Original Article

Possible Protective Role of Levothyroxine Sodium and Nigella Sativa Oil on Cerebellar Cortex of Adult Male Albino Rat in Carbimazole-induced Hypothyroidism: Histo-Biochemical Study

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ABSTRACT

Background: The brain damage induced by hypothyroidism affects numerous important areas. Although the management of hypothyroidism by levothyroxine sodium (thyroxine), is achieved, it still needs close monitoring for fear of hyperthyroidism development. Therefore, it is crucial for seeking natural products like black seeds that has a lot of evidence which suggests its pharmacological efficacy. The current study aimed to determine the scavenging role of thyroxine and nigella, on the cerebellar cortex of adult male albino rats in the hypothyroidism induced by carbimazole.

Materials and Methods: Sixty adult male albino rats were equally distributed into six groups; 10 rats in each (control, sham control, carbimazole, Levo thyroxine sodium, nigella sativa oil and combined group). Hormonal assay was done by measurements of serum TSH, T3 andT4, oxidation marker malondialdhyde (MDA) and glutathione (GSH) were also performed. Histological and immunohistochemical staining by Glial fibrillary acidic protein (GFAP) & caspase-3 immunostaining was carried out and followed by histomorphometric analysis. Expression of α-Synuclein gene was done using real-time polymerase chain reaction (PCR).

Results: Pointed out that thyroxine and nigella individually ameliorated the histopathological alteration like vacuolations and dark nuclei, gliosis as well as apoptosis induced by carbimazole. These changes were subjected to phenotypic quantitation. In the combined therapy group, the improvement of these changes were remarkable.

Conclusion: Hypothyroidism induced degenerative changes in the cerebellar cortex, that were ameliorated by receiving either thyroxine or nigella individually, with more pronounced results achieved in thyroxine versus nigella. In addition, combined treatment reversed the mentioned parameters to nearly its normal level.

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Key Words: Carbimazole, hypothyroidism; levothyroxine; nigella.

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INTRODUCTION

Hypothyroidism induced brain lesion especially in the frontal lobe, hippocampus as well as the cerebellar cortex[1]. As a result, granule cell neurons diminish neural connection, which affects the synapsis of Purkinje cell (Pc) neurons[2].

Due to the high cerebellar need of oxygen, it is especially vulnerable to oxidation stress and its biochemical integrity is crucial for its normal function. Increased lipid peroxidation and decreased levels of tissue antioxidants may promote reactive oxygen species (ROS) creation and cellular damage[3].

All clinical circumstances, including primary (thyroidal), secondary (pituitary), and tertiary (hypothalamic) hypothyroidism, thyroxine is the medication of choice. It is a synthetic T4 hormone that is identical to the natural one in terms of biochemistry and physiology[4].

Despite effectiveness of thyroxine in treatment of hypothyroidism, symptoms as persistency of tiredness, cognitive dysfunction and mood disorders still remain in some patients even with reaching the euthyroid state[5]. Serum TSH concentrations are usually used to monitor thyroxine therapy in hypothyroid patients as long term synthetic thyroid drug therapy might lead to hyperthyroidism[6].

Nigella sativa (Black seed) has many nutritional and medicinal uses, it has repeatedly been outlined and suggested in Tibb-e-Nabawi, Unani medicines, Arabic, Chinese and African medicines[7]. Many naturally occurring elements, such as carbohydrates, proteins and vitamins, such as thiamine, niacin, riboflavin, pyridoxine and folic acid, have been scientifically proved to be present in the black seed oil[8].

Because of the large number of significant ingredients especially thymoquinone (TQ) in its fixed oil, it has been discovered that it affects various body parts and has several pharmacological effects, used against different viruses or bacteria and wound healing effects[9].

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The current study aimed to determine the possible scavenging role of thyroxine and nigella, on the cerebellar cortex of adult male albino rats in hypothyroidism induced by carbimazole.

MATERIALS AND METHODS

The experimental design was performed according to the regulations of the Cairo University Institutional Animal Care and Use Committee (IACUC) (CU III F, 1 21).

Animals

The study was performed on sixty adult male albino rats with weight ranged from 180-200 gm. All rats were obtained from the experimental Animal House, Faculty of Medicine, Cairo University. The rats were kept in metal cages, about 5 rats per cage under standard laboratory conditions with allowance of free access to drink water and rat pellet diet. The rats were exposed to 12 hours light/dark cycles. The rats were acclimatized in the laboratory for a period of two weeks before carrying out the experiment.

Experimental design

The animals were equally distributed into six groups, ten rats per group:

Group I (Control Group): rats obtained no medications for 7 weeks.

Group II (Sham Group): 5 rats, each obtained 0.5 ml distilled water by gastric gavage daily for 3 weeks.

5 rats, each obtained 0.5 ml normal saline by gastric gavage daily for 4 weeks.

Group III (Carbimazole Group): rats obtained a daily dose of 1.35 mg/kg of carbimazole by gastric gavage for 3 weeks[10]. At the beginning of the 4th week[11] hormonal levels (TSH, T3 and T4) were assessed to confirm the incidence of hypothyroidism. Another four weeks were spent with the rats without any further administration.

Group IV (Thyroxine Group): rats received carbimazole at the same dosage and time of group III. At the beginning of the 4th week, the rats received 20 µg/kg of thyroxine everyday by gastric gavage for 4 weeks[12].

Group V (Nigella Group): rats received carbimazole at the same dosage and time of group III. At the beginning of the 4th week, the rats received 2 ml/kg of nigella everyday by gastric gavage for 4 weeks[11].

Group VI (Combined Group): rats received carbimazole at the same dosage and time of group III. At the beginning of the 4th week, the rats received combined thyroxine and nigella at the same doses and duration of groups IV and group V.

Chemicals

Carbimazole was purchased as (NeoMercazole® 5 mg tablets, Amdipharm, Dublin, Ireland), it was administered everyday by gastric gavage at a dose of 1.35 mg/kg/day dissolved in 1 ml distilled water.

Thyroxine was purchased as (Eltroxin® 50 µg tablets, Glaxo Welcome Co., Egypt), that was administered everyday by gastric gavage at a dose of 20 microgram/kg/day dissolved in normal saline (20 µg/mL).

Nigella was purchased from Kahira pharmaceutical and chemical industries Co., Cairo, Egypt. It was given by gastric gavage at a dose of 2 ml/kg/day.

Methods

One day after the end of the experiment (7 weeks), all the experimental animals were euthanized by ether anaethesia followed by cervical dislocation[13]. The cerebellum specimens were immediately excised carefully and distributed into two sections, one part was rapidly fixed in 10% formal saline solution and subjected to histological and immunohistochemical studies and the other part was taken as tissue homogenate for biochemical and gene expression.

Measurement of hormonal levels: blood sample from tail vein was collected from rats of all groups to measure blood level of TSH, T3 and T4, both at the beginning of the fourth week to ensure hypothyroid state and after seven weeks to investigate the persistence of hypothyroid state and the protective effects.

Oxidative stress determinatio[14]

Lipid perioxidation was assessed spectrophotometrically to express MDA (lipid peroxidation end product) concentration, the MDA molecular absorbance coefficient (1.56 u 10-5 mol/cm) was used. Measurements of GSH (antioxidant enzyme): content was calculated using a colorimetric method as nmol GSH/mg protein.

Histological parameters

- Hematoxylin and eosin (H&E)[15].
- Immunohistochemical Study:
    1. Glial fibrillary acidic protein (GFAP): marker that differentiate between astrocytes from other glial cells (gliosis)[16]. It was used as 0.1 ml of the 1ry rabbit monoclonal antibody, (MS-1376-PO) (Lab Vision Corporation, USA)
    2. Caspase3 immunostaining[17], is the marker for apoptosis. A rabbit monoclonal antibody (700182, 9H19L2), (Lab Vision Corporation, USA) was used.

Positive brown reaction of the cytoplasm was shown in the apoptotic cells and astrocytes. Negative reaction was observed by omitting the 1ry antibody.

Phenotypic Quantitation

The area% of dark nuclei and that of vacuolations were performed and that of GFAP and caspase 3 positive immunoexpression (IE) were measured using binary menu. Five non-overlapping fields were picked from five

slides belonging to various groups in order to conduct the measurements on Leica Qwin 500 LTD (Cambridge, UK) image analyzer.

Quantitative polymerase chain reaction (qPCR) for α-synuclein gene: part of the cerebellar homogenate of animals of all groups was used in real time polymerase chain reaction (RTPCR) to evaluate the change in α-Synuclein gene expression[18].

Data analysis[19]

It was done using statistical package for the social sciences (SPSS) version 21.0 statistical software (IBM Corporation, Somers, New York, USA). The data were expressed as means ± standard deviation (SD). Using ANOVA. Any significant difference was determined by *P-values* <0.05.

RESULTS

Serological results

After two weeks, there was significant increase in TSH and significant decrease in T3 &T4 in groups III, IV, V and VI versus groups I and II.

After seven weeks, there was significant increase in TSH and significant decrease in T3 and T4 in group III against all groups. Group V showed significant increase in TSH & significant decrease in T3 and T4 against groups I, II, IV and VI, in group IV with groups I, II and VI (Tables 1,2, Histograms 1,2).

Biochemical results of oxidative stress

There was significant increase in MDA & α-synuclein gene expression levels & significant decrease in GSH level in group III with all groups. In group V a significant increase and decrease was found with groups I, II, IV and VI, in group IV against groups I, II and VI (Table 3, Histogram 3).

Histological examination

By histological examination of stained cerebellar sections from different groups, H&E stained sections of control and sham groups showed normal histological architecture of the grey and white matter. Grey matter forming cerebellar cortex consisted of three indistinct layers, molecular (ML), ganglion cell layer (GCL) and granular layer (GL). Medulla is located deep to the cerebellar cortex and formed mainly of dense nerve fibers intermingled with medullary cells. ML exhibited pleomorphic neurons and few microglia with dark elongated nucleus, while GCL revealed one layer of large pyriform neurons and GL was formed of small neurons. Sections from the carbimazole group showed vacuolations in the three layers, dark nuclei in ML and GL, few neurons in GCL and congested vessels in the GL. These changes were slightly ameliorated in the thyroxine or nigella group, by the presence of few vacuolations and some dark nuclei in GCL and GL. Further improvement was achieved in the combined thyroxine and nigella groups (Figures 1,2).

Immunohistochemical results

Detection of astrocytes immunoreactivity in the astrocytes process, as it was scattered reaction in ML and GL in groups I, II &VI (Figures 3 a,e). While extensive reaction was observed in group III (Figure 3b), that appeared less obvious in group IV (Figure 3c) but obvious in group V (Figure 3d) and minimal in group VI (Figure 3e).

The apoptosis marker, caspase-3 reaction was negative in group I (Figure 3f) and in group VI (Figures 3 h,j). In carbimazole group extensive reaction was seen in the three layers (Figure 3g). In group IV less obvious reaction was seen, while in group V (Figure 3i) the reaction was obvious in the three layers and minimal in ML and GCL of group VI.

Morphometric assessment of area% of dark nuclei and vacuolations, GFAP and caspase3 IE

The values denoted significant increase in group III versus the other groups, in group V versus groups I, II, IV and VI, in group IV versus groups I, II and VI (Table 4, Histogram 4).

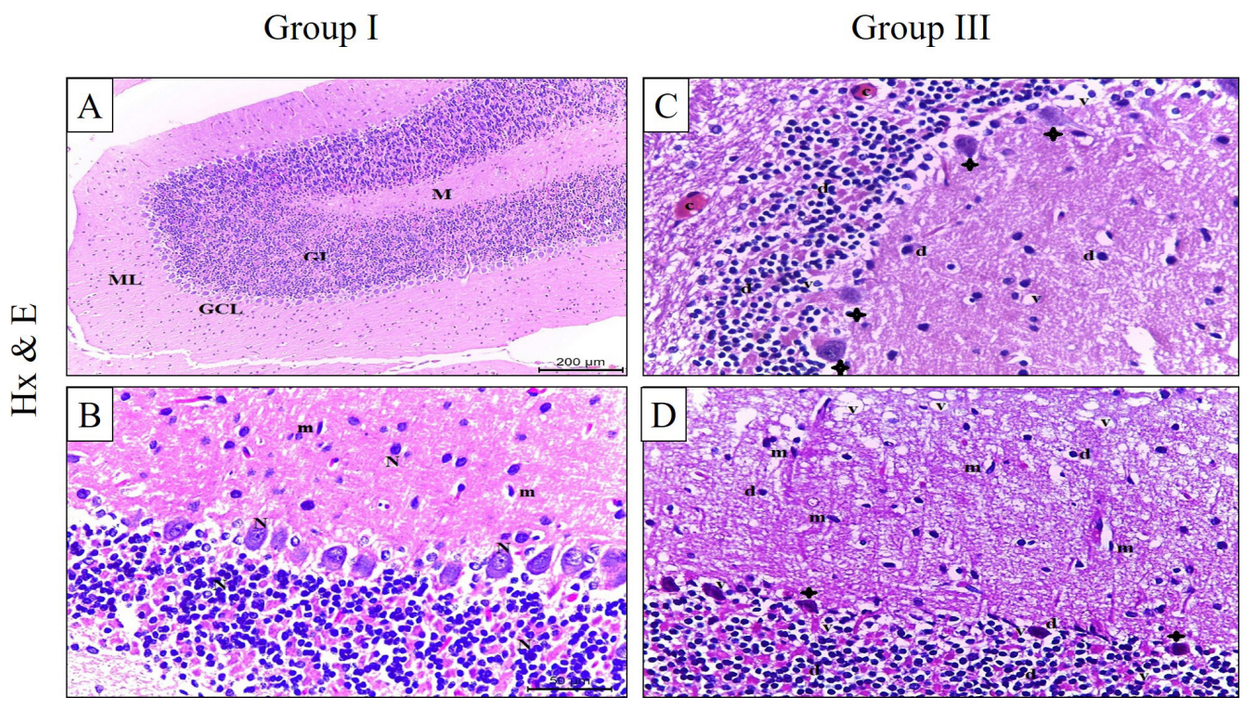


Fig. 1: H&E, showing: In Group I (a): molecular layer (ML), ganglion cell layer (GCL) and granular layer (GL) of cerebellar cortex and medulla (M) (x100), (b): ML exhibiting pleomorphic neurons and few microglia (m) with dark elongated nucleus, GCL one layer of large pyriform neurons and GL of small neurons. All neurons have pale nuclei (N). In Group III (c): vacuolations (v) in the 3 layers, dark nuclei (d) in ML and GL, few neurons (*) in GCL and congested vessels (c) in GL, (d): obvious vacuolations (v) in the 3 layers, dark nuclei (d) in most neurons of the 3 layers, multiple microglia (m) in ML and few neurons (*) in GCL.

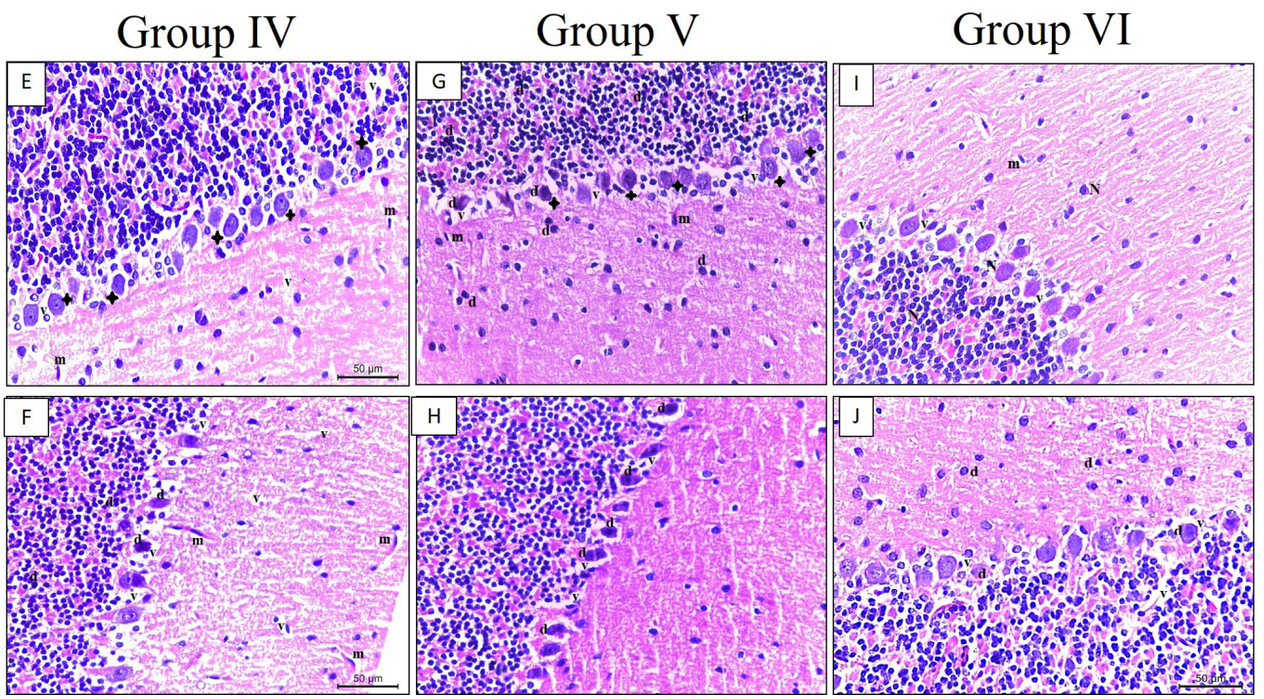


Fig. 2: H&E, showing: In Group IV (e): few vacuolations (v) in the 3 layers, few microglia (m) in ML and multiple neurons (*) in GCL, (f): some vacuolations (v) in ML and GCL, few microglia (m) in ML, few dark nuclei (d) in GCL and GL (x400). In Group V (g): few dark nuclei (d) and few microglia (m) in ML, multiple neurons (*), few dark nuclei (d) and some vaculations in GCL and multiple dark nuclei (d) in GL, (h): multiple dark nuclei (N) and some vacuolations (v) in GCL. In Group VI (i): few microglia (m) in ML, few vacuolations (v) in GCL and pale nuclei (N) in the 3 layers, (j): few vacuolations (v) the 3 layers, few dark nuclei (d) in GCL and ML (x400).

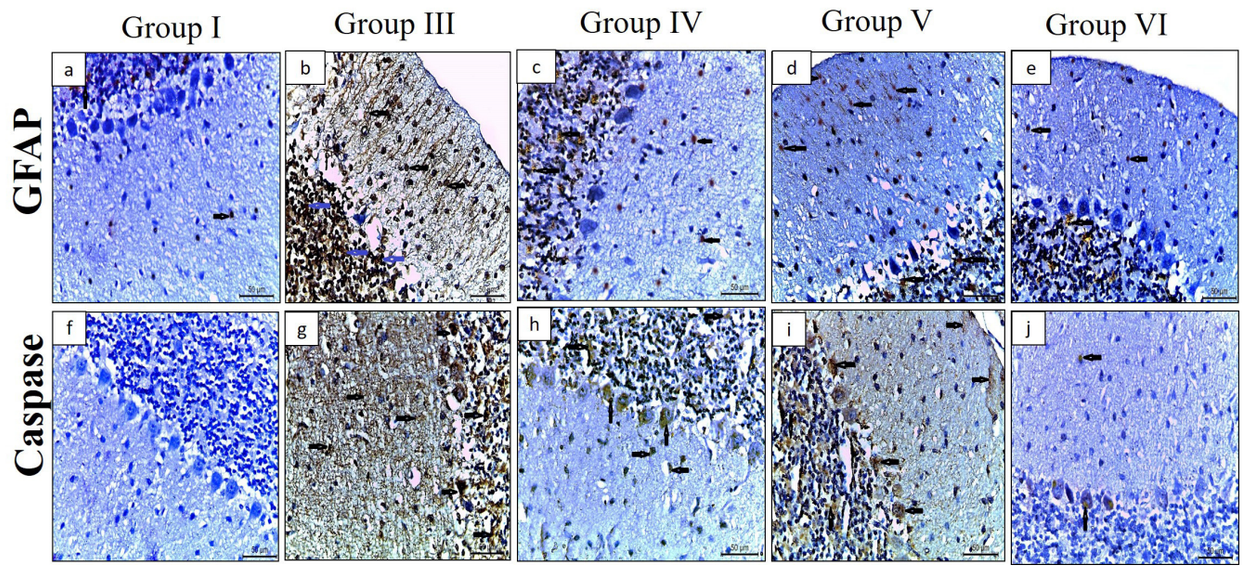


Fig. 3: GFAP immunostaining (x400) showing: In Group I (a): scattered reaction in ML and GL. In Group III (b): extensive reaction (arrows) in ML and GL. In group IV (c): less obvious reaction (arrows) in the ML and GL. In group V (d): obvious reaction (arrows) in ML and GL. In group VI (e): scattered reaction (arrows) in ML and GL. caspase3 immunostaining (x400) showing: In Group I (f): -ve reaction in the 3 layers. In Group III (g): extensive reaction (arrows) in the 3 layers. In group IV (h): less obvious reaction (arrows) in the 3 layers. In group V (i): obvious reaction (arrows) in the 3 layers. In group VI (j): minimal reaction (arrows) in ML and GCL.

Table 1: Mean ± SD TSH, T3 and T4 levels after two weeks:

Group	TSH	T3	T4
Group I	1.867± 0.356	4.185 ± 0.874	90.808 ± 9.071
Group II	2.069± 0.356	4.052 ± 1.008	93.731 ± 10.507
Group III	9.148± 1.572*	0.848 ± 0.154	50.394 ± 5.357
Group IV	10.251±1.978*	0.892 ± 0.213	51.882 ± 8.112
Group V	9.165± 1.834*	0.865 ± 0.237	44.755 ± 5.067
Group VI	8.808± 1.925*	0.985 ± 0.204	50.097 ± 8.947

* Significant ≤0.05 against groups I and II.

Table 2: Mean ± SD TSH, T3 and T4 levels after seven weeks:

Group	TSH (µg\dl)	T3 (ng\dl)	T4 (µg\dl)
Group I	1.907± 0.505	4.867± 0.622	102.983± 9.007
Group II	1.996± 0.433	4.936 ± 0.651	101.942 ± 10.9
Group III	9.488± 1.729*	1.083 ± 0.327*	39.522 ± 4.636*
Group IV	3.052± 0.730@	3.812 ± 0.768@	89.557 ± 8.851@
Group V	4.173± 0.961^	1.809 ± 0.455^	69.246± 10.069^
Group VI	2.399± 0.652	4.829 ± 0.508	104.31± 10.04

* Significant ≤0.05 differences with other groups ^ against groups I, II, IV and VI @ against groups I, II, VI

Table 3: Mean ± SD MDA, GSH levels and α-synuclein gene expression:

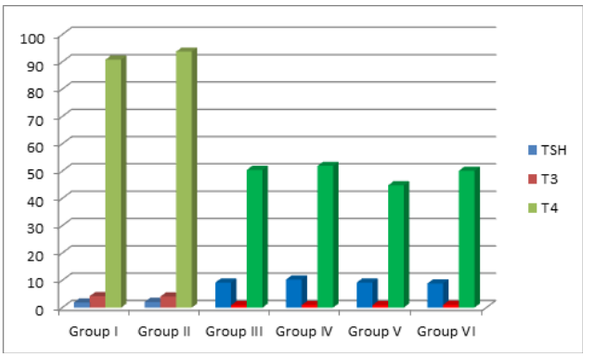
Group	MDA(nMol/gm)	GSH (µg/gm)	α-synuclein gene (ng/ml)
Group I	45.175 ± 6.636	116.344 ± 6.429	0.863 ± 0.218
Group II	46.388 ± 6.87	114.42 ± 7.018	0.953 ± 0.115
Group III	166.875 ± 11.103*	38.494 ± 6.867*	6.561 ± 0.703*
Group IV	56.423 ± 8.238@	85.976 ± 6.629@	3.493 ± 0.427@
Group V	81.181 ± 9.727^	94.972 ± 6.831@	3.628 ± 0.437@
Group VI	42.057 ± 8.651	112.894 ± 7.643	1.003 ± 0.138

* Significant ≤0.05 differences against other groups ^ against groups I, II, IV and VI @ against groups I, II, VI

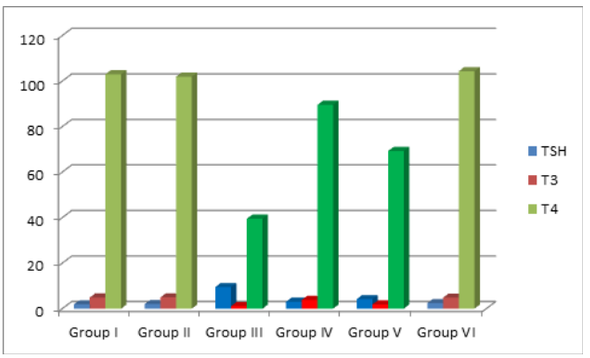
Table 4: Mean ± SD area% of dark nuclei and vacuolations, GFAP and caspase3 expression:

Group	Dark nuclei	Vacuolations	GFAP	Caspase3
Group I	0	0	9.32±1.66	1.30±0.94
Group II	0	0	9.61±1.58	1.71±1.18
Group III	20.32±2.97*	21.99±3.86	38.22±6.54*	41.54±13.71*
Group IV	4.28±0.53@	3.67±0.52	15.83±1.43@	10.75±2.15@
Group V	9.91±1.58^	5.22±1.05	19.05±1.45^	18.78±2.34^
Group VI	2.22±0.32	1.97±0.23	10.74±1.69	2.26±1.28

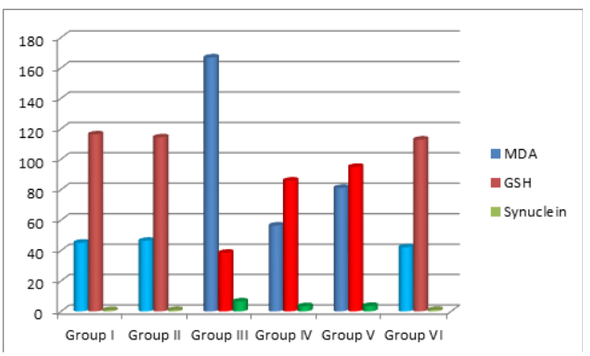
* Significant ≤0.05 differences against other groups ^ against groups I, II, IV and VI @ against groups I, II, VI.



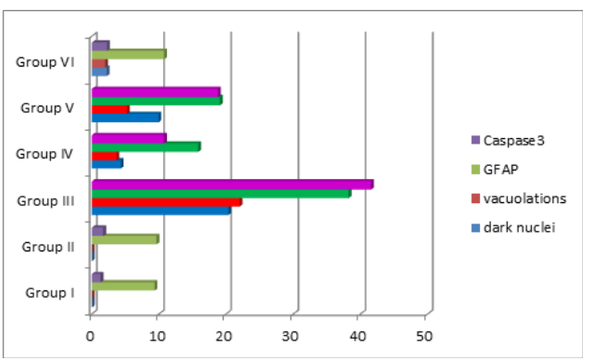
Histogram 1: TSH, T3 and T4 levels after two weeks



Histogram 2: TSH, T3 and T4 levels after seven weeks



Histogram 3: Mean MDA, GSH levels and α-synuclein gene expression



Histogram 4: Mean area% of dark nuclei and vacuolations, GFAP and caspase3 expression

DISCUSSION

Thyroid dysfunction considered as the prevalent endocrine conditions that has an impact on the architecture and operations of the brain. From early childhood to old life, hypothyroidism has an effect on the cerebellum[20]. It is exceedingly difficult to have a positive therapeutic outcome, especially when using monotherapy. Here, it was hypothesized that the effects of levothyroxine sodium, one of the most often prescribed medications for hypothyroidism, could be increased through various pathways when combined with other antioxidants. Nigella has numerous nutritional and therapeutic applications, and the FDA has approved its usage in food[21]. Therefore we aimed to study the histological, immune-histochemical and biochemical parameters to point out the scavenging role of thyroxine and nigella, on the cerebellar cortex of adult male albino rats in the hypothyroidism induced by carbimazole.

In the current work, serological examination of either individual thyroxine or Nigella groups showed significant decrease of the mean TSH level, significant increase in the mean T3 and T4 levels versus carbimazole group. In addition, a significant difference was found in the nigella versus the control, sham, thyroxine and combined therapy groups. Similarly, nigella failed to normalize previous hormonal levels with continuous propylthiouracil administration[22]. Furthermore, our results revealed that, the combined thyroxine and nigella treated group showed restoration of hormonal levels, which was better than using thyroxine alone. In agreement, combined usage of nigella with several conventional agents, proved to have synergistic benefits[23].

In the present study, early restoration of the equilibrium between oxygen free radicals and antioxidant synthesis was found. In carbimazole group there was significant increase of MDA and significant decrease of GSH levels. Similarly, greater MDA concentrations in the cortical and hippocampus tissues of the hypothyroid rats was postulated[24]. It has been proved that hypothyroidism leads to inhibition of Gamma glutamyl transpeptidase activity that diminish the glutamate reservoir essential for GSH synthesis[25].

The effect of thyroxine was more pronounced than Nigella, both drugs in combination exerted the best ameliorative effect on MDA level in cerebellum. The previous findings were in agreement with authors who studied the effect of 10 mg/kg of thymoquinone administration on the hippocampus and cerebral cortex of hypothyroid rats[26]. In accordance, it was added that concomitant administration of L-thyroxin and selenium as an antioxidant to hypothyroid patients had a synergistic effect[27].

Furthermore, significant decrease of the mean GSH value was recorded in carbimazole group versus treated groups. On the other hand, sig increase of mean GSH was noted in combined group compared to thyroxine group,

which proved a significant sig increase versus Nigella group. In support, it was reported that thyroid hormones are necessary to maintain GSH homeostasis in astrocytes in order to shield the brain from oxidative stress[28].

The current work showed down-regulation of α-synuclein gene expression, in combined group more than thyroxine group which exerted more effective diminution effect than in nigella group. This confirmed the improvement of cerebellar function. In addition to the well-known symptoms of hypothyroidism, neurological symptoms such as cerebellar ataxia[29]. Consequently, it was advised that measurements of hormonal assay should be performed to exclude hypothyroidism in patients presented with cerebellar ataxia. Impairment of the cerebellar function in hypothyroid patient is closely related to α-synuclein gene expression. As a proof of principle, the gene overexpression increase the cell damage and injury in human[30]. Improvement of gene expression level in the combined group, run with authors who proved that thymoquinone which is the active ingredient of Nigella can protect the cultured hippocampus against α- synuclein -induced synapse lesion[31].

Regarding the histological changes in carbimazole group, histological alternations of all layers of the cerebellar cortex were observed, dark nuclei in ML and GL, congested vessels in GL, obvious vacuolations and multiple microglia in ML. In addition, few Purkinje cells were found. These changes were attributed to the deterioration in the antioxidant defense system in the hypothyroid state[32]. In agreement, this was referred to the neurotoxic effect of hypothyroidism which enhance apoptosis as well as demyelination[33].

These previous histological changes were improved in Nigella group. In thyroxine group few vacuolations in the three layers, few microglia in ML and multiple neurons in GCL, some vacuolations in ML and GCL, few microglia in ML, few dark nuclei in GCL and GL were found, Moreover, marked improvement in the combined group was recorded. It was confirmed that the nigella has protective effect on the nervous system against the damaging effect of nitric oxide overproduction[34]. In accordance, it was proved that sig improvement of myocardial damage induced by hypothyroidism was observed upon receiving a mixture of thyroxine and vitamin E. Those authors added that the hormone compensation only increased the production of oxygen free radical thus restoration of the antioxidant balance by administration of antioxidants could prevent such adverse effects[35].

The previous histological changes were confirmed by the values of area% of dark nuclei and vacuolations that denoted significant increase in group III versus the other groups, in group V versus groups I, II, IV and VI, in group IV versus groups I, II and VI.

In the current work GFAP IE, revealed extensive reaction in carbimazole group both ML and GL. In thyroxine group it was less obvious, while in Nigella group it was obvious and scattered in combined therapy group. In support, it was clarified that as a result of any brain insults, astrocyte generation and hypertrophy is accompanied with gliosis and increased GFAP IE[36]. Regarding caspase3 IE, it was extensive in carbimazole group, less obvious in thyroxine group, while obvious in Nigella group and minimal in combined therapy group. This was in alignment, with authors who observed that administration of thyroid hormones suppress the apoptosis process through elevation of the anti-apoptotic Bcl-2 protein level in the hippocampus[37], It was added that astudy recommended simultaneous usage of antioxidant herbs besides the traditional thyroid medications in hypothyroidism to improve results[38].

The previously mentioned immunohistochemical results were confirmed by phenotypic quantitation as increase in the area % of both GFAP and Caspase-3 in carbimazole group. Amelioration was found in thyroxine more than nigella treated group and remarkable regression of changes was found in combined therapy group.

CONCLUSION

Hypothyroidism has an adverse effect on the cerebellar cortex histologically, immunohistochemically and biochemically. These adverse effects were ameliorated by receiving either thyroxine or nigella individually. However better results were achieved in thyroxine versus nigella. Moreover, combined administration of both medications reversed the mentioned parameters to nearly its normal level.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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الملخص العربى

الدور الوقائي المحتمل لليفوثيروكسين الصوديوم وزيت حبة البركة على قشرة المخيخ لدى ذكور الفئران البيضاء البالغة في قصور الغدة الدرقية الناجم عن الكاربيمازول: دراسة هستوكيميائية حيوية

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مقدمه: يتأثر المخيخ بالاضطرابات الهرمونية. لذلك، كان الهدف من هذه الدراسة هو تقييم التغيرات النسيجية والهستوكيميائية المناعية والكيميائية الحيوية في قشرة مخيخ ذكور الفئران البيضاء البالغة ذات القصور في الغدة الدرقية المستحث بعقار كاربيمازول والدور الوقائي لليفوثيروكسين صوديوم أو زيت حبة البركة أو الإثنين معا في تخفيف هذه التغييرات.

لخلفية: يؤثر تلف الدماغ الناجم عن قصور الغدة الدرقية على العديد من المجالات الهامة. على الرغم من تحقيق السيطرة على قصور الغدة الدرقية بواسطة ليفوثيروكسين الصوديوم (ثيروكسين)، إلا أنها لا تزال بحاجة إلى مراقبة دقيقة خوفًا من تطور فرط نشاط الغدة الدرقية. لذلك، من الضروري البحث عن المنتجات الطبيعية مثل الحبة السوداء التي لديها الكثير من الأدلة التي تشير إلى فعاليتها الدوائية. هدفت الدراسة الحالية إلى تحديد الدور للثيروكسين والحبة السوداء في القشرة المخيخية لذكور الجرذان البيضاء البالغة في قصور الغدة الدرقية المحدث بالكاربيمازول.

المواد والطرق: تم توزيع ستين من ذكور الجرذان البيضاء البالغة بالتساوي على ست مجموعات؛ ١٠ فئران في كل منها (الضابطة، ، الكاربيمازول، ليفو ثيروكسين الصوديوم، زيت حبة البركة والمجموعة المركبة). تم إجراء الفحص الهرموني عن طريق قياسات TSH وT٣ وT٤ في الدم، كما تم إجراء تعريض أحد الجزئين لدراسات نسيجية (H & E)، ودراسات كيميائية مناعية (GFAP وCaspase-٣) وقياس النسيجي للتفاعل المناعي الايجابي لـ GFAP و Caspase -٣ باستخدام محلل الصورة. وقد تم استخدام الجزء الأخر من المخيخ كتجانس انسجة للدراسات البيوكيميائية (قياس مستويات MDA وGSH) والتعبير الجيني (α-Synuclein).

النتائج: أشارت إلى أن هرمون الثيروكسين والحبة السوداء ساهما بشكل فردي في تحسين التغيرات النسيجية المرضية مثل الفجوات والنوى الداكنة، الدباق وكذلك موت الخلايا المبرمج الناجم عن الكاربيمازول.. وفي مجموعة العلاج المشترك، كان تحسن هذه التغييرات ملحوظا.

الاستنتاج: قصور الغدة الدرقية يسبب تغيرات تنكسية في قشرة المخيخ، والتي تم تحسينها عن طريق تناول إما هرمون الغدة الدرقية أو حبة البركة بشكل فردي، مع نتائج أكثر وضوحا تم تحقيقها في هرمون الغدة الدرقية مقابل حبة البركة. بالإضافة إلى ذلك، أدى العلاج المشترك إلى عكس المعلمات المذكورة إلى مستواها الطبيعي تقريبًا. يوصى باستخدام زيت حبة البركة كعلاج عشبي مصاحب مع علاج ليفوثيروكسين الصوديوم التقليدي في حالات قصور الغدة الدرقية. حيث إن زيت حبة البركة قد يتآزر مع العلاج الهرموني مما يساعده على إعادة TSH و T٣ و T٤ إلى مستوياتها الطبيعية حتى مع الجرعات العلاجية المنخفضة من ليفوثيروكسين صوديوم