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Melatonin Improves Blood Biochemical Parameters and DNA Integrity in the Liver and Kidney of Hyperthyroid Male Rats

Hager M Ramadan*, Nadia A Taha and Hodallah H Ahmed

Department of Physiology, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt
***Corresponding author:** hmagdy515@yahoo.com

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ABSTRACT

The present study was carried out to investigate and compare the effect of hyperthyroidism either alone or in combination with melatonin on some blood biochemical parameters and DNA integrity in liver and kidney of male rats. For this purpose 75 mature male rats weighing 120-140g were randomly divided into five groups (control, hyperthyroid, hyperthyroid plus 1, 5 or 10 mg/kg bwt of melatonin, respectively). Hyperthyroidism was experimentally induced by daily I/P injection of L-thyroxine (0.2 mg/kg body weight). The melatonin treated groups were injected with the same dose of L-thyroxine followed by I/P injection of melatonin (1, 5 or 10 mg/kg, respectively). The study was carried out for 21 days. The last blood and tissue samples were collected one day after the end of the last injection (on 22nd day). The results revealed that hyperthyroidism significantly increased the levels of urea and the activities of GOT, GPT, ALP as well as the percentage of DNA fragmentation in the liver and kidney. At the same time hyperthyroidism induced a significant decrease in level of creatinine. Treatment with the 3 doses of melatonin completely ameliorated the hyperthyroidism- induced increase in GPT and ALP, while 5 and 10 mg could completely counteract the hyperthyroidism- induced increase in urea and only the 10 mg melatonin could ameliorate the hyperthyroidism-induced increase in GOT activity. It is concluded that melatonin can ameliorate the hyperthyroidism-induced disturbance in blood biochemical parameters and DNA fragmentation.

Key words: Melatonin, L-thyroxine, Biochemical parameters, DNA, Rats.

INTRODUCTION

Thyroid hormones were found to play an important role in the regulation of energy metabolism in almost all mammalian tissues (Ourique *et al.*, 2013). However, the hypermetabolic state in hyperthyroidism is accompanied with increased free radical production and lipid peroxidation (Varghese *et al.*, 2001). The hyperthyroidism-induced oxidative stress was found to be accompanied with oxidative damage in macromolecules such as lipid, proteins and DNA (Karbownik-Lewińska and Kokoszko-Bilska, 2012) with hepatic (Messarah *et al.*, 2010) and renal injury (Basu and Mohapatra, 2012). In addition peroxidation of membrane lipids was found to disturb the structure of cell membranes which will affect membrane fluidity, the dynamics of lipid-lipid and lipid-protein interaction, membrane permeability, nutrient and ion transport as well as metabolic process leading to cell death (Volinsky and Kinnunen, 2013).

Melatonin was reported to play an important role in the endocrine control of some physiological and metabolic actions and to affect the capacity of nutrient benefits (Çalislar *et al.*, 2018). It was found to have many characters which make it of special importance: a) it can easily cross cell membranes and blood brain and placental barriers, b) it can be absorbed if it is administered by any route, c) protects mitochondrial function and has low toxicity (Acuña-Castroviejo *et al.*, 2001). Melatonin was also found to d) increase the ability of antioxidants such as vitamin E, vitamin C and glutathione synergistically (Gitto *et al.*, 2001), e) prevent the renal injury and protect the kidney from oxidative damage (Kilic *et al.*, 2013), f) control oxidative damage induced by hyperthyroidism (Mogulkoc *et al.*, 2006) and g) have hepatoprotective effect (Oleshchuk *et al.*, 2019).

The aim of the present study was to evaluate and compare the effect of different levels of melatonin on the adverse effect induced by hyperthyroidism on some blood biochemical parameters and DNA damage in the liver and kidney of male rats with experimentally-induced hyperthyroidism.

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MATERIALS AND METHODS

Animals and treatments

Seventy-five mature male rats weighing 120-140g were obtained from the animal house colony of Abou-Rawish, Giza, Egypt. The experimental protocols and procedures were approved by the Institutional Animal Care and Use Committee of the Faculty of Veterinary Medicine Cairo University (VET. CU. IACUC) (Egypt) (VetCU/10102019095). The rats were fed on standard granulated ration containing crude protein not less than 21%, crude fat not less than 4.43% and crude fibers not more than 2.94%. The rats were kept under 12h light/dark cycle and had free access to food and water.

Drugs

a) L-thyroxine: (levothyroxine, sigma, Egypt) prepared by daily dilution of the stock solution (L-thyroxine dissolved in 4M ammonium hydroxide in methanol) to the desired concentration with distilled water (Shinohara *et al.*, 2000) (Sigma, catalog number T-2376).

b) Melatonin: It was obtained from Memphis Company for pharmacy and chemical industry in Egypt. It was dissolved every day in a minimum volume of absolute ethanol and further diluted in saline to give a final concentration of 1% ethanol (Hermoso *et al.*, 2016). The purity and strength of melatonin was tested by using HPLC (Perkin-Elmer, Binary LC Pump USA) in Micro analytical center Cairo University and it was 97%.

Experimental design

Rats were randomly divided into 5 groups with 3 replicate cages having 5 rats in each as follow: The first group was kept as a control group and received 2 I/P injections of the vehicles used for dissolving of L-thyroxine and melatonin. In the second group (Hyperthyroid group): hyperthyroidism was induced by daily I/P injection of 0.2 mg L-thyroxine/kg body weight. In the 3rd, 4th and 5th groups (Hyperthyroidism and melatonin groups): the rats received daily I/P injection of the same dose L-thyroxine followed by I/P of melatonin (1, 5 or 10 mg/kg body weight, respectively). The study was carried out for 21 days (Mogulkoc *et al.*, 2006). At the end of the experiment, blood and tissue samples were collected 24h after the end of the last injection (on the 22nd day).

Blood samples

Fasting blood samples were collected twice by orbital sinus puncture under isoflurane anesthesia. The 1st blood sample was collected on the morning of 11th day and the second was collected on the morning of 22nd day (24h after last injection). Sera were stored at -20°C until assays were carried out.

Tissue samples

At the end of experiment after collection of the 2nd blood samples all rats were sacrificed by cervical dislocation and tissue samples from liver and kidney were obtained and used for estimation of the percentage of DNA damage.

Measured parameters

Hormones

To confirm that the used dose of L-thyroxine induced hyperthyroidism thyroid hormones (T3 and T4) and TSH were estimated as follow: Thyroxine (T4), Triiodothyronine (T3) and Thyroid stimulating hormone (TSH) were measured using automatic ELISA reader (EZ Read 400, Microplate Reader, Biohrome, England). T4 and T3 were estimated by using commercial kits purchased from Chemux Company-Egypt. TSH was measured by using rat specific commercial kits purchased from EIAab Company-Egypt.

Biochemical parameters

All kits used in this part were purchased from Spectrum Company, Egypt. Biochemical parameters in serum were measured using UV spectrophotometer (Jasco,V-730., Japan), biochemical study included measurements of Liver enzymes activity by: a) Aspartate amino transferase (AST), b) Alanine amino transferase (ALT) and c) Alkaline phosphatase (ALP) with kidney functions by: d) Creatinine (SC) and e) Urea nitrogen concentration (SUN).

Determination of DNA damage in liver and kidney tissues of male rats

At the 22nd day apoptotic changes in the liver and kidney tissue were evaluated colorimetrically by DNA fragmentation percentage using the diphenylamine (DPA) assay and DNA laddering assay using agarose gel electrophoresis according to the method described by Perandones *et al.* (1993).

Statistical analysis

All data were presented as Mean±SE. They were subjected to one-way analysis of variance test (ANOVA), followed by the Tukey-Kramer multiple comparison test using statistical analysis system program (Instat-3). The GraphPad prism software, version 6 (GraphPad Software Inc., San Diego, CA, USA) was used to draw the attached figures.

RESULTS

Thyroid activity

Figures (1 and 2) indicates that the hyperthyroid group showed a significant increase in the level of serum T4 and T3 ($P<0.001$) with a significant decrease in the level of TSH ($P<0.001$) vs control group during all sampling periods.

Effect of L-thyroxine alone and in combination with melatonin on blood biochemical parameters of male rats

Liver function tests

a) Glutamic oxaloacetic transaminase (GOT, U/L) and Glutamic pyruvate transaminase (GPT, U/L): The data represented in table (1) show that GOT and GPT activities in the hyperthyroid group were significantly higher than control group ($P<0.001$) and all groups treated with melatonin ($P<0.001$) through all sampling periods. At the 11th day melatonin treated groups induced a significant decrease in GOT and GPT activities vs the hyperthyroid

Table 1: Effect of L-thyroxine either alone or in combination with melatonin on liver function tests in the serum of male rats

Groups time	Control	L-thyroxine (L-thy. 0.2 mg/kg)	L-thy. + melatonin (1mg/Kg)	L-thy. + melatonin (5mg/Kg)	L-thy. + melatonin (10 mg/Kg)
			GOT (U/L)		
11th day	20.9±1.3^{abcd}	100.8±3.8^{aefg}	69.5±1.7^{behi}	55.6±2.97^{cfhj}	37.8±1.6^{dgij}
22nd day	49.2±1.9^{ab}	110.9±6.3^{acde}	74.5±2.9^{bcfg}	54.4±3.1^{df}	41.0±2.6^{eg}
			GPT (U/L)		
11th day	4.7±0.6^{abcd}	31.4±1.5^{aefg}	20.9±0.8^{beh}	21±1.3^{cfi}	14.8±1.2^{dghi}
22nd day	12±0.8^{abc}	30.2±1.2^{adef}	21.6±1.1^{bdg}	19.9±0.8^{ceh}	7.9±1.2^{fgh}
			ALP (U/L)		
11th day	36.2±1.89^{abc}	68.7±2.5^{adef}	55.9±1.99^{bdg}	48.96±2.02^{ceh}	39.5±1.9^{fgh}
22nd day	35.05±1.4^{a}	73.2±4.5^{abcd}	34.9±2.9^{b}	37.6±2.3^{c}	35.16±2.4^{d}

Each value is expressed as Mean±SE. Means having the same letter in the same row are significantly different.

Table 2: Effect of L-thyroxine either alone or in combination with melatonin on kidney function tests in the serum of male rats.

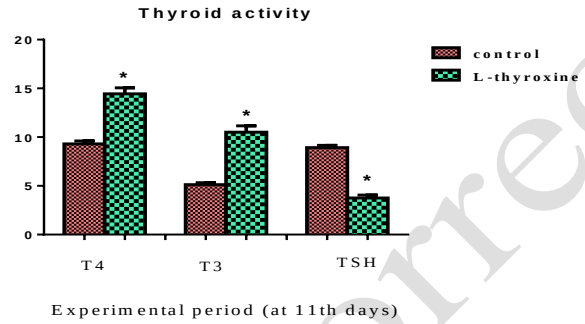
groups time	Control	L-thyroxine (L-thy. 0.2mg/kg)	L-thy. + melatonin (1mg/Kg)	L-thy.+ melatonin (5mg/Kg)	L-thy. + melatonin (10 mg/Kg)
Creatinine (mg/dL)					
11th day	0.97±0.1^{abcd}	0.45±0.03^{a}	0.47±0.03^{b}	0.47±0.04^{c}	0.5±0.04^{d}
22nd day	0.89±0.054^{abcd}	0.42±0.029^{ade}	0.57±0.028^{b}	0.68±0.051^{cd}	0.64±0.026^{de}
Urea (mg/dL)					
11th day	39.2±1.8^{ab}	61±3.8^{acde}	48.7±1.79^{bcf}	46.96±0.57^{dg}	36.9±2.4^{efg}
22nd day	30.1±1.80^{ab}	45.8±1.22^{acde}	38.3±1.81^{bcfg}	31.9±1.41^{df}	26.6±1.3^{eg}

Each value is expressed as Mean±SE. Means having the same letter in the same row are significantly different.

Table 3: Effect of L-thyroxine alone and in combination with melatonin on level of DNA damage (%) in the kidney and liver tissue of male rats at the 22nd day.

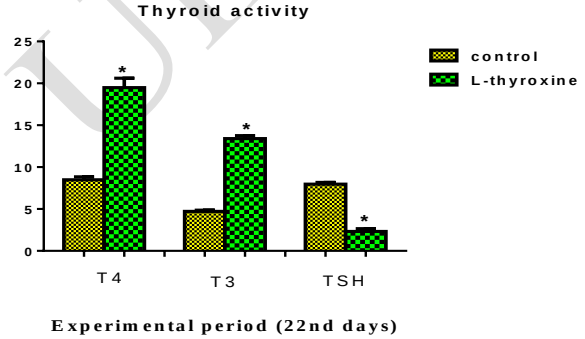
Organs	Control	L-thyroxine (L-thy. 0.2mg/kg)	L-thy. + melatonin (1mg/Kg)	L-thy.+ melatonin (5mg/Kg)	L-thy. + melatonin (10 mg/Kg)
Kidney	18.7±2$^{\mathbf{abcd}}$	60.7±4.9$^{\mathbf{aefg}}$	46±1.5$^{\mathbf{beh}}$	33.7±1.5$^{\mathbf{cfh}}$	45.3±1.2$^{\mathbf{dg}}$
Liver	12.7±2.8$^{\mathbf{abcd}}$	57.7±2.6$^{\mathbf{aefg}}$	43.7±1.8$^{\mathbf{be}}$	34.7±1.8$^{\mathbf{cf}}$	45±2.3$^{\mathbf{dg}}$

Each value is expressed as Mean±SE. Means having the same letter in the same row are significantly different



Groups having (*) are significantly different vs their corresponding controls.

Fig. 1: Effect of L-thyroxine either alone or in combination with melatonin on serum T4, T3 and TSH of male rats at 11th day of experiment.



Groups having (*) are significantly different vs their corresponding controls.

Fig. 2: Effect of L-thyroxine either alone or in combination with melatonin on serum T4, T3 and TSH of male rats at 22nd day of experiment

group; however, they were still significantly higher than control group. At the end of experiment the 5 and 10 mg/kg melatonin completely counteracted the hyperthyroid-induced increase in serum GOT activity while, the increase in GPT activity was completely counteracted by the 10 mg/kg dose of melatonin only.

b) Alkaline phosphatase (ALP) (U/L)

Table 1 shows that ALP activity in the hyperthyroid group was significantly higher than control and all melatonin treated groups all over the sampling periods. The ALP activity in the control group was significantly lower than groups treated with 1 and 5 mg melatonin on the 11th day of the experiment, while, no significance difference was recorded between control group and groups treated with 10 mg melatonin. However, no significance difference was found between control group and all melatonin treated groups at the end of the experiment.

Kidney function tests

a) Serum creatinine level (SC, mg/dL)

Table 2 clarifies that serum creatinine level was significantly higher in the control group vs all treated groups ($P<0.001$) during the whole sampling periods. No significant difference was recorded between hyperthyroid group and melatonin treated groups at the 11th day of the experiment. However, at the end of experiment the 5 and 10mg/kg melatonin groups exhibited a significant increase ($P<0.001$ and $P<0.01$, respectively) in creatinine level vs hyperthyroid group.

b) Serum urea nitrogen level (SUN, mg/dL)

It is clear from table 2 that SUN in the hyperthyroid group was significantly higher than control group (P<0.001) and all melatonin treated groups (P<0.01, P<0.001 and P<0.001 in the 1, 5 and 10 mg, respectively) at all sampling periods. Melatonin treated groups produced a dose dependent decrease in SUN level vs hyperthyroid group. No significant difference was found between control group and groups treated with 5 and 10 mg melatonin at the 11th and 22nd days of the experiment.

Effect of L-thyroxine alone and in combination with melatonin on DNA fragmentation in liver and kidney tissues of male rats

From table 3 it is clear that hyperthyroidism induced the highest percentage of DNA damage in the tissues of liver and kidney versus control group (P<0.001 and P<0.001, respectively). The 3 doses of melatonin partially ameliorated the hyperthyroid induced increase in the percentage of DNA damage in both tissues. Melatonin at 1, 5 and 10 mg significantly decreased the percentage of DNA damage in the liver (P<0.01, P<0.001 and P<0.05, respectively) and kidney (P<0.05, P<0.001 and P<0.05, respectively) tissues vs hyperthyroid group. However, the control group exhibited the lowest percentage of DNA damage in both organs. It was significantly lower than hyperthyroid and all melatonin supplemented groups.

DISCUSSION

The effect of hyperthyroidism on liver enzymes in the present data revealed that hyperthyroidism induced about 5 fold increase in serum AST (382.2%) vs control group; however, addition of melatonin in combination with L-thyroxine induced a significant dose dependent decrease in AST activity and the 10 mg dose of melatonin produced the highest decrease (-166.6%) vs hyperthyroid group. Meanwhile, ALT activity in the hyperthyroid group was significantly higher than control group (580%). The 3 doses of melatonin significantly decreased the hyperthyroidism-induced increase in ALT activity and also the 10 mg dose of melatonin produced the highest decrease. Concerning ALP activity, the present results revealed that hyperthyroid group significantly increased ALP activity vs control group (89.8%) and the 3 doses of melatonin produced a dose dependent decrease in ALP activity and still the 10 mg dose producing the highest decrease. It returned ALP activity back to the normal activity in the control group and completely counteracted the hyperthyroidism induced increase in ALP activity. No available data could be found regarding the effect of melatonin on the hyperthyroidism-induced increase in liver enzymes. However, Messarah *et al.* (2010) recorded higher activities of liver enzymes in the serum in response to oxidative stress induced by hyperthyroidism in rats. The lowering effect of melatonin reported in the present study might be explained by the study of Oleshchuk *et al.* (2019) who attributed the hepatoprotective effect of melatonin to its ability to reduce free radical generation, metal detoxification and to regulate mitochondrial homeostasis and antioxidant system balance in rats.

Concerning the effect of hyperthyroidism on kidney function the present study revealed that hyperthyroid group produced a significant increase in serum urea nitrogen (SUN) concentration vs control group (55.6%) and all L-thyroxine plus melatonin treated groups. Addition of melatonin to L-thyroxine induced a significant dose dependent decrease in SUN level. The 10 and 5 mg of melatonin produced the highest decrease (-65.3% and -30.0%, respectively) in SUN and completely counteracted the hyperthyroidism-induced increase in SUN level and returned it back to the normal level in the control group.

The study of Abdella *et al.* (2013) recorded an increase in serum urea level in hyperthyroid patients. Moreover, Aizawa *et al.* (1986) in his earlier study attributed the increase in BUN to the increase in UN production as results of the increase in protein catabolism with insufficient renal excretion. The hyperthyroidism-induced increase in SUN could also be attributed to the oxidative stress produced as a result of the effect of hyperthyroidism on the kidney tissue. Mogulkoc *et al.* (2005) demonstrated that hyperthyroidism induced by L-thyroxine produced oxidative stress in kidney tissue and melatonin could inhibit this stress. Khodadadi *et al.* (2016) added that melatonin was able to prevent renal tubular injury by protecting the kidney from oxidative damage by activating several antioxidant enzymes and reducing lipid peroxidation.

Meanwhile, the obtained data concerning the effect of hyperthyroidism on serum creatinine level indicated that the control group showed the highest concentration of serum creatinine. It was significantly higher than hyperthyroid group (53.6%) and all groups treated with L-thyroxine plus melatonin. The three doses of melatonin used in the present study could not produce any significant changes in the hyperthyroidism-induced decrease in serum creatinine level at the 11th day; however, at the end of the experiment the 5 and 10 mg doses of melatonin significantly increased creatinine level vs hyperthyroid group and partially counteracted the hyperthyroid-induced decrease in serum creatinine level. The effect of hyperthyroidism on serum creatinine level in the present study is in agreement with Den Hollander *et al.* (2005) and Basu and Mohapatra (2012) who detected a decrease in serum creatinine level in hyperthyroid patients. The reduction in serum creatinine level could be due to the increase in renal plasma flow and glomerular filtration rate (Den Hollander *et al.*, 2005). Moreover, Den Hollander *et al.* (2005) added that creatinine production in muscles depends on thyroid condition and in hyperthyroidism both lean body mass and muscle mass are decreased resulting in lowering of creatinine level.

The present data also revealed that hyperthyroidism exhibited a significant increase in the percentage of DNA damage in liver (57.6%) and kidney (60.7%) vs controls in both organs (12.7 and 18.7%, respectively). However, all groups treated with L-thyroxine plus melatonin (1, 5 or 10 mg/kg) showed a significant decrease in the percentage of DNA damage in the liver (43, 34.7 and 50%, respectively) and kidney (46, 33.7 and 45.3%, respectively) vs hyperthyroidism in both organs. The obtained results on the hyperthyroidism-induced DNA damage are in agreement with Leo *et al.* (2012) who recorded an oxidative DNA damage in the liver and kidney of mice after thyroid hormones administration.

Moreover, Pascual and Aranda (2013) reported that treatment with thyroid hormones produce formation of DNA damage foci in the liver and kidney of mice. In the same concern, the study of Zambrano *et al.* (2014) demonstrated that binding of T3 to its receptors (THRB) produces DNA damage in culture cells of hyperthyroid mice. Rodier *et al.* (2009) added that T3 could generate DNA damage which result in induction of cellular senescence. The DNA damage reported in the current study could be attributed to the hyperthyroidism-induced free radical formation. The earlier studies of many investigators attributed the DNA damage induced by hyperthyroidism to the hypermetabolic condition which is accompanied with an increase in lipid peroxidation and free radical formation (Venditti *et al.*, 1997). Also Das and Chainy (2001) stated that ROS induces oxidative damage in the macromolecules of the cell as lipids, proteins and DNA. Moreover, Taleux *et al.* (2009) indicated that thyroid hormones are involved in the modulation of mitochondrial respiration process. No available data could be found regarding the effect of melatonin on the hyperthyroidism induced increase in DNA damage. However, Liu *et al.* (2013) reported that melatonin was able to protect DNA against oxidative damage and increase DNA repair capacity. Melatonin hormone was also found to be involved in the regulation of DNA synthesis, cell cycle and apoptosis (Cos *et al.*, 2006). Furthermore, melatonin was demonstrated to protect cells against oxidative DNA damage by stimulating anti-oxidative enzymes, scavenging reactive oxygen species as well as inactivating hydrogen peroxide (Osseni *et al.*, 2000). In the same regard Liu *et al.* (2013), added that melatonin was reported to suppress apoptosis, help in inactivation of the DNA-damaging agent and to stimulate DNA repair capacity by affecting several key genes involved in DNA damage repair pathways.

Conclusions

The present study provides a new understanding about the role of melatonin in the protection against the hyperthyroidism-induced change in DNA and blood biochemical parameters and the 10 mg dose of melatonin produced the highest effect in most cases.

Authors contribution

Hodallah H. Ahmed was the supervisor of this work; she designed the protocol of experiment and shared in collection the scientific material, formulating results and discussing it and writing the manuscript. Nadia A. Taha: She shared in collecting of samples, formulating results and writing the manuscript. Hager M. Ramadan: she was responsible for taking care of the animals and shared in collection of samples, applying statistics, collection of literature and writing of the manuscript.

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