

Effect of Melatonin and/ or Propylthiouracil on Hyperthyroidic Male Rats

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Abstract: The mutual relationships between the pineal gland and the thyroid have for a long time been a subject of intensive research. Thus, this study was performed in order to determine the effect of melatonin and/or propylthiouracil on oxidative stress in a model of hyperthyroidism. This study was carried out on 35 adult male albino rats (245-276 g) divided into 4 groups: G1: control rats, G2: hyperthyroid rats fed with standard chow diet, G3: hyperthyroid rats treated with propylthiouracil, G4: hyperthyroid rats treated with propylthiouracil & melatonin. At the end of the experimental period, rats were anaesthetized and electrocardiograph (ECG) recordings were obtained. Hearts & liver were subjected to histopathological examinations. Blood was collected for determination of hemoglobin (Hb), serum total tT_3 , tT_4 , thyroid stimulating hormone (TSH), and blood superoxide dismutase (SOD), plasma glutathione (GSH) and malondialdehyde (MDA). ECG recording revealed significant increase in HR and R voltage in G2 compared to control rats. Biochemical studies showed significant decrease in blood SOD, Hb and serum TSH in the three experimental trial groups compared to the control group. Plasma MDA level showed significant increase in G2 and G3 compared to control group, as well as significant decrease was observed in G4 compared to G2 and G3. Concerning GSH a significant decrease was observed in G2 compared to control group, upon addition of melatonin, significant increase was observed compared to G2 and G3. As regard serum tT_4 , significant increases was observed in the three trial groups compared to control group. Concerning serum tT_3 level, a significant decrease in its level in treated hyperthyroid rats (G3) compared to G2. Histopathological examination of hearts of G2 showed vacuolation of cardiac myocytes and myolysis. These changes were ameliorated upon addition of melatonin to propylthiouracil than propylthiouracil alone. Histopathological examination of liver of hyperthyroid rats showed kupffer cells activation, focal area of hepatic necrosis and leucocytic cell infiltration. Rats treated with propylthiouracil showed only congestion of central vein. Upon addition of melatonin to propylthiouracil, no change was observed in the liver except a slight congestion of central vein. These findings indicate that hyperthyroid associated-oxidative stress contributes to early cardiac & hepatic complications of hyperthyroidism and that addition of melatonin to anti-thyroid drugs could be beneficial in amelioration of these complications. [Journal of American Science 2010;6(10):904-914]. (ISSN: 1545-1003).

Key words: Propylthiouracil, melatonin, oxidative stress

1. Introduction:

Hypertrophic cardiomyopathy is a major heart disease which causes a large number of deaths globally (Ghosh *et al.*, 2007). Extrinsic stimuli include pressure overload, volume overload, abnormal hormone levels, intrinsic stimuli include contractile abnormalities (Feldman *et al.*, 1993); as in prolonged hyperthyroidism (Cohn 1996).

In hyperthyroidism, cardiac hypertrophy is accompanied by an overall increase in metabolic rate and enhanced lipolysis (Woeber 1992). Accumulating evidence has suggested that the hyper-metabolic state in hyperthyroidism is associated with increases risk in free radical production and lipid peroxide levels in several target tissues (Videla 2000). Recent study on liver, muscle, and heart suggest that this oxidative stress is due to increased reactive oxygen species released from mitochondria (Venditti *et al.*, 2003).

Despite methimazole and propylthiouracil having been used for more than a century to treat

hyperthyroidism, controversy still exists in antithyroid drug therapy (Nakamura *et al.*, 2007).

Melatonin (N-acetyl-S methoxy tryptamine), the main secretory product of the pineal gland is a well known antioxidant and free radical scavenger, widely distributed in the organism. Mutual relationships between the pineal gland and the thyroid have for long time been a subject of intensive research. It is highly probable that under physiological conditions, melatonin and possibly other antioxidants regulate ROS generation for thyroid hormone synthesis (Karbownik and Lewinski, 2003). Thus, this study was performed in order to determine the effect of melatonin and/or thiouracil on oxidative stress in a model of medical hyperthyroidism.

2. Material and Methods

Experimental animals:

This study was carried out on 35 adult male albino rats weighing 245- 276 gm at start of the study,

rats were purchased from Experimental Animal farm of Helwan and housed individually in suspended wire-mesh cages and maintained in Nutrition National Institute animal house under standard conditions of boarding. All rats were fed standard rat chow before starting the experiment. The standard rat chow diet (AIN-93 M diet formulated for adult rodents) was prepared according to the National Research Council (NRC) 1978 and Reeves *et al.*, (1993).

Experimental protocol:

The rats were allocated into 4 groups

- Group 1 include control rats (n=8). These rats were treated with intraperitoneal injection of normal saline in a similar volume as test group.
- Group 2 include untreated hyperthyroid rats (n=10). Hyperthyroidism was induced by daily intraperitoneal injection of tetra-iodo-thyronine in a dose of 10 µg/100 g BW for 30 days (40 µg dissolved in 1 ml normal saline) as described by Venditti *et al.*, (2006).
- Group 3 (n=9): Hyperthyroid rats treated with propylthiouracil; hyperthyroidism was induced as previous group for 30 days. Propylthiouracil therapy was started in the last 10 days of the study at a dose of 0.04 g/100 ml drinking water, as described by Delp *et al.*, (1995).
- Group 4 (n=8): Hyperthyroid rats treated with propylthiouracil and melatonin. Hyperthyroidism and propylthiouracil therapy was started as the previous group. Melatonin was given by intraperitoneal injection in a dose of 10 mg/kg started in the last 10 days of the experiment as described by Baydas & Merai (2005).

Tetra-iodo-thyronine, Propylthiouracil and Melatonin were purchased from (Sigma- Aldrich, St Louis, MO, USA)

Experimental procedure:

At the end of the experimental period, all rats were fasted overnight, weighed and anaesthetized with intraperitoneal thiopental sodium (40 mg/Kg BW). Height (from the tip of the nose to the anus) was measured and ECG was recorded for each rat, a midline incision was made, then the abdominal aorta was exposed and blood samples were collected in three centrifuge tubes; plain tube to obtain serum and 2 Na₂EDTA tubes to obtain whole blood and plasma. Tubes were centrifuged at 4000 r.p.m. for 15 minutes for separation of serum and plasma and were stored at -20° till used for determination of biochemical measurements.

1-ECG recording:

Needle electrodes were placed under the skin of the 4 limbs of the animal near the paws, and connected through an ECG coupler to a 2 channel oscillograph

(Cardimax FX 121, Fukuda Denshi Co, LTD, Tokyo, Japan). The electro-cardiographic tracing was recorded using standard limbs. From lead II-ECG tracing with paper speed of 25 mm/sec, heart rate (HR), P-R interval, QRS duration, QT interval, Q wave voltage, R wave voltage and ST segment deviation were measured. The heart rate was calculated using the following formula:

$$\text{Heart rate (HR)} = 7500 / \text{Distance in mm between 6 successive peaks of R waves}$$

2-Organs:

Organs as hearts and livers were removed, washed with sterilized saline, dried between filter papers and weighed.

3-Histopathological examination:

Hearts and livers were kept in 10% formaline for histopathological examination, dehydrated, cleared in zylol and embedded in parablax. Paraffin sections were cut serially at 6 µm thickness and stained by hematoxylin and eosin (Hx & E) as described by Drury and Wallington (1980).

4-Biochemical measurements:

- Serum total T₃ (tT₃) (Monobind USA, ELISA microwells: 225-300), according to the method described by Gharib *et al.*, (1971) and Chopra (1977).
- Serum total T₄ (tT₄) (Monobind USA, ELISA microwells: 225-300), according to the method described by Chopra *et al.*, (1971).
- Serum thyroid stimulating hormone (TSH) (RIA), Rat TSH kit (Amersham life Science, Buckinghamshire, UK).
- Blood superoxide dismutase (SOD), according to the method described by Concetti *et al.*, (1976).
- Plasma malonyldialdehyde (MDA), according to the method described by Draper and Hadley (1990).
- Plasma glutathione (GSH), according to the method described by Beutler *et al.*, (1963).
- Haemoglobin (Hb) level was measured using the cyanomethaemoglobin method using Randox kits, Randox: Laboratories, USA, as described by Dacie and Lewis (1975).

Statistical Analysis (Armitage and Berry 1987):

All statistical data and significance tests were performed by using SPSS (Statistical Program for Social Science) statistical package (SPSS Inc) version 11. Statistical significance was determined by one-way ANOVA (analysis of variance) for differences between means of different groups; further analysis was made by LSD (least significance difference) multiple-range test to find inter-group differences; a probability of P< 0.05 was considered statistically significant.

3. Results

Results encountered in the present study are displayed in tables (1-5) and figures (1-12). Results are expressed as Mean \pm SEM.

1-Measured parameters:

Concerning ECG parameters, significant increase in HR was observed in untreated hyperthyroidism compared to control rats. In addition, significant increase ($p < 0.001$) in R voltage in untreated hyperthyroidism and hyperthyroidism + thiouracil compared to control one. Upon addition of melatonin to propylthiouracil, significant decrease ($P < 0.001$) in R voltage was observed compared to untreated and treated hyperthyroidism with propylthiouracil, although the level was significantly higher ($P < 0.001$) than the control group (Table 1, figure 3 a, b, c, d).

Body weight and diet intake along the period of the study are shown in figures (1&2). Despite matched body weight at start of the study ($P > 0.05$), table (2) demonstrated significant decrease ($P < 0.001$) in the body weight and body mass index in the three trial groups compared to the control one.

As regard liver weights; a significant increase was observed in untreated hyperthyroidism compared to control one, and to treated hyperthyroidism. Upon calculation LW/BW ratios, a significant increase were found in the three trial groups compared to control one.

Concerning heart weights; a significant increase ($P < 0.001$) was observed in untreated hyperthyroidism and treated hyperthyroidism with thiouracil group compared to control one, upon addition of melatonin significant decrease ($P < 0.001$) was observed compared to untreated hyperthyroidism group. Upon calculation HW/BW ratio, significances mentioned before were still present, in addition significant decrease in HW/BW ratio was found upon addition of melatonin to antithyroid drug compared to treated hyperthyroidism with thiouracil.

2-Biochemical studies:

As regard plasma SOD; significant decrease ($P < 0.001$) was observed in the three trial groups compared to the control one. Plasma MDA level showed significant increases in untreated hyperthyroidism and treated hyperthyroidism with

thiouracil groups compared to control group. Upon addition of melatonin, a significant decrease was observed in MDA level to match the level recorded in the control group. Concerning GSH, significant decrease was observed in untreated hyperthyroidism and treated hyperthyroidism with thiouracil groups compared to control group, upon addition of melatonin, significant increase was observed to match the level recorded in the control group.

Concerning Hb level, a significant decrease ($P < 0.001$) was observed in the three trial groups compared to control group. However, upon addition of melatonin a significant increase in Hb level was observed compared to untreated hyperthyroid group.

As regard serum tT_3 and tT_4 ; significant increase was observed in untreated hyperthyroidism and treated hyperthyroidism with thiouracil groups compared to control group, upon addition of melatonin, tT_3 level showed significant decrease ($P < 0.001$) compared to untreated hyperthyroidism and to treated hyperthyroidism with thiouracil. While, serum TSH levels showed significant decrease ($P < 0.05$) in the three trial groups compared to control one.

Histopathological examination of hearts isolated from control rats revealed normal cardiac myocytes (Fig. 4). Meanwhile, hearts of untreated hyperthyroid rats showed vacuolation of cardiac myocytes (Fig. 5), and myolysis of individual cardiac myocytes (Fig. 6). On the other hand hearts of rats isolated from thiouracil treated group revealed only slight congestion of intermuscular blood capillaries (Fig. 7) while hearts isolated from hyperthyroid rats treated by melatonin and thiouracil showed no apparent histopathological changes (Fig. 8).

Histopathological examination of livers isolated from control rats revealed normal histological structure of hepatic lobule (Fig. 9). Untreated hyperthyroid rats showed kupffer cells activation & focal area of hepatic necrosis and leucocytic cell infiltration (Fig. 10). Rats treated with propylthiouracil showed only congestion of central vein (Fig. 11). Upon addition of melatonin to propylthiouracil, no changes were observed in the liver except slight congestion of central vein (Fig. 12).

Table (1): Changes in ECG parameters; heart rate (HR, beats/min), P-R interval (msec), QRS wave (msec), QT (msec), Q wave (μ v), R wave (μ v), and ST segment elevation (μ v) in the four studied groups.

Groups	No	HR (beats/min)	P-R (msec)	QRS (msec)	QT (msec)	Q (μ v)	R (μ v)	ST (μ v)
Control	8	474 \pm 11	52.5 \pm 3.1	50 \pm 4.2	75 \pm 5.4	65.6 \pm 4.5	406.3 \pm 25.8	50 \pm 4.7
Hyperthyroid	10	527 \pm 17*	54 \pm 3.1	44 \pm 2.7	79 \pm 2.3	57.5 \pm 3.8	805 \pm 13.8*	62.5 \pm 4.2
Hyperthyroid+thiouracil	9	459 \pm 18**	61.1 \pm 2.6	45.6 \pm 2.9	80 \pm 3.7	58.3 \pm 4.2	677.8 \pm 29*,**	58.3 \pm 4.2
Hyperthyroid+thiouracil+melatonin	8	452 \pm 16**	61.3 \pm 3.0	50 \pm 4.2	76.3 \pm 3.8	62.5 \pm 4.7	537.5 \pm 28*, **, ***	56.3 \pm 4.1
P		< 0.05	NS	NS	NS	NS	< 0.001	NS

P:Significance by one-way ANOVA among the 4 studied groups. *: Significance by LSD at P< 0.05 from control group;

** : Significance by LSD at P< 0.05 from untreated hyperthyroidism; ***: Significance by LSD at P< 0.05 from hyperthyroidism+ thiouracil.

In parenthesis is the number of rats. NS: not significant. Results are expressed as Mean \pm SEM.

Table (2): Changes in body weight (BW, g), height (cm), body mass index (BMI, Kg/m²), in the four studied groups.

Groups	BW (g)	Height (cm)	BMI (Kg/m ²)
Control (8)	318.3 \pm 7.3	20 \pm 0.3	8.0 \pm 0.2
Hyperthyroid (10)	214.8 \pm 4.8*	20.1 \pm 0.3	5.4 \pm 0.2*
Hyperthyroid +thiouracil (9)	206.9 \pm 4.8*	19.9 \pm 0.3	5.2 \pm 0.2*
Hyperthyroid+thiouracil+ melatonin (8)	217.5 \pm 3.2*	20.3 \pm 0.2	5.3 \pm 0.1*
P	< 0.001	NS	< 0.001

P:Significance by one-way ANOVA among the 4 studied groups. *: Significance by LSD at P< 0.05 from control group;

** : Significance by LSD at P< 0.05 from untreated hyperthyroidism; ***: Significance by LSD at P< 0.05 from hyperthyroidism+ thiouracil.

In parenthesis is the number of rats. NS: not significant. Results are expressed as Mean \pm SEM.

Table (3): Changes in liver weight (LW,g), liver weight / body weight (LW/BW), heart weight (HW, g), heart weight/ body weight (HW/BW) in the four studied groups.

Groups	LW (g)	LW/BW	HW (g)	HW/BW
Control (8)	5.8 \pm 0.3	18.2 \pm 0.7	0.663 \pm 0.04	2.1 \pm 0.08
Hyperthyroid (10)	7.9 \pm 0.4*	36.7 \pm 1.6*	1.11 \pm 0.03*	5.1 \pm 0.1*
Hyperthyroid + thiouracil (9)	5.2 \pm 0.4**	24.9 \pm 1.7*,**	0.809 \pm 0.04*,**	3.9 \pm 0.2*,**
Hyperthyroid + thiouracil+melatonin (8)	5.5 \pm 0.4**	25.4 \pm 2.1*,**	0.755 \pm 0.03**	3.5 \pm 0.1*,**,***
P	< 0.001	< 0.001	< 0.001	< 0.001

P:Significance by one-way ANOVA among the 4 studied groups. *: Significance by LSD at P< 0.05 from control group;

** : Significance by LSD at P< 0.05 from untreated hyperthyroidism; ***: Significance by LSD at P< 0.05 from hyperthyroidism+ thiouracil.

In parenthesis is the number of rats. NS: not significant. Results are expressed as Mean \pm SEM.

Table (4): Changes in plasma superoxide dismutase (SOD, μ g/ml), plasma malondialdehyde (MDA, μ mol/L), plasma glutathione peroxidase (GSH, mg/dl), Hb level (g/dl) in the four studied groups .

Groups	Plasma SOD (μ g/ml)	Plasma MDA (μ mol/L)	Plasma GSH (mg/dl)	Hb (g/dl)
Control (8)	54.8 \pm 1.5	74.2 \pm 3.9	45.7 \pm 1.8	12.5 \pm 0.34
Hyperthyroid (10)	39.4 \pm 1.1*	106.9 \pm 2.8*	35.9 \pm 1.3*	9.7 \pm 0.37*
Hyperthyroid +thiouracil (9)	49.5 \pm 1.0*,**	98.1 \pm 1.6*,**	39.5 \pm 0.9*,**	10.4 \pm 0.36*
Hyperthyroid+ thiouracil+melatonin (8)	40.9 \pm 0.6*,***	79.4 \pm 1.0**,***	43.6 \pm 0.8**,***	11.1 \pm 0.35*,**
P	< 0.001	< 0.001	< 0.001	< 0.001

P:Significance by one-way ANOVA among the 4 studied groups. *: Significance by LSD at P< 0.05 from control group;

** : Significance by LSD at P< 0.05 from untreated hyperthyroidism;

***: Significance by LSD at P< 0.05 from hyperthyroidism+ thiouracil. In parenthesis is the number of rats. NS: not significant.

Table (5): Changes in serum tri-iodo-thyronine (tT₃, ng/ml), serum tetra-iodo-thyronine (tT₄, μ g/dl), and serum thyroid stimulating hormone (TSH, μ IU/ml) levels in the four studied groups.

Groups	tT ₃ (ng/ml)	tT ₄ (μ g/dl)	TSH (μ IU/ml)
Control (8)	4.07 \pm 0.15	6.00 \pm 0.60	0.41 \pm 0.04
Hyperthyroid (10)	7.1 \pm 0.16*	9.8 \pm 0.3*	0.32 \pm 0.02*
Hyperthyroid+ thiouracil (9)	4.8 \pm 0.15*,**	8.3 \pm 0.44*,**	0.35 \pm 0.01*
Hyperthyroid+ + thiouracil+melatonin (8)	4.3 \pm 0.09**,***	9.1 \pm 0.57*	0.35 \pm 0.009*
P	< 0.001	< 0.001	< 0.05

P:Significance by one-way ANOVA among the 4 studied groups. *: Significance by LSD at P< 0.05 from control group;

** : Significance by LSD at P< 0.05 from untreated hyperthyroidism; ***: Significance by LSD at P< 0.05 from hyperthyroidism+ thiouracil.

In parenthesis is the number of rats. NS: not significant. Results are expressed as Mean \pm SEM.

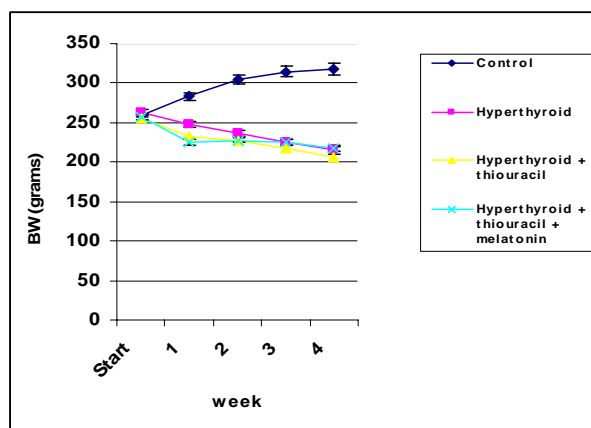


Figure (1): Chart of body weight (BW, grams) throughout the period of the study (4 weeks) showing Mean \pm SEM in the 4 studied groups.

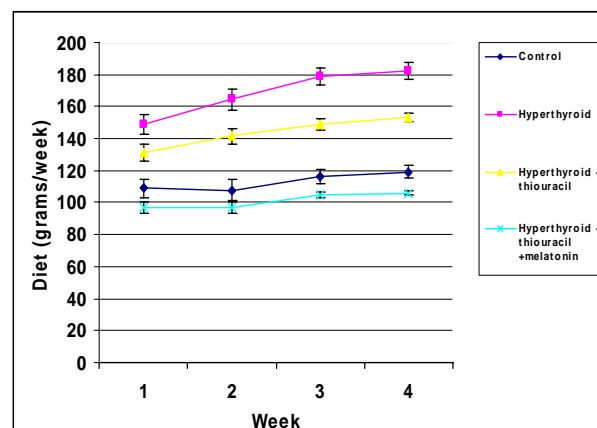
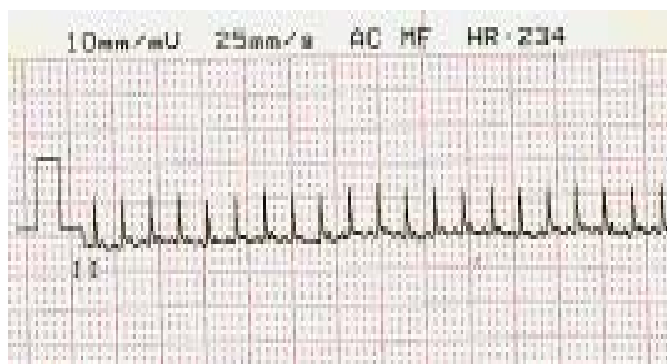
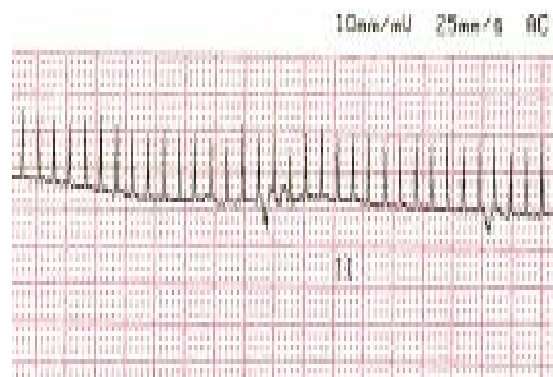


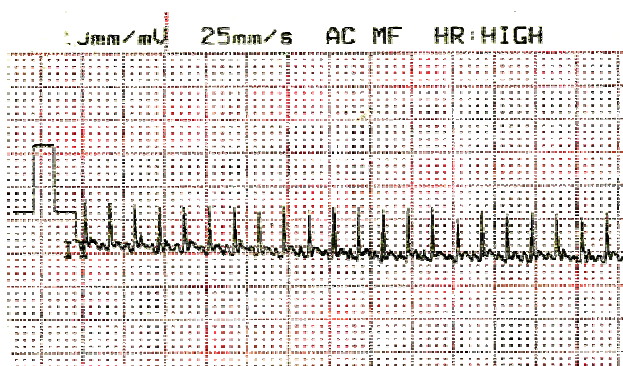
Figure (2): Chart of diet intake (diet, grams/week) throughout the period of the study (4 weeks) showing Mean \pm SEM in the 4 studied groups.



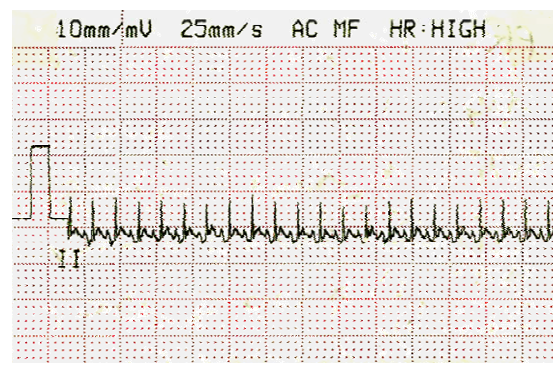
(A)



(B)



(C)



(D)

Figure (3): ECG records (lead II) of: a)Control, b) Untreated Hyperthyroidism, c) Hyperthyroidism + Thiouracil, d) Hyperthyroidism + Thiouracil + Melatonin.

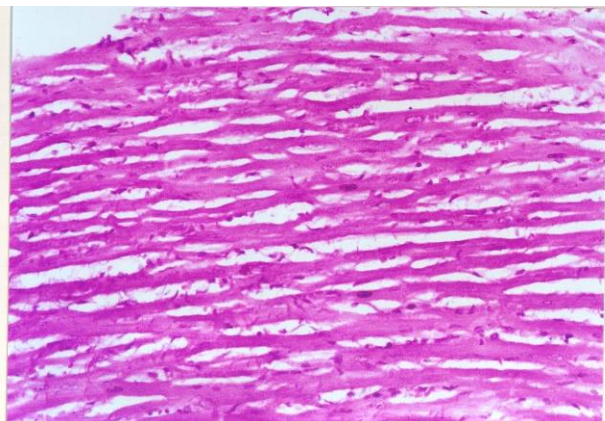


Fig (4): Heart of control rat showing normal cardiac myocytes (Hx & E X 200)

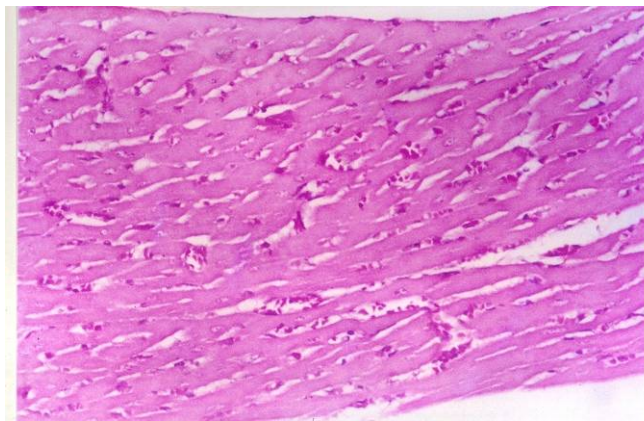


Fig. (7): Heart of hyperthyroid rat + thiouracil showing slight congestion of intermuscular blood capillaries (Hx & E X 200).

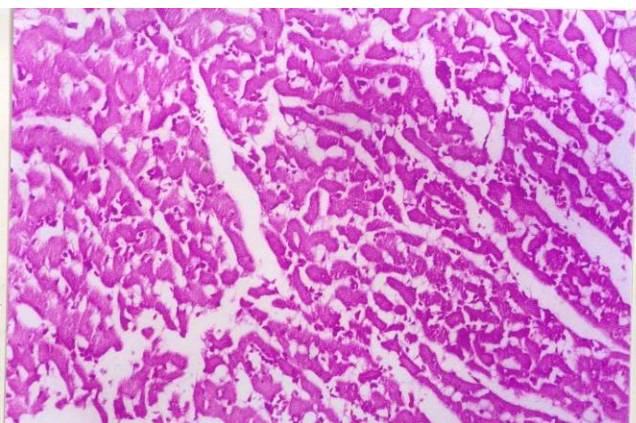


Fig. (5): Heart of untreated hyperthyroid rat showing vacuolations of cardiac myocytes (Hx & E X 200).

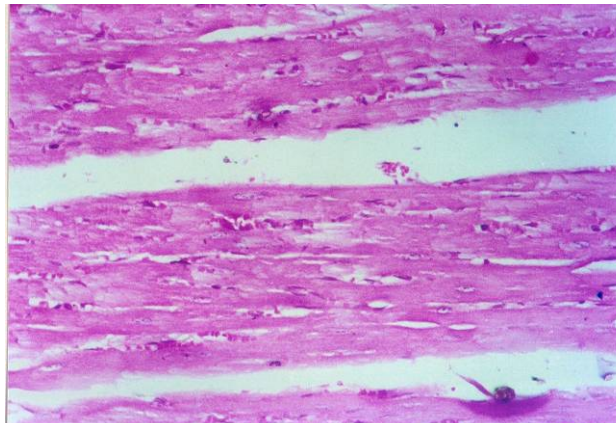


Fig. (8): Heart of hyperthyroid rat + thiouracil + melatonin showing no apparent histopathological changes of cardiac myocytes (Hx & E X 200).

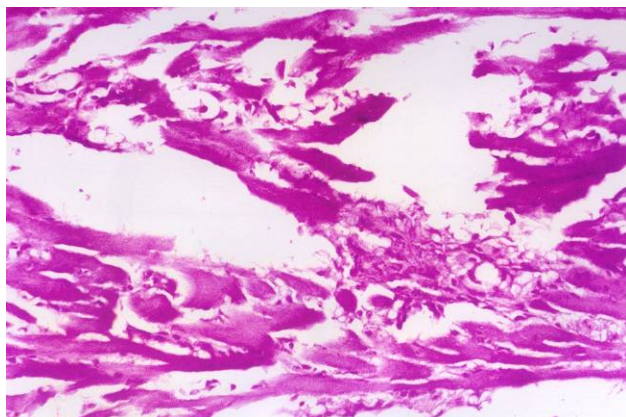


Fig. (6): Heart of untreated hyperthyroid rat showing myolysis of individual cardiac myocytes (Hx & E X 200).

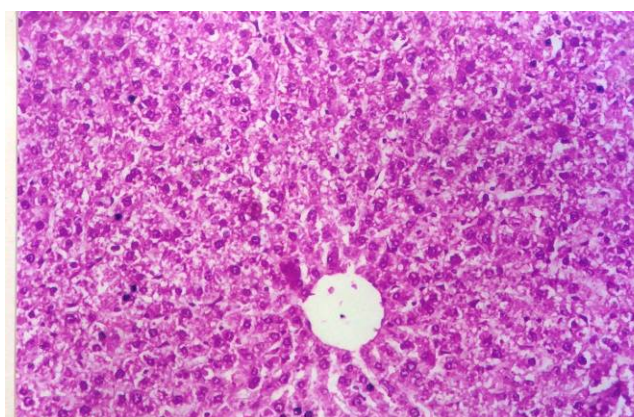


Fig (9): Liver of control showing the normal histological structure of hepatic lobule (Hx & E X 200)

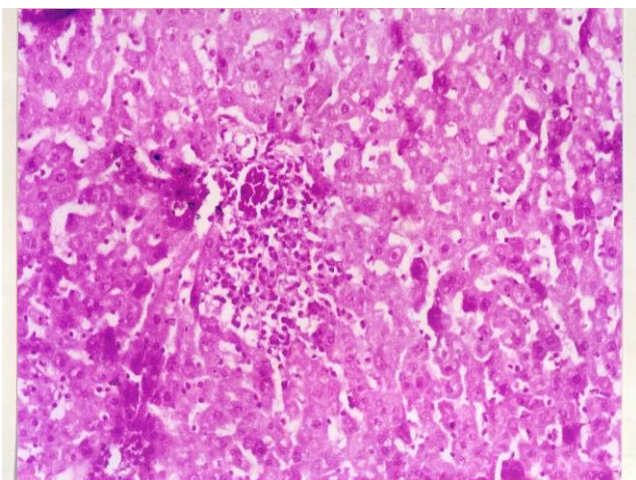


Fig. (10): Liver of untreated hyperthyroid rat showing focal area of hepatic necrosis replaced by leucocyte cell infiltration (Hx & E X 200).

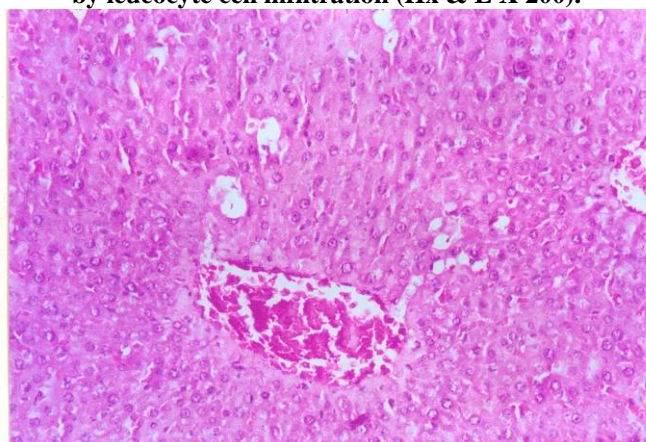


Fig. (11): Liver of untreated hyperthyroid rat + thiouracil showing congestion of central vein (Hx & E X 200).

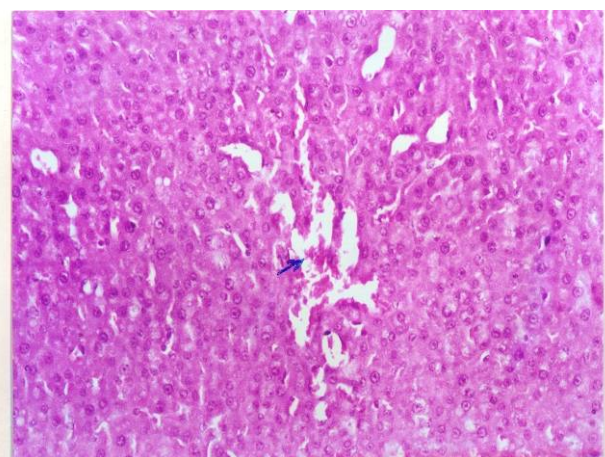


Fig. (12): Liver of hyperthyroid rat + thiouracil + melatonin showing slight congestion of central vein (Hx & E X 200).

4. Discussion:

The present study demonstrates the contribution of free radicals to cardiac & hepatic complications of hyperthyroidism and the possible beneficial effects of adding an antioxidant like melatonin to the antithyroid drug propylthiouracil.

Untreated hyperthyroid rats in the present study had significant increases in serum tT_4 & tT_3 as well as significant decrease in serum TSH. Untreated hyperthyroid rats showed significant decreases in body weight throughout the period of the study and body weight and BMI at the end of the study, despite significant increase in food intake. These findings indicate negative energy balance due to increased metabolic rate & energy expenditure (Ganong, 2010).

Hyperthyroid state for four weeks caused significant anemia despite balanced diet formula & increased food intake. This anemia is difficult to explain in the context of the present study, but we may predict that its cause may be due to a relative nutritional deficiency due to the hypermetabolic state which is associated with increased in free radicle production (Ganong, 2010) or due to liver damage as shown in histopathological examination of these rats (Venditti *et al.*, 2003 & 2006). The improvement of anemia with propylthiouracil treatment inferred that control of the hyperthyroid state resulted in sparing of dietary elements needed for erythropoiesis. Addition of melatonin to antithyroid therapy ameliorated hemoglobin level suggesting that oxidative stress might contribute to hyperthyroid associated-anemia. In a study by Snyder and Reddy (2009), they reported that microcytic anemia accompany hyperthyroidism, which could be due to increased 2, 3 DPG which decreases oxygen affinity to hemoglobin, thus decreasing the drive for enhanced erythropoiesis (Snyder and Reddy, 2009). Another mechanisms; could be expanded blood volume, hemodilution & accelerated but ineffective erythropoiesis (Ganong, 2010).

Hyperthyroid untreated rats exhibited significant sinus tachycardia & significant increase in R-voltage. Effects of an excess thyroid hormone on heart rate are related, in part, to an indirect adrenergic effect and, in part, to a direct intrinsic positive chronotropic effect (Abe *et al.*, 1998). The increase in R-voltage could be explained by the decrease in the body weight at the end of the present study (Goldberger 2006).

Regarding relative cardiac weights, a significant increase was observed in untreated hyperthyroid rats which could be due to the significant decrease in body weight (Ghosh *et al.*, 2007). However, the significant increase in absolute

cardiac weights indicates hyperthyroid induced-cardiomyopathy (Liu *et al.*, 1984) via increasing arterial natriuretic peptide (ANP) mRNA production (Ghosh *et al.*, 2007). Histopathological examination of the livers and hearts isolated from untreated hyperthyroid rats inferred hepatocyte and cardiac myocyte damage. Initial hypertrophy of cardiac myocytes followed by apoptosis was reported in 4 week-hyperthyroid rats which may explain heart failure frequently encountered in hyperthyroid patients (Wang *et al.*, 2010). Amelioration of cardiac enlargement in rats treated with propylthiouracil and melatonin provides evidence that inhibition of oxidative stress reduces myocyte hypertrophy and restores the activity of the antioxidant enzymes; SOD & GSH which suggest that a feedback inhibitory mechanism of endogenous antioxidant system operates against the deleterious effects of hyperthyroidism on cardiac muscle (Ghosh *et al.*, 2007 & Martinez-Cruz *et al.*, 2002). As melatonin has no morphophysiological barriers and is readily available in the cytosol (Martinez-Cruz *et al.*, 2002), it is likely that melatonin is an effective antioxidant in the cytosol (Baydas *et al.*, 2002). Microscopic examination of livers isolated from untreated hyperthyroid rats revealed hepatic damage & congestion which may be due to heart failure that was reported to occur in hyperthyroidism (Wang *et al.*, 2010).

Increased oxidative stress & decreased antioxidant potential in untreated hyperthyroid rats in the present study came in accordance with previous literatures and could explain early hepatic & cardiac derangement associated with hyperthyroidism (Kumar *et al.*, 2007, and Chattopadhyay *et al.*, 2010). The hypermetabolic state in hyperthyroidism results in oxidative damage due to increased activity of mitochondrial respiratory chain components that would result in increased generation of superoxide at the site of ubiquinone (Sugden *et al.*, 1999). tT3 induced-liver free radical generation was reported to occur in concomitance with enhanced respiratory burst activity and chemiluminescent response in both experimental and human studies (Fernandez and Videla 1995). Superoxide radical can lead to the formation of many other reactive species; including hydroxyl radicals, which can readily start the free radical process of lipid peroxidation with increased production of ROS in several tissues including the heart via lipid peroxidation and protein oxidation (Venditti *et al.*, 2003). ROS markedly influence cardiac function in hyperthyroidism via inhibition of important metabolic genes such as GLUT4 (Ghosh *et al.*, 2007). This may lead to an energy crisis & cardiac dysfunction because of reduced uptake of

glucose by hypertrophic cardiomyocyte (Liu *et al.*, 1994), which is mainly stimulated by insulin & intracellular storage sites (Young *et al.*, 1997). Thus, repression of GLUT4 expression takes place in the hyperthyroid rat heart in situation when the demand for energy is high to meet the increased heart rate & contractile activities in the hyperthyroid state.

Treatment of hyperthyroid rats with propylthiouracil alone resulted in amelioration of hyperthyroid induced complications including oxidative stress, hepatic & cardiac myocyte damage due to inhibition of peripheral conversion of T₄ to T₃ (Geffner *et al.*, 1975). Addition of melatonin to propylthiouracil almost normalized all these complications. This indicates that oxidative stress associated with the hyperthyroid state contributes to or aggravates the hyperthyroid induced-complications, and that melatonin inhibits oxidative stress which may be explained by its inhibition to deiodinase 2 expression which is responsible for formation of deiodinase 2 which converts T₄ into active T₃, thus controlling local T₃ concentrations as previously reported by Lechan and Fekete (2005).

Melatonin: The main secretory product of pineal gland can directly neutralize a number of free radicals and reactive oxygen species (Reiter *et al.*, 2002). Melatonin is more effective in neutralizing hydroxyl radicals (HO[•]), the free radical normally responsible for more than half of all free radical damage in the body (causing lipid peroxidation, DNA damage and protein oxidation] as well as superoxide, singlet oxygen, hydrogen peroxide & hypochlorous acid (Leon *et al.*, 2005). Moreover, Melatonin stimulates gene expression & the activity of the antioxidant enzymes glutathione peroxidase, superoxide dismutase & catalase (Reiter *et al.*, 2000 and Rodriguez *et al.*, 2004). As well, it is well known that melatonin function as free radical scavengers (Onuki *et al.*, 2005). Free radical scavengers and antioxidants neutralize and/or metabolically remove reactive species from cells before they carry out their destructive activities (Reiter *et al.*, 2002). While the antioxidative actions of most molecules are limited by their specific intercellular distribution, antioxidative actions of melatonin include the protection of lipids in cell membranes, proteins in cytosol and DNA in nuclei. Furthermore, melatonin can enter all cells in the organism (Reiter *et al.*, 1999). Restoration of glucose uptake upon administration of melatonin in Ghosh *et al.*, (2007) study; clearly indicates that this antioxidant may improve the cardiac function by correcting defects in GLUT4 gene expression (Ghosh *et al.*, 2007).

Results of the present study demonstrate that oxidative stress contributes to early

manifestations of hyperthyroidism like anemia, early cardiac & hepatic damage. The use of propylthiouracil alone produced partial improvement, while addition of melatonin to propylthiouracil restored the balance between oxidants and antioxidants and was more effective in reversing the complications of hyperthyroidism.

Thus, we can conclude from the present study, that antioxidant treatment added to antithyroid drug makes hyperthyroid cardiomyopathy a potentially curable form of heart failure.

Note: This study was approved by the high society of scientific ethic committee of GOTH (General Organization for Teaching Hospitals and Institutes) no. IN 000033.

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