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Biochemical and Histopathological Effects Induced by Methimazole in Rats Treated by vitamin C

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Abstract

In order to investigate some biochemical and histopathological changes effects of Methimazole (ME)in rats twenty-eight white female rats were equally divided into four target groups, (ME) The 1st group, was given sterile saline (0.9%) and served as a control (c). The 2nd group, was given Methimazole at dose 0.04 mg/kg B.W. orally (positive control). The 3rd group, was given Methimazole at dose 0.04 mg/kg B.W. and Vitamin C 200 mg/kg B.W. orally. The 4th group, was given only Vitamin C 200 mg/kg B.W. orally. Results aimed in the present study on the effect of Methimazole at daily intake doses to investigate possible adverse effects and modulate its possible mechanism by measuring the oxidant and antioxidant impact in brain and Ameliorative trail of vit. C. Vit C antioxidant, such as super oxide, increase super oxide elimination. examination revealed that the (ME) histopathological figures showed very small size thyroid gland that displayed atrophy, composed numerous small size follicles with normal follicular cells and C-cells. Study concludes, (vit c) can decreased thyroid damage caused by oxidative effects triggered by (ME) and linked to their antioxidant effects.

Keyword: Methimazole, thyroid, biochemical, histopathological changes.

I. INTRODUCTION

Actually, Methimazole (MMI) is an anti-thyroid drug that belongs to drug class thionamides. The primary mechanism of action of methimazole is to block thyroid hormone production from the thyroid gland. It interferes with the step that causes the iodination of tyrosine residues in thyroglobulin, mediated by the enzyme thyroid peroxidase, thus preventing the synthesis of thyroxine (T4) and triiodothyronine (T3) (Marriott Mp et al., 2020). Methimazole affects the production of thyroid hormone and is useful in treating conditions related to thyroid hormone, especially thyrotoxicosis. Thus, it is

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considered a thyroid blocking agent. It most frequently occurs in the first three months of starting therapy but can occur even after a year or more of exposure or during repeated exposures when treating a relapse. The hepatic toxicity of methimazole is more of a cholestatic process than allergic hepatitis seen in propylthiouracil and recovers slowly after discontinuing the drug. Otherwise, the biological efficacy of Ascorbic acid (vitamin C) is used as a dietary supplement as an antioxidant to protect the cells against free radicals, which may play a role in heart disease, cancer and other diseases. Al-saadi, R. N. (2007) Vitamin C is an essential nutrient involved in the repair of tissue and the enzymatic production of certain neurotransmitters. (Marriott Mp et al., 2020). Al-saadi, 2007 found the group that treated with vitamin C in mice treated with nitrate there was no no pathological changes in the 3rd and 4th group were recorded. Also, there was Vitamin C has affected Some Physiological and Reproductive Characters in Adult male and rabbit (AL-Ma'atheedi and A.A. Hassan,2012; Dawood, 2015). And on the reproductive hormones (Azeez, 2022) and some biochemical parameters in in rabbit feed with potassium nitrate (Jassim Alrawi, 2017).

found the purpose of this investigation was experimentally to measure the proportional different between Artemisia and methimazole on the female rat liver through calculation of possible changes in biochemical and histopathological factors.

II. MATERIALS AND METHODS

Animals and treatments: 28 adult Wistar albino female rats aged 40 days, weighting 250 ± 10 gm, were collected via (Animal Housing Colony of the Veterinary College of AL Muthanna University). Female animals were stored in temperature monitored rooms (24 ° ±3oC) and humidity (40-70%) and a 12h/12h light / dark period prior to being used in experimental procedures. To accommodate the workshop conditions, rats were allowed to participate in the experiment for one week. Rats were divided into four separate groups (methimazole group) The first group, was given sterile saline (0.9%) and served as a control (c). The second group, was given Methimazole at dose 0.04 mg/kg B.W. orally (positive control). (Ennas Mohamed., *et al.*, 2018). The third group, was given Methimazole at dose 0.04 mg/kg B.W. and Vitamin C 200 mg/kg B.W. orally. (Deshpande UR., *et al.*, 2002). The fourth group, was given only Vitamin C 200 mg/kg B.W. orally. (Deshpande UR., *et al.*, 2002). The doses that used for chronic treatment of Methimazole were prepared by dissolving 1 tablet of captopril (5 mgs) in (100 ml) distilled water gradually to prepare concentration (0.04 mg/ml) and to be given at dose volume 0.2 ml/ 100 g. of body weight of animal. The 10% Artemisia was obtained from the regional market. Another group was administered for 40 days only Vitamin C 200 mg/kg B.W. orally. (Deshpande UR., *et al.*, 2002). The animals' blood was collected after 40 days. Blood collection was completed on each rat and used for a serological examination.

Superoxide Dismutase Activity kit:

The Superoxide Dismutase Activity kit is designed to quantitatively measure SOD activity in a variety of samples. The assay measures all types of SOD activity, including Cu/Zn, Mn, and FeSOD types. A bovine erythrocyte SOD standard is provided to generate a standard curve for the assay and all samples should be read off of the standard curve. Samples are diluted in our specially colored sample diluent and added to the wells. The substrate is added followed by Xanthine Oxidase Reagent and incubated at room temperature for 20 minutes. The xanthine oxidase generates superoxide in the

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presence of oxygen, which converts a colorless substrate in the detection reagent into a yellow-colored product. The colored product is read at 450 nm. Increasing levels of SOD in the samples causes a decrease in superoxide concentration and a reduction in yellow product.

Concentration of MDA

Calculation; The concentration of MDA (nmol/ml) was calculated by using the following formula: Concentration of the test= Abs (test) – Abs (blank) / 1.56 x 1000000 Determination of reduced glutathione (GSH). Serum (0.2 ml) were used in the assay.

Histopathological study:

liver was taken and fixed in 10% of formalin, for hematoxylin and eosin (H & E) (8). As well as being examined with a light microscope (Leica, Germania).

Data analysis:

statistical analysis was consistently administered using ANOVA and the study was performed to compare the data from the control group with the experimental groups. The findings were described as mean ± S.E.M (standard mean error). A P-value of less than 0.05 was considered to be important and is described in brackets.

III. RESULTS

The result in list table (1): showed significance increase in G2 of SOD level compared with control group and significance decrease in G3 and G4 compared with G2, significance decrease P (≤0.05) in G3 and G4 compared with G2. whereas showed significance decrease P (≤0.05) in G2 of MDA level compared with G3 and G4.

Table (1) Evolution serum levels with different parameters in relation with Methimazole drug and vit. C:

Parameters / Groups		Serum level parameters	
		MDA (unit pg/mg)	SOD
G1	Control	6.2±0.85 c	96.1±1.05 c
G2	Methimazole	6±0.81 c	130.2±0.94 a

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G3	**Methimazole + Vit c**	**7.5±0.98 b**	**101.0±0.87 b**
G4	**Vit c**	**8.9±0.86 a**	**98.1±0.85 b**
LSD		**1.40**	**10.2**

*** Different small letters mean significant (p<0.05) results between groups.**

IV. HISTOPATHOLOGICAL

The histopathological figures showed very small size thyroid gland that displayed atrophy, composed numerous small size follicles with normal follicular cells and C-cells (1 & 2). The histopathological figures showed hypertrophied thyroid gland with normal mass of para thyroid tissue (fig. 3). The thyroid follicles revealed normal appearance filled with colloid, normal appearance of the follicular cells and normal C-cell (fig.4&5).

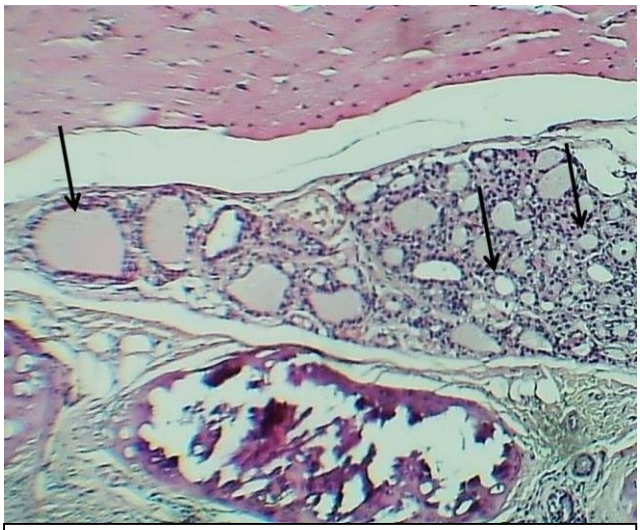

Figure 1: section of thyroid gland (G1S1) shows very small size thyroid gland with very small follicles (arrows) H&E stain.100x.

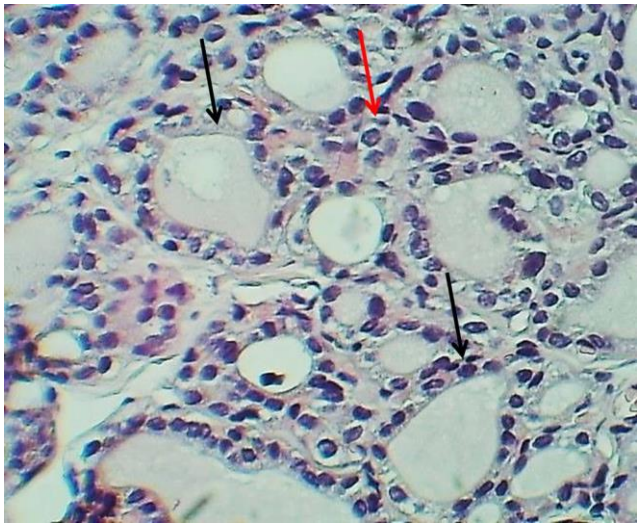

Figure 2: section of thyroid gland (G1S1) shows very small size thyroid follicles with normal follicular cells (Black arrows) and C-cells (red arrow) H&E stain.200x.

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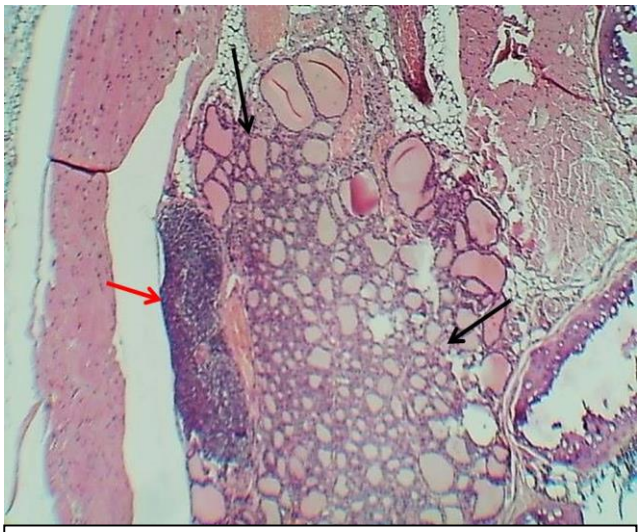

Figure 3: section of thyroid gland (G1S2) shows very hypertrophied thyroid gland with normal para thyroid tissue (red arrow) and normal appearance of follicles (Black arrows) H&E stain.100x.

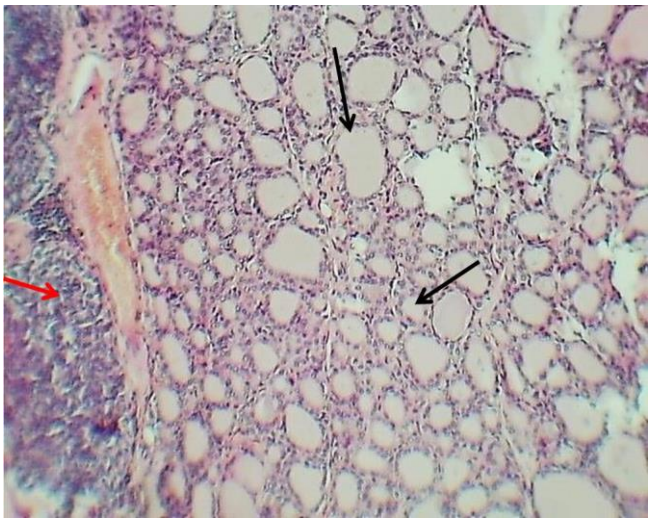

Figure 4: section of thyroid gland (G1S2) shows normal appearance of thyroid follicles (Black arrows0 with normal para thyroid tissue (red arrow) H&E stain.100x.

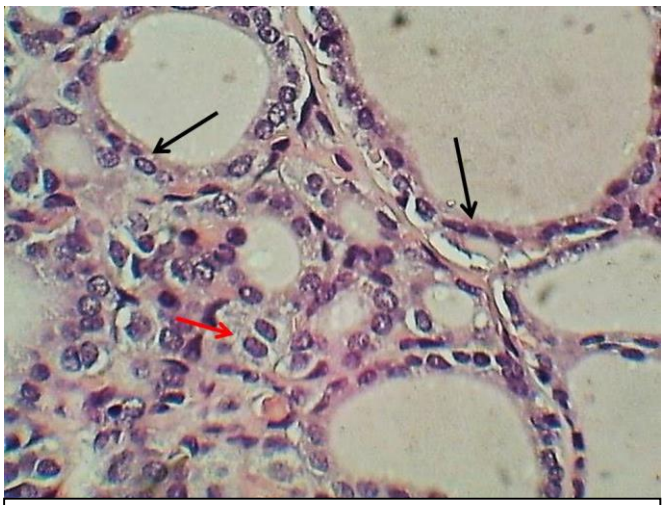

Figure 5: section of thyroid gland (G1S2) shows normal appearance of follicular cells (Black arrows0 with normal C-cell (Red arrow). H&E stain.400x.

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V. DISCUSSIONS

Revealed that Methimazole exposure different antioxidant enzyme activities and antioxidant stages, indicating that MDA were elevated as defensive mechanisms. In aerobic organisms, a certain number of reactive oxygen species (ROS) are created as by-products of normal metabolic processes, such as superoxide anions O2, hydrogen peroxides (H2O2), and hydroxyl radicals (_OH), and a balance is maintained between ROS generation and clearance (Ozcan & Ogun,2015). Organisms can respond to increased ROS generation by up regulating antioxidant defense play a critical role in cellular detoxification metabolism for anti-oxidation and detoxification (Hejazian *et al*., 2021). Lipid peroxidation, a process between free radicals and unsaturated fatty acids in cellular membranes, produces MDA (Taso *et al*., 2019) Increased MDA level is an important indicator of lipid peroxidation The Histopathological study showed decrease acinar sizes in the thyroid gland of G2 because the free radicals are directly cytotoxic. Prolonged oxidative stress can result in oxidative damage to tissues. These findings are consistent with (Hussein *et al*.,2022), who discovered that an increase in the concentration of apoptosis markers, which may lead to an increase in the levels of thyroid autoantibodies. showed follicular loss of normal architecture with lymphocytic inflammatory infiltrate. Some appeared degenerated, others appeared with exfoliated desquamated cells in the lumen and some appeared fused (Fouad *et al*.,2022). Similar thyroid lesions were also reported by Banu and Sharma, (Melina et al.,2021) concluded ROS induces the expression of CAT, GPx-1, and SOD-1. The activity of these enzymes may contribute to the protection of PBMC from the harmful effect of free radicals on cell viability. Increased expression of DNMT-1 may be associated with aberrant methylation patterns in immunoregulatory genes contributing to autoimmunity in Graves' disease. While the G3 demonstrates only minor histopathological changes in the thyroid. The thyroid protective properties of vit C may be attributed to variations in oxidative parameters compared to those in the group 2 this result agreed with (Melike et al., 2021) who found the use of natural antioxidants is inevitable in order to minimize the harmful effects of these toxic substances.

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VII. DESCRIPTIVES

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
ALP	1.0	7	218.571	5.1270	1.9378	213.830	223.313	213.0	226.0
	2.0	7	139.143	15.0491	5.6880	125.225	153.061	119.0	158.0
	3.0	7	96.429	22.8025	8.6185	75.340	117.517	77.0	141.0
	Total	21	151.381	54.0486	11.7944	126.778	175.984	77.0	226.0
ALT	1.0	7	106.857	15.1814	5.7380	92.817	120.898	78.0	119.0
	2.0	7	50.143	7.8619	2.9715	42.872	57.414	39.0	62.0
	3.0	7	34.143	2.1157	.7997	32.186	36.100	31.0	37.0
	Total	21	63.714	33.3319	7.2736	48.542	78.887	31.0	119.0
AST	1.0	7	95.2857	12.88040	4.86833	83.3733	107.1981	70.00	110.00
	2.0	7	42.4571	6.32952	2.39233	36.6033	48.3110	34.00	51.00
	3.0	7	20.5871	2.06892	.78198	18.6737	22.5006	18.00	24.22
	Total	21	52.7767	33.09776	7.22252	37.7107	67.8426	18.00	110.00
Bilirubin	1.0	7	1.6357	1.53902	.58170	.2124	3.0591	.33	3.33
	2.0	7	2.5700	.46332	.17512	2.1415	2.9985	1.86	2.90
	3.0	7	.5557	.12700	.04800	.4383	.6732	.31	.69
	Total	21	1.5871	1.22110	.26647	1.0313	2.1430	.31	3.33
Total protein	1.0	7	5.4800	.25697	.09713	5.2423	5.7177	5.14	5.91
	2.0	7	6.6386	.26131	.09876	6.3969	6.8802	6.32	6.99
	3.0	7	7.6029	.21554	.08147	7.4035	7.8022	7.30	7.88
	Total	21	6.5738	.91928	.20060	6.1554	6.9923	5.14	7.88