International Journal of Pharmacology and Clinical Research
Online ISSN: 2664-7621; Print ISSN: 2664-7613
Received: 03-03-2019; Accepted: 04-04-2019; Published: 11-04-2019
www.pharmacologyjournal.in
Volume 1; Issue 1; 2019; Page No. 12-24

Melatonin reduces toxicity induced by HAART in mice and HIV patients

JB Borges[1], FR Nerone[2], GF Messias[3], ALF Martins[4], M Spack Jr[5], ART Pupulin[6*]
[1-5] Department of Basic Health Sciences, State University of Maringa, Maringá, PR, Brazil
[6] Dra Áurea Regina T Pupulin. Av. Colombo, Maringá-Pr Brazil

DOI: https://doi.org/10.33545/26647613.2019.v1.i1a.3

Abstract
Aims: Highly Active Antiretroviral Therapy (HAART) is the current care standard for treating patients with HIV/AIDS. Although HAART has is the only regimen potent enough to decrease viral load, adverse events may limit its efficacy. HAART-associated major toxic effects: neuropathy, myopathy, pancreatitis, hepatic steatosis, lactic acidosis and lipoatrophy, metabolic complications (insulin resistance and hyperlipidemia).Melatonin (N-acetyl-5-methoxytryptamine), the neuro-hormone synthetized during the night, has seen an unexpected extension of its functional implications toward type 2 diabetes development, sleep disturbances and depression. Melatonin has been shown to reduce the toxicity and increase the efficacy of a large number of drugs.
Objective: This study evaluated the effects of melatonin supplementation (6mg / day / 30 days) in mice treated with antiretroviral therapy and AIDS patients using antiretroviral therapy (HAART).
Material and Methods: For animal experiments mice were divided into experimental groups with 12 animals each: (I) animals treated with antiretroviral therapy for 15 days, (II) animals untreated animals treated with antiretroviral therapy and melatonin 6 mg/kg/day for 15 days, (III) untreated animals. Body weight, water intake and ration, excretion products and behavior were clinically assessed before and after treatment; further, serum cholesterol, triglycerides, hepatic enzymes (AST, ALT, GGT), creatinine, were evaluated by specific methods. For patient evaluation the study was carried out in a double-blind, placebo-controlled and completely randomized design. AIDS patients who had metabolic alterations were selected. Patients were divided into two groups: Group I (HAART) consisted of patients receiving placebo once a day in the evening. Group II (HAART+ Melatonin) comprised patients who received the melatonin (6 mg) once a day in the evening for one month. Clinical, emotional and laboratorial evaluation was performed before and after 30 days. Clinical evaluation was performed to assess the patients´ overall clinical state. Patients were instructed to report any complications. Laboratorial evaluation was performed. Glucose levels were determined by glucose oxidase method and ELISA (Genway Biotechnology, USA), respectively, following manufacturer's instructions. Plasma levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and gamma glutamyl transferase (GGT) were performed by the kinetic colorimetric method; triglycerides, total cholesterol and creatinine were performed by enzymatic colorimetric method, both provided by Gold Analisa Diagnóstica Ltda.
Results: Animals treated with antiretroviral therapy and melatonin (II) had higher body weight gain, less hepatomegaly, less anxiety, lower levels of triglycerides, cholesterol and hepatic enzymes when compared to animals treated with antiretroviral therapy. Current study revealed that 40% (12/30) of the patients had changes in AST liver enzymes (> 38 U/I), 30% (9/30) had changes in ALT levels (> 38 U/I) and 30% (9/30) had GGT levels (> 40 U/I). Results obtained after the use of melatonin suggest melatonin activity on the liver. Significant differences between groups in plasma cholesterol indicate that melatonin exerted better improvement of blood lipid composition. Melatonin would lower cholesterol in liver and decrease plasma cholesterol. Above all, melatonin could decrease oxidative stress and improve dyslipidemia.
Conclusion: Considering the low toxicity of melatonin and its ability to reduce the side effects and increase the efficacy of the drugs, its use may be important and significant as a combination therapy with HAART. Current study which investigated the effect of melatonin associated with antiretroviral treatment demonstrated that melatonin reduces toxicity induced by HAART. Melatonin improves metabolic abnormalities and emotional disturbs in animals and patients.

Keywords: melatonin, HIV/AIDS, antiretroviral, toxicity

1. Introduction
Highly Active Antiretroviral Therapy (HAART) is the current care standard for treating patients with HIV/ AIDS. Although HAART is the only regimen potent enough for viral decrease, its efficacy may be limited by adverse events. Nucleoside reverse transcriptase inhibitors may cause mitochondrial toxicity and anemia, non-nucleoside reverse transcriptase inhibitors are associated with rash and central nervous system disturbance, protease inhibitors elicit gastrointestinal adverse effects and metabolic abnormalities including lipodystrophy syndrome, hyperlipidemia and insulin resistance [1, 2]. The above complications significantly increase morbidity and mortality in those requiring long-term treatment for HIV-infection. Moreover, abnormalities also impact adherence to treatment [3].
Prevalence of cardiovascular diseases in HIV patients

increases with aging and duration of the disease. Hypertension, high cholesterol level, obesity, diabetes, tobacco and use of alcohol are among the traditional risk factors that contribute towards cardiovascular diseases [4]. Three-year outcome antiretroviral therapy in adult's demonstrated good virological response featuring high toxicity rates [5].

Severe hepatotoxicity in HIV-infected patients treated with highly active antiretroviral therapy occurs and mortality is significantly higher in individuals with pre-existing liver disease [6]. Liver transaminases should be closely monitored after the start of highly active antiretroviral therapy [7]. Light to moderate transaminase rise may improve under continued therapy, but life-threatening liver injury associated with jaundice or high transaminase rates above 10 times the upper normal limit makes the de-continuation of antiretroviral therapy mandatory [8].

Hepatotoxicity has been associated with the use of human immunodeficiency virus-protease inhibitors. However, the complexity of the HIV-infected patient´s conditions and the combination of medicines to treat HIV complicate the evaluation of the effects of drug-induced liver injury [9].

A retrospective cohort study investigated whether particular antiretroviral agents were associated with a higher risk for the development of grade 4 liver enzyme elevations in patients with human immunodeficiency virus type 1 infection who started receiving HAART. Regimen of nevirapine or high-dose ritonavir was a risk factor [10].

Melatonin (N-acetyl-5-methoxytryptamine) is a molecule with a very wide phylogenetic distribution from plants to man. In vertebrates, melatonin was initially thought to have an exclusive pineal origin but recent studies have shown that melatonin synthesis may occur in a variety of cells and organs. It has been shown that melatonin has several functions and research during the last decade has proven the indole to be a direct free radical scavenger and indirect antioxidant [. Due to the above activities and others that may be defined in the future, melatonin reduces toxicity and increases the efficacy of a large number of many side effects, as documented in [11, 12, 13, 14].

Melatonin was chosen for its antioxidant and anti-apoptotic activity on renal tissue [15] and injury on myocardic cells [16]. Melatonin reduces obesity and improves the metabolic profile in experimental model. Melatonin also lowers mitochondrial oxidative status by reducing nitrite levels and by increasing superoxide dismutase activity. The above results demonstrate that chronic oral melatonin improves mitochondrial respiration and reduces the oxidative status and susceptibility to apoptosis in white and beige adipocytes [17].

There are many beneficial effects of melatonin when combined with the following drugs: doxorubicin, cisplatin, epirubicin, cytarabine, bleomycin, gentamicin, cyclosporine, indomethacin, acetylsalicylic acid, ranitidine, omeprazole, isoniazid, iron and erythropoietin, phenobarbital, carbamazepine, haloperidol, capside-50, morphine, cyclophosphamide and L-cysteine [18]. While most studies were conducted on animals, several investigations were also done on humans [19].

Many physiological and pathological conditions may alter melatonin levels. Decrease in indoleamine levels has been reported in subjects with low tryptophan intake, and people suffering from insomnia, depression, coronary heart disease, rheumatoid arthritis and liver cirrhosis [20, 21].

Therapies based on the administration of melatonin in high concentrations result in different modulations in the immune response [22], such as increased proliferation of T-lymphocytes, the antigen provided by macrophages and phagocytic activity of these defense cells; increase in the activity of lymphoid cell system, spleen and bone marrow [23]; stimulation of several cytokines synthesis such as IL-2, IFN-γ and IL-6, and the regulation of nitric oxide synthesis by endothelial cells [24].

Finally, the number of mechanisms for melatonin's reduction of molecular destruction and cellular dysfunction due to oxygen-and nitrogen-based reactants is extensive. These activities have been registered in in vitro and in vivo conditions.

Considering the low toxicity of melatonin and its ability to reduce the side effects and increase the efficacy of some drugs, its use as a combination therapy with these agents seems important [18].

Current study evaluates the effect of the use of oral melatonin in mice undergoing HAART therapy and the effect of the use of oral melatonin in HIV patients undergoing antiretroviral therapy.

2. Material and Methods

2.1 Animal experiments

2.1.1 Animals

Four-week-old male Swiss Webster mice, weighing approximately 28-30 g, retrieved from the Central Animal Laboratory of the State University of Maringá, were used for the experiments. Protocol n. 7968200115/2015 for the experiments was approved by the Committee for Ethics in Animal Experiments/ State University of Maringa.

Animals, kept in cages with food and water *ad libitum*, were monitored daily for 7 days for clinical assessment. The animals were kept in a vivarium of the Laboratory of Parasitology / DBS/UEM under best temperature conditions at 22ºC ± 2ºC, 70% humidity and photoperiod (light / dark cycle 12 h).

2.1.2 Preparation of Melatonin

Melatonin® 3 mg Spring Valley was administered at a 6mg / kg dose diluted in water: 0.2 ml were injected intragastrically in each animal. Treatment was performed in the morning due to lower plasma melatonin levels.

2.1.3 Preparation of HAART

Protocol was based on a standard therapeutic regimen of patients from Brazil. The calculation of dose for humans was proportional to that in animals. The animals received treatment for 5 mg / kg atazanavir sulfate, 5 mg / kg tenofovir disoproxil fumarate, 1.67mg / kg ritonavir and 2.5mg / kg lamivudine, diluted in 1.2mL of water.

The treatment period was 15 days and the drug was dispensed always at 9 a.m.

2.1.4 Treatment Schedule

Each experimental group comprised 12 animals: (I) animals

treated with HAART diluted in 0.2 mL water by gavage + water 0.2mL by gavage/day; (II) animals treated with HAART diluted in 0.2 mL water gavage/day + Melatonin in water 0.2 mL once a day by gavage, (III) untreated (control group) received 0.2 mL water + 0.2 mL water by gavage/day. The experimental groups were treated for 15 days.

2.1.5 Evaluation

2.1.5.1 Assessment of body weight

Animals were weighed in semi-analytical balance BL320H Mars Shimadzu before the start of treatment and at the end of the experiment. The results were expressed in mean groups.

2.1.5.2 Clinical evaluation

Qualitative parameters such as physical appearance of animals during the treatment period, hair bristling and irritability were assessed. Feed and water intake by the animals were measured until the end of the experiment. Excreta production was evaluated by weighing shaver on alternate days.

2.1.5.3 Laboratory evaluation

Plasma levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and gamma glutamyl transferase (GGT) were measured by kinetic colorimetric method; triglycerides, total cholesterol and creatinine were calculated by the enzymatic colorimetric method, both provided by GOLD ANALISA DIAGNÓSTICA LTDA.

2.1.5.4 Macroscopic evaluation of organs (liver and spleen): Liver and the spleen of all animals were examined macroscopically and weighed at the end of the experiments.

2.1.5.5 Emotional assessment

The open field method was employed to evaluate emotional assessment. Animals were kept in a quiet environment, in a low light and at room temperature one hour before evaluation. Each animal was placed in the center of an arena, 40 cm in diameter and 30 cm high, and its behavior was recorded for 5 minutes using a video camera connected to a computer located in a separate room. The videos were converted by the program Format Factory 3.0 and later analyzed by Noldus Etho-Vision® software, for motor and exploratory activity of animals, assessing distance from the edge (cm), moves away (cm), velocity (cm / s) and motion (%).

2.2 Patients Experiments

2.2.1 Subjects

HIV / AIDS patients were treated at the Center for Studies and Support to HIV Patients of the State University of Maringá - Department of Basic Health Sciences. The Center attends approximately 200 patients who freely seek the sector to participate in the project. All patients are evaluated clinically and laboratory tests are performed prior to their participation in the projects.

2.2.2 Study Model

Current study was carried out in a double-blind, placebo-controlled and completely randomized design. AIDS patients who had metabolic alterations were selected. The patients were divided into two groups: Group I (HAART) consisted of patients receiving placebo once a day, in the evening. Group II (HAART+ Melatonin) comprised patients who received melatonin (6mg) once a day, in the evening, for one month.

2.2.3 Preparation of Melatonin

Melatonin® 3 mg was manipulated by Rexall Sundown, USA and dispensed by Medformula/Maringá/Brazil.

2.2.4 Antiretroviral Therapy

The therapeutic scheme follows the standard protocol used in Brazil (ritonavir 100mg+zidovudine 300mg+lamivudine 150 mg+ atazanavir 300 mg/day).

2.2.5 Clinical and Laboratorial Evaluation

Clinical and laboratorial evaluation was performed before and after 30 days

2.2.5.1 Assessment of body weight

Patients were weighed on scale Sensimax 130 before the start of treatment and at the end of the experiment.

2.2.5.2 Clinical evaluation

Clinical evaluation was performed to assess the overall clinical state of the patient and patients were instructed to report any complications.

2.2.5.3 Laboratory evaluation

After an approximately 10-h overnight fast, 5 ml of venous blood were obtained from each patient. Glucose levels were determined respectively by glucose oxidase method and ELISA (Genway Biotechnology, USA), following the manufacturer's instructions. Plasma levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and gamma glutamyl transferase (GGT) were assessed by the kinetic colorimetric method; triglycerides, total cholesterol and creatinine were evaluated by the enzymatic colorimetric method, both provided by Gold Analisa Diagnóstica Ltda.

2.2.5.4 Emotional assessment

Hamilton (1959) [25], retrieved from the *Guide to the Use of Assessment Tools: Detecting Depression* (*and Anxiety*) *in the Elderly Patient*, was used to evaluate and measure depression. Hamilton Scale covers items related to depressed mood, guilt, suicide, insomnia (initial, intermediate, late), work and other activities, retardation, agitation, anxiety (psychic, somatic, gastrointestinal), general somatic symptoms, hypochondria, loss of weight, awareness, depersonalization, loss of reality and rancid symptoms, obsessive and compulsive symptoms. Each item is scored according to Likert scale, ranging from zero to four, and interpreted as a total score: score 8 to 13 indicates mild depression; 14 to18, moderate depression; 19 to 22, severe depression; over 23, very severe depression Montgomery and Asberg depression scale (MADRS) [26] was applied as a confirmatory test, with scores for each item, ranging between zero and six (one, three and five were intermediate values). Total score from 13 to 24 indicated mild depression; from 25 to 30, moderate depression; from 31 to 43, a worsening depression; score over 44 indicated very severe depression. Each instrument was administered by a single evaluator, which ensured uniformity in contact and in

data collection. The interviews, with an average duration of twenty minutes, were individual and agreed to on a previously prepared schedule. Analyses were performed according to the standardization and validation of each instrument.

2.2.5.5. Sleep Quality Assessment: Was assessed through the Pittsburgh Sleep Quality Index Questionnaire (PSQI) [27]. The PSQI assesses sleep quality and disturbances over a period of one month, being a standardized questionnaire, simple and well accepted by patients. The instrument consists of 19 self-report questions and five questions directed to the spouse or roommate. The last five questions are used for clinical practice only, not contributing to the total score of the index. The 19 questions are categorized into seven components, graded in scores from zero (no difficulty) to three (severe difficulty). The components of PSQI are: C1 subjective sleep quality, C2 sleep latency, C3 sleep duration, C4 habitual sleep efficiency, C5 sleep changes, C6 use of sleeping medication C7 daytime sleep dysfunction. The sum of the values attributed to the seven components varies from zero to twenty-one in the total score of the questionnaire indicating that the higher the number, the worse the quality of sleep. A total score greater than five indicates that the individual is presenting major dysfunction in at least two components, or moderate dysfunction in at least three components.

Statistical Analysis

Group-comparing statistics were performed with Graph Pad Prism 6.0 (Graph Pad, San Diego, CA, USA) by Student´s *t* test; p values <0.05 were statistically significant.

3. Results

3.1 Animals experiments

Figure 1 shows the results of melatonin on weight gain of mice submitted to HAART and Figure 2 shows the consumption of water and feed by these animals.

Results in current experiments demonstrate lower weight gain, or rather, 90% less (p=0.006) in the group treated with HAART, whereas the group treated with HAART and melatonin showed 43% less weight gain as that of control group.

Figure 2 revealed that intake of food and water rates in the group treated with HAART + Melatonin was close to control group, while the group treated with HAART revealed a significant difference from the others (p = 0.02).

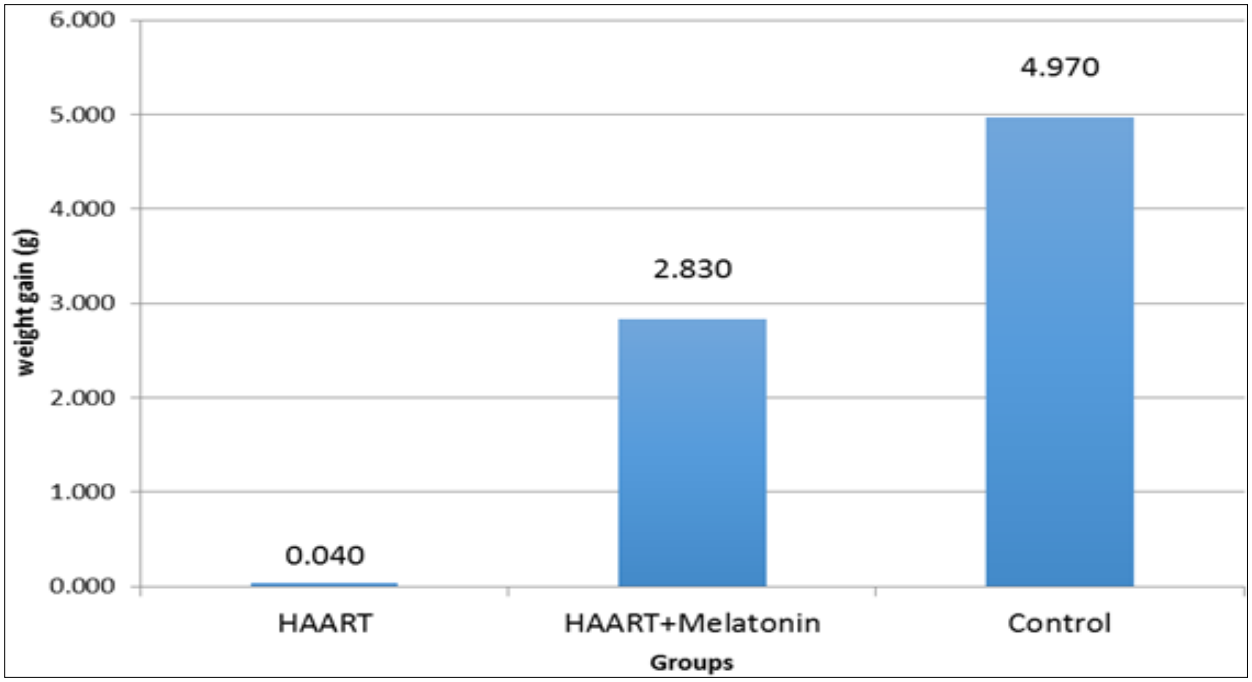

Fig 1: Weight gain (g) of Swiss mice in experimental and control groups after 15 days of treatment.

Comparison between the experimental groups: Group I treated with HAART (5mg / kg atazanavir + 5mg / kg tenofovir disoproxil fumarate + 1.67mg / kg ritonavir + 2.5mg / kg lamivudine, diluted in 1.2mL of water/day/15 days; Group II treated with HAART (5mg / kg atazanavir + 5mg / kg tenofovir disoproxil fumarate + 1.67mg / kg ritonavir + 2.5mg / kg lamivudine, diluted in 1.2 mL of water + melatonin 6mg/Kg /day/15 days; Group III: non-treated group (control group). Results are expressed by mean ± SD of 12 animals.

Figure 2 shows ration (g) and water (mL) consumption in Swiss mice on the experimental and control groups after 15 days of treatment

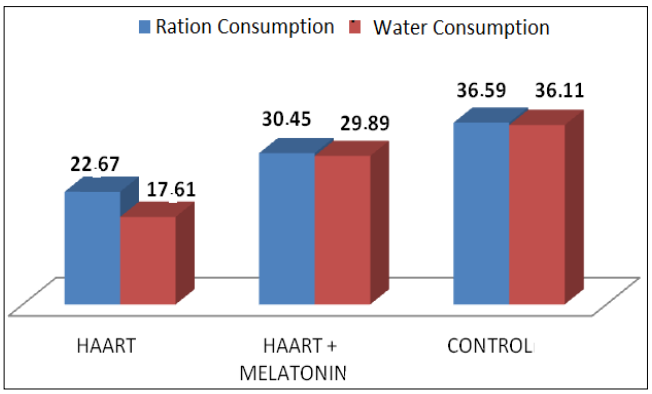

Fig 2: Ration (g) and water consumption (mL) of Swiss mice after 15 days.

Comparison between experimental groups: Group I treated with HAART (5mg / kg atazanavir + 5mg / kg tenofovir disoproxil fumarate + 1.67mg / kg ritonavir+ 2.5mg / kg lamivudine, diluted in 1.2mL water/day/15 days; Group II treated with HAART (5mg / kg atazanavir + 5mg / kg tenofovir disoproxil fumarate + 1.67mg / kg ritonavir + 2.5mg / kg lamivudine, diluted in 1.2 mL of water + melatonin 6mg/kg /day/15 days; Group III: non-treated group (control group). Results are expressed by mean ± SD of 12 animals.
Table 1 and Figure 3 show emotional assessment results of the experimental groups.

Table 1: Motor and exploratory activity of Swiss mice in the experimental and control groups after 15 days of treatment.

Parameters	Haart	Haart+Melatonin	Control
Distance from the edge (cm)	7.11 ± 0.99	5.73 ± 1.15*	7.15 ± 3.08
Moved away (cm)	416.41 ± 162.97*	125.31 ± 133.06*	122.48 ± 73.91
Velocity(cm/s)	7.55 ± 1.05	7.42 ± 1.40	6.88 ± 0.67
Motion (%)	85.65 ± 10.70*	66.42 ± 6.79*	68.56 ± 4.19

Rates = mean ± SD, n=12 per group. Group I treated with HAART (5mg / kg atazanavir+ 5mg / kg tenofovir disoproxil fumarate + 1.67mg / kg ritonavir+ 2.5mg / kg lamivudine, diluted in 1.2mL of water/day/15 days; Group II treated with HAART (5mg / kg atazanavir + 5mg / kg tenofovir disoproxil fumarate + 1.67mg / kg ritonavir + 2.5mg / kg lamivudine, diluted in 1.2 mL of water + melatonin 6mg/Kg /day/15 days; Group III non-treated group (control group).* p>0.05

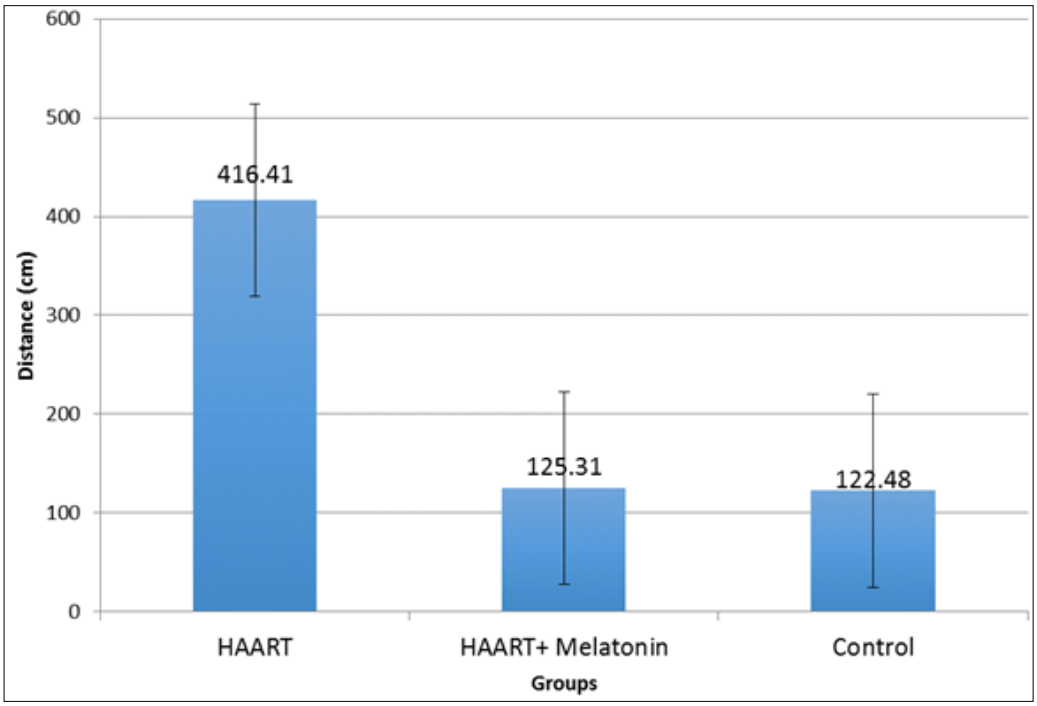

Fig 3: Distance traveled (cm) by Swiss mice. The distance traveled corresponds to degree of anxiety and was reported by video tracking software Noldus Etho-Vision®.

Comparison between the experimental groups Group I treated with HAART (5mg / kg atazanavir + 5mg / kg tenofovir disoproxil fumarate + 1.67mg / kg ritonavir + 2.5mg / kg of lamivudine, diluted in 1.2mL of water/day/15 days; Group II treated with HAART (5mg / kg of atazanavir + 5mg / kg of tenofovir disoproxil fumarate + 1.67mg / kg of ritonavir + 2.5mg / kg lamivudine, diluted in 1.2 mL of water + melatonin 6mg/Kg /day/15 days; Group III non-treated group (control group). Results are expressed by mean ± SD of 12 animals.
Behavioral assessment was performed by an open field test on

the 15th day of treatment in a specific place intended for this type of analysis and performed by a single handler. Test consisted of measuring behavioral variables for effects of stress and anxiety. Analysis of the open field activity parameters comprised: distance from edge (corresponds to exploratory capacity); total distance (corresponds to the distance spent by the animal in the act of placing the four paws in one of the divisions of the arena, that is, total mobility of the mouse (cm); mean speed: average speed traveled by the animal in the arena (cm / s); moving time: the time the animal moved in the area of the open field arena (%).

Table 1 reveals a significant difference with regard to the parameter "distance from the edge" in the melatonin-treated group (group II) compared to the group treated only with HAART (group I) and control group (group III), with a decrease of 24% and 20% respectively.

In the case of total distance moved by the animals in the open field, there was a reduction of 232% in the group treated with HAART + melatonin when compared with the HAART-treated group.

Total distance traveled corresponds to positive and negative symptom spectrum (hyperactivity, curiosity, anxiety) and may be monitored. Results in Figure 3 demonstrate that animals treated with melatonin moved in the same way as control animals, whereas HAART-treated animals have higher rates demonstrating higher levels of anxiety or hyperactivity.

Table 2 shows the evaluation of laboratory parameters in the experimental groups.

Table 2: Laboratory parameters in the experimental groups

Group Parameters	Haart	Haart+ Melatonin	Control
Total Cholesterol (mg/dL)	95.15± 23.79	94.43±15.68	98.60±11.36
Tryglicerides (mg/dL)	218.80±55.54	185.20±37.47	199.60±50.96
AST (U/l)	105.50±27.41	97.16±32.74	90.20±39.00
ALT (U/l)	95.97±19.94	75.22±82.93	79.11±16.83

Values are mean ± SD, n=12 per group. Group I treated with HAART (5mg / kg of atazanavir+ 5mg / kg of tenofovir disoproxil fumarate+ 1.67mg / kg of ritonavir+ 2.5mg / kg of lamivudine, diluted with 1.2mL of water/day/15 days; Group II treated with HAART (5mg / kg of atazanavir+ 5mg / kg of tenofovir disoproxil fumarate + 1.67mg / kg of ritonavir + 2.5mg / kg of lamivudine, diluted with 1.2 mL of water + melatonin 6mg/Kg /day/15 days; Group III non-treated group (control group)

HAART increases lipids in the bloodstream and reduces the peripheral storage of these molecules which, accumulated in the plasma and associated to arterial inflammation from HIV infection, may clog the arteries and facilitate the formation of fat plaques, leading to the development of atherosclerosis and its complications, such as myocardial infarction and peripheral vascular disease. Current study demonstrated that the concomitant use of melatonin and HAART enhanced an 18% decrease in triglycerides levels when compared to the group treated with HAART.

AST and ALT are intracellular enzymes present in large amounts in the cytoplasm of hepatocytes. When liver cells are injured or destroyed, these enzymes are released into the circulation. ALT is mainly found in the cytoplasm of the hepatocyte, while 80% of AST are present in mitochondria. In light hepatocellular damage, cytoplasmic enzyme (ALT) is predominantly found in the serum. However, mitochondrial enzyme (AST) is released in serious injury. The dosage of enzymes AST and ALT was used to indicate the extent of liver cell damage in these experiments. Current results demonstrate a reduction in plasma levels of AST and ALT in animals treated with HAART + melatonin and suggest a direct effect on the liver damage by HAART.

3.2 Patients Experiments

Sixty patients with certain metabolic abnormalities (glucose levels above 100.0 mg/ dL or total cholesterol above 200mg/dL or triglycerides above 200mg/dL) participated. All patients had been using HAART therapy for at least five years and their average infection period was 15 years. Patients´ age ranged between 35 and 49 years, with mean 43.7 years. Patients were separated into two groups (treated with and without melatonin) with 30 patients each, in a paired form for age and gender. All patients were followed up by a clinical evaluation during the 30 days of treatment.

Effect of Melatonin on Fasting Blood Glucose

Diabetes and changes in glucose tolerance are common in adult populations.

They are associated with increased mortality from cardiovascular disease and microvascular complications. The diagnosis may be made early. Fasting plasma glucose measurement is used as a fast and easy test, with altered rates when higher than 110mg / dL. On the other hand, rates over 100mg / dL should be monitored. The introduction of antiretroviral therapy has triggered the emergence of some metabolic complications, including hyperglycemia and diabetes mellitus, among HIV-positive patients. Hyperglycemia, especially pre-diabetes is very frequent among people with HIV. Forty-three percent of patients (13/30) in current study presented changes in glycemic levels (> 100mg / dL) at the start of the study.

Table 3 shows fasting blood glucose, was significantly lower in subjects contained in Group I treated with melatonin when compared to subjects included in the control group who were not treated with melatonin after one month of treatment. Levels of blood glucose were 23% lower in patients who used melatonin reference values after one month of treatment

Table 3: Blood glucose levels in HIV/AIDS patients treated by antiretroviral therapy with and without melatonin

Patients HIV/AIDS	Glucose Levels (mg/dL)Before 30days	After 30 days	p
HAART	107 ± 15.53	113.8± 11.1	0.5486
HAART + melatonin	105 ± 17.76	80.8 ± 7.7*	0.0264

Table 3: Glucose levels in the experimental groups after 30 days. Comparison between experimental groups: Group I treated with HAART (ritonavir 100mg+zidovudine 300mg+lamivudine 150 mg+ atazanavir 300 mg/day); Group II treated with HAART (ritonavir 100mg+zidovudine 300mg+lamivudine 150 mg+ atazanavir 300 mg/day) + Melatonin 6mg, once a day. Results are given as mean ± SD of 30 patients. *p<0.05

Our results demonstrate a beneficial effect of melatonin on blood glucose levels in patients using HAART therapy.

Effect of the Melatonin on Hepatic Enzymes

The clinical use of lopinavir/ritonavir as a component of antiretroviral regimens was found to be associated with the occurrence of hepatotoxicity ranging between 1% and 9.5% in clinical trials. Hepatotoxicity, characterized by increase in hepatocellular cytolysis rates and significant increases in serum transaminase levels, is a common complication in HIV-positive patients receiving HAART. An increase in hepatic enzymes has been registered in about 6% to 30% of patients receiving antiretroviral therapy. Severe hepatotoxicity (defined as an increase in transaminases levels five times greater than the normal limit) has been reported in less than 10% of patients receiving treatment [28].

In current study, 40% (12/30) of patients had changes in AST liver enzymes (> 38 U/I); 30% (9/30) had changes in ALT levels (> 38 U/I); and 30% (9/30) in GGT levels (> 40 U/I). Results obtained after the use of melatonin suggest the impact of melatonin on the liver (table 2).

Table 4: Hepatic enzymes in HIV/AIDS patients treated by antiretroviral therapy with and without melatonin

Patients HIV/AIDS	Hepatic enzymes					
	AST(U/I) Before 30days	After 30 days	ALT(U/I) Before 30days	After 30 days	GGT(U/I) Before 30days	After 30 days
HAART	57.33±13.19	64.21±33.43	37.33±7.34	36.67±15.7	51.29±14.28	58.14±35.73
HAART + melatonin	41.79±5.17	24.85±3.48*	34.03±5.15	25.34±1.7*	45.93±6.37	22.10±19.46

Table 4: Hepatic enzymes in experimental groups after 30 days. Comparison between experimental groups: Group I treated with HAART (ritonavir 100mg+zidovudine 300mg+lamivudine 150 mg+ atazanavir 300 mg/day); Group II treated with HAART (ritonavir 100mg+zidovudine 300mg+lamivudine 150 mg+ atazanavir 300 mg/day) + Melatonin 6mg, once a day. Results are given as mean ± SD of 30 patients. *p<0.05

The liver is a vital organ of the human body and is responsible for several fundamental and important roles, including digestive and excretory functions, nutrient storage and metabolic functions, synthesis of new molecules and purification of toxic chemical. Liver steatosis, fatty liver, hepatitis, fibrosis, cirrhosis and hepatocellular carcinoma are the most prevalent liver diseases. They have also been investigated extensively.

The results of this study demonstrate an improvement in liver enzyme levels in patients using melatonin.

Effect of the Melatonin on Lipid profile

Diabetes and elevated triglycerides were the metabolic syndrome components most strongly linked with global increase in HIV patients. In current study, 83% of patients (25/30) presented changes in triglycerides levels (> 200mg / dL) and 76% (23/30) presented changes in total cholesterol levels (> 200mg / dL) at the start of the study.

Table 5: Metabolic parameters in HIV/AIDS patients treated by antiretroviral therapy with and without melatonin

Patients HIV/AIDS	Lipid profile					
	Total Cholesterol (mg/dL) Before 30days	After 30 days	p	Triglycerides (mg/dL) Before 30days	After 30 days	P
HAART	255.3±18.37	261.7±43.3	0.7310	364.8±49.84	353.7±88.63	0.8240
HAART + melatonin	205.0±14.29	157.7±9.34*	0.0102	272.0±19.45	242.8±18.49	0.2910

Table 5: Lipid profile in the experimental groups after 30 days. Comparison between experimental groups: Group I treated with HAART (ritonavir 100mg+zidovudine 300mg+lamivudine 150 mg+ atazanavir 300 mg/day).; Group II treated with HAART (ritonavir 100mg+zidovudine 300mg+lamivudine 150 mg+ atazanavir 300 mg/day) + Melatonin 6mg, once a day. Results are given as mean ± SD of 30 patients. *p<0.05

Table 5 shows significant differences between groups in plasma cholesterol and indicates that melatonin exerts better improvement of blood lipid composition. Melatonin may lower cholesterol in liver and decrease plasma cholesterol. Above all, melatonin may decrease oxidative stress and improve dyslipidemia.

Effect of Melatonin on Renal Function

Acute kidney and chronic kidney diseases are more common in the HIV-infected population than in the general population. Renal dysfunction is common in HIV-positive patients who receive antiretroviral therapy. Glomerular and tubular diseases are often identified in HIV-infected patients.

In current study, 10% of patients (3/30) presented high creatinine levels (> 1.2mg / dL) at the start of the study. The authors would like to highlight a patient who had presented a rate of 5.25mg/dL. Rates were within the normal range (1.1mg/dL) after the use of melatonin (table 6).

Table 6: Renal Function in HIV/AIDS patients treated by antiretroviral therapy with and without melatonin

Patients HIV/AIDS	Renal function		
	Creatinine(mg/dL) Before 30days	After 30 days	p
HAART	0.8567±0.11	0.8913±0.25	0.7564
HAART + melatonin	1.471±0.44	1.135±0.57*	0.0482

Table 6: Creatinine levels in the experimental groups after 30 days. Comparison between experimental groups: Group I treated with HAART (ritonavir 100mg+zidovudine

300mg+lamivudine 150 mg+ atazanavir 300 mg/day); Group II treated with HAART (ritonavir 100mg+zidovudine 300mg+lamivudine 150 mg+ atazanavir 300 mg/day) + Melatonin 6mg, once a day. Results are given as mean ± SD of 30 patients. *p<0.05

Effect of Melatonin on Emotional Parameters

The evaluation of depression indices showed that 23% (7/30) of patients had a scale score compatible with mild depression (8-13 points) and 16% (5/30) with moderate depression (14-18 points). At the end of treatment, all participants showed improvement in the depression parameter. Patients who previously had mild depression scored below 8 points and those who presented with moderate depression scored between 8 and 13, configuring mild depression. These results suggest that melatonin has a positive effect on depression in patients using HAART therapy.

Effect of Melatonin on Sleep Quality

After the use of melatonin for 30 consecutive days, it was observed that 30% (9/30) of participants had between 30 minutes and one hour more sleep and 16% (5/30) reported 4 or 5 hours more sleep. Fewer sleep interruptions per night were observed in 30% (9/30) and minus two interruptions in 30% (9/30) of participants. As for dreams, 30% (9/30) of participants reported having fewer dreams / nightmares and 16% (5/30) had more nightmares. Only 1 participant reported increased daytime sleepiness and 46% (14/30) reported feeling more willing during the day.

4. Discussion

Besides providing health benefits, HAART may have a negative impact on the patient's life quality. Identification and treatment of these complications have important implications on patient survival.

Long-term complications of this disease are multifactorial and can be related to the virus itself or to adverse effects of antiretroviral therapy. Each drug class has side effects: nucleoside/nucleotide reverse transcriptase inhibitors are associated with lactic acidosis, lipodystrophy and hyperlipidemia, non-nucleoside reverse transcriptase inhibitors are associaterd with meuropsychiatric symptons, rash, liver toxicity, and lipid abnormalities and protease inhibitors are associated with gastrointestinal intolerance and glucose and lipid abnormalities [36].

The results obtained in this study in both animals and humans demonstrate a direct effect of HAART inducing significant metabolic changes.

Melatonin has been shown to have beneficial effects when combined with several drugs. Studies were conducted with animals and humans [17]. Considering low toxicity of melatonin and its ability to reduce the side effects and increase the efficacy of these drugs, its use as a combination therapy with HAART may be important and significant. Current study investigates the effect of melatonin associated with antiretroviral treatment in animals and humans demonstrated a beneficial effect.

Results in animals experiments demonstrate lower weight gain in the group treated with HAART, whereas the group treated with HAART and melatonin showed weight gain as that of control group. Similarly water and food intake (figure 2) revealed that intake of food and water rates in the group treated with HAART + Melatonin was close to control group, while the group treated with HAART revealed a significant difference from the others.

Melatonin may be found in the intestinal lumen at higher concentrations than those in nocturnal peak of the substance in the circulation. Such high concentrations of melatonin in the intestinal lumen derives from various sources, namely, pineal from the circulation itself, extra pineal from bile which also has high concentrations of the hormone, and food which contain melatonin [37]. Melatonin in the digestive tract, especially in the large intestine, is related to modulation of intestinal motility, intestinal regeneration processes and especially antioxidants [38]. Current study demonstrates that exogenous melatonin use associated to HAART is beneficial (Figure 1 and 2) since it decreases the effects of gastrointestinal intolerance induced by HAART [39].

Several studies demonstrate neuroprotective effect of melatonin in mice with cognitive deficits and anxiety [40], prevention of delirium following cardiac surgery [41], melatonin-improved sleep in post-cardiac surgery patients more than that observed with oxazepam [42]. Current results of experiments with mice undergoing HAART + melatonin demonstrate possible protective effect of melatonin which may be explained by its antioxidant and neuroprotective effects.

The percentage of depression detected in this population was high but it is within the expected levels for this population. A depressed patient tends not to take prescribed medications and to disregard medical advice, in addition to the increased risk of suicide.

Poor sleep quality has a prevalence of 8-18% in the general population and 50-70% in the elderly population is strongly associated with cardiovascular disease and the total mortality [43]. Studies suggest that poor sleep quality is a factor risk of worsening cardiovascular disease, and may also be an important marker of cardiovascular health. There is a proven relationship between poor quality and sleep duration with a number of independent risk factors for coronary artery disease [44], such as systemic arterial hypertension [45], diabetes mellitus and obesity [46].

Sleep disorders are more common in people living with HIV than in the general population, with a prevalence of 30 to 100% depending on the methodology used for evaluation. These disorders have been reported since the beginning of the epidemic, but they have become important in patients on antiretroviral therapy that has made HIV infection a chronic disease. In addition, sleep disorders may play an important regular role in the immune system [47]. Studies evaluating sleep quality in HIV patients have shown that there is a decrease in sleep efficiency with increased sleep fragmentation and increased sleep. Latency period when compared to a non-HIV control group [48].

The application of the sleep quality assessment instrument showed that most patients had rates considered low and after the use of melatonin showed improvement of these parameters.

Melatonin is currently used by millions of people around the world as a natural supplement for circadian and sleep

disturbance. However, the mechanisms responsible for the beneficial effect of melatonin are still not fully understood. Hundreds of reports have appeared lately that documented melatonin's ability to neutralize directly free radicals and related toxicants. The first indication that melatonin may be a direct free radical scavenger actually appeared in 1991 [49], after which definitive evidence that melatonin functioned as a direct scavenger of hydroxyl radicals was provided [50].

This study demonstrates that animals treated with antiretroviral therapy and melatonin had higher body weight gain, less hepatomegaly, less anxiety, lower levels of triglycerides, cholesterol and hepatic enzymes, when compared to animals treated with antiretroviral therapy.

The study performed on patients shows a positive change for the parameters blood glucose, liver enzyme levels and lipid profile when melatonin was used.

Animal models of diabetes have been used to examine the influence of diabetes on melatonin levels and, in turn, the effect of melatonin treatment on glucose homeostasis and the diabetic status in these animal models. A combined treatment of insulin and melatonin of STZ-induced diabetic rats had beneficial effects on blood glucose levels and body weight. Melatonin, or insulin alone, provided limited protection against hyperglycemia and induced oxidative damage in diabetic rats, whereas combined treatment with insulin and melatonin suppressed hyperglycemia, prevented oxidative damage, and restored endothelium function. Beneficial effects of the combined treatment are thus demonstrated [51].

In STZ-rats insulin-positive, β-cells appeared degranulated and degenerated or necrotic, revealing decreased insulin secretion and increased blood glucose levels. Melatonin administration, however, ameliorated the diabetic phenotype by causing a partial regeneration/proliferation of pancreatic β-cells, a decrease in serum glucose and an increase in insulin concentrations [52]. Treatment with melatonin resulted in the appearance of high-intensity insulin and anti-apoptotic Bbcl-xL-positive cells in the pancreas, whereas the number of apoptotic cells decreased [53].

Pineal hormone melatonin exerts its influence on the periphery through the activation of two specific trans-membrane receptors: MT1 and MT2. The two isoforms, expressed in the islets of Langerhans, are involved in the modulation of insulin secretion from β-cells and in glucagon secretion from α-cells. De-synchrony of receptor signaling may lead towards the development of Type 2 diabetes [54] [55].

Most authors agree that the pineal gland has a suppressive effect on the activity of the pancreatic insulin-producing β-cell, because melatonin reduces insulin levels and glucose tolerance in rats [56]. Due to increased insulin level that exerts an inhibitory effect on the melatonin synthesis of the pineal gland, a functional antagonism between insulin and melatonin is presumed. This antagonism is in line with the fact that, in humans, low insulin levels at night and high levels during the day coincide with elevated nocturnal melatonin concentrations and reduced levels during the day [57]. Moreover, diabetic patients generally lack a circadian melatonin rhythm [58].

Melatonin is chemically characterized as an amphiphilic molecule due to methoxy groups at carbon 5, and to acetyl grouping linked to the nitrogen of the amino group. The molecule diffuses with equal ability in the two media as hydrophilic lipophilic. Thus, once produced in the pineal gland, melatonin is secreted and may immediately be found in all compartments of the body, whether intracellular or even in cell nuclei. Another feature is its high antioxidant or reducing capacity conferred by carbons 2 and 3 of pyrrole ring with its high ability to donate electrons. Consequently, melatonin is considered one of the most powerful natural antioxidants [59].

Ohta *et al*. [60] found that melatonin might prevent serious injury induced by carbon tetrachloride (CCl_4) in the liver of rats, attenuating increased lipid peroxidation and reducing the depletion of glutathione in its reduced form. The authors concluded that therapeutic doses of melatonin, a one-time post-treatment with CCl_4, attenuated the decrease of ascorbic acid concentration and activity of superoxide dismutase, catalase and glutathione reductase, coupled to an increase in the activity of xanthine oxidase in rat liver subjected to this treatment.

Current results demonstrate a reduction in plasma levels of AST and ALT in animals and patients treated with HAART + melatonin and suggest a direct effect on the liver damage by HAART. Although there are few studies on the bioavailability of melatonin, it is completely absorbed by the gastrointestinal tract. Its plasma peak occurs 60 minutes after administration [61]. Oral doses generally use 2 - 4mg, and only 15% reach the circulatory system, probably due to first-pass hepatic metabolism [62].

There are four mechanisms of liver damage associated with the use of antiretroviral drugs: hypersensitivity reactions, direct toxicity of pharmacologic and / or its metabolite, mitochondrial toxicity and immune reconstitution inflammatory syndrome. Ozturk *et al*. [63] reported increased activity of superoxide dismutase antioxidant enzyme in rat liver after administration of melatonin at a concentration of 10 mg / kg for 7 days. Another study reported increased superoxide dismutase activity in the kidney, liver and brain after a single injection of melatonin at concentrations of 5mg / kg animal [64].

Current results demonstrate that the use of melatonin associated with HAART may have a direct effect on the liver, probably mediated by the union of the indolamine to specific receptors in the body.

Therefore, it may be suggested that the addition of melatonin for scavenger free radical may act through different receptors and also include neuroendocrine regulatory functions of the liver.

HAART increases lipids in the bloodstream and reduces the peripheral storage of these molecules [65] which, accumulated in the plasma and associated to arterial inflammation from HIV infection, may clog the arteries and facilitate the formation of fat plaques, leading to the development of atherosclerosis and its complications, such as myocardial infarction and peripheral vascular disease [66]. Current study demonstrated that the concomitant use of melatonin and HAART decrease in triglyceride levels when cmpared to the group treated with HAART.

Experiments performed by Melchiorri *et al*. [67] established the antioxidant properties of melatonin against liver lipid peroxidation by the herbicide Paraquat (20 to 70mg / kg) which induces oxidative damage on the liver and lung (measured by malonaldehyde and 4-hydroxyalquens 4HDA).

The authors reported that melatonin doses of 10mg / kg previously administered by Paraquat decreased oxidative changes caused by the herbicide. In fact, melatonin protected hepatic nuclear DNA from the effects of free radicals produced by carcinogenic safrole. Rats treated with 300mg / kg of safrole and later with melatonin (0.2 to 0.4 mg / kg) revealed the protective effect of the hormone on nuclear DNA from the effects of safrole. This protection is a consequence of the detoxification of free radicals within the nucleus, indicating that melatonin acts on the core to reduce DNA damage.

The significance of melatonin as an OH scavenger is related to the fact that the reactant is generally considered to be the most damaging of all endogenously generated reactive agents.

The mechanisms whereby melatonin stimulates enzyme activity that detoxify oxygen-based reactants remain unknown but it is likely to be mediated via specific receptors. Although membrane receptors for melatonin have been identified in many cells, nuclear binding sites for the indole have also been documented [68].

Finally, the importance of a number of endogenously generated melatonin metabolites in terms of their scavenging action should not be overlooked, since they likely contribute, via the antioxidant cascade defined above, towards the total capacity of melatonin to reduce oxidative damage.

When organisms are exposed to drugs or other free radical generating agents or processes (excessive exercise, hyperoxia, ionizing radiation, inflammation, ischemia, toxins, trauma), the number of reactants produced overwhelms the capacity of the system to defend itself. In such situations, supraphysiological levels of an antioxidant must be given to prevent plundering by the large number of induced toxic reactants. So that an antioxidant neutralizes a toxic reactant before it mutilates a bystander molecule, it must be very near to where the reactant was generated. Since it is amphiphilic, melatonin seems to be ubiquitously distributed, albeit unevenly, in subcellular compartments.

Another important factor in melatonin's role as an antioxidant within a vertebrate organism is its ability to transverse established morphophysiological barriers. Melatonin penetrates in the brain minutes after its administration [69].

The authors assume that physiological levels of melatonin have a function similar to those of the pharmacological concentrations used. It may be relevant that endogenous melatonin levels change substantially throughout a lifetime. There are sometimes substantial differences in the quantity of melatonin produced by different individuals [70].

Studies on melatonin´s bioavailability have shown that it is completely absorbed in the gastrointestinal tract. Its plasma peak occurs 60 minutes after its administration. The usual oral dosages range between 2 and 4 mg, with only 15% reaching the circulatory system, probably due to first-pass hepatic metabolism [71].

Studies on the administration of melatonin in normal subjects indicate the absence of significant adverse effects [72].

Considering melatonin´s low toxicity and its ability to reduce side effects and increase the efficacy of the drugs, its use as a combination therapy with HAART may be important and significant. In fact, current analysis on melatonin's effects associated with antiretroviral treatment has proved to have a good effect on the metabolic abnormalities in AIDS patients.

4. Conclusion

Current study suggests that Melatonin reduced the toxic effects of HAART in mice and patients. Decrease in the effects of gastrointestinal intolerance induced by HAART, decrease in triglyceride levels, higher weight gain and better AST and ALT levels were reported. The evaluated emotional parameters indicated that melatonin might decrease HAART-induced anxiety. Current study suggests that melatonin may be an adjuvant treatment which minimizes the side effects of HAART.

Competing interests

Authors declared no competing interests.

Authors' Contributions

Author ARTP designed the study, performed the statistical analysis, wrote the protocol and the first draft of the manuscript. Authors JBB conducted the experimental analyses in animals. FRN, GFM, ALFM and MSJ focused on the clinical and laboratorial evaluation of the study. Author MSJ searched the literature involved. All authors read and approved the final manuscript.

Ethical approval

All authors hereby declare that "The principles of laboratory animal care" (NIH publication n. 85-23, revised 1985) and specific Brazilian laws, where applicable, were complied with. All experiments have been examined and approved by the appropriate committee for ethics. The protocol nº 7968200115/2015 for the animal experiments was approves by the Ethics Committee of Animal Experiments. All human experiments have been examined and approved by the appropriate Committee for Ethics (CAAE: 68289417.8.0000.0104)

References

1. Narciso P, Tozzi V, D'Offizi G, de Carli G, Orchi N, Galati V. *et al*. metabolic and morphologic disorders in patients treated with hightly active antiretroviral therapy since primary HIV infection. Ann N Y Acad Sci, 2001; 946:214-22.
2. Nduka C, Sarki A, Uthman O, Stranges S. Impact of antiretroviral therapy on serum lipoprotein levels and dyslipidemias: a systematic review and meta-analysis. Int J Cardiol. 2015; 199:307-18.
3. Lo Re Rd V, Zeldow B, Kallan MJ, Tate JP, Carbonari DM, Hennessy S. *et al*. Risk of liver descompensation with cumulative use of mitochondrial toxic nucleoside analogues in HIV/hepatites C virus coinfection. Pharmacoepidemiol Drug Saf, 2017, 19:4258
4. Rogalska-Plonska M, Rogalski P, Leszczyszyn-Pynka M, Stempkowska J, Kocbach P, Kowalczuk-Kot A. *et al*.

Hypertension, dyslipidemia and cardiovascular risk in HIV infected adults in Poland. Kardiol Pol, 2017; 199:307-18.
5. Castelnuovo B, John L, Lutwama F, Ronald A, Spacek LA, bates M. *et al*. Three years outcome data of second-line anti-retroviral therapy an Ugandan adults; good virological response but high rate of toxicity. J Int Assoc Physicians AIDS care. 2009; 8(1):52-9.
6. Chalasani N, Bonkovsky HL, Fontana R, Lee W, Stolz A, Talwalkar J. *et al*. Features and outcomes of 899 patients with drug-induced liver injury; The DILIN prospective study. Gastroenterology. 2015; 148(7):1340-52.
7. Vogel M, Rockstroh JK. Hepatotoxicity and liver disease in the context of HIV therapy. Curr Opin HIV AIDS. 2007; 2(4):306-13.
8. Sulkowski MS. Drug-induced liver injury associated with antiretroviral therapy that includes HIV-1 protease inhibitors.Clin Infect Dis. 2004; 38(2):90-7.
9. Wit FW, Weverling GJ, Weel J, Jurriaans S, Lange JM. Incidence of and risk factors for severe hepatotoxicity assicated with antiretroviral combination therapy. J Infect Dis. 2002; 186(1):23-31.
10. Echenique IA, Rich JD. EFV/FTC/TDF-associated hepatotoxicity: A case report and review. AIDS Patient Care STDS. 2013; 27(9):493-7.
11. Morishima I, Matsui H, Mukawa A, Hayashi K, Toki Y, Okumura K. *et al*. Melatonin, a pineal hormone with antioxidant property, protects against adriamycin-induced cardiomyopathy in rats. Life sci. 1998; 68:511-521.
12. Anwar MM, Mahfouz HA, Sayed AS. Potential protective effects of melatonin on bone marrow of rats exposed to cytotoxic drugs. Comp Biochem Physiol. 1998; 119(1)493-501.
13. Alarcon de la Lastra C, Motilva V, Marin MJ, Nieto A, Barranco MD, Cabezza J. *et al*. Protective effect of melatonin on indomethacin-induced gastric injury in rats. J Pineal Res. 1999; 26:101-107.
14. Li Z, Nickkholgh A, Yi X. Melatonin protects kidney grafts from ischemia/reperfusion injury through inhibition of NF-kB and apoptosis after experimental kidney transplantation. J Pineal Res. 2009; 46:365-72.
15. Petrosillo G, Di Venosa N, Pistolese M. protective effect of melatonin against mitochondrial dysfunction associated with cardiac ischemia-reperfusion: role of cardiolipin. FASEB J. 2006; 20:269-76.
16. Jimenez-Arant A, Fernandez-vazquez G, Serrano MM, Reiter RJ, Agil A. melatonin improves mitochondrial function in inguinal white adipose tissue of Zucker diabetic fatty rats. J Pineal res, 2014; 57(1):103-9.
17. Reiter JR, Tan D, Sainz RM, Mayo JC, Lopez-Burillo S. Melatonin: reducing the toxicity and increasing the efficacy of drugs. Pharmacy and Pharmacology. 2002; 54(10):1299-321.
18. Li Z, Nickkholgh A, Yi X. Melatonin protects kidney grafts from ischemia/reperfusion injury through inhibition of NF-kB and apoptosis after experimental kidney transplantation. J Pineal Res, 2009; 46:365-72.
19. Petrosillo G, Di Venosa N, Pistolese M. protective effect of melatonin against mitochondrial dysfunction associated with cardiac ischemia-reperfusion: role of cardiolipin. FASEB J, 2006; 20:269-76.
20. Huether G. Melatonin synthesis in the gastrointestinal tract and the impact for nutritional factors on circulating melatonin. Ann N Y Acad Sci, 1994; 729:146-58.
21. Bubenik GA. Gastrointestinal melatonin localization, function and clinical relevance. Dig Dis Sci. 2002; 47(10):23362348.
22. Carrilo-Vico A, Calvo Jr, Aberu P, Lardone PJ, Garcia-Maurino S, Reiter RJ. *et al*. Evidence of Melatonin Synthesis by Human Lymphocytes and its Physiological significance-possible role as intracine, autocrine and/or paracrine substance faseb J. 2009; 18(3):537-539.
23. Tan DX, Manchester LC, Reiter RJ, Zhang M, Weintraub ST, Cabrera J. *et al*. Identification of highly elevated levels of melatonin in bone marrow: its origin and significance. Biochem Biophyd Acta, 1999; 1472:206-214.
24. Miller Sc, Pandi-Perumal SR, esquifino AI, Cardinali DP, maestroni GJ. The role of melatonin in immuno-enhancement: potential application in cancer. Int J Exp Pathol. 2006; 87(2):81-87.
25. Hamilton M. The assessment of anxiety states by rating. British Journal medical Psychology, 1959; 32:50-55
26. Montgomery SA, Asberg M. A new depression scale designed to be sensitive to change. British Journal Psychiatry 1979; 134:382-389.
27. Buysse DJ, Reynolds CF, Monk TH, Berman SR, Kupfer DJ. The Pittsburg Sleep Quality Index: A new Instrument for Psychiatric Practice and Research. Journal of Psychiatric Research. 1989; 28(2):193-213.
28. Sulkowski MS, Thomas DL, Chaisson RE, Moore RD. Hepatotoxicity associated with antiretroviral therapy in adults infected with human immunodeficiency virus and the role of hepatitis C or B virus infection. JAMA, 2000; 283:74-80.
29. Bubenik GA. Gastrointestinal melatonin localization, function and clinical relevance. Dig Dis Sci, 2002, 47(10):2336-2348.
30. Reijers MH, Weigel HM, Hart AA, Ten Kate RW, Mulder JW, Reiss P. *et al*. Toxicity and drug exposure in a quadruple drug regimen in HIV-1 infected patients participating in the ADAM study. AIDS. 2000; 14(1):59-67.
31. Tonolini M, Bianco R. Acute HIV-related gastrointestinal disorders and complications in the antiretroviral era: spectrum of cross-sectional imaging findings. Abd Imaging. 2013; 38(5):994-1004.
32. Ma P, Liu X, Wu J, Zhang Y, Lu Y, Wu Y. *et al*. Cognitive deficits and anxiety induced by diisononyl phthalate in mice and the neuroprotective effects of melatonin. Sci Rep. 2015; 1(5):14676.
33. Ford AH, Flicker L, Passage J, Wibrow B, Ansley M, Edwards M. *et al*. Trials. 2016; 17(1):55.
34. Dianatkhah M, Ghaeli P, Hajhossein Talasaz A, Karimi A, Salehiomran A, Bina P. *et al*. Evaluating the potential effect of melatonin on the post-cardiac surgery sleep disorder. J Tehran Heart Cent. 2015; 10(3):122-8.
35. Malbergier A, Schoffel AC. Tratamento de depressão em indivíduos infectados pelo HIV. Revista Bras de Psiquiatria. 2001; 23(3):160-7. portuguese

36. Reust CE. Common adverse effects of antiretroviral therapy for HIV disease. Am Fam Physician. 2011; 84(2):1443-51.
37. Huether G. Melatonin synthesis in the gastrointestinal tract and the impact for nutritional factors on circulating melatonin. Ann N Y Acad Sci, 1994; 729:146-58.
38. Bubenik GA. Gastrointestinal melatonin localization, function and clinical relevance. Dig Dis Sci. 2002; 47(10):2336-2348.
39. Buysse DJ, Reynolds CF, Monk TH, Berman SR. The Pittisburg Sleep Quality Index; A New Instrument for Psychiatric Practice and Research. Psychiatry Research, 1988; 28;193-213
40. Ma P, Liu X, Wu J, Zhang Y, Lu Y, Wu Y. *et al*. Cognitive deficits and anxiety induced by diisononyl phthalate in mice and the neuroprotective effects of melatonin. Sci Rep. 2015; 1(5):14676.
41. Ford AH, Flicker L, Passage J, Wibrow B, Ansley M, Edwards M. *et al*. Trials, 2016; 17(1):55.
42. Dianatkhah M, Ghaeli P, Hajhossein Talasaz A, Karimi A, Salehiomran A, Bina P. *et al*. Evaluating the potential effect of melatonin on the post-cardiac surgery sleep disorder. J Tehran Heart Cent. 2015; 10(3):122-8.
43. Grandner MA, Halr L, Moore M, Patel NP. Mortality with short sleep duration: the evidence, the possible mechanism and the future. Sleep med Rev. 2010; 14(3):191-203.
44. Vgontzas AN, Liao D, Bixler EO, Chrousos GP, Vela-Bueno A. Insomnia with objective short sleep duration is associated with a high risk for hypertension. Sleep. 2009; 32(4):491-7
45. Spiegel K, Knutson K, Leproult R, Tasali E, Van Cauter E. Sleep loss: a novel risk factor for insulin resistance and type 2 diabetes. J Appl Physiol. 2005; 99(5):1008-19.
46. Watanabe M, Kikuchi H, Tanaka K, Takahashi M. Association of short sleep duration with weght gain and obesity and 1 year follow-up: a large scale prospective study. Sleep. 2019; 33(2):161-7.
47. Babson KA, Heinz AJ, Bonn-Miller MO. HIV medication adherence and HIV symptom severity: the roles of sleep quality and memory
48. Reid S, Dweyer J. Insomnia in HIV infection a systematic review of prevalence, correlates and management. Psychomosome. 2005; 67(20):260-269.
49. Ianas O, Olinescu R, Balescu I. Melatonin involved in oxidative processes. Rom Endocrinol, 1991; 29:147-153.
50. Paskaloglu K, Sener G, Ayangolu-Dulger G. Melatonin treatment protects against diabetes-induced functional and biochemical changes in rat aorta and corpus cavernosum. Eur J Pharmacol, 2004; 499:345-354.
51. Kanter M, Uysal H, Karaca T, Sagmanligil HO. Depression of glucose levels and partial restoration of pancreatic β-cells damage by melatonin in streptozotocin-induced diabetic rats. Arch Toxicol, 2006; 80:362-369.
52. Simsek N, Kaya M, Kara A, Can I, Karadeniz A, Kalkan Y. *et al*. Effects of melatonin on islet neogenesis and β cell apoptosis in streptozotocin-induced diabetic rats: an immunohistochemical study. Domest Anim Endocrinol, 2012; 43:47-57.
53. Reppert SM, Weaver DR, Ebisawa T. Cloting and characterization of a mammalian melatonin receptor that mediates reproductive and circadian responses. Neuron, 1994; 13:1177-85.
54. Reppert SM, Godsonr C, Mahle CD, Weaver DR, Slaugenhaupt SA, Gusella JF. *et al*. Molecular characterization of a second melatonin receptor ecxpressed in human retina and brais: The Mel 1b melatonin receptor. Proc Natl Acad Sci USA, 1995; 92:8734-38.
55. Rasmussen DD, Boldt BMI, Wilkinson CW, Yellon SM, Matsumoto AM. Daily melatonin administration at middle age suppresses male rate visceral fat, plasm leptin and plasma insulin to youthful levels. Endocrinology, 1999; 140:1009-1012.
56. Boden G, Ruiz J, Urbain JL, Chen X. Evidence for a circadian rhythm of insulin secretion. Am J Physiol, 1996; 271:E246-E252.
57. O'Brien IA, Lewin IG, O'Hare JP, Arendt J, Corrall RJ. Abnormal circadian rhythm of melatonin in diabetic autonomic neuropathy. Clin Endocrinology, 1986; 24:359-364.
58. Tan DX, Reiter RJ, Sainz SM, Mavo JC, Lopez-Burillo S. Melatonin: Reducing the toxicity and increasing the efficacy of drugsd. J Pharm Pharmacol. 2002; 54(10):1299-321
59. Ohta Y, Kongo-Nishimura M, Hayashi T, Kishikawa T. Effect of Orengedoku-to (Huanglian-Jie-Du-Tang) extract on disruption of hepatic antioxidant defense system in rats treated with D-galactosamine. Journal of Ethnopharmacology, 2004; 94:323-329.
60. Geoffriau M, Brun J, Chazot G, Claustrat B. The physiology and pharmacology of melatonin in humans. Hom Res. 1998; 49(3-4):136-141.
61. Demuro RI, Nafziger AN, Blask DE, Menhinick AM, Bertino JS. The absolute biovalibity of oral melatonin. J Clin Pharmacol, 2000; 40:781-784.
62. Ozturk G, Conskun S, Erbas D, Hasanoglu E. The effect of melatonin on liver superoxide dismutase activity, serum nitrate and tyroid hormone levels. Japanese jornal of Physiology, 2000: 50:149-153
63. Liu F. Effect of pineal indoles on activities of the antioxidant defense enzymes superoxide dismutase, catalase and glutathione reductase and levels of reduced and oxidized glutathione in rat tissues. Biochemistry and cell biology, 2000; 78:447-453.
64. Naji LA, Carrilo-Vico J, Guerrero J, Calvo. Expression of membrane and nuclear melatonin receptors in mouse peripheral organs. Life Sci, 2004; 74:2227-2236
65. Menendez-Pelaez AR, Reiter R. Distribution of melatonin in mammalian tissues: the relative importance of nuclear versus cytosoloc localization. J Pineal Res, 1993; 15:59-69.
66. Kramer AS, Lazzarotto AR, Sprinz E, manfroi WC. Alterações metabolicas, terapia antiretroviral e doença cardiovascular em idosos portadores de HIV. Arq Brás Cardiol. 2009; 93(5):561-568.
67. Melchiorri DR, Reiter A, Attia M, Hara A, Burgos G. potent protective effect of melatonin on in vivo paraquat

Induced oxidative damage in rats. Life Sci. 1995; 56:83-89.
68. Dubocovich ML, Mansana MI, Benloucif S. Molecular pharmacology and function of melatonin receptors. Adv Exp Med Biol, 1999; 460:181-191.
69. Menendez—Pelaez A, Reiter RJ. Distribution of melatonina in mammalian tissues: relative importance of nuclear versus cytosolic localization J Pineal Res, 1993; 15:59-69.
70. Arendt J. Melatonin and the mammalian pineal gland. London Chapman and Hall, 1993, p. 331.
71. Lane EA, Moss, HB. Pharmacokinetics of melatonin in man: first pass hepatic metabolism. J Clin Endocr Metab. 1985; 61(6):1214-1216
72. Seabra MLV. Randomized, double blind clinical trial, controlled with placebo, of the toxicology of chronic melatonin treatment. J Pineal. Res, 2000; 29:193-200.