

# Investigating the impact of resveratrol on thyroid function, structure, and metabolic alterations in hyperthyroidism model: in vivo study

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## Abstract

**Background:** Thyroid diseases have effects on metabolism and inflammation. The mechanism of these effects is not clear. Recently, there are several reports suggesting this interrelation between adipocytokines and thyroid dysfunction. Resveratrol is a natural polyphenol with antioxidant, anti-inflammatory, and antiproliferative properties. Previous studies showed that resveratrol down-regulates the expression of the thyroid-stimulating hormone receptor (TSHR in the FRTL-5 rat thyroid cell line).

**Objective:** To investigate the effects of resveratrol alone and in combination with methimazole on the function and structure of the thyroid gland and metabolic alteration in hyperthyroid male rats induced by levothyroxine.

**Methods:** Forty rats were divided randomly into five groups, each consisting of 8 male rats: Group-I (Healthy control): healthy rats receive 10% DMSO (5ml/kg) orally for two weeks and then continue for the next three weeks. Group-II (induction group): hyperthyroid-induced animals (received thyroxine 600µg/kg, in 10% DMSO) for two weeks, then 10% DMSO (5ml/kg) for the next three weeks, orally. Group-III (standard therapy): hyperthyroid-induced animals (received thyroxine 600µg/kg, in 10% DMSO) for two weeks orally, then treated with standard drug (Methimazole 10mg/kg in 10%DMSO for the next three weeks, orally. Group-IV (resveratrol treatment group): hyperthyroid-induced animals (Received thyroxin 600µg/kg, in 10% DMSO) for two weeks orally, then treated intraperitoneally with Resveratrol (100 mg/kg, in 10% DMSO) for the next three weeks. Group-V (combination of resveratrol and methimazole): hyperthyroid-induced animals (received thyroxine-600µg/kg, in 10% DMSO) for two weeks orally, then treated intraperitoneally with Resveratrol (100mg/kg, in 10% DMSO) and (Methimazole 10mg/kg in 10%DMSO orally) for the next three weeks. On day 21, the rats were euthanized, and whole blood was collected to examine thyroid hormones (TSH, T3, and T4), sodium iodide symporter, and metabolic markers (glucose, cholesterol, leptin, adiponectin), and the thyroid gland was harvested for histopathological examination.

**Results:** Resveratrol cause significantly decrease the serum levels of thyroid hormones (T3, T4) and increase the serum levels of (TSH). Also, resveratrol cause a significant decrease in serum levels of (glucose, cholesterol, leptin) and increase the level of adiponectin. In addition, resveratrol show significant improvement in histological changes that are associated with hyperthyroidism. The use of combination therapy (resveratrol and methimazole) cause a greater reduction in thyroid hormones (T3, T4) than each one alone.

**Conclusion:** resveratrol produces an antithyroid effect and show significant improvement in metabolic alteration and histological changes at 100 mg/kg in hyperthyroid male rats induced by levothyroxine. Combination therapy (methimazole and resveratrol) exhibit a greater reduction in levels of thyroid hormones (T3, T4) than each one alone.

**Keywords:** Resveratrol, Thyroid hormones, Hyperthyroidism, Methimazole, Metabolic marker

## INTRODUCTION

Thyroid hormones (THs) are important determinants of cellular metabolism

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of carbohydrates, lipids, and proteins in multiple target tissues. Notably, hyperthyroidism generates a hypermetabolic state marked by increased resting energy expenditure, decreased cholesterol levels, enhanced lipolysis and gluconeogenesis, and weight loss. (1). The isoforms of the thyroid hormone receptor (TR) are variably expressed in tissues and have diverse functions in TH signaling. The conversion of thyroxine (T4) to triiodothyronine (T3) by 5'-deiodinase type 2 (D2) is a crucial process for TH control of metabolism. D2 is essential for adaptive thermogenesis and is abundant in the hypothalamus, white fat, brown adipose tissue (BAT), and skeletal muscle. There is a central modulation of appetite by dietary signals like leptin and appetite-regulating peptides. Through the epigenetic change of histones, the cellular nutritional state gives feedback on TH signaling pathways (2). Integration of TH signaling with the adrenergic nervous system occurs both peripherally, in the liver, white fat, and BAT, and centrally, within the hypothalamus (3). TR modulates cholesterol and glucose metabolism by direct effects on gene expression and cross-talk with other nuclear receptors, such as peroxisome proliferator-activated receptor (PPAR), liver X receptor (LXR), and bile acid signaling pathways. TH affects hepatic insulin sensitivity, which is particularly crucial for gluconeogenesis suppression (4).

Resveratrol (3, 4',5-trihydroxystilbene) is a naturally occurring polyphenol found in grapes, berries, peanuts, and some other plants, where it functions as a phytoalexin to defend the plant against pathogens (5). Multiple investigations have demonstrated that resveratrol possesses numerous therapeutically significant qualities, including antioxidant, anti-inflammatory, and antiproliferative effects. (6). For these reasons, there is considerable interest in the use of resveratrol in a variety of chronic diseases, including cancer and diabetes, as well as neurological and cardiovascular problems. Indeed, resveratrol is provided as a dietary supplement, and its application for various illnesses is being examined in several number of completed and current clinical trials (7). Regarding the thyroid, there is little data on resveratrol's impacts on thyroid dysfunction. Some studies show that resveratrol inhibits the proliferation of thyroid tumor cell lines (8). Only one study evaluated the impact of resveratrol on thyroid cells using a nontransformed thyroid cell line. After 6 to 12 hours of therapy, resveratrol stimulates NIS protein expression and iodide uptake, but this effect is temporary, as it is no longer visible after 24 hours. However, they provide no information regarding the impact of resveratrol on sodium iodide symporter mRNA (9).

The activation of the aryl hydrocarbon receptor also is a working hypothesis (AhR). In fact, resveratrol is an AhR regulator with agonist or antagonist effects in different cell types, and previous studies have shown that stimulation of AhR can influence thyroid function by lowering the expression of the sodium iodide symporter (NIS) gene (10), this study aims to determine the effect of resveratrol alone and in combination with methimazole on the thyroid function, structure, and metabolic alteration in hyperthyroid

male rats.

## MATERIALS AND METHODS

### Chemicals, Reagents and Kits

All chemicals and reagents from the good origin (levothyroxine, methimazole, and resveratrol) were purchased from Sigma Aldrich (Merck). ELISA kits (TSH, T3, T4, sodium iodide symporter) were purchased from MyBioSource/USA. Ketamine injection 10 % and xylazine vial were purchased from Alfasan/Netherlands. Hematoxylin and Eosin stain was purchased from Juku/Japan.

### Experimental animals

In this study 40 male albino rats, weighing between 160 and 200 grams, purchased from the National Center for Drug Control and Research/Ministry of Health. The scientific and animal ethical committees of Mustansiriyah University's College of Pharmacy gave their permission before the study could be performed. The animals were kept in an experimental cage (20x25x35 cm) at 22°C 3° with a regular light/dark cycle, where ventilation was good and temperature and humidity were controlled. They also had free access to food and water.

### Dose selection

Animal's doses selection were based on previous studies for methimazole (11) and levothyroxine (LT4) (12) and a pilot study for the dose of resveratrol.

### Study design

Forty rats were divided randomly into five groups, each consisting of eight male rats:

-Group-I (Healthy control): healthy rats receive 10% DMSO (5ml/kg) orally for two weeks and then continue for the next three weeks.

-Group-II (induction group): hyperthyroid-induced animals (received thyroxine 600µg/kg, in 10% DMSO) for two weeks, then 10% DMSO (5ml/kg) for the next three weeks, orally.

-Group-III (standard therapy): hyperthyroid-induced animals (received thyroxine 600µg/kg, in 10% DMSO) for two weeks orally, then treated with standard drug (Methimazole 10mg/kg in 10%DMSO for the next three weeks, orally).

-Group-IV (resveratrol treatment group): hyperthyroid-induced animals (Received thyroxin 600µg/kg, in 10% DMSO) for two weeks orally, then treated intraperitoneally with Resveratrol (100 mg/kg, in 10% DMSO) for the next three weeks.

-Group-V (combination of resveratrol and methimazole): hyperthyroid-induced animals (received thyroxine-600µg/kg, in 10% DMSO) for two weeks orally, then treated intraperitoneally with Resveratrol (100mg/kg, in 10%

Yasir Hassan Hadi *et al.*: Investigating the impact of resveratrol on thyroid function, structure, and metabolic alterations in hyperthyroidism model: in vivo study (Table 1).

DMSO) and (Methimazole 10mg/kg in 10%DMSO orally) for the next three weeks.

### Sample Collection and Preparation

A cardiac puncture was used to get a blood sample, which was then placed in a plain gel tube and centrifuged at 3000x for fifteen minutes to prepare the serum for ELISA analysis, it was kept at -80°C.

### Thyroid status measurement

Thyroid hormones (TSH, T3, T4, and NIS) were measured by the ELISA technique according to the manufacturer's instructions (My BioSource - USA).

### Metabolic markers measurement

Metabolic markers (glucose, cholesterol, leptin, adiponectin) were measured by the ELISA technique according to the manufacturer's instructions (My BioSource - USA).

### Organ harvest

Under anesthesia, the thyroid gland was harvested. After that, washing organs with distilled water then transferred to 10 % buffered formalin for microscopical examination of histopathological changes by Hematoxylin and Eosin stain according to the manufacturer's instructions (13).

### Statistical Analysis

The statistical analysis was done using the 16.0 version of SPSS. Every single result was described as a mean  $\pm$  standard error mean (M $\pm$ SEM). The One-way ANOVA test was utilized to differentiate between group results, and a post hoc LSD test followed. Statistically significant differences were confirmed for the data when the P<0.05.

## RESULTS

### A. Effect of resveratrol on serum levels of thyroid status markers

The descriptive statistics were described as mean  $\pm$  SEM

**Table 1:** Effect of resveratrol on mean serum levels of thyroid status markers in hyperthyroid rats induced by levothyroxine.

Groups	N	TSH (ng/ml)	T3 (ng/ml)	T4 (ng/ml)	NIS (ng/ml)
Healthy control	8	7.52 $\pm$ 0.61a	2.10 $\pm$ 0.30bc	118.38 $\pm$ 4.81b	3.13 $\pm$ 0.12b
Induction	8	4.19 $\pm$ 0.85b	8.05 $\pm$ 0.40a	225.89 $\pm$ 11.71a	1.66 $\pm$ 0.14c
Standard Therapy	8	7.22 $\pm$ 1.01a	1.53 $\pm$ 0.14cd	131.78 $\pm$ 4.61b	3.59 $\pm$ 0.10a
Resveratrol	8	8.24 $\pm$ 1.00a	2.84 $\pm$ 0.48b	131.81 $\pm$ 4.08b	0.77 $\pm$ 0.03d
Combination therapy	8	7.69 $\pm$ 0.58a	0.86 $\pm$ 0.08d	72.55 $\pm$ 4.17c	1.00 $\pm$ 0.06d
LSD		2.39	0.92	19.21	0.29

Table (1) illustrated the status of thyroid hormones for the studied groups, where an elevation in the mean levels of thyroid hormones (T3, T4), and a reduction in the mean levels of thyroid-stimulating hormones (TSH) was observed in rats given just levothyroxine, while a significant improvement in the status of these hormones was reported with the administration of standard therapy, resveratrol, or their combination.

Mean serum levels of TSH were significantly lowered (P-value  $\leq$  0.05) in the induction group (L-T4) alone in comparison with the healthy control, standard therapy, resveratrol and combination groups, where the levels of this hormone were not significantly differ among these groups (p>0.05), figure(1A)

Meanwhile, mean serum levels of T3 were increased significantly (P-value  $\leq$  0.05) within the induction group (LT4) alone when compared with other groups, while the level of this hormone did not differ significantly between the standard and combination therapy (p>0.05). Rats that were treated with resveratrol plus methimazole (combination group) exhibited a significant (P-value  $\leq$  0.05) decrease in serum T3 levels when compared to the control or resveratrol group. The levels of this hormone in rats given just resveratrol were approach to that of control (P-value  $\leq$  0.05), figure (1B).

Figure (1C) presents a statistically significant increase (P-value  $\leq$  0.05) in T4 mean levels within the induction group (LT4)alone compared to the healthy control, standard and resveratrol groups, where the levels of this hormone did not differ significantly among these groups (p>0.05). Rats that were treated with resveratrol plus methimazole in combination group exhibited a significant (P-value  $\leq$  0.05) decrease in serum T4 levels when compared to the control, standard, and resveratrol groups.

In this study, the mean serum level of NIS within the induction group was significantly lower (P-value  $\leq$  0.05) than that of healthy control and standard therapy groups. Results from resveratrol and combination groups showed a significant decrease (p<0.05) in NIS level when compared with healthy control, induction, and standard therapy groups where the levels of NIS do not differ significantly between these two groups (p>0.05), figure (1D).

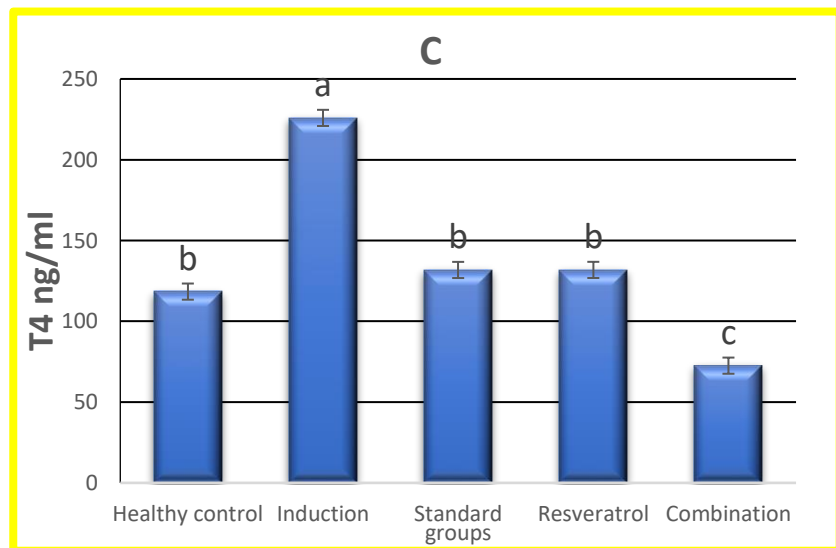
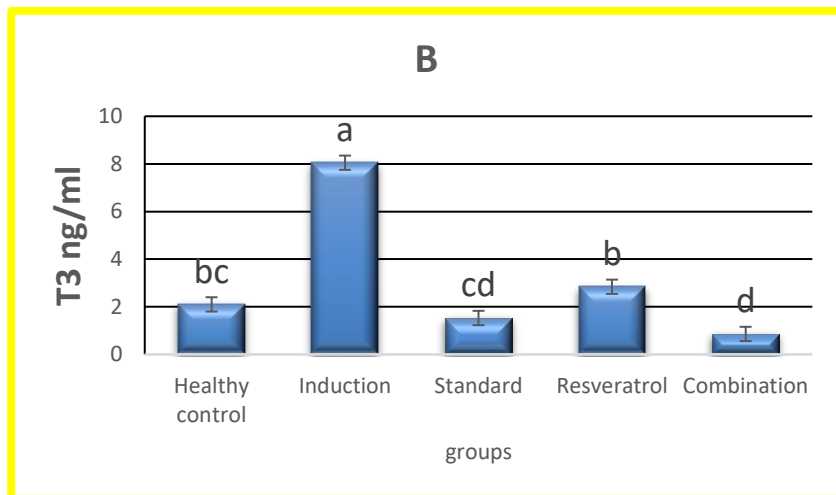
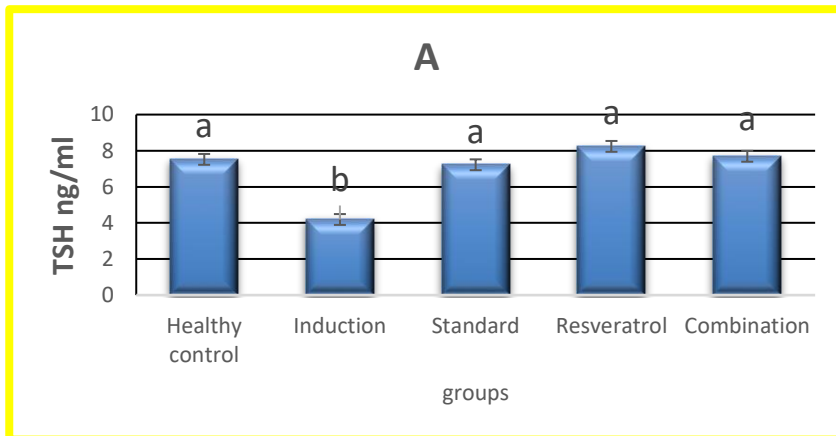
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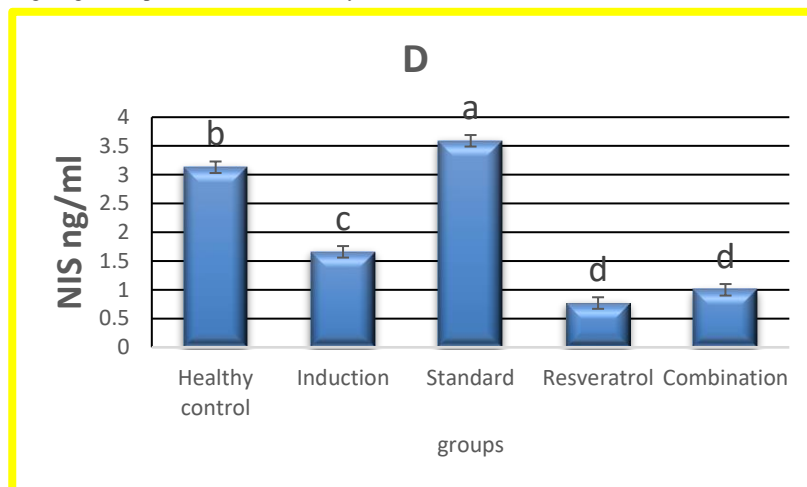
Statistical difference was represented by a different superscript small letter.

p-value <0.05 mean statistically significant difference.

N: number of rats in each group. NIS:sodium iodide symporter

LSD: Least Significant Difference.





**Fig.1:** Effect of resveratrol on mean serum levels of thyroid status markers in hyperthyroid rats induced by levothyroxine. (A): TSH (B): T3 (C): T4 (D): NIS

The data were represented by mean  $\pm$  SEM.

Statistical difference was represented by a different small letter.

The p-value  $<0.05$  mean statistically significant difference.

## B. Effect of resveratrol on serum levels of metabolic markers

Table (2) show the following:

Regarding the effect on metabolic markers after exposure to levothyroxine (LT4), glucose levels were significantly increased ( $P$ -value  $\leq 0.05$ ) in the induction group (L-T4) alone when compared with the healthy control, standard, Resveratrol and combination groups. Meanwhile, the effect of levothyroxine on glucose levels was significantly reversed ( $P$ -value  $\leq 0.05$ ) within the standard, resveratrol and combination-treated groups, presented by a reduction of glucose mean levels. Also, glucose level was significantly higher ( $P$ -value  $\leq 0.05$ ) in standard therapy when compared with healthy control, and resveratrol groups. Rats that were treated with resveratrol exhibited a significant decrease in serum level of glucose ( $P$ -value  $\leq 0.05$ ) than that of standard, combination, and healthy control groups ( $26.64 \pm 6.03$  versus  $155.35 \pm 23.28$ ,  $79.79 \pm 15.94$ ,  $75.82 \pm 7.29$ ) respectively, as shown in figure (2A).

Considering serum cholesterol, its levels were decreased significantly ( $P$ -value  $\leq 0.05$ ) in the induction group (L-T4) alone when compared with the healthy control and standard

therapy groups, where the levels of this parameter do not differ significantly between these groups ( $p > 0.05$ ). Rats that were treated with resveratrol and combination therapy exhibited a significant ( $P$ -value  $\leq 0.05$ ) decrease in serum levels of cholesterol when compared to healthy control, induction, and standard therapy groups, where there's no significant difference in cholesterol levels between these two groups ( $p > 0.05$ ), as shown in figure (2 B).

Regarding leptin levels, the induction group (L-T4) alone elevated leptin levels significantly compared with other groups ( $P$ -value  $\leq 0.05$ ). Meanwhile, the effect of levothyroxine on leptin levels was significantly reversed within the standard, resveratrol and combination-treated groups ( $P$ -value  $\leq 0.05$ ), presented by reduction of leptin mean levels. Also, the levels of this marker were significantly decreased ( $P$ -value  $\leq 0.05$ ) in resveratrol and combination groups when compared with standard group, although the levels of this marker were not significantly differ ( $p > 0.05$ ) between these two groups, they were still significantly greater than the control group, as presented in figure (2 C).

Meanwhile, levels of adiponectin were significantly increased ( $P$ -value  $\leq 0.05$ ) in the induction group (L-T4) alone when compared with the healthy control, standard, and combination group. On the other side, the levels of this marker were significantly higher ( $P$ -value  $\leq 0.05$ ) in standard and resveratrol treated groups compared to the healthy group and the levels of this marker did not differ between these two groups significantly ( $p > 0.05$ ), as shown in figure (2D).

**Table 2:** Effect of resveratrol on mean serum levels of metabolic markers in hyperthyroid rats induced by levothyroxine.

Groups	N	Glucose (nmol/L)	Cholesterol (nmol/L)	Leptin (ng/ml)	Adiponectin (µg/ml)
Healthy control	8	75.82±7.29c	161.21±9.54a	7.32±1.21d	12.44±0.53c
Induction	8	312.43±19.45a	80.60±2.97b	31.07±2.61a	27.32±2.61a
Standard Therapy	8	155.35±23.28b	149.91±6.80a	19.38±0.84b	21.42±1.10b
Resveratrol	8	26.64±6.03d	58.22±1.76c	13.65±1.93c	25.56±1.54ab
Combination therapy	8	79.79±15.94c	61.12±2.38c	13.41±1.45c	21.81±0.99b
LSD	8	45.66	15.99	4.95	4.39

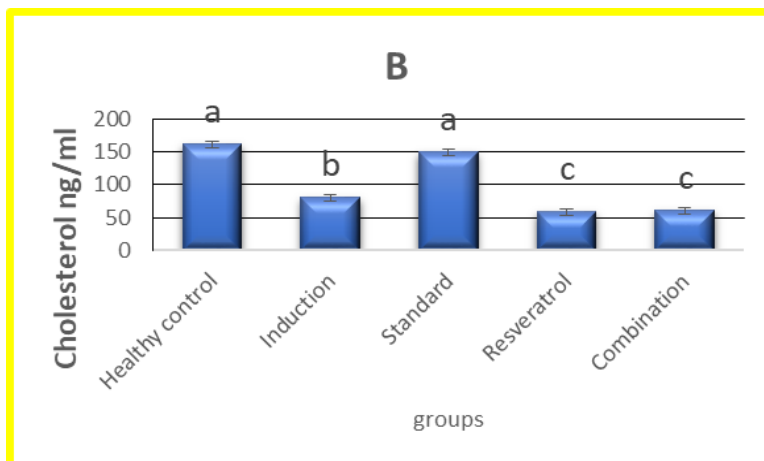
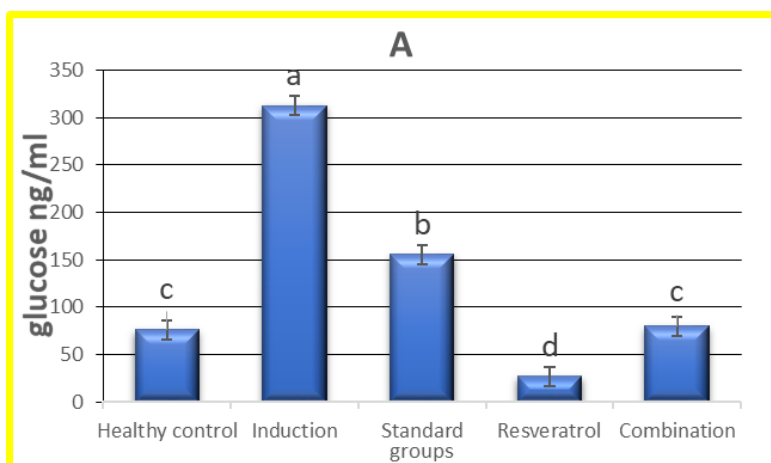
The data were represented by mean ± SEM.

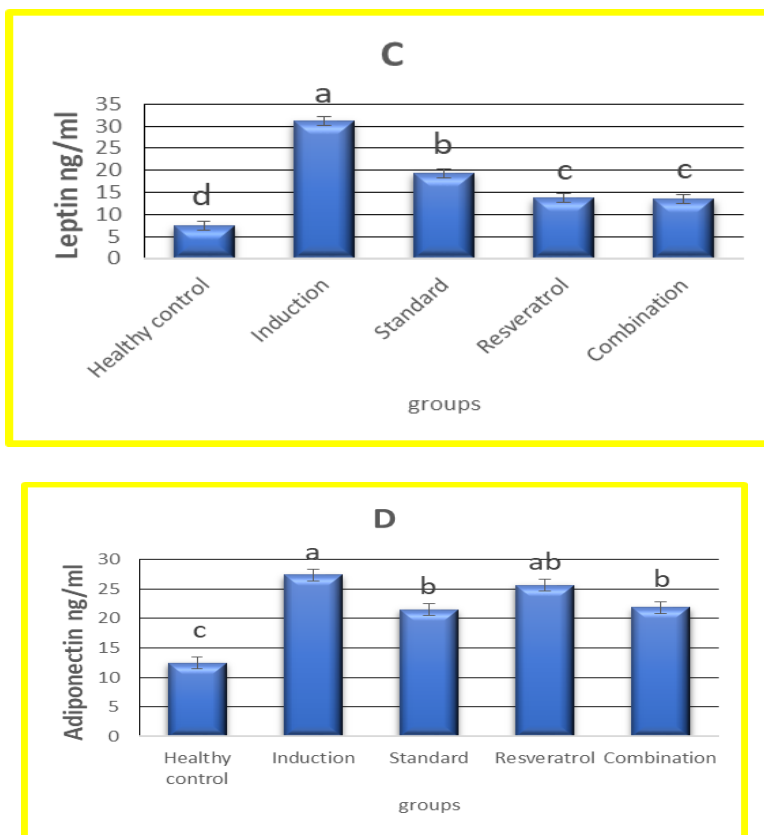
Statistical difference was represented by different superscript small letter.

p-value <0.05 mean statistically significant difference.

N: number of rats in each group.

LSD: Least Significant Difference.





**Fig.2:** Effect of resveratrol on mean serum levels of metabolic markers in hyperthyroid rats induced by levothyroxine. A: glucose B: cholesterol C: leptin D: adiponectin

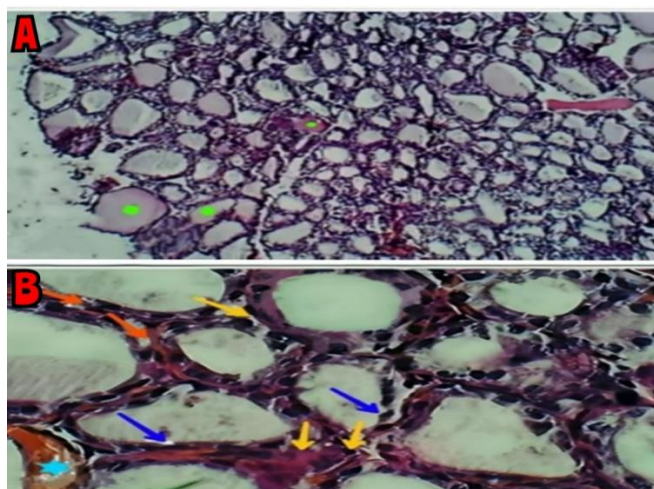
The data were represented by mean ± SEM.

Statistical difference was represented by a different small letter.

p-value <0.05 mean statistically significant difference.

**C- Effect of resveratrol on thyroid gland histopathological examination after hyperthyroidism induction by levothyroxine.**

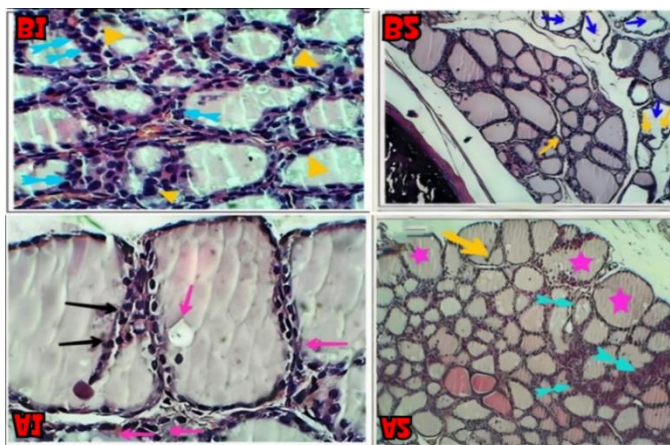
Microscopically, the parenchyma of the thyroid gland in the healthy control group consisted of numerous normal follicles of variable sizes. Thyroid follicles were composed of simple cuboidal follicular cells, which showed intense eosinophilic cytoplasm and a large central nucleus. Interfollicular tissue was composed of a thin layer of fibrous tissue that showed fibroblasts, interfollicular sinusoidal capillaries, and clear cells (figure 3A).



**Fig.3A:** Light microscopic section of the rat thyroid gland in the healthy control group shows the normal appearance of follicles of the thyroid gland (green circles), follicular cells (blue arrows), interfollicular fibroblasts (red arrows), C-cells (yellow arrows) & interfollicular sinusoidal capillaries (blue asterisk).

Magnification (A) 100X, (B) 400X, staining: H & E.

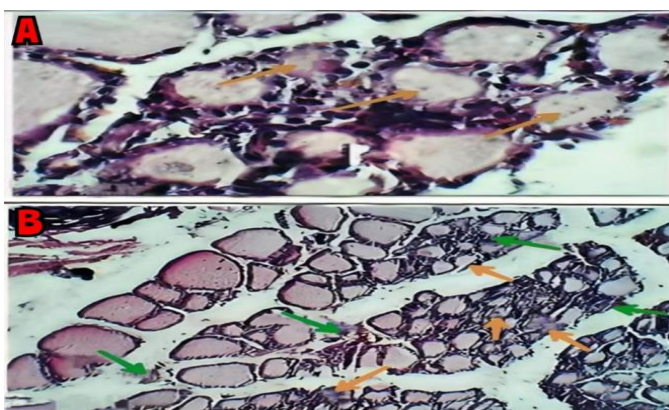
On other hand, light microscopy of thyroid tissue revealed that the induction group that received just levothyroxine for 14 days showed marked hypertrophy of the thyroid gland and moderate hyperplasia of new thyroid follicles, which led to moderate papillary infolding. Soft epithelial papillae formation lacks a fibrovascular core, colloid resorption, nuclear enlargement, multinucleation, and nuclear pleomorphism (figure 3B).



**Fig.3B:** Light microscopic section of the rat thyroid gland of the induction group shows hypertrophy of follicles (pink asterisks), epithelial papillary (yellow Arrows), multinucleation (celestial blue arrows), follicular hyperplasia (blue arrows), and nuclear enlargement (yellow triangles).

Magnification: (A1-2) 100X, (B1-2) 400X ,staining: H &E.

The thyroid gland of the standard therapy (methimazole) group showed mild inter-follicular interstitial edema, vascular congestion, follicular disruption, and atrophy of the central region of the gland. The periphery of the gland showed normal glandular tissue (figure 3C).

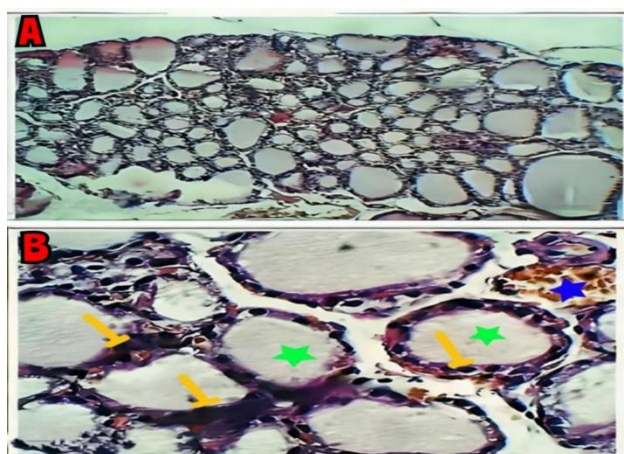


**Fig.3C:** Section of the thyroid gland of standard therapy (methimazole) shows mild interfollicular edema and vascular congestion (green arrows) with central follicular disruption, necrosis, and atrophy (orange arrows).

Magnification: (A) 100X, (B) 400X ,staining: H &E.

In addition, the resveratrol treated group shows normal appearance and size of the thyroid gland. Follicular cells had a normal appearance and showed a tall cuboidal shape that revealed foamy cytoplasm, which is the feature of colloid resorption. Sections of thyroid parenchyma revealed mild interfollicular vascular congestion, Sections of thyroid parenchyma revealed mild interfollicular vascular congestion, reducing the sizes of most thyroid follicles associated with active colloid resorption by follicular cells (figure 3D)

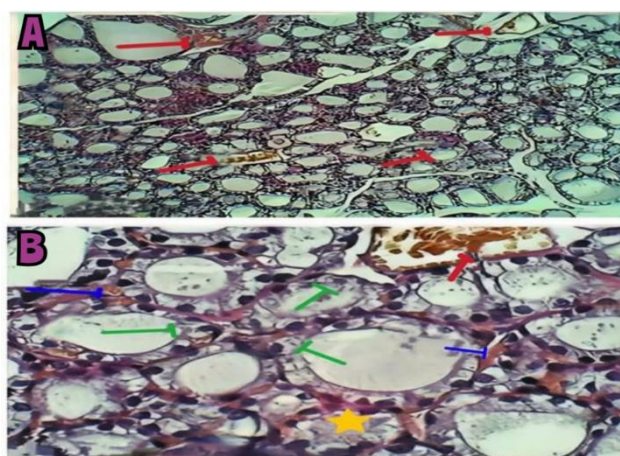
Finally, the thyroid gland of the combination group (resveratrol-methimazole) showed normal appearance and size of thyroidal cells, similar to those in the healthy control group (figure 3E).



**Fig.3D:** Section of the thyroid gland of the resveratrol group shows mild sinusoidal dilation (Red line) and marked reduced sizes of most follicles associated with active colloid resorption (yellow asterisks), foamy cytoplasm (Green line), and interfollicular congestion (blue line).

Magnification: (A) 100X, (B) 400X, staining: H &E.

## DISCUSSION



**Fig.3E:** Section of the thyroid gland for the combination (methimazole+resveratrol) shows normal follicles (green asterisks), normal sinusoidal capillary (blue asterisk), and normal follicular cells (yellow arrows).

Magnification:(A) 100X, (B) 400X ,staining: H &E.

Resveratrol exhibited an antithyroid effect in this study by a significant reduction in serum levels of (T3, T4, NIS) and a significant elevation in (TSH) levels (like methimazole), as shown in table(1)and figures(1 A, B, C, D). This outcome was in line with Kweon *et.al.*study (2014), who was found that the activation of sirtuins is another hypothesized mechanism of action for the new antithyroid agents (14). Resveratrol acts as a stimulator for sirtuin 1, a protein deacetylase that controls the production of many transcription factors. Sirtuin 1 is expressed at high levels in a variety of organs, one of which being the thyroid gland (15). These findings disagree with the outcome of a study conducted by Ge *et.al.* (2016), who showed that sirtuin-1 activators for a short length of time (sixteen days) in rats did not have an impact on blood T3 levels but caused a decrease in the serum of TSH levels (16). According to the findings of Amakura *et.al.* (2008) (17), and Xu *et.al.* (2016) (18), resveratrol is a regulator of the aryl hydrocarbon receptor (AhR). This means that it can have agonist or antagonist properties in various cells that may contribute to the antithyroid effect. Activation of AhR can reduce the expression of the sodium iodide symporter (NIS) gene, hence impairing thyroid function (19).

The resveratrol group showed a significant reduction in serum NIS levels compared to the induction group with no significant difference when compared with the combination group, as shown in table (1) and figure (1D). These results agreed with a previous study stating that stilbenoid (natural phenols) caused a significant decrease in NIS levels, as reported by Giuliani *et.al.* (2014) (20), but were inconsistent with Sebai H *et.al.*(2010) study, who evaluated the influence of resveratrol on FRTL-5 cells, where the results showed that after 6 to 12 hours of treatment, resveratrol enhance NIS gene expression and iodine uptake. This increase was transient and it was no longer detected following 24 hours. The researchers did not reveal any information about resveratrol's long-term impact on NIS mRNA (21).

Numerous processes are affected by an increase in the levels of thyroid hormones, such as increased serum levels of glucose, leptin and decreased serum levels of adiponectin and cholesterol.in addition to histological changes in the thyroid gland (22).

In the present study, the mean serum glucose level within resveratrol group is significantly decreased compared to other studied groups, as shown in table (2) and figure (2A), where the use of resveratrol alone gave a beneficial effect over the combination and standard therapy groups. The results of the current work were in line with Goh *et.al.*study (2018), who was reported that resveratrol might reduce blood glucose in type 2 diabetes patients through stimulation of SIRT1 or AMPK (23) and agree with Movahed *et.al.* (2019), who showed that 1 gram of resveratrol administered for 45 days significantly decreases fasting blood glucose, insulin, and systolic blood pressure (24). There may be several mechanisms involved in resveratrol's ability to control glucose levels. Resveratrol promotes *in vivo* Sirtuin 1 expression, which operates downstream of calorie

restriction and has a favorable effect on glycemic control (91). It may enhance glucose absorption by elevating the expression of the insulin-dependent glucose transporter GLUT4. In the absence of insulin, resveratrol can stimulate glucose absorption (25).

In the present study, resveratrol significantly diminished cholesterol levels compared to the induction group (LT4 alone) with no significant difference compared with the combination group, as shown in table (2) and figure (2B), These findings were agree with Aleksandra Rakovi *et.al.*study (2019), who found that resveratrol supplements significantly reduced rats' plasma lipid profile and glucose intolerance in rats induced with type 2 diabetes (26).

Thyroid hormones control adipokine levels, thus managing the body's energy balance. Thyroid-stimulating hormone (TSH) receptors are located in adipose tissues, suggesting that they have a role in controlling adipocytokines, which are important in energy balance regulation. Moreover, leptin raises thyroid hormone levels. It has indirect impacts on thyroid metabolism and may have acute effects on the thyroid axis (27). By elevating thyroid releasing hormone (TRH) expression within the hypothalamus, leptin delivery reverses the fasting-induced inhibition of the hypothalamus-pituitary-thyroid axis at the central level (28). In addition, TSH increases leptin release by a direct action on adipocytes, possibly via TSH-receptors on the surfaces of adipocytes; thus, the direct impact of TSH on leptin production by adipocytes can generate a positive relationship between leptin and TSH (29). Leptin modulates the activity of central and peripheral iodothyronine deiodinase and the conversion of T4 to T3; it also enhances central D2 action and causes an increase in T3. Thyroid hormones and leptin interact and may modulate body composition and metabolism through various pathways (30).

Serum levels of leptin in the resveratrol group showed a significant reduction when compared to the induction group, as shown in table (2) and figure (2C). These findings are similar to those of Müller *et al.*study (2015), who demonstrated that glucose catabolism increases ATP production in adipocytes and that resveratrol-induced attenuation of leptin efflux may be due to glucose transport and metabolism modifications (31).

Adiponectin is an adipocytokine that reduces insulin resistance and is anti-inflammatory and anti-atherogenic. Hyperthyroidism is associated with increased serum levels of adiponectin levels by various mechanisms (32). In the current data regarding the resveratrol group, serum levels of adiponectin showed no significant difference compared to the induction group. These findings are consist with Yoshizaki *et.al.*results (2010), who discovered that resveratrol treatments boosted adiponectin levels in rats that had been injected with resveratrol. This was the mechanism that was responsible for the reduction in both blood glucose levels and body weight (33). Ji H *et.al.* (2015) found that resveratrol ameliorated alcoholic fatty liver in mice by raising hepatic AdipoR1/R2 expression and reducing diabetic neuropathy by

enhancing renal AdipoR1 expression in mice with streptozotocin-induced diabetes (34).

In the current study, Administered high doses of LT4 for prolonged times could cause marked thyroid gland hypertrophy and mild hyperplasia of new thyroid follicles, leading to mild papillary infolding, soft epithelial papillae formation lacks a fibrovascular core, colloid resorption, nuclear enlargement, multinucleation, and nuclear pleomorphism. The protective effect of resveratrol against levothyroxine histological changes within the thyroid gland may be attributed to its antioxidant defense mechanism (figures 3D, E). This finding was consistent with the finding of Meltem Kurus *et al.* (2009), who found that resveratrol has healing effects on the damage of thyroid tissue in rats exposed to cigarette smoke (35) and with Chaitali Sarkar *et al.* (2014), who found that Resveratrol treatment in fluoride-exposed rats significantly reduced fluoride-induced metabolic damage and normalized both the functional capacity and ultrastructural organization of the thyroid gland (36).

## CONCLUSION

Resveratrol produces an antithyroid effect by significantly decrease the serum levels of thyroid hormone (T3, T4) and increase the serum levels of (TSH). The proposed mechanism of this action is due to inhibition of NIS and the antioxidant properties of resveratrol Also, resveratrol shows significant improvement in metabolic and histological changes of the thyroid gland associated with hyperthyroidism. The use of combination therapy produce significant reduction in levels of thyroid hormones (T3, T4) than each one alone.

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