American Journal of Biomedical Sciences
ISSN: 1937-9080
nwpii.com/ajbms

Melatonin and Alpha Lipoic Acid Restore Gonadal Hormones of Lopinavir/ritonavir-Treated Male Albino rats

Elias Adikwu[1*], Nelson Braimbaifa[2], Wolfe Atuboyedia Obianime[2], Owunari. A. Georgewill[2]

[1] Department of Pharmacology/Toxicology, Faculty of Pharmacy, Niger Delta University, Bayelsa State, Nigeria
[2] Department of Pharmacology, Faculty of Basic Clinical Sciences, University of Port Harcourt, Rivers State, Nigeria
[*]**Corresponding Author**
Dr Elias Adikwu
Department of Pharmacology/Toxicology, Faculty of Pharmacy, Niger Delta University
Bayelsa State
Nigeria
Email: adikwuelias@gmail.com

Received:06 November 2021; | Revised:27 November 2021; | Accepted:15 March 2022

Abstract

Background: The use of lopinavir/ritonavir (LPV/r) may cause gonadal hormone dysfunction as an adverse consequence. Melatonin (MT) and alpha lipoic acid (ALA) have been associated with the regulations of gonadal functions. **Objectives:** The current study investigated the protective effects of MT and ALA against LPV/r-induced alterations in gonadal hormones of male albino rats. **Methods:** Rats were randomized into groups and were orally supplemented with MT (10mg/kg), ALA (10mg/kg) and MT + ALA prior to treatment with LPV/r (22.9/5.71-91.4/22.9 mg/kg) daily for 90 days, respectively. Rats were sacrificed, blood samples were collected, and sera were extracted and evaluated for luteinizing hormone (LH), follicle - stimulating hormone (FSH), testosterone (T), prolactin (PRL) and estradiol (ED). **Results:** Significant ($p<0.001$) decreases in serum LH, FSH, and T levels with significant ($p<0.001$) increases in PRL and ED levels in a dose-related fashion occurred in LPV/r-treated rats when compared to control. However, serum LH, FSH, T, PRL and ED levels were significantly restored in rats supplemented with MT ($p<0.01$), ALA ($p<0.01$) and MT+ALA ($p<0.001$) when compared to LPV/r-treated rats. Conclusion: This study showed that MT and ALA restored serum gonadal hormones of LPV/r-treated rats.

Keywords: Lopinavir/ritonavir; testis, hormones, antioxidants, protection

1. Introduction

It is widely recognized that gonadal function depends on the coordinated action of multiple factors influencing the syntheses and secretion of gonadal hormones. These hormones include luteinizing hormone (LH), follicle-stimulating hormone (FSH), which regulate the synthesis of testosterone (T).The syntheses and secretion of these hormones are regulated by the hormones produced by the pituitary, hypothalamus and gonads. [1] Optimal levels of gonadal hormones are very imperative for sustained fertility and procreation. However, abysmal levels of gonadal hormones attributed to toxicological insults from medications, substance abuse and chemicals may

lead to hypogonadism with catastrophic consequences on fertility and procreation. [2]

LPV/r is an essential component of highly active antiretroviral therapy (HAART) that has a strong track record as salvage therapy in cases of resistance to other antiretroviral regimens due to higher genetic barrier to resistance. [3] Accessibility to LPV/r regimen has improved health, quality of life, and survival rate; however it may have adverse influence on the fertility determinants of patients. Morphologic changes in testis and endocrine perturbations are complications that may occur with the use of LPV/r containing regimen. [4] Gonadal dysfunction characterized by impaired FSH, LH and T functions and hyper activities of estradiol (ED) and prolactin (PRL) are serious therapeutic predicaments that may arise with the use of LPV/r containing HAART. [5,6] The mechanism of hypgonadism due to HAART is not clear, but mechanisms ranging from testicular oxidative stress, mitochondria damage and the inhibition of hypothalamic-pituitary-gonadal axis (HPG) were proposed. [7]

Melatonin (MT) is an antioxidant and anti-inflammatory agent known as "sleep hormone" because of its regulatory effect on circadian rhythm. It is a hormone primarily produced in the pineal gland. [8] In addition to its pharmacologic activities, there are growing reports on its regulatory effect on the hypothalamus-pituitary-gonadal (HPG) axis. [9] In humans, MT can regulate the release of gonadotropins by the anterior pituitary, as well as the functions of the gonads and gonadal adnexa, through its specific receptors present in reproductive system. Experimentally it has protected the testes from assaults associated with some xenobiotics. [10] It has stabilized antioxidant status and decreased inflammatory reactions in testes exposed to toxic substances. [11]

Alpha lipoic acid (ALA) is a disulfide compound that functions as a coenzyme in pyruvate dehydrogenase and alpha-ketoglutarate dehydrogenase mitochondrial reactions in the synthesis of cellular energy (ATP).[12] ALA and its reduced dithiol form, dihydrolipoic acid (DHLA), are powerful anti-oxidative and anti-inflammatory agents. Experimentally, it has some potential health benefits such as the protection of testis from chemical-induced toxicity. [13] It has protected against lipopolysaccharide-induced damage in rat Sertoli cells and restored serum levels of reproductive hormones in cypermetrin-induced toxicity in the testis. [14, 15] This study was conducted in order to investigate the protective effects of MT and ALA on the gonadal hormones of LPV/r-treated male rats.

2. Materials and Methods

2.1 Animals, drugs and chemicals

Eighty five male albino rats (180 ± 20 g) were taken from the animal house of the Department of Pharmacology, Faculty of Basic Clinical Sciences, University of Port Harcourt, Nigeria and were randomized into groups of n=5/per group. The rats were kept under standard laboratory conditions, in separated metal cages at room temperature of 25 ± 3 ° C with a 12 h dark/light cycle. The rats were allowed to acclimatize for two weeks prior to the experiment. The rats were maintained on standard laboratory feed and water *ad libitum* and were handled according to the guidelines and regulations by the Canadian Council on Care for Animals used for research. The ALA and MT used were sourced from AO Pharm Import and Export Co Ltd, China whereas LPV/r was manufactured by Myland Laboratories Ltd, India. The doses of LPV/r used represent 2, 4 and 8 times the clinical dose. [16] MT (10mg/kg) and ALA (10mg/kg) were used for this study. [17] MT was dissolve in 1% ethanol and diluted with normal saline, LPV/r was dissolved in 1% ethanol whereas ALA was dissolved in water. [18,19]

2.2 Treatment procedure

Group A1: Placebo control was orally treated with normal saline (0.2ml) daily for 90 days

Group A2: Solvent control was orally treated with 1% ethanol daily for 90 days

Group B (B1-B3) was orally treated with MT (10mg/kg), ALA (10mg/kg) and MT+ALA daily for 90 days, respectively.

Group C (C1-C3) was orally treated daily with LPV/r (22.9/5.71, 45.6/11.4 94 and 91.4/22.9 mg/kg) daily for 90 days, respectively

Group D (D1-D3) was orally supplemented with MT (10mg/kg) before treatment with LPV/r

(22.9/5.71, 45.6/11.4 94 and 91.4/22.9 mg/kg) daily for 90 days, respectively.

Group E (E1-E3) was orally supplemented with ALA (10mg/kg) before treatment with LPV/r (22.9/5.71, 45.6/11.4 94 and 91.4/22.9 mg/kg) daily for 90 days, respectively

Group F (F1-F3) was orally supplemented with MT +ALA before treatment with LPV/r (22.9/5.71, 45.6/11.4 94 and 91.4/22.9 mg/kg) daily for 90 days, respectively

2.3 Collection of blood sample

After treatment, blood samples were taken through cardiac puncture and were immediately collected into clean and screw capped non heparinized tubes. Thereafter, blood samples were centrifuged at 3000 rpm for 15 min and sera were separated and evaluated for hormonal parameters.

2.4 Hormonal assay

Testosterone, luteinizing hormone, follicle-stimulating hormone, estradiol and prolactin were measured using specific Enzyme Immuno Assay (EIA) test kits (Biocheck, Inc, USA). The assay was performed according to the manufacturer's instructions.

2.5 Statistical analysis

The results are expressed as mean ± SEM (Standard error of mean). Statistical analysis was evaluated using one way analysis of variance (ANOVA) followed by Tukey's post hoc test. Values were considered statistically significant at $p<0.05$; $p<0.01$ and $p<0.001$.

Table 1: Effects of melatonin and alpha lipoic acid on serum gonadal hormones of rats

Parameters	Control	MT	ALA	MT+ALA
T(μg/mL)	3.40 ± 0.14	3.49 ± 0.05	3.40 ± 2.43	3.58 ± 0.07
FSH (μg/mL)	2.85 ± 0.03	2.91 ± 0.05	2.85 ± 0.23	3.01 ± 0.06
LH (μg/mL)	10.7± 0.03	10.9 ± 0.22	10.8 ± 0.41	11.0 ± 1.11
ED (μg/mL)	12.5 ± 0.02	12.0 ± 1.33	12.2 ± 1.22	11.9 ± 1.37
PRL (μg/mL)	0.28 ± 0.05	0.27 ± 0.02	0.26 ± 0.03	0.24 ± 0.02

T=Testosterone, FSH=Follicle stimulating hormone, LH= Luteinizing hormone, ED= Estradiol, PRL=Prolactin, MT=Melatonin, ALA=Alpha lipoic acid, Data as mean ± SEM (Standard error of mean), n=5.

Table 2: Effects of melatonin and alpha lipoic acid on lopinavir/ritonavir-induced serum testosterone levels in rats

	Serum testosterone (μg/mL)			
Dose (mg/kg)	LPV/r	MT+LPV/r	ALA+LPV/r	MT+ALA+LPV/r
Control	3.40 ± 0.14	3.40 ± 0.14	3.40 ± 0.14	3.40 ± 0.14
22.8/5.71	2.00 ± 0.03	2.61 ± 0.15*	2.53 ± 0.01*	3.20 ± 0.01*
45.6/11.4	1.51± 0.24	2.09 ± 0.11*	2.00 ± 0.02*	3.15 ± 0.05**
91.2/22.9	1.01 ± 0.02	1.50 ± 0.23*	1.46 ± 0.01*	3.00 ± 0.04**

LPV/r= Lopinavir/ritonavir. MT= Melatonin. ALA=Alphalipoic acid. Data as mean ± SEM (Standard error of mean), n=5, *Significant ($p<0.01$) difference when compared to LPV/r, **Significant ($p<0.001$) difference when compared to LPV/r.

Table 3: Effects of melatonin and alpha lipoic acid on lopinavir/ritonavir- induced serum luteinizing hormone levels in rats

Serum luteinizing hormone (μg/mL)				
Dose (mg/kg)	LPV/r	MEL+LVP/r	ALA+LVP/r	MEL+ALA+LPV/r
Control	2.85 ± 0.03	2.85 ± 0.03	2.85 ± 0.03	2.85 ± 0.03
22.8/5.71	1.20 ± 0.07	2.00 ± 0.06*	1.91± 0.09*	2.60 ± 0.07**
45.6/11.4	0.71 ± 0.03	1.51 ± 0.10*	1.40 ± 0.02*	2.53 ± 0.05**
91.2/22.9	0.40 ± 0.07	1.21 ± 0.13*	1.25 ± 0.05*	2.30 ± 0.04**

LPV/r= Lopinavir/ritonavir. MT= Melatonin. ALA=Alpha lipoic acid. Data as mean ± SEM (Standard error of mean), n=5, *Significant ($p<0.01$) difference when compared to LPV/r, **Significant ($p<0.001$) difference when compared to LPV/r.

Table 4: Effects of melatonin and alpha lipoic acid on lopinavir/ritonavir- induced serum follicle stimulating hormone levels in rats

Serum follicle stimulating hormone (μg/mL)				
Dose(mg/kg)	LPV/r	MT+LPV/r	ALA+LPV/r	MT+ALA+LPV/r
Control	10.7 ± 0.03	10.7 ± 0.03	10.7± 0.03	10.7 ± 0.03
22.8/5.71	4.10 ± 0.01	6.18 ± 0.03	6.05 ± 0.05*	10.31 ± 0.06**
45.6/11.4	2.70 ± 0.08	4.30 ± 0.07*	4.20 ± 0.04*	10.30 ± 0.02**
91.2/22.9	1.91 ± 0.04	3.68 ± 0.03*	3.55± 0.01*	10.00 ± 0.01**

LPV/r= Lopinavir/ritonavir, MT= Melatonin, ALA=Alpha lipoic acid, Data as mean ± SEM (Standard error of mean), n=5, *Significant ($p<0.01$) difference when compared to LPV/r, **Significant ($p<0.001$) difference when compared to LPV/r.

Table 5: Effects of melatonin and alpha lipoic acid on lopinavir/ritonavir- induced serum estradiol levels in rats

Estradiol (μg/mL)				
Dose(mg/kg)	LPV/r	MT+LPV/r	ALA+LPV/r	MT+ALA+LPV/r
Control	12.48 ± 1.25	12.48 ± 1.25	12.48 ± 1.25	12.48 ± 1.25
22.8/5.71	33.55 ± 3.60	17.55 ± 1.24*	18.15 ± 1.15*	10.18 ± 1.33*
45.6/11.4	47.45 ± 3.52	20.45 ± 2.03*	20.65 ± 1.30*	10.43 ± 1.30*
91.2/22.9	69.10 ± 4.20	27.01 ± 2.31*	30.26 ± 3.07*	11.38 ± 1.14**

LPV/r= Lopinavir/ritonavir, MT= Melatonin, ALA=Alpha lipoic acid, Data as mean ± SEM (Standard error of mean), n=5, *Significant ($p<0.01$) difference when compared to LPV/r, **Significant ($p<0.001$) difference when compared to LPV/r.

Table 6: Effects of melatonin and alpha lipoic acid on lopinavir/ritonavir- induced serum prolactin levels in rats

	Prolactin (µg/mL)			
Dose(mg/kg)	LPV/r	MT+LPV/r	ALA+LPV/r	MT+ALA+LPV/r
Control	0.28 ± 0.05	0.28 ± 0.05	0.28 ± 0.05	0.28 ± 0.05
22.8/5.71	1.57 ± 0.60	1.10 ± 0.04*	1.00 ± 0.05*	0.32 ± 0.06**
45.6/11.4	2.85 ± 0.74	1.03 ± 0.08*	1.10 ± 0.32*	0.35 ± 0.81**
91.2/22.9	4.63 ± 0.25	2.17 ± 0.45*	2.23 ± 0.31*	0.38 ± 0.14**

LPV/r= Lopinavir/ritonavir, MT= Melatonin, ALA=Alpha lipoic acid, Data as mean ± SEM (Standard error of mean), n=5, *Significant ($p<0.01$) difference when compared to LPV/r, **Significant ($p<0.001$) difference when compared to LPV/r.

3. Results

Serum levels of FSH, LH, PRL, ED, and T were normal ($p>0.05$) in rats treated with individual doses of MT and ALA when compared to control. Also, effect on serum FSH, LH, PRL, ED, and T levels were normal ($p>0.05$) in rats treated with combined doses of MT and ALA when compared to control (Table 1). On the other hand, serum levels of FSH, LH, and T were significantly ($p<0.001$) increased in a dose-dependent fashion in LPV/r-treated rats when compared to control. At LPV/r (99.2/22mg/kg) decreases represent FSH (450.6%), LH (550.9%), and T (470.3%) (Tables 2-4). However, the serum levels of FSH, LH and T were significantly ($p<0.01$) restored in rats supplemented with individual doses of MT and ALA when compared to LPV/r-treated rats. The restored levels of FSH, LH, and T were highest ($p<0.001$) in rats supplemented with combined doses of MT and ALA when compared to LPV/r-treated rats (Tables 2-4). Furthermore, serum levels of PRL and ED were significantly ($p<0.001$) increased in a dose-related manner in LPV/r-treated rats when compared to control. However, the serum levels of PRL and ED were significantly ($p<0.01$) restored in rats supplemented with individual doses of MT and ALA when compared to LPV/r-treated rats. The serum levels of PRL and ED were best ($p<0.001$) restored in rats supplemented with combined doses of MT and ALA when compared to LPV/r-treated rats (Tables 5 and 6).

4. Discussion

The current study assessed the protective effects of MT and ALA on altered levels of gonadal hormones in LPV/r-treated rats. The development of the male reproductive tract is a dynamic process, requiring the interaction of many factors and gonadal hormones. The activations of specific pathways are required, involving the productions FSH, LH and T that are essential for the development of the male internal and external reproductive system.[20, 21] Studies have shown that testicular morphologic changes as consequences of altered gonadal hormones are potential targets for HAART. [22] In the current study, serum FSH, LH, T, PRL and ED levels were normal in MT and ALA-treated rats. On the other hand, this study demonstrated decreased serum FSH, LH and T levels in LPV/r-treated rats in a dose-dependent fashion. Interestingly, the serum levels of FSH, LH and T were restored in MT and ALA supplemented rats. This study observed abysmal increases in serum PRL and ED levels in a dose-related fashion in LPV/r-treated rats. This finding is consistent with elevated PRL and ED levels associated with HAART. [23] However, serum ED and PRL levels were reversed in MT and ALA supplemented rats. The restored serum levels of FSH, LH, T, PRL and ED were most in rats supplemented with combined doses of MT and ALA in comparison to their individual doses. The mechanisms associated with altered levels of gonadal hormones in LPV/r-treated rats are not well understood, but LPV/r might have inhibited the functions of HPG axis leading to impaired production of gonadal hormones. [24] The hormonal regulation of spermatogenesis and testicular androgen production involves the HPG axis, and the testes. Testicular regulation involves three sets of hormones; gonadotropin-releasing hormone (GRH), FSH and LH. GRH is released from the hypothalamus and stimulates the pituitary

to produce gonadotropins. FSH and LH are gonadotropins, which stimulate and sustain intragonadal T production and spermatogenesis. [25] The reduced serum T observed in LPV/r- treated rats may be due to direct damage or oxidative damage to Leydig cells responsible for the synthesis of T. [26] Also, studies have shown that elevations in PRL and ED can inhibit hypophyseal gonadotrophins and other hypothalamic factors leading to impaired gonadotrophin synthesis. [27] The testicular protective effect of MT observed in this study can be correlated with the protective effect of MT against xenobiotic-induced testicular toxicity. [28] Also, the observed protective effect of ALA can be correlated with its protective effect against acrylamide and bi-n-butyl phthalate-induced testicular toxicities in rats. [29] The restored serum levels of FSH, LH, T, PRL and ED by MT and ALA observed in the current study may be due to their counter effects on the activity of LPV/r on HPG axis through their antioxidant effects and the modulation of other antioxidants activities through increased antioxidant gene expression. [30] MT and ALA might have terminated the oxidative effect of LPV/r at the testicular axis.[31] Also, studies have shown that MT and ALA can sustained the levels of cholesterol and sex hormone binding globulin (SHBG) by facilitating and stimulating signal transduction mechanisms necessary for normal function of hypothalamus-testicular axis leading to normal secretion of T and sperm production. [32] Furthermore, MT and ALA might have provided dual protection to create a robust shield on the testicular cells along with the liquid that surrounds the cells to tolerate higher volumes of free radical attack. [33] Inflammatory reactions through the production of pro-inflammatory cytokines which include tumour necrosis factor and interleukin 1 beta have been implicated in xenobiotic-induced testicular toxicity. [34] This showed that the testicular protective effects of MT and ALA observed in the current study may also be attributed to their anti-inflammatory effects. [35] In addition, the most testicular protective effect observed in rats supplemented with combined doses of MT and ALA can be attributed to pharmacologic interaction leading to increased effect.

Conclusion: The current study showed that dose-dependent alterations in gonadal hormones of LPV/r-treated rats were abrogated by MT and ALA supplementations. Abrogation was best in rats supplemented with combined doses of MT and ALA.

Acknowledgments: The authors appreciate Mr Joshua Isirima of the Department of Pharmacology, Faculty of Basic Clinical Sciences, University of Port Harcourt, Rivers State.

Funding: None

Conflict of interest: None

References

1 Manjithaya RR, Dighe RR. Regulation of Gonadotropins Synthesis. ***Proceed Ind Nat Sci Acad.*** 2006; 72 (1): 1-12

2 Sansone A, Di Dato C, de Angelis C, Menafra D, Pozza C, Pivonello R, Isidori A, Gianfrilli D. Smoke, alcohol and drug addiction and male fertility. ***Reprod Biol Endocrinol*** 2018; 16(1): 3 DOI: 10.1186/s12958-018-0320-7

3 Paton NI, Kityo C, Hoppe A, Reid A, Kambugu A, Lugemwa A, van Oosterhout JJ, Kiconco M, Siika A, Mwebaze R, Abwola M, Abongomera G, Mweemba A, Alima H, Atwongyeire D, Nyirenda R, Boles J, Thompson J, Tumukunde D, Chidziva E, Mambule I, Arribas JR, Easterbrook PJ, Hakim J, Walker AS, Mugyenyi P, Team ET. Assessment of second-line antiretroviral regimens for HIV therapy in Africa. ***N Engl J Med*** 2014; 371(3): 234-247 [PMID: 25014688 DOI: 10.1056/NEJMoa1311274]

4 Azu OO. Highly active antiretroviral therapy (HAART) and testicular morphology: current status and a case for a stereologic approach. ***J Androl*** 2012; 33(6): 1130-1142 [PMID: 22700761 DOI: 10.2164/jandrol.112.016758]

5 Collazos J. Sexual dysfunction in the highly active antiretroviral therapy era. ***AIDS Rev*** 2007; 9(4): 237-245 [PMID: 18219367]

6 Benveniste O, Simon A, Herson S. Successful percutaneous dihydrotestosterone treatment of gynecomastia occurring during highly active

antiretroviral therapy: four cases and a review of the literature. ***Clin Infect Dis*** 2001; 33(6): 891-893 DOI: 10.1086/322637

7 Fronczak CM, Kim ED, Barqawi AB. The insults of illicit drug use on male fertility. ***J Androl*** 2012; 33(4): 515-528 [PMID: 21799144 DOI: 10.2164/jandrol.110.011874]

8 Hardeland R, Balzer I, Poeggeler B, Fuhrberg B, Uria H, Behrmann G, Wolf R, Meyer TJ, Reiter RJ. On the primary functions of melatonin in evolution: mediation of photoperiodic signals in a unicell, photooxidation, and scavenging of free radicals. ***J Pineal Res*** 1995; 18(2): 104-111 DOI: 10.1111/j.1600-079x.1995.tb00147.x

9 Lukaszyk A. Melatonin as a modulator of testosteorne secretionin vivo and in vitro. Advances in pineal research. ***John Lbbey and CO. Ltd.*** Pp 1990. 217-224.

10 Torabi F, Malekzadeh Shafaroudi M, Rezaei N. Combined protective effect of zinc oxide nanoparticles and melatonin on cyclophosphamide-induced toxicity in testicular histology and sperm parameters in adult Wistar rats. ***Int J Reprod Biomed*** 2017; 15(7): 403-412 [PMID: 29202124 PMCID: PMC5601931]

11 Mirhoseini M, Talebpour Amiri F, Karimpour Malekshah AA, Rezanejad Gatabi Z, Ghaffari E. Protective effects of melatonin on testis histology following acute torsion-detorsion in rats. ***Int J Reprod Biomed*** 2017; 15(3): 141-146 [PMID: 28580446 PMCID: PMC5447830]

12 Hamzawy,M.A.,El-Denshary ESM,Bahgat AK, Hassan NS, Aly SE, Abdel-Wahhab MA. Hepatoprotective effect of estradiol and-Lipoic Acid in Rats. ***Global J Pharm.*** 2014. 8 (4): 694-702. DOI:10.5829/idosi.gjp.2014.8.4.91118

13 El-Beshbishy HA, Mariah RA, Al-Azhary NM, Aly HA, Ozbak HA, Baghdadi HH. Influence of lipoic acid on testicular toxicity induced by bi-n-butyl phthalate in rats. ***Food Chem Toxicol*** 2014; 71: 26-32 [PMID: 24912129 DOI: 10.1016/j.fct.2014.05.024]

14 Aly HA, Lightfoot DA, El-Shemy HA. Modulatory role of lipoic acid on lipopolysaccharide-induced oxidative stress in adult rat Sertoli cells in vitro. ***Chem Biol Interact*** 2009; 182(2-3): 112-118 [PMID: 19699728 DOI: 10.1016/j.cbi.2009.08.013]

15 Sedky A, Ali A. Protective Effects of α-lipoic acid on Biological changes Induced by -cypermethrin in Testis Rats. ***J Bios and App Res.*** 2018 4 (2): 84-97 DOI: 10.21608/jbaar.2018.129883

16 Hull MW, Harris M, Lima V, Guillemi S, Harrigan PR, Montaner JS. Lopinavir/ritonavir pharmacokinetics in a substitution of high-dose soft-gelatin capsule to tablet formulation. J Clin Pharmacol 2009; 49(2): 155-161 [PMID: 19179294 DOI: 10.1177/0091270008329550]

17 Bilginoglu A, Aydin D, Ozsoy S, Aygun H. Protective effect of melatonin on adriamycin-induced cardiotoxicity in rats. ***Turk Kardiyol Dern Ars*** 2014; 42(3): 265-273 [PMID: 24769819 DOI: 10.5543/tkda.2014.36089]

18 Reyskens KM, Fisher TL, Schisler JC, O'Connor WG, Rogers AB, Willis MS, Planesse C, Boyer F, Rondeau P, Bourdon E, Essop MF. Cardio-metabolic effectsof HIV protease inhibitors (lopinavir/ritonavir). ***PLoS One*** 2013; 8(9): e73347 DOI: 10.1371/journal.pone.0073347

19 Shagirtha K, Muthumani M, Prabu SM. Melatonin abrogates cadmium induced oxidative stress related neurotoxicity in rats. ***Eur Rev Med Pharmacol Sci*** 2011; 15(9): 1039-1050 [PMID: 22013727]

20 Wilson JD. Sexual differentiation. ***Annu Rev Physiol*** 1978; 40: 279-306 [PMID: 345951 DOI: 10.1146/annurev.ph.40.030178.001431]

21 Bispo AC, Galvão TC, Tamoyese VM, Costa GA, Ramos SP, Salles MJS. Effects of Isotretinoin on the Reproduction of Pubertal Male Mice and Malformations in the Offspring ***Am J Biomed Sci.*** 2017; 9(4) : 225-236; DOI:10.5099/aj170400225

22 Barboza JM, Medina H, Doria M, Rivero L, Hernandez L, Joshi NV. Use of atomic force microscopy to reveal sperm ultrastructure in HIV-patients on highly active antiretroviral therapy. ***Arch Androl*** 2004; 50(2): 121-129 [PMID: 14761843]

23 Lamba H, Goldmeier D, Mackie NE, Scullard G. Antiretroviral therapy is associated with sexual dysfunction and with increased serum oestradiol levels in men. ***Int J STD AIDS*** 2004;

15(4): 234-237 [PMID: 15075015 DOI: 10.1258/095646204773557749]
24 Ghanbarzehi A ,Fanaei H , Shahraki MK , Niafar MB. Effect of iranian crack on testosterone and gonadotropin levels in addicted men. ***IIOAB J.*** 2017; 8 (1) :7-10 http://eprints.zaums.ac.ir/id/eprint/528
25 Corradi PF, Corradi RB, Greene LW. Physiology of the Hypothalamic Pituitary Gonadal Axis in the Male. ***Urol Clin North Am*** 2016; 43(2): 151-162 [PMID: 27132572 DOI: 10.1016/j.ucl.2016.01.001]
26 Aguilar-Mahecha A, Hales BF, Robaire B. Chronic cyclophosphamide treatment alters the expression of stress response genes in rat male germ cells. ***Biol Reprod*** 2002; 66(4): 1024-1032 DOI: 10.1095/biolreprod66.4.1024
27 Srilatha B, Adaikan PG. Estrogen and phytoestrogen predispose to erectile dysfunction: do ER-alpha and ER-beta in the cavernosum play a role? ***Urology*** 2004; 63(2): 382-386 DOI: 10.1016/j.urology.2003.08.042
28 Gholami M, Ahmadi SAY, Abaszadeh A, Khaki A. Protective effects of melatonin and ghrelin on spermatogenesis: A narrative review of the literature. ***Int J Reprod Biomed*** 2017; 15(5): 265-272 [PMID: 28744521 PMCID: PMC5510579]
29 Lebda M, Gad S, Gaafar H. Effects of lipoic Acid on acrylamide induced testicular damage. ***Mater Sociomed*** 2014; 26(3): 208-212 DOI: 10.5455/msm.2014.26.208-212
30 Alp BF, Kesik V, Malkoc E, Yigit N, Saldir M, Babacan O, Akgul EO, Poyrazoglu Y, Korkmazer N, Gulgun M, Erdem O. The effect of melatonin on procarbazine induced testicular toxicity on rats. ***Syst Biol Reprod Med*** 2014; 60(6): 323-328 [PMID: 25140409 DOI: 10.3109/19396368.2014.930212]
31 Adikwu E, Brambaifa N, Obianime WA. Melatonin and alpha lipoic acid as possible therapies for lopinavir/ritonavir-induced hepatotoxicity in albino rats. ***Phys and Pharm,*** 2016; 20; 287-295
32 Othman AI, El-Missiry MA, Koriem KM, El-Sayed AA. Alfa-lipoic acid protects testosterone secretion pathway and sperm quality against 4-tert-octylphenol induced reproductive toxicity. ***Ecotoxicol Environ Saf*** 2012; 81: 76-83 [PMID: 22560493 DOI: 10.1016/j.ecoenv.2012.04.018]
33 Cronan JE, Zhao X, Jiang Y. Function, attachment and synthesis of lipoic acid in Escherichia coli. ***Adv Microb Physiol*** 2005; 50: 103-146 DOI: 10.1016/S0065-2911(05)50003-1
34 Sherif IO, Abdel-Aziz A, Sarhan OM. Cisplatin-induced testicular toxicity in rats: the protective effect of arjunolic acid. ***J Biochem Mol Toxicol*** 2014; 28(11): 515-521 [PMID: 25130312 DOI: 10.1002/jbt.21593]
35 Adikwu E, Brambaifa N, Obianime WA. Beneficial effects of melatonin and alpha lipoic acid on lopinavir/ ritonavir-induced alterations in serum lipid and glucose levels of male albino rats. ***Maced Pharm Bull.*** 2016; 62 (1): 47–55
DOI:10.33320/maced.pharm.bull.2016.62.01.004

