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Melatonin Improves Metabolic Abnormalities Induced by HAART in Mice

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Authors' contributions

This work was carried out in collaboration between all authors. Author ARTP designed the study, wrote the protocol, and wrote the first draft of the manuscript. Author MSJ managed the literature searches. Authors JBB and TSJ managed the experimental process and author LC managed the emotional assessment. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Highly Active Antiretroviral Therapy (HAART) is associated with metabolic complications. An unexpected extension of functional implications of melatonin, the neuro-hormone synthetized during the night, has been reported on the development of type 2 diabetes, sleep disturbances and depression. Melatonin has been shown to reduce the toxicity and increase the efficacy of a large number of drugs.
Objective: Current study evaluated the effect of Melatonin on mice treated with antiretroviral therapy.
Materials and Methods: Animals were divided into experimental groups with 12 animals each: (I) animals treated with antiretroviral therapy for 15 days, (II) 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. Results were analyzed with GraphPad Prism by Student´s *t* test.
Results: Animals treated with antiretroviral therapy and melatonin (II) had higher body weight gain,

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less hepatomegaly, less anxiety, lower levels of triglycerides, cholesterol and hepatic enzymes when compared to animals treated with antiretroviral therapy.
Conclusion: Due to the low toxicity of melatonin and its ability to reduce the side effects and increase the efficacy of drugs its use as a combination therapy with antiretroviral therapy seems important and worthy of pursuit.

Keywords: Melatonin; HIV/AIDS; antiretroviral; metabolic abnormalities.

1. INTRODUCTION

Highly Active Antiretroviral Therapy (HAART) is the current care standard for the treatment of 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. The above complications significantly increase morbidity and mortality in those requiring long-term treatment for HIV-infection. Moreover, abnormalities also impact adherence to treatment [1].

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 [2]. Because of these activities and others that may be defined in the future, melatonin reduces toxicity and increases the efficacy of a large number of drugs as documented in [3,4,5,6].

Melatonin was chosen for its antioxidant and anti-apoptotic activity on renal tissue [7] and injury on myocardic cells [8]. 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 [9].

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 [10].

Therapies based on the administration of melatonin in high concentrations result in different modulations in the immune response [11], 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 [12]; stimulation of several cytokines synthesis such as IL-2, IFN-γ and IL-6, and the regulation of nitric oxide synthesis by endothelial cells [13].

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 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 [14].

Thus the aim of this study was to evaluate the effect of the use of oral melatonin in mice undergoing HAART therapy.

2. MATERIALS AND METHODS

2.1 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 22℃ ± 2℃, 70% humidity and photoperiod (light / dark cycle 12 h).

2.2 Preparation of Melatonin

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

2.3 Preparation of HAART

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

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

2.4 Treatment Schedule

Each experimental group comprised 12 animals: (I) animals treated with HAART diluted in 0.2 mL water by gavage + water 0.2 mL 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.

2.5 Evaluation

2.5.1 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.

2.5.2 Clinical evaluation

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

2.5.3 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.

2.5.4 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.

2.5.5 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 (%).

2.6 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 AND 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. Melatonin has been shown to have beneficial effects when combined with the following drugs; doxorubicin, cisplatin, epirubicin, cytarabine, bleomycin, gentamicin, ciclosporin, indometacin, acetylsalicylic acid, ranitidine, omeprazol, isoniazid, iron and erythropoietin, phenobarbital, carbamazepine, haloperidol, caposide-50, morphine,

cyclophosphamide and L-cysteine. Studies were conducted with animals and humans [14]. 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.

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

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

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.

Being either overweight or underweight at HAART initiation was associated with heightened systemic inflammation, weight gain among underweight persons predicted reduced inflammation [15].

On the other hand animals subjected to environmental stress showed less weight gain and disturbances in melatonin production [16].

Fig. 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).

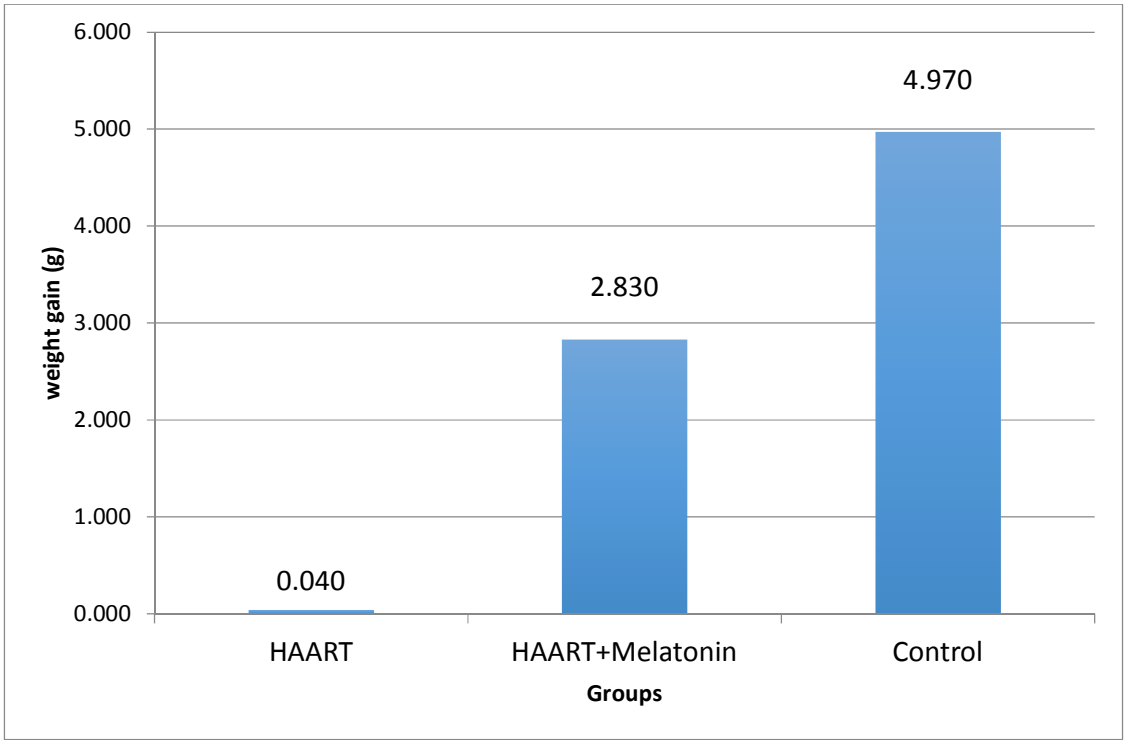

Fig. 1. Weight gain (g) of Swiss mice in experimental and control groups after 15 days of treatment

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

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 [17]. Melatonin in the digestive tract, especially in the large intestine, is related to modulation of intestinal motility, intestinal regeneration processes and especially antioxidants [18]. Current study demonstrates that exogenous melatonin use associated to HAART is beneficial (Figs. 1 and 2) since it decreases the effects of gastrointestinal intolerance induced by HAART [19].

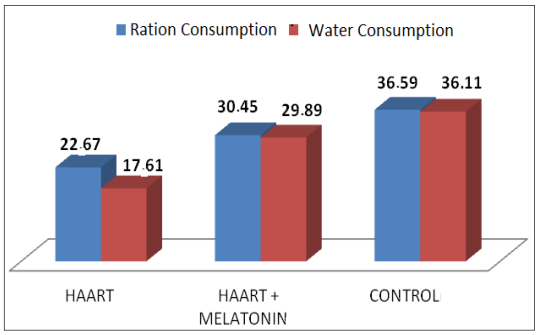

Fig. 2. Ration (g) and water consumption (mL) of Swiss mice after 15 days

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

Table 1 and Fig. 3 show behavioral evaluation results of the experimental groups.

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

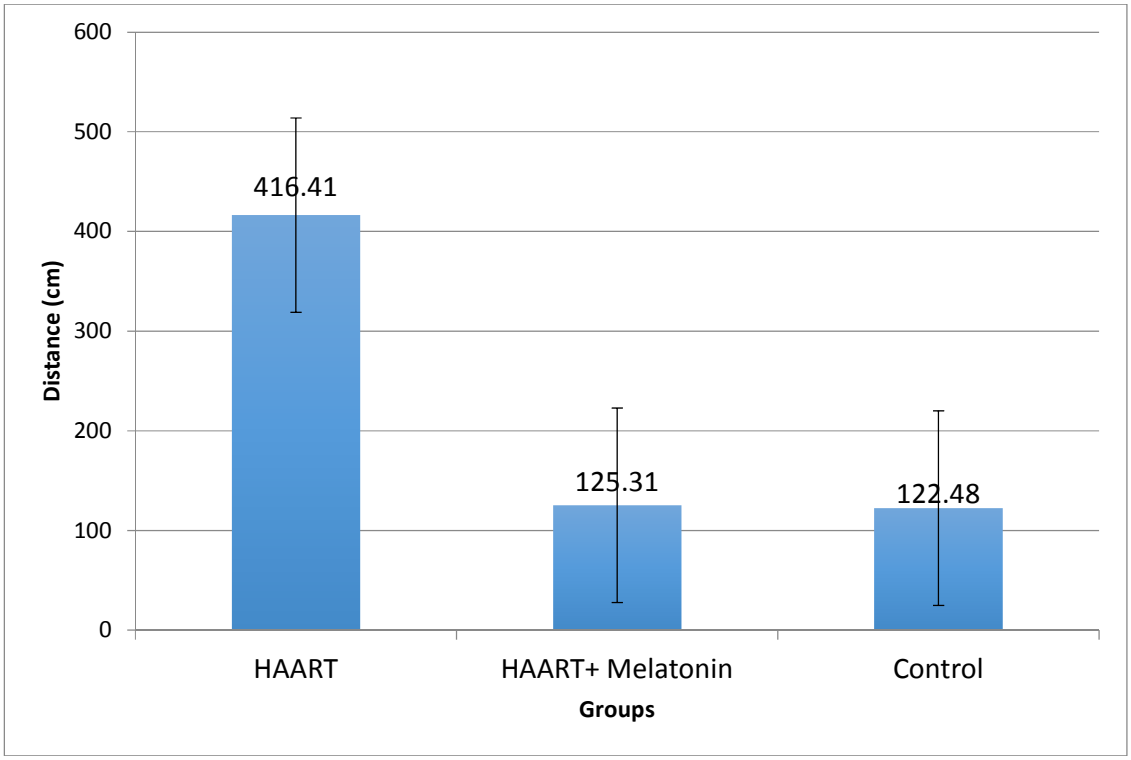

Fig. 3. Distance traveled (cm) by Swiss mice. The distance traveled corresponds to degree of anxiety and was reported by video tracking software Noldus Etho-Vision®

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

Several studies demonstrate neuroprotective effect of melatonin in mice with cognitive deficits and anxiety [20], prevention of delirium following cardiac surgery [21], melatonin-improved sleep in post-cardiac surgery patients more than that observed with oxazepam [22]. 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.

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 (5 mg / kg of atazanavir+ 5 mg / kg of tenofovir disoproxil fumarate+ 1.67 mg / kg of ritonavir+ 2.5 mg / kg of lamivudine, diluted with 1.2 mL of water/day/15 days; Group II treated with HAART (5 mg / kg of atazanavir+ 5 mg / kg of tenofovir disoproxil fumarate + 1.67 mg / kg of ritonavir + 2.5 mg / kg of lamivudine, diluted with 1.2 mL of water + melatonin 6 mg/Kg /day/15 days; Group III non-treated group (control group)

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.

Melatonin may reduce tissue destruction during inflammatory response either directly by scanning free radicals or indirectly by decreasing the production of cytokines and adhesion molecules which contribute to cellular damage [14].

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 [23].

Ohta et al. [24] 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 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 [25]. Oral doses generally use 2 – 4 mg, and only 15% reach the circulatory system, probably due to first-pass hepatic metabolism [26].

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 [27].

Ozturk et al. [28] 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 [29].

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. In fact, receptors for melatonin, such as MLT1 and RORoCj, a nuclear receptor described in liver cells of mice, have been described [30].

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.

Experiments performed by Melchiorri et al. [31] established the antioxidant properties of melatonin against liver lipid peroxidation by the herbicide Paraquat (20 to 70 mg / 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 [32]. 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 [33].

HAART increases lipids in the bloodstream and reduces the peripheral storage of these molecules [34] 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 [35]. Current study demonstrated that the concomitant use of melatonin and HAART enhanced an 18% decrease in triglyceride levels when compared to the group treated with HAART.

4. CONCLUSION

Current study suggests that Melatonin reduced the toxic effects of HAART in mice. 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.

Extant analyses on the administration of melatonin in normal subjects indicate no significant adverse effects [34]. Current study suggests that melatonin may be an adjuvant treatment which minimizes the side effects of HAART.

CONSENT

It is not applicable.

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 in animal experimentation.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Teodor A, Teodor D, Luca V. Side effects of antiretroviral therapy. Rev Med Chir Soc Med Nat Iasi. 2004;108(1):23-6.
2. Cadenas E, Packer L, (eds). Handbook of antioxidants. 2nd Edition, New York, Marcel Dekker. 2002;712.
3. Morishima I, Matsui H, Mukawa A, Hayashi K, Toki Y, Okumura K, Ito T, Hayakawa T. Melatonin, a pineal hormone with antioxidant property, protects against adriamycin-induced cardiomyopathy in rats. Life Sci. 1998;68:511-521.
4. Lissoni P. Is there a role for melatonin in supportive care? Support Care Cancer. 2002;10:110-116.
5. 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.
6. Alarcon de la Lastra C, Motilva V, Marin MJ, Nieto A, Barranco MD, Cabezza J, Herrerias JM. Protective effect of melatonin on indomethacin-induced gastric injury in rats. J Pineal Res. 1999;26:101-107.
7. 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.
8. 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.
9. 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.
10. Cagnacci A. Melatonin in relation to physiology in adult human and rat plasma proteins. Endocrinology. 1972;91:1213-1218.
11. Carrilo-Vico A, Calvo JR, Aberu P, Lardone PJ, Garcia-Maurino S, Reiter RJ, Guerrero JM. Evidence of melatonin synthesis by human lymphocytes and its physiological significance-possible role as intracrine, autocrine and/or paracrine substance. FASEB J. 2009;18(3):537-539.
12. Tan DX, Manchester LC, Reiter RJ, Zhang M, Weintraub ST, Cabrera J, Sainz RM, Mayo JC. Identification of highly elevated levels of melatonin in bone marrow: Its origin and significance. Biochem Biophyd Acta. 1999;1472:206-214.

13. 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.
14. 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.
15. Mave V, Erlandson KM, Gupte N, Balagopal, Campbell TB, Smeaton L, et al. Inflammation and change in body weight with antiretroviral therapy initiation in a multinational cohort of HIV-infected adults. J Infect Dis. 2016;214(1):65-72.
16. Yuan M, Liu LJ, Xu LZ, Guo TY, YUE XD, LI SX. Effects of environmental stress on the depression like behaviors and the diurnal rhythm of corticosterone and melatonin in male rats. Sheng Li Xue Bao. 2016;68(3):215-23.
17. 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.
18. Bubenik GA. Gastrointestinal melatonin localization, function and clinical relevance. Dig Dis Sci. 2002;47(10):2336-2348.
19. Reijers MH, Weigel HM, Hart AA, Ten Kate RW, Mulder JW, Reiss P, Schuitemaker H, Hoetelmans RM, Weverling GJ, Lange JM. 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.
20. 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.
21. Ma P, Liu X, Wu J, Zhang Y, Lu Y, Wu Y, Liu C, Guo J, Nanberg E, Bornehag CG, Yang X. Cognitive deficits and anxiety induced by diisononyl phthalate in mice and the neuroprotective effects of melatonin. Sci Rep. 2015;5:14676.
22. Dianatkhah M, Ghaeli P, Hajhossein Talasaz A, Karimi A, Salehiomran A, Bina P, Jalali A, Ghaffary S, Shahmansouri N, Vejdani S. Evaluating the potential effect of melatonin on the post-cardiac surgery sleep disorder. J Tehran Heart Cent. 2015;10 (3):122-8.
23. 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.
24. 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.
25. Geoffriau M, Brun J, Chazot G, Claustrat B. The physiology and pharmacology of melatonin in humans. Hom Res. 1998; 49(3-4):136-141.
26. Demuro RI, Nafziger AN, Blask DE, menhinick AM, Bertino JS. The absolute biovalibity of oral melatonin. J Clin Pharmacol. 2000;40:781-784.
27. Brasil. Ministerio da Saude, Secretaria de Vigilancia em Saude, Departamento de DST, AIDS e hepatitis Virais. Alterações hepáticas relacionadas ao HIV. Brasília; 2013.
28. 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
29. 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.
30. 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.
31. 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.
32. Tan DX, Reiter RJ, sainz SM, Mavo JC, Lopez-Burillo S. Melatonin: reducing the toxicity and increasing the efficacy of drugs. J Pharm Pharmacol. 2002;54(10): 1299-321.
33. 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.

34. 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.

35. Seabra MLV. Randomized double blind clinical trial controlled with placebo of the toxicity of chronic melatonin treatment. 2000;29:193-2000.

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