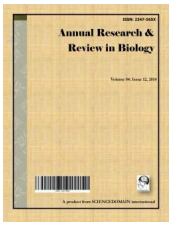

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Changes in Lipid Peroxidation, Free Radical Scavengers and Tumour Necrosis Factor-alpha in Serum of Wistar Rats with Induced Thyroid Dysfunction

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Authors' contributions

This work was conducted in collaboration between both authors. Author MHY designed the study, wrote the protocol and approved the final manuscript. Author SI did the literature searches and wrote the first draft of the manuscript. The collection of samples and analysis were handled by all the authors jointly. Both authors read, reviewed and approved the final manuscript.

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ABSTRACT

Aim: To assess the changes in lipid peroxidation, free radical scavengers and tumour necrosis factor-alpha in serum of Wistar rats with induced thyroid dysfunction.
Study Design: An experimental animal study was conducted in which Wistar rats with induced thyroid dysfunction were studied.
Place and Duration of Study: Animal House, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto and Department of Chemical Pathology, Faculty of Medical Laboratory Sciences, Usmanu Danfodiyo University, Sokoto, between June, 2016 and December, 2016.
Methodology: Twenty-one (21) male Wistar rats weighing 140 - 180 grams were randomly divided into three groups. Therefore, each group consists of 7 rats. Euthyroid (control): untreated receiving

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daily intraperitoneal injection of 0.9% normal saline solution; hypothyroid: treated with daily oral administration of 6-propyl-2-thiouracil (5 mg/100 g) and hyperthyroid: treated with daily intraperitoneal injection of L-thyroxine (0.1 µg/g). At the end of the 30 days treatment, rats were fasted for 12 hours and blood samples were collected under chloroform anaesthesia for the estimation of serum total triidothyronine (tT3), total tetraiodothyronine (tT4), thyroid stimulating hormone (TSH), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), malondialdehyde (MDA) and tumour necrosis-alpha (TNF-α) using standard techniques. The rats were then scarified by cervical decapitation and slices of liver tissue were made for histological examination.

Results: The result indicated that final body weight, and serum tT_3, tT_4, SOD, CAT and GPX were significantly ($P<0.05$; $P< 0.001$) lower in hypothyroid and hyperthyroid rats while, serum MDA and TNF-α were significantly ($P<0.05$; $P< 0.001$) higher in hypothyroid and hyperthyroid compared with euthyroid rats. Serum TSH was significantly ($P< 0.001$) higher in hypothyroid compared with euthyroid and hyperthyroid rats. Histological examination of the hepatocellular tissue of euthyroid rat revealed normochromic and normocytic cellular architecture. There was polymorphocytic infiltration with mild inflammation and hypochromatic liver in hypothyroid rats while, conspicuous infiltrations of polymorphs in all fields were observed in hyperthyroid rats.

Conclusion: In this study, serum MDA and TNF-α were significantly higher, and SOD, CAT and GPX activities were lower in experimental hypothyroid and hyperthyroid rats. The result therefore suggests that a decreased antioxidant capacity coupled with increased oxidative stress and TNF-α may play an important role in the pathogenesis of hepatic injury due to thyroid dysfunction and underscores the role of antioxidants in reducing oxidative stress associated with thyroid dysfunction.

Keywords: Antioxidants; oxidative stress; thyroid dysfunction; TNF-α; wistar rats.

1. INTRODUCTION

The thyroid gland is one of the endocrine glands and because of its size; it is thought to be the largest and most responsive in human body [1]. Two major hormones are secreted by follicular cells in the thyroid gland. Thyroid-stimulating hormone (TSH) stimulates the secretion of thyroid hormones, including triidothyronine (T_3) and thyroxin (T_4). In the tissues, T_4 is converted to T_3 by 5'-deiodinase enzyme [2,3]. These hormones are both produced from iodine, an essential mineral and tyrosine, an amino acid [2]. Thyroid hormones are involved in the regulation of basal metabolic state and in oxidative metabolism [1]. They can cause many changes in the number and activity of mitochondrial respiratory chain components. This may result in the increase generation of reactive oxygen species (ROS) [2,3]. One of the major effects of thyroid hormones is the acceleration of mitochondrial respiration which is accompanied by excessive production of reactive oxygen species (ROS) leading to oxidative stress and damage to membrane lipids [4,5].

Hyperthyroidism occurs when the gland produces excessive amounts of thyroid hormones, the most common cause being Graves' disease, an autoimmune disorder. In contrast, hypothyroidism is a state of insufficient thyroid hormone production. Worldwide, the most common cause is iodine deficiency. Basal metabolism decreases in hypothyroidism and increases in hyperthyroidism. Thyroid hormones are important for development, and hypothyroidism secondary to iodine deficiency remains the leading cause of preventable intellectual disability [6]. Thyroid disease, constitutes the most common endocrine abnormality in recent years, diagnosed either in subclinical or clinical form and is associated with various metabolic abnormalities, due to the effects of thyroid hormones on nearly all major metabolic pathways [7].

Oxidative stress is a general term used to describe an imbalance between the formation of reactive oxygen/nitrogen species and the rate at which they are scavenged. In physiological conditions mitochondrial tissues are the main source of reactive oxygen species (ROS) in an organism owing to the chain of tissue oxidation, which is located in them. The leakage of electrons from mitochondria from the respiratory chain can cause a one-electron reduction of oxygen which leads to the generation of a superoxide anion radical (O_2^-), a highly reactive form of oxygen. The magnitude of superoxide anion radical production is directly derived from mitochondrial oxygen consumption [8]. Oxidative stress occurs when excessive production of free

radicals overwhelms the antioxidant defence system and has been implicated in the aetiology of many diseases including arthritis, cancer, coronary heart disease, cataract, diabetes and degenerative disease [9]. In order to cope with an excess of free radicals produced upon oxidative stress, humans have developed sophisticated mechanisms in order to maintain redox homeostasis [10]. Enzymatic antioxidants such as catalase (CAT), glutathione peroxidase (GPX) and superoxide dismutase (SOD), and transition-metal binding proteins, ceruloplasmin, ferritin and transferrin, rapidly inactivate or prevent the production of free radicals. Substances known as ''Scavengers'' molecules, including both hydrophilic, such as, ascorbic, uric acid, bilirubin, and thiols, and hydrophobic, such as Vitamins A and E, and coenzyme Q10 (CoQ10), interrupt the chain of lipid peroxidation of polyunsaturated fatty acids by reacting with and neutralizing the intermediate radicals [4,11].

Oxidative stress resulting from overproduction and/or inadequate removal of reactive oxygen species is a deleterious process and plays a significant role in the pathogenesis of thyroid dysfunction and complications [12]. Thyroid hormones are also involved in the synthesis and degradation of enzymatic antioxidants, including CAT, SOD, GPX as well as the non-enzymatic antioxidants, such as glutathione, vitamins E and C, uric acid, ceruloplasmin and ferritin [13]. Alterations in these antioxidants can affect the redox balance in the body. Oxidative stress has been reported to be common in the thyroid tissue and consistently associated with hyperthyroidism or hypothyroidism [4,11].

Previous studies have reported contradictory results about antioxidant enzymes activities in both hypothyroidism and hyperthyroidism. In some studies, it was demonstrated that serum activities of CAT in hypothyroid and hyperthyroid rats were decreased [14-16]. On the contrary, other studies reported that CAT activity is increased in patients with hypothyroidism and hyperthyroidism [17,18].

Similarly in some studies, increased SOD activity in the liver of hyperthyroid rats was reported [19,20]. Decreased SOD activity which occurs particularly in the peripheral tissues was also reported in hypothyroid rats [21,22]. Some studies reported no significant changes in the SOD and GPX activities [23,24], while others have reported an increase of the enzyme activity in hypothyroid rats [20,25,26].

Superoxide dismutase activity was also decreased in the blood samples of patients with hyperthyroidism [4]. Studies of Pasupathi et al. [14] and Sahoo et al. [15] also revealed a significant decrease in SOD activity among patients with hypothyroidism as compared to healthy controls. On the other hand, another study indicated no significant difference in the SOD activity between hypothyroid patients and controls or between hyperthyroid patients and controls [27].

Several studies have reported conflicting results about GPX activities in thyroid dysfunction. A lower serum activity of GPX in rats with thyroid dysfunction has previously been reported [28]. In their study [29] also demonstrated decreased serum activity of GPX in patients with Grave's disease. However, GPX activities in other studies contrasted with the previous study of [30, 31, 32, 33] who reported increased GPX activities in experimental hypothyroid and hyperthyroid rats or patients.

Increased lipid peroxidation had previously been reported in the tissues of experimental hyperthyroid rats. Tapia et al. [34] in their study demonstrated that an increased oxygen consumption and progressive accumulation of protein oxidation and lipid peroxidation products occurred in the liver of T_3 induced hyperthyroid rats. The accumulation of malondialdehyde (MDA) in the liver, ventricular tissue of the heart and cerebral cortex in hyperthyroid rats has also been reported [35]. Studies of Aslan et al. [36] also revealed a significant increase in total oxidant status with concomitant decrease in antioxidant capacity in hyperthyroid patients. In a similar study, serum MDA was significantly higher while, total antioxidant capacity levels were significantly lower in both hypothyroid and hyperthyroid rats [5].

Tumour necrosis factor-alpha (TNF-α) a pro-inflammatory cytokine may play a pivotal role in thyroid dysfunction. Recent findings suggest that mitochondrial reactive species are signalling molecules that mediate the production of proinflammatory cytokines, thus connecting oxidative stress and inflammation. Oxidative stress is associated with increased production of TNF-α by the Kupffer cells of the liver [37]. Several studies have reported increased levels of TNF-α in thyroid dysfunction. In their study, Diez et al. [38] demonstrated elevated plasma levels of TNF-α and soluble TNF-α-receptor in patients with hyperthyroidism and hypothyroidism. A similar study also reported increased levels of

TNF-α and TNF-α-receptor in the liver of hyperthyroid rats [5,39].

There is paucity of knowledge on oxidative stress, enzymatic antioxidant status and tumour necrosis alpha in subjects with thyroid dysfunction in the study environment. The current study was undertaken to assess oxidative stress, enzymatic antioxidant status and tumour necrosis alpha and investigates the relationship between thyroid hormones (tT3, tT4, and TSH), MDA, antioxidant enzymes (SOD, CAT and GPX) and TNF-α in Wistar rats with experimentally induced thyroid dysfunction.

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

Analytical graded chemicals and reagents were used for this research. L-thyroxine and propylthiouracil (6-propyl-2-thiouracil) were purchased from Sigma-Aldrich®, Saint Louis, MO, USA. The kits for serum tT_3, tT_4 and TSH were purchased from Monobind Inc. Lake forest, CA 92630, USA. SOD, GPX and CAT assay kits were procured from Enzo Life Sciences International, Inc. 5120 Butler Pike, Plymouth Meeting, USA, while MDA assay kit was procured from Cayman Chemical Company, Ann Arbor, Michigan, USA. TNF-α ELISA kit was procured from Sigma-Aldrich®, Saint Louis, MO, USA.

2.2 Experimental Animals

A total of 21 male Wistar rats weighing between 140 – 180 grams were purchased from National Veterinary Research Institute Vom, Jos, Nigeria. The rats were housed in well aerated cages under hygienic conditions in the Animal House, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto. The rats were allowed to acclimatize for a period of 2 weeks before the commencement of the experiment. The rats were fed pelletized growers feed (Vital®), obtained from Grand Cereal Soil Mills Limited, Jos, Nigeria. They were also allowed access to clean drinking water *ad libitum* throughout the experimental period. Cleaning of the animal cages was carried out daily, and on regular basis. They were maintained in clean metabolic cage-sand, placed in a well-ventilated room conditions with a temperature of 26℃ to 28^{o}C, photoperiods of 12 hours light and 12 hours darkness and humidity of 40% to 60% as described by Aniagu et al. [40]. All the experimental protocols were in compliance with our Institutional Animal Ethics Committee guidelines as well as internationally accepted practices for use and care of laboratory animals as contained in US guidelines (National Institute of Health).

2.3 Experimental Design

The animals were randomly divided into three (3) groups of seven (7) rats each: Euthyroid group (control): untreated receiving daily intraperitoneal injection of 0.9% normal saline solution; hypothyroid group: treated with 6-propyl-2-thiouracil and hyperthyroid group: treated with L-thyroxine.

2.4 Induction of Thyroid Dysfunction

Hypothyroidism was induced by administering propylthiouracil (PTU; 6-propyl-2-thiouracil) at a dose of 5 mg/100 g body weight per day through oral route by the use of oral cannula (a feeding needle) for 30 days according to Petrulea et al. [41]. Hyperthyroidism was induced by daily intraperitoneal injection of L- thyroxine (T_4) at a dose of 0.1 µg/g body weight per day for 30 days. The control group received daily intraperitoneal injections of normal saline (0.9%) solution for 30 days [5].

2.5 Measurement of Body Weight

The body weight of the rats in all the groups were taken using a sensitive balance, once before the commencement of PTU or L- thyroxine (T_4) dosing and once on the day of animals sacrifice.

2.6 Blood Samples Collection and Processing

After 30 days period, the animals were fasted for 12 hours, and anaesthetized in a glass jar containing wool soaked with chloroform. About five millilitres (5 ml) of blood samples were collected from the animals through cardiac puncture, into clean, plain Vacutainer tubes. The samples collected were allowed to clot at room temperature and later centrifuged at 4000 revolution per minute (4000 rpm) for 10 minutes. The sera were then transferred into labelled sterile serum bottles and tightly caped and stored at-20℃ until the time for the estimation of biochemical parameters.

2.7 Biochemical Estimations

ELISA method was used to estimate serum tT_3, tT_4 and TSH [42], SOD [43] and GPX [44], while CAT was estimated by fluorometric method [45] using ELISA kits (Enzo Life Sciences International, Inc. 5120 Butler Pike, Plymouth Meeting USA). Serum MDA was estimated by the method of [46] using Lipid Hydroperoxide (LPO) ELISA kit (Cayman Chemical Company, Ann Arbor, Michigan, USA) and Rat TNF-α by the method of Yener et al. [47] using ELISA kit (Sigma-Aldrich®, Saint Louis, MO, USA).

2.8 Histological Study

The animals were sacrificed by cervical decapitation and slices of liver tissue were made. Then, specimens were taken rapidly, fixed in 10% formalin and processed in paraffin wax, cut at 6 microns. The sections were stained by haematoxylin and eosin stain for microscopic examination [48].

2.9 Statistical Analysis

Results were expressed as mean ± standard error of mean for 7 rats in each group. The body weight and biochemical parameters were analysed statistically using one way analysis of variance (ANOVA), followed by Bonferroni's multiple comparison test using GraphPad Instat software. Differences were considered significant when $P < 0.05$.

3. RESULTS

Table 1 shows the initial and final body weight in Wister rats with induced thyroid dysfunction. The result indicated significantly ($p<0.05$) decrease of final body weight in hypothyroid and hyperthyroid rats as compared with euthyroid rats. The administration of 5mg/100g PTU to rats (Table 2) significantly ($P< 0.05$; $P< 0.001$) decrease serum tT_3 and tT_4 (0.95±0.06 ng/ml and 0.54±0.07 µg/dl respectively) while, serum TSH (0.13±0.00 µIU/ml) significantly ($P<0.001$) increase in hypothyroid rats compared with euthyroid (1.53±0.05 ng/ml, 3.62±0.25 µg/dl and 0.05±0.00 µIU/ml respectively). Administration of L-thyroxine (0.1 µg/g) to rats significantly ($P<0.001$) increased serum tT_3, and tT_4 (2.60±0.21 ng/ml and 12.91±0.57 µg/dl respectively) and significantly ($P< 0.001$) decreased serum TSH (0.02±0.00 µIU/ml) in hyperthyroid rats compared with euthyroid rats (1.53±0.05 ng/ml, 3.62±0.25 µg/dl and 0.05±0.00 µIU/ml respectively).

Table 1. Initial and final body weight in Wistar rats with induced thyroid dysfunction

Group	N	Initial body weight (g)	Final body weight (g)
I	7	128.00 ± 1.46	161.57 ± 8.22
II	7	129.71 ± 2.08	141.43 ± 2.59
III	7	130.86 ± 2.72	136.71 ± 2.99
P-value		>0.05	<0.01
Post-hoc analysis, bonferroni			
Group I Vs II		P=1.000	P=0.043
Group I Vs III		P=1.000	P=0.011
Group II Vs III		P=1.000	P=1.000

Values are expressed as mean ± SEM, N= number of rats; Group I=euthyroid; Group II=hypothyroid; Group III=hyperthyroid; p = p-value

Table 2. Serum tT_3, tT_4 and TSH in Wistar rats with induced thyroid dysfunction

Group	N	tT_3 (ng/ml)	tT_4 (µg/dl)	TSH (µIU/ml)
I	7	1.53 ± 0.05	3.62 ± 0.25	0.05 ± 0.00
II	7	0.95 ± 0.06	0.54 ± 0.07	0.13 ± 0.00
III	7	2.60 ± 0.21	12.91 ± 0.57	0.02 ± 0.00
P-value		<0.001	<0.001	<0.001
Post-Hoc analysis, bonferroni				
Group I Vs II		P=0.015	P< 0.001	P< 0.001
Group I Vs III		P< 0.001	P<0.001	P< 0.001
Group II Vs III		P< 0.001	P< 0.001	P< 0.001

Values are expressed as mean ± SEM; N= number of rats; tT_3 = total triidothyronine; tT_4= total thyroxine; TSH=thyroid stimulating hormone; Group I=euthyroid; Group II=hypothyroid; Group III=hyperthyroid; p = p-value

The result of serum activities of enzymes SOD, CAT, GPX in Wistar rats with induced thyroid dysfunction is presented in Table 3. The results indicated that serum activities of SOD, CAT, GPX significantly ($p<0.001$) decrease in hypothyroid rats (2.41 ± 0.08 U/ml, 3.94 ± 0.28 U/ml and 14.75 ± 0.36 nmol/min/ml respectively) and hyperthyroid rats (1.36 ± 0.08 U/ml, 5.17 ± 0.27 U/ml and 14.02 ± 0.38 nmol/min/ml respectively) compared with euthyroid rats (3.95±0.36 U/ml, 7.43±0.28 U/ml and 25.03±0.37 nmol/min/ml respectively). A significantly higher SOD activity and a significantly lower CAT activity were observed in the hypothyroid rats in comparison to the hyperthyroid rats.

Serum levels of MDA and TNF-α in Wistar rats with induced thyroid dysfunction are presented in Table 4 and Figs. 1-2. The results indicated that serum MDA levels in hypothyroid and hyperthyroid rats (9.87±1.89 µm and10.95±2.02 µm respectively) were significantly ($p=0.038$; $p=0.014$ respectively) higher than those of the euthyroid rats (3.50±0.54 µm). However, the serum MDA levels were not significantly ($p>0.05$) different between the hypothyroid and hyperthyroid rats. The result also indicated significantly ($p<0.001$) increased serum TNF-α in hypothyroid and hyperthyroid rats (256.51±26.80 pg/ml and 182.83±6.22 pg/ml respectively) compared with euthyroid rats (52.64±13.01 pg/ml). In the hypothyroid rats, the TNF-α levels were significantly ($p<0.05$) higher as compared to similar values in hyperthyroid thyroid rats.

There were no significant correlations between various oxidative stress markers and thyroid profiles for either the hypothyroid, hyperthyroid or the euthyroid group (Table 5). However a significant negative correlation was established between TSH and TNF-α among the hyperthyroid group of rats.

Histological examination of the hepatocellular tissue of euthyroid rat revealed normochromic and normocytic cellular architecture (Fig. 3). Fig. 4 shows a photomicrograph of hepatocellular tissue of hypothyroid rat. The result indicated mild inflammation in hypochromatic rat liver with polymorphocytic infiltrates (blue arrow). White arrow points central vein. Histopathological changes in the hepatic tissue of hyperthyroid rat (Fig. 5) indicated conspicuous infiltration (blue arrow) of polymorphs in all fields.

Table 3. Serum activities of SOD, CAT and GPX in Wistar rats with induced thyroid dysfunction

Group	N	SOD (U/ml)	CAT (U/ml)	GPX (nmol/min/ml)
I	7	3.95±0.36	7.43±0.28	25.03±0.37
II	7	2.41±0.08	3.94±0.28	14.75±0.36
III	7	1.36±0.08	5.17±0.27	14.02±0.38
P-value		P<0.001	P<0.001	P<0.001
Post-Hoc analysis, bonferroni				
Group I Vs II		P<0.001	P<0.001	P<0.001
Group I Vs III		P<0.001	P<0.001	P<0.001
Group II Vs III		P<0.001	P=0.015	P=0.542

Values are expressed as mean ± SEM; N= number of rats; SOD= superoxide dismutase CAT= catalase GPX= glutathione peroxidise; Group I=euthyroid; Group II=hypothyroid; Group III=hyperthyroid; p = p-value

Table 4. Serum MDA and TNF-α in Wistar rats with induced thyroid dysfunction

Group	N	MDA	TNF-α (pg/ml)
I	7	3.50±0.54	52.64±13.01
II	7	9.87±1.89	256.51±26.80
III	7	10.95±2.02	182.83±6.22
P-value		P=0.009	P<0.001
Post-hoc analysis, bonferroni			
Group I Vs II		P=0.038	P<0.001
Group I Vs III		P=0.014	P<0.001
Group II Vs III		P=1.000	P=0.025

Values are expressed as mean ± SEM; N= number of rats; MDA=malondialdehyde; TNF-α=tumour necrosis factor-alpha; Group I=euthyroid; Group II=hypothyroid; Group III=hyperthyroid; p = p-value

Table 5. Pearson correlation coefficients between measured parameters in Wistar rats with induced thyroid dysfunction

Group		SOD (U/ml)	CAT (U/ml)	GPX (nmol/min/ml)	MDA (µm)	TNF-α (pg/ml)
Euthyroid (n=7)	tT_3 (ng/ml)	r= 0.302	r= 0.316	r= -0.749	r= 0.352	r= 0.043
		p= 0.510	p= 0.490	p= 0.053	p= 0.438	p= 0.928
	tT_4 (µg/dl)	r= -0.378	r= -0.065	r= -0.116	r= -0.161	r= -0.012
		p= 0.403	p= 0.890	p= 0.804	p= 0.730	p= 0.980
	TSH (µIU/ml)	r= 0.157	r= 0.351	r= -0.119	r= 0.618	r= 0.739
		p= 0.737	p= 0.440	p= 0.799	p= 0.139	p= 0.058
Hypothyroid (n=7)	tT_3 (ng/ml)	r= 0.256	r= -0.217	r= 0.495	r= 0.624	r= 0.467
		p= 0.580	p= 0.639	p= 0.258	p= 0.134	p= 0.291
	tT_4 (µg/dl)	r= 0.447	r= -0.082	r= -0.239	r= 0.202	r= 0.299
		p= 0.315	p= 0.861	p= 0.606	p= 0.664	p= 0.515
	TSH (µIU/ml)	r= -0.314	r= -0.054	r= 0.167	r= 0.229	r= -0.641
		p= 0.492	p= 0.908	p= 0.720	p= 0.622	p= 0.121
Hyperthyroid (n=7)	tT_3 (ng/ml)	r= 0.345	r= -0.418	r=0.321	r= 0.687	r= 0.339
		p= 0.449	p= 0.351	p=0.483	p= 0.088	p= 0.456
	tT_4 (µg/dl)	r= 0.578	r= 0.350	r=-0.004	r= 0.177	r= 0.004
		p= 0.174	p= 0.442	p=0.992	p= 0.704	p= 0.376
	TSH (µIU/ml)	r= 0.431	r= 0.056	r=0.600	r= -0.129	r= -0.873
		p= 0.335	p= 0.905	p=0.154	p= 0.782	p= 0.010

r=Pearson correlation coefficient; p= p value; correlation is significant for p<0.05

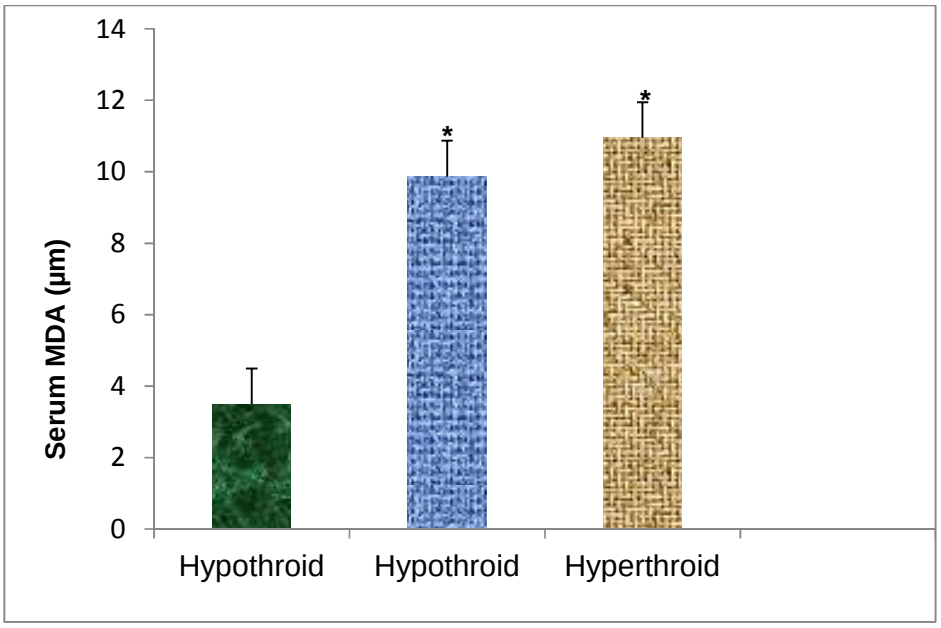

Fig. 1. Serum MDA in euthyroid, hypothyroid and hyperthyroid rats
***P < 0.05 when compared with euthyroid rats*

4. DISCUSSION

Oxidative stress resulting from overproduction and/or inadequate removal of reactive oxygen species is a deleterious process and plays a significant role in the pathogenesis of thyroid dysfunction and complications [12]. One of the major effects of thyroid hormones is the acceleration of mitochondrial respiration which is accompanied by excessive production of reactive oxygen species (ROS) leading to oxidative stress and damage to membrane lipids [4,5]. Thyroid hormones are also involved in the synthesis and degradation of enzymatic antioxidants, including

CAT, SOD, GPX as well as the non-enzymatic antioxidants, such as glutathione, vitamin E and C, uric acid, ceruloplasmin and ferritin [13]. Alterations in these antioxidants can affect the redox balance in the body. Oxidative stress has been reported to be common in the thyroid tissue and consistently associated with hyperthyroidism or hypothyroidism [4].

In the current study, the decrease of serum levels of tT_3, tT_4 and increase of TSH confirmed the establishment of hypothyroid state in rats. The results are in concordance with the studies of Zbucki et al. [49] and Khalawi et al. [50] who independently reported significant decrease in the plasma levels of tT_3, tT_4 with concomitant increase in TSH in experimental hypothyroid rats compared with controls. It also agreed with results of Haiying et al. [51] who reported that hypothyroid subjects were diagnosed having plasma concentrations of tT_3 and tT_4 below the normal ranges, and TSH above the normal range. The mechanism by which propylthiouracil (PTU) induce hypothyroid state in rats could be due to its inhibition of thyroid hormone synthesis by interfering with thyroid peroxidase–mediated iodination of tyrosine residues in the thyroid gland at both steps of iodine organification and

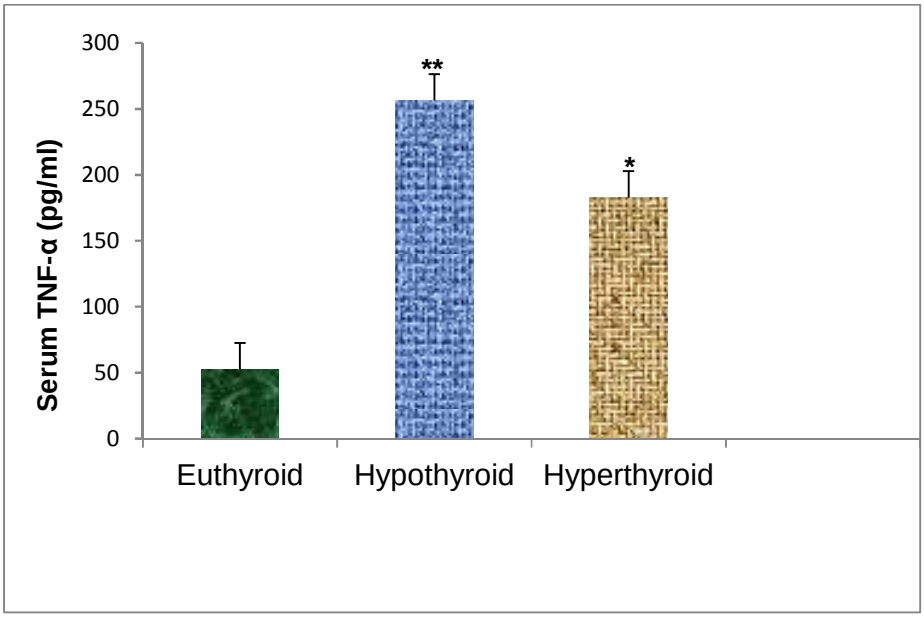

Fig. 2. Serum TNF-α in euthyroid, hypothyroid and hyperthyroid rats
**P < 0.05 and ** P < 0.001 when compared with euthyroid rats*

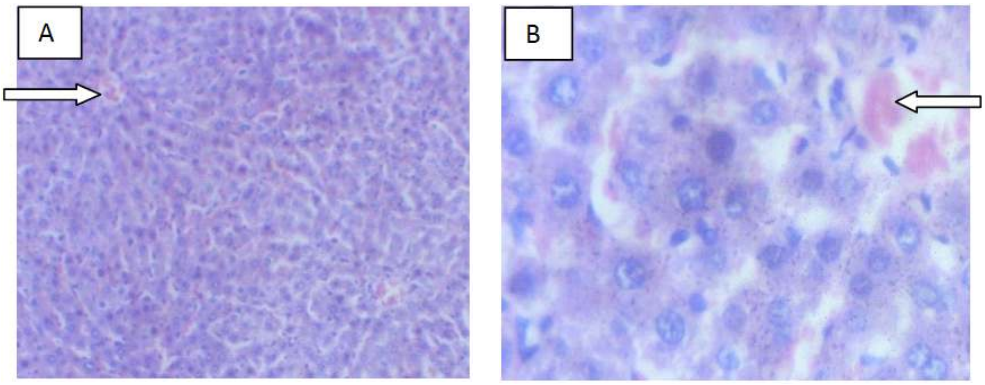

Fig. 3. Photomicrograph of hepatocellular tissue of euthyroid rat showing normochromic and normocytic cellular architecture. Central vein is indicated by white arrow (H&E; A ×100; B ×400)

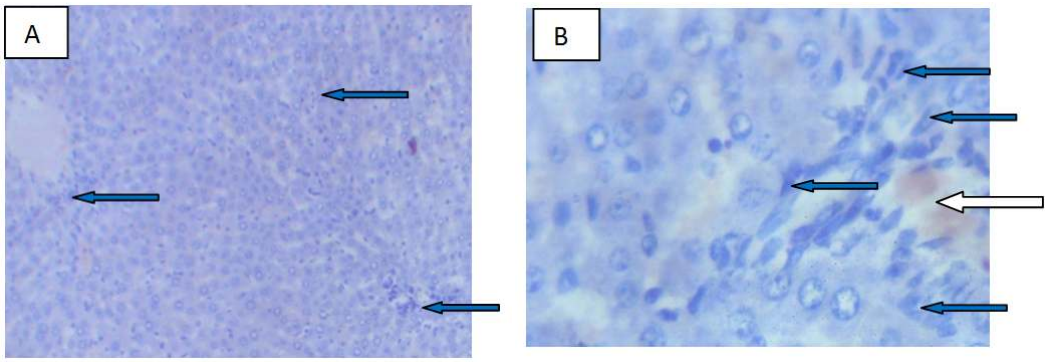

Fig. 4. Photomicrograph of hepatocellular tissue of hypothyroid rat showing mild inflammation in hypochromatic rat liver with polymorphocytic infiltrates (blue arrow). White arrow points central vein (H&E; A × 100; B × 400)

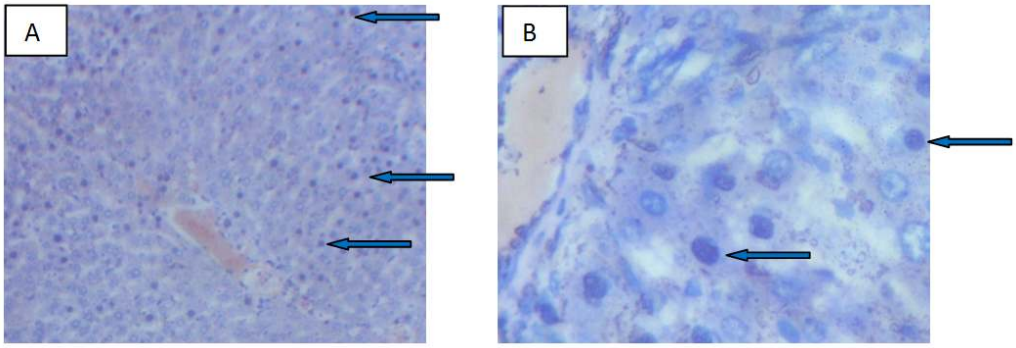

Fig. 5. Photomicrograph of hepatocellular tissue of hyperthyroid rat showing conspicuous infiltration (blue arrow) of polymorphs in all fields (H&E; A ×100; B ×400)

iodotyrosine coupling as well as inhibiting the conversion of tT_4 to tT_3 in extra thyroidal tissues [52].

The hyperthyroid state in the rats was confirmed by increase of tT_3 and tT_4 and simultaneous decrease of TSH compared to controls. The elevation of serum tT_3 and tT_4 are indicative of hyperthyroidism, while a low serum TSH in the presence of elevated thyroid hormones is logical because secretion of TSH from the anterior pituitary is regulated by negative feedback from the serum free thyroid hormone concentrations [5, 53]. There was no significant difference in the body weight of the rats at baseline. However after 30 days of PTU and L-thyroxine treatment, the final body weight of rats in both hyper- and hypothyroid groups were significantly lower than that of the control. The result in this study corroborated with previous studies [50,54]. The mechanism responsible for the increase in body weight in hypothyroid rats could be due to a reduction in the basal metabolic rate which the rats undergo following the administration of PTU [50]. However, following 30 days treatment with L-thyroxine, hyperthyroid rats significantly gained body weight. Our result contrasted with the studies of Ai et al. [55] and Yi et al. [56] who reported a decrease in the mean final body weight of hyperthyroid rats.

The current study showed that the propylthiouracil (PTU) induced- hypothyroid and L-thyroxine-induced hyperthyroid states in rats were associated with antioxidant deficiency as indicated by significant decrease in the serum activities of antioxidant enzymes (SOD, CAT and GPX) in both hypothyroid and hyperthyroid rats. In order to defend and protect against the effects of oxidative stress, SOD activity is expected to increase as a protection mechanism. However in our study, the decrease of serum activities of SOD in both hypothyroid and hyperthyroid rats are in agreement with Erdamar et al. [4] who reported decrease of SOD activity in hyperthyroid patients. Decrease of SOD activity in patients

with hypothyroidism compared with controls has previously been reported by several studies [14, 15]. On the contrary, several researchers [16,19, 20] independently reported increase of SOD activity in the liver of rats with induced hyperthyroidism while, other studies indicated no significant difference in the SOD activity between hypothyroid or hyperthyroid patients and controls [26-28].

The decrease of serum activity of CAT in both hypothyroid and hyperthyroid rats observed in the current study is in agreement with previous studies [14-16]. However, our results are not in concordance with the findings of Vassev et al. [17] and Bednarek et al. [18] who reported that CAT activity is increased in patients with hypothyroid and hyperthyroidism.

In our study, the decrease of activity of GPX in the serum of 6-propyl-2-thiouracil-treated and L-thyroxine–treated rats is in agreement with previous studies [29,31,32]. Significantly lower erythrocyte GPX activity in patients with hyperthyroidism has been reported [57, 58] while, others [28,59] showed that it significantly increased in hyperthyroidism. The decrease of GPX activity observed in the present study might be the result of increasing utilization of the enzyme in the liver and muscles due to increased thyroid hormone levels. Intracellular GPX degrades hydrogen peroxide (H_2O_2) and hydroperoxides, whereas in plasma GPX catalyzes breakdown of H_2O_2 and hydroperoxides of phospholipids. The decrease of serum activities of SOD, CAT and GPX in hyperthyroid rats may be due to high metabolic state in hyperthyroidism, alongside with increasing free radical production and lipid peroxide levels. While in hypothyroid induced rats, failure of the redox potential leads to free radicals chain reaction and metabolic suppression of antioxidant enzymes, SOD, CAT and GPX [60] may be responsible.

In the current study, serum MDA levels significantly increased in both hypothyroid and hyperthyroid rats compared to euthyroid rats. These results suggested that, oxidative stress increases in thyroid dysfunction. The elevated serum MDA concentrations observed in the PTU and L-thyroxine administered groups as compared with the control group were similar with the findings of [5]. Studies of Venditti et al. [26] demonstrated significant increase in MDA in rat tissues following the daily intraperitoneal administration of triidothyronine for ten days. In addition, oxidative stress has also been demonstrated by Aslan et al. [36] who observed significant decrease in the total antioxidant capacity and significant increase in the total oxidant status in hyperthyroid patients compared to their controls. However the results in this study are not in concordance with the findings of Petrulea et al. [58] who reported the MDA levels in L-thyroxine-treated rats to be similar with the corresponding values in euthyroid rats.

Enhance generation of reactive oxygen species with peroxidation of membrane lipids and depression of the antioxidant scavenger status have been described [5,58]. Another explanation for the elevated MDA level in the serum of hyperthyroid rats could be that hyperthyroidism is known to be a hypermetabolic state that increases mitochondrial respiration in the electron transport chain. This may promote the excessive production of reactive oxygen species which will in turn initiate a free radical mediated chain reaction to produce MDA, an index of lipid peroxidation that can lead to oxidative damage to membrane lipids and other cellular structures. Oxidative stress was demonstrated in the liver of hyperthyroid rats [61] and was also implicated for enhancing the lipid peroxidation in the liver and heart of experimentally induced hyperthyroid rats [62].

The mechanisms for the occurrence of oxidative stress in both hyperthyroid and hypothyroid rats may differ. Excessive production of reactive oxygen species that overwhelmed the antioxidant capacity is responsible for hypothyroidism; while antioxidant deficiency may be responsible for the occurrence of oxidative stress in hyperthyroidism [11,31]. However, the controversial findings in the oxidative stress biomarkers such as SOD, CAT, GPX and MDA may be due to differences in experimental conditions and different analytical assays used in the study.

The discrepancies in serum values of SOD, CAT, GPX and MDA were probably due to type of samples analyzed (serum, plasma, tissue or exudates), the dose and the duration of treatment, grade of hyperthyroidism/ hypothyroidism, methods of determination and result expression (enzyme activity or concentration, expression of enzyme concentration or activity per protein or tissue mass) [58]. For example the ELISA method used for the estimation of serum SOD, CAT and GPX was accurate and précised as compared with colorimetric method. Likewise the method of

serum MDA measurement using Lipid Hydroperoxide ELISA kit is accurate in comparison with thiobarbituric acid reactive substances (TBARS) which is inaccurate, and results may differ according to experimental and assay conditions used.

Tumour necrosis-alpha (TNF-α), an inflammatory cytokine majorly produced by activated macrophages, lymphocytes and natural killer cells [5] play an essential role in inflammatory and immunological functions. TNF-α has been shown to have varied effects on thyroid function. TNF-α induced inhibition of thyroid function in rats is considered to play a pivotal role in the pathogenesis of many inflammatory and autoimmune diseases [63]. In this study, the increased levels of TNF-α in serum of hypothyroid and hyperthyroid rats is in agreement with previous studies [5,37-39]. In pathophysiological conditions, polymorphonuclear leucocytes are attracted to the site of inflammation by TNF-α. This triggers the respiratory burst with excessive generation of reactive oxygen species which overwhelmed the antioxidant capacity leading to oxidative stress and excessive production of TNF- α in the Kupffer cells of the liver [5,37].

In the current study, histological examination of the hepatic tissue revealed polymorphocytic infiltration with mild inflammation and hypochromatic liver in hypothyroid rats while, conspicuous infiltration of polymorphs in all fields were seen in hyperthyroid rats. These findings were consistent with the reports of previous studies [5,64] who found cellular hyperplasia with mild lobular inflammatory infiltrates that majorly comprised of polymorphic neutrophils, eosinophils and lymphocytes, which aroused due to nuclear changes and Kupffer cell hyperplasia.

5. CONCLUSION

In this study, serum MDA and TNF-α were higher, and SOD, CAT and GPX activities were lower in experimental hypothyroid and hyperthyroid rats. The result therefore suggests that a decrease of antioxidant capacity coupled with increase of oxidative stress and TNF-α may play an important role in the pathogenesis of hepatic injury due to thyroid dysfunction and underscores the role of antioxidants in reducing oxidative stress associated with thyroid dysfunction.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. As per international standard or university standard written ethical approval has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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