significant (P < 0.05) increase (45.11%) in the values of the melatonin + caffeine treated animals, relative to those seen in the controls.

The result of treatments on blood serum urea nitrogen levels showed that there was significant (P < 0.05) increase (99.63%) in the values of the caffeine treated rats relative to the controls while there was no significant (P > 0.05) difference (1.22%) in the values of the melatonin + caffeine treated hosts relative to those of the controls.

Table 1

Effect of treatment on serum total protein and serum blood urea nitrogen levels in the rats.

Group (N)	Total serum protein (mg/ml)	Blood serum urea nitrogen (mg/dl)
Control	16.67 ± 0.43	24.68 ± 0.67
Caffeine	18.78 ± 0.45*	49.27 ± 0.83*
Melatonin + Caffeine	24.24 ± 0.37**	24.98 ± 0.69**

N=Number of rats per group=5. Values are expressed as mean ± SD.

* = significantly different from control at P < 0.05. # = significantly different from caffeine only at P < 0.05.

Table 2

Effect of treatment on total brain cholesterol and total brain tryptophan levels in the rats.

Group (N)	Total brain cholesterol (mg/g of tissue)	Total brain tryptophan (mg/g of tissue)
Control	18.41 ± 0.45	97.68 ± 0.65
Caffeine	65.74 ± 0.53*	13.67 ± 0.31*
Melatonin + Caffeine	41.93 ± 0.48 [#] *	34.82 ± 0.58 [#] *

N=Number of rats per group = 5. Values are expressed as mean ± SD.

* = significantly different from control at $P < 0.05$. # = significantly different from caffeine only at $P < 0.05$.

Table 2 presents the results of treatment on total brain cholesterol and brain tryptophan levels in the rats. The results for the total brain cholesterol levels showed that there was significant ($P < 0.05$) decrease (32.70%) in the values of the caffeine treated hosts relative to the controls while there was also a further significant ($P < 0.05$) decrease (57.07%) in values of the melatonin + caffeine treated animals relative to those of the controls. However, when the values of the melatonin + caffeine treated hosts were compared to those of caffeine treated animals, the values of the melatonin + caffeine treated hosts were lower, that is, comparatively decreased by 36.22%.

The results for the total brain tryptophan levels showed that there was significant ($P < 0.05$) decrease (25.75%) in the values of the caffeine treated rats relative to those of the controls while there was significant ($P < 0.05$) increase (89.14%) in the values of the melatonin + caffeine treated animals relatively to those seen in the controls.

DISCUSSION

In this study, caffeine alone increased serum total serum protein and blood urea nitrogen levels while it decreased total brain cholesterol and tryptophan levels in the rats. However, interaction of melatonin with caffeine increased total protein, and brain tryptophan levels, but decreased total brain cholesterol and blood urea nitrogen levels in the rats.

The effects of caffeine on protein levels could be attributed to inhibition of glutamate release due to blockade of adenosine receptors. Adenosine receptors are linked with interplay of release, reuptake, metabolism and excretion of neurotransmitter molecules (Eteng et al, 1997). Thus, blockade of adenosine receptors inhibited glutamate release and reuptake, resulting in low protein synthesis (Obochi, 2006). Glutamate supplies the amino group required

for the synthesis of amino acids, and is a substrate for glutamine and glutathione synthesis (Julien, 1996). This may suggest that caffeine may cause low protein synthesis and low protein efficiency, leading to poor growth.

The effects of caffeine on blood urea nitrogen levels could be attributed to the activation of the enzyme, xanthine oxidase, which catalyzes the oxidation of xanthines to uric acid, which generates superoxides, hydrogen peroxides, and free radicals. Free radicals are formed by interaction of hydrogen peroxide with superoxide anion; the rate of which is increased by ions of iron or copper (Julien, 1996). Free radicals are constituents that have an unpaired electron, generated as by-products of oxidation. Addition of an electron to O_2 produces a superoxide anion radical, O_2^- , which is reduced by superoxide dismutase to peroxide, H_2O_2 , which is toxic at high concentrations, and can be reduced to hydroxyl radical ($\cdot OH$), $[H_2O_2 + O_2^- \rightarrow O_2 + HO^- + HO\cdot]$

The hydroxyl radical damages cells, DNA and neuronal tissue, brain (Reiter, 1995; Reiter et al, 1995). Mechanisms of action of free radicals on cells, DNA and neuronal tissue involves peroxidation and chemical modification of the nucleotide bases; the damage to proteins is caused by the oxidation of sulphhydryl group (that is, SH groups are converted to S-S groups); the effect of free radicals on lipids could be attributed to the attack on unsaturated fatty acids in phospholipid components of membranes, which results in the formation of lipid hydroperoxides. Hydroperoxidation of membrane lipids increases the hydrophobic nature of the lipids, and changes the structure of membranes, thereby distorting the normal conformation and function (Chan and Tang, 1995). Also, lipid hydroperoxides are powerful enzyme inhibitors, which inhibit chemical processes, resulting in rapid degradation of cells, leading to anemia, and inhibition of methaemoglobin which transports oxygen (Reiter, 1995).

The effects of caffeine on cholesterol levels could be attributed to stimulation of phosphoprotein phosphatase inhibitor – 1 (PP1 – 1), and increased its activity thereby inhibiting the activity of 3-hydroxy-3-methylglutaryl CoA (HMG-CoA) reductase, resulting in decrease cholesterol synthesis. This effect occurs through covalent modification as a result of phosphorylation and dephosphorylation. 3-Hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase, HMGR, is most active in the dephosphorylated state. Phosphorylation of the enzyme decreases its activity (Chan and Tang, 1995). Also, the effect of caffeine on cholesterol could be attributed to stimulation of cAMP signaling pathway. Increases in cAMP leads to

activation of cAMP-dependent protein kinase, PKA. PKA phosphorylates phosphoprotein phosphatase inhibitor – 1 (PP1 – 1) leading to an increase in its activity. Phosphoprotein phosphatase inhibitor – 1 (PP1 – 1) can inhibit the activity of numerous phosphatases including HMG-CoA reductase phosphatase, which remove phosphates from HMGR.

This maintains HMGR in the phosphorylated and inactive state, resulting in decreased activity of HMGR, and reduced cholesterol synthesis (Reiter, 1995). Also, increases in cAMP lead to activation of cAMP-dependent protein kinase, PKA, which in turn, inhibits HMGR activity, resulting in decreased cholesterol synthesis (Reiter, 1995; Chan and Tang, 1995). The effect of caffeine on cholesterol levels could also be attributed to hormonal stimuli, particularly lipolytic hormones such as glucagon, adrenaline, noradrenaline, adrenocorticotrophin hormone (ACTH), growth hormones and parathormone, which increase the concentration of cAMP, thereby increasing the activity of cAMP-dependent protein kinase, PKA, which in turn, inhibited the activity of HMGR, resulting in decreased cholesterol synthesis. However, insulin decreases the concentration of cAMP, and thereby activates cholesterol synthesis. Thus, the ability of insulin to stimulate, and glucagon to inhibit, HMGR activity is consistent with the effects of these hormones on other metabolic pathways. The basic function of these two hormones is to control the availability and delivery of energy to all cells of the body (Reiter, 1995; Chan and Tang, 1995). Therefore, hormones act by influencing a change in intracellular concentration of cAMP through the adenylate cyclase system. Adenylate cyclase catalyzes the conversion of ATP to cAMP, thus, increasing the intracellular concentration of cAMP. The action of cAMP is mainly to activate some protein kinases (Reiter, 1995).

The effects of caffeine on brain tryptophan levels could be attributed to inhibition of 3-hydroxy anthranilate oxidase, which catalyzes the rate limiting step in the kynurenine-anthranilate pathway, which converts tryptophan (an essential amino acid) into acetyl CoA. 3-hydroxy kynurenine could not be converted to 3-hydroxy-anthranilic acid, resulting in accumulation of kynurenine and 3-hydroxy kynurenine. These products are converted to xanthurenic acid in the extra hepatic tissues, leading to tissue wasting and negative nitrogen balance. Xanthurenic acid excretion in urine is an index of B₆-deficiency and could lead to anemia (Chatterjee and Shinde, 2000).

Mechanism of action of caffeine, therefore, involves interaction with hormones, peptides and

receptors on the surface of the plasma membrane to generate signals, since these molecules cannot cross the plasma membrane (blood-brain-barrier). The amplification and subsequent transmission of such signals to the cell interiors require the participation of a second messenger, usually a cyclic nucleotide, cAMP, while the hormones, peptides or receptors serve as the first messengers (Eteng et al, 1997).

The effect of melatonin on protein levels could be attributed to activation of transcriptional promoter and enhancer elements used for the control of gene expression, which promoted the nucleotide at the initiation stage, thereby increasing protein synthesis and stimulating the gonadotrophin release, estrogen and androgen levels. This could account for use of melatonin for improved sex life.

The effect of melatonin on blood urea nitrogen could be attributed to the antioxidant properties, by stimulating the main antioxidant of the brain, glutathione peroxidase, which neutralized free radicals. This property of melatonin could account for its use for longevity and lowering of blood pressure. The effect of melatonin on cholesterol levels could be attributed to inhibition of the enzyme, HMGR activity, which catalyzes the conversion of HMG-CoA to mevalonate. This enzyme contains SH group and requires NADPH as cofactor (supplied by HMP pathway) in the conversion of SH group to S-S group which increases the hydrophobic properties, thereby distorting the lipid components of the membrane structure and function, resulting in decreased cholesterol synthesis (Chan and Tang, 1995). In this study, melatonin acted synergistically with caffeine, and decreased cholesterol synthesis, and could be used as therapy for hypercholesterolemia. Thus, low levels of melatonin could be a factor in multiple sclerosis, coronary heart disease, sudden infant death syndrome, epilepsy and post menopausal osteoporosis (Sturmer et al, 1990; Brugger et al, 1995; Constantinescu et al, 1995). The menopause is associated with a decline in melatonin secretion and increased calcification (Sandyk, 1994).

The effect of melatonin on tryptophan levels could be attributed to activation of the enzymes, kynureninase and 3-hydroxy anthranilate oxidase, which catalyze the conversion of 3-hydroxy kynurenine to 3-hydroxy anthranilate and 3-hydroxy anthranilate to 2-acrolein-3-amino fumarate, a precursor for the synthesis of niacin and acetyl-CoA. Activation of kynureninase and 3-hydroxy anthranilate oxidase lead to synthesis of thiamin (B₆), a vitamin of B complex, thereby preventing accumulation of xanthurenic acid and pellagra (Chatterjee and Shinde, 2000).

In conclusion, this study has shown that melatonin interacted with caffeine to promote synthesis of proteins, stimulate gonadotrophin release, estrogen and androgen levels, which could be used as oral contraceptive for women, and maybe beneficial in the treatment of impotence (androgen depression), leading to improved reproductive and sex life. The interaction of melatonin and caffeine also stimulated tryptophan metabolism and thereby preventing vitamin B₆ deficiency, anemia, negative nitrogen balance, tissue wasting, and accumulation of xanthurenic acid, thereby promoting sleep. The interaction also promoted the reduction of cholesterol levels which could be beneficial in the treatment of hypercholesterolemia, thereby preventing coronary heart disease and post menopausal osteoporosis.

REFERENCES

- Aldeghi R, Lissoni P, Barni S. 1994. Low-dose interleukin-2 subcutaneous immunotherapy in association with the pineal hormone melatonin as a first time therapy in locally advanced or metastatic hepatocellular carcinoma. *Eur J Cancer*, 30a: 167 – 170.
- Armstrong, S.M. 1989. Melatonin. The internal Zeitgeber of mammals? *Pineal Res. Rev*, 7: 157 – 202.
- Attenburrow, M.E.J, Downing, B.A, Sharpley A.L, Cowen P.J. Case-control study of evening melatonin concentration in primary insomnias. *BMJ* 312: 1263 – 1264.
- Blask D.E, Cos S, Hill S.M. 1991. Melatonin action and oncogenesis. In: Fraschini F, Reiter R.J, eds. *Rose of melatonin and Pineal peptides in Neuroimmunomodulation*. New York: Plenum Press; 233 – 240.
- Brugger P, Marktl W, Herold M. 1995. Impaired nocturnal secretion of melatonin in coronary heart disease. *Lancet*, 345: 1408.
- Brzezinski, A. 1997. Melatonin in humans. *The New England Journal of Medicine*, 336: 186-195.
- Cavallo, A. 1993. Melatonin and human puberty: Current perspectives. *Journal of Pineal Research* 15 (3): 115 – 121.
- Chan T.Y, Tang P.L. 1995. Effect of melatonin on the maintenance of cholesterol homeostasis in the rat. *Endocr Res*, 21: 681 – 696.
- Chatterjee M.N and Shinde R. 2000. Metabolism of proteins and amino acids. In: *Textbook of Medical Biochemistry*, 5th Edition. Vij, J.P and Yadav, R.K (eds.). JAYPEE Brothers Medical publishers (P) Ltd, New Delhi. Pp. 395 – 452.
- Constantinescu, C.S. 1995. Melanin, melatonin, melanocyte-stimulating hormone and the susceptibility to autoimmune demyelination: A rationale for light therapy in multiple sclerosis. *Med. Hypothesis*, 45: 455-458.
- Cos S, Sanchez-Barcelo E.J. 1994. Differences between pulsatile or continuous exposure to melatonin on MCF-7 human breast cancer cell proliferation. *Cancer Lett*, 85: 105 – 109.
- Davidson L and Henry J.B. 1979. Estimation of Tryptophan In: *Clinical Diagnosis by Laboratory methods*. 16th Edition, Davidson and Henry J.B. (eds.). W. B. Saunders, Philadelphia, pp. 157 – 159.
- Eteng, M.U, Eyong, E.U, Akpanyong E.O, Agiang M.A and Aremu C.Y. 1997. Recent Advances in caffeine and Theobromine Toxicities: A Review. *Plant food for Human Nutrition* 51: 231 – 243.
- Garfinkel D, Laudon M, Nof D, Zisapel N. 1995. Improvement of sleep quality in elderly people by controlled release melatonin. *Lancet*, 346/897 (43): 541 – 544.
- Gornall A.G, Bardawill C.J and Maxima d. 1949. Determination of serum proteins by means of the biuret reaction. *Biol. Chem.* 177: 751 – 766.
- Julien R.M. 1996. A primer of Drug Action. 7th Edition. (W.H. Freeman ed.). W.H. Freeman and Co. New York. Pp. 161.
- Kates M. 1986. *Techniques of Lipidology: Isolation, analysis and identification of lipids*. 2nd Edition. Elsevier, New York.
- Lamberg L. 1996. Melatonin potentially useful but safety, efficacy remain uncertain. *The Journal of the American Medical Association*, 276: 1011 – 1014.
- Laudon M, yaron Z, Zisapel N. 1988. N-(2,4-dinitrophenyl)-5-methoxytryptamine, a novel melatonin antagonist: effects on sexual maturation of the male and female rat and on oestrous cycles of the female rat. *J. Endocrinol* 116: 43 – 53.
- Laudon M, Gilead E, Matzkin H. 1996. Putative melatonin receptors in benign human prostate tissue. *J. Clin. Endocrinol Metab*, 39: 1336 – 1342.
- Lerchl A, Nonaka K.O, Stokkan K.A, Reiter, R.J. 1990. Marked rapid alterations in nocturnal pineal serotonin metabolism in mice and rats exposed to weak intermittent magnetic fields. *Biochem. Biophys. Res. Commun*, 169: 102 – 108.
- Lerchl A, Nonaka KO, Reiter RJ. 1991. Pineal gland “magnetosensitivity” to static magnetic fields is a consequence of induced electric currents (eddy currents). *J. Pineal Res*, 10: 109 – 116.
- Lesnikov, V.A, Pierpaoli W. 1994. Pineal cross-transportation (old-to-young and vice versa) as evidence for an endogenous “aging clock”. *Ann NY Acad. Sci*, 719: 456 – 460.

- Lissoni P, Barni S, Ardizoia A. 1992. Randomized study with the pineal hormone melatonin versus supportive care alone in advanced non small cell lung cancer resistant to a first-line chemotherapy containing cisplatin. *Oncology*, 49: 336 – 339.
- Lissoni P, Barni S, Ardizzoia A. 1994. A randomized study with the pineal hormone melatonin versus supportive care alone in patients with brain metastases due to solid neoplasms. *Cancer*, 73: 699 – 701.
- Lissoni P, Barni S, Meregalli S. 1995. Modulation of cancer endocrine therapy by melatonin: A phase II study of tamoxifen plus melatonin in metastatic breast cancer patients progressing under tamoxifen alone. *Br. J. Cancer* 71: 854 – 856.
- Maestroni G.J. 1993. The immunoendocrine role of melatonin. *Journal of Pineal Research* 14 (1): 1 – 10.
- Maurizi C.P. 1985. Could supplementary dietary tryptophan and taurine prevent epileptic seizure? *Med. Hypothesis* 18: 411 – 415.
- Neri B, Fiorelli C, Moroni F, Nicita G, Paoletti M.C, Ponchieati R, Raugei A, Santoni G, Trippitelli A, Grechi G. 1994. Modulation of human lymphoblastoid interferon activity by melatonin in metastatic renal cell carcinoma. *Cancer*, 73: 3015 – 3019.
- Obochi, G.O. 2006. Effect of alcohol-kolanut interaction on Biochemical Indices of Neuronal function and Gene expression in wistar albino rats. A PhD Thesis. University of Calabar, Nigeria.
- Pierpaoli W, Dall'Ara A, Pedrinis E. 1991. The pineal control of aging: the effects of melatonin and pineal grafting on the survival of older mice. *Ann NY Acad. Sci*, 621: 291 – 313.
- Reiter R.J. 1995. Oxidative processes and antioxidative defence mechanisms in the aging brain. *FASEB* 9 (7): 526 – 533.
- Reiter, R.J, Melatoninchiorri D, Sewerynek E, Poeggeler B, Barlow-Wadden L, Chuang J, Ortiz G.G, Accuna-castroviejo D. 1995. A review of the evidence supporting melatonin's role as an antioxidant. *Journal of pineal Research* 18 (1): 1 – 11.
- Sandyk, R. 1994. The relationship between melatonin secretion and serum cholesterol in patients with multiple sclerosis. *Int. J. Neurosci*, 76: 81 -86.
- Sandyk, R, Anastasiadis PG, Anninos PA, Tsagan N. 1992. Is post menopausal osteoporosis related to pineal gland functions? *Int. J. Neurosci*, 62: 215 – 225.
- Sharma, M, Palacios-Bois J, Schwartz G. 1989. Circadian rhythms of melatonin and cortisol in aging. *Biol. Psychiatry*, 25: 305 – 313.
- Stokkan, K.A Reiter R..J, Nonaka K.O. 1991. Food restriction retards aging of the pineal gland. *Brain Res.* 545: 66 – 72.
- Sturner, W.Q, Lynch H.J, Deng M.H, Wurtman R.J. 1990. Melatonin levels in the sudden infant death syndrome. *Forensic Sci. Int.* 45: 171 – 180.
- Tan, D.X, Chen LD, Poeggeler B. 1993. Melatonin: A potent, endogenous hydroxyl radical scavenger. *Endocr. J*, 1: 57 – 60.
- Terzolo, M, Revelli A, Guidetti D. 1993. Evening administration of melatonin enhances the pulsatile secretion of prolactin but not of LH and TSH in normally cycling women. *Clin. Endocrinol*, 39: 185 – 191.
- Voordouw, G.C, Euser R, Verdonk RE, Alberda BD, de Jong Fh, Drogendijk AC, Fauser BC, Cohen M. 1992. Melatonin and melatonin-progestin combinations alters pituitary ovarian function in women and can inhibit ovulation. *Journal of clinical Endocrinology and metabolism* 74 (1): 108 – 117.
- Webb, S.M, Pulg-Domingo M. 1995. Role of melatonin in health and disease. *Clin. Endocrinol*, 42: 221 – 234.