

Hematological Studies on the Effect of Some Agents Used in the Treatment of Thyroid Disorders in Rats

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Abstract.

The present research aimed to examine the effects of levothyroxine and ashwagandha root extract (ARE) on hypothyroidic rats. In this study, sixty male albino rats were split into six equal groups. Group I: (CON) was the control group; it received 0.5 ml/kg b.w. of saline 0.9% NaCl solution orally for eight weeks. Group Δ I: (ARE) got 500 mg/ kg b.w. of ashwagandha root extract through oral tradition for 8 weeks. Group Δ II: (LEV) got 20 μ g/kg b.w. of levothyroxine orally for 8 weeks. Group IV: (PTU) hypothyroidic control; it received 0.05% (w/v) Propylthiouracil orally for 8 weeks. Group V: (PTU+ARE) hypothyroidic rats treated with ARE for 4 weeks. Group VI: (PTU+LEV), Group VI: (PTU+LEV) hypothyroidic rats treated with LEV for 4 weeks. The results showed that PTU-induced rats had signs of normocytic normochromic anemia. Also, they showed significant leukopenia, lymphopenia, and monocytopenia, while neutrophils and eosinophils had non-significant changes. Moreover, they showed a considerable thrombocytopenia. However, hypothyroidic rats treated with either ARE or LEV showed improvement in the hematological parameters when compared to the hypothyroidic untreated group. This suggested that ARE could be used as a protective agent against hypothyroidism, probably due to its antioxidant properties.

Keywords:

Ashwagandha root extract, Levothyroxine, Antioxidant, Hematology, Rats.

Introduction

Thyroid gland is one of the important endocrine glands. Thyroid follicles, which make and store

thyroid hormones, make up the thyroid gland. Thyroxine is released by thyroid follicular cells in response to TSH stimulation, and

triiodothyronine or T₃ (*Pirahanchi et al., 2018*). T₄ is the primary hormone secreted by the thyroid gland in terms of quantity, but T₃ is more active in terms of biology and is mostly produced by peripheral deiodination of T₄ (*Köhrle and Frädrich, 2022*). Through their direct impact on the metabolism of fat, protein, and carbohydrates or through their indirect impact on other regulatory hormones like insulin or catechol amines, thyroid hormones control the baseline energy requirement (*Mullur et al., 2014*).

Inadequate thyroid hormones production is an indication of hypothyroidism, commonly referred to as the underactive thyroid hormones (T₃ and T₄). One of the most important diagnostic criteria for hypothyroidism is the TSH concentration, which is produced when the negative feedback system produces too much thyroid hormone (TSH) (*Chiovato et al., 2019*).

Propylthiouracil (PTU) or thio carbamide is a thiouracil-derived drug which inhibits the synthesis of thyroid hormones stops T₄ from being converted to its active form, T₃, in peripheral tissues of the thyroid gland, so, T₄ and T₃ levels decrease in serum (*Hassan et al., 2013*). It is used to induce hypothyroidism in laboratory animals (*Xu et al., 2017*).

Within the Solanaceae family, ashwagandha (*Withania somnifera Dunal*) is one of the few iodine-free herbal remedies that has been shown

to boost thyroid hormones. It has saponin, alkaloids, and steroidal components. By changing T₄ into T₃, these substances contribute to the synthesis of more T₄ hormone (*Rafieian-Kopaei, 2018*). According to *Verma and Kumar (2011)*, steroidal lactones known as withanolides and steroidal alkaloids are the main biochemical components of ashwaganda root.

Levothyroxine is the exogenous synthetic levoisomer form of T₄ that is similar to the endogenous hormone produced by the thyroid gland which is metabolized to its active form, T₃. It is used in the form of tablets for treatment of hypothyroidism, goiter and thyroid cancer (*Eghtedari and Correa, 2021*).

Accordingly, the purpose of this research is to investigate the effect of ashwagandha root extract and levothyroxine as anti-hypothyroidic hematological disorders in rats.

Material and methods

Lab animals

A total of sixty normal male albino rats, weighing between 110-130 g were taken from the Suez Canal University Faculty of Veterinary Medicine's laboratory animal housing. Before being used in experiments, the animals were given a week for adaptation to their new surroundings at the laboratory animal house.

Rats were kept in a constant temperature of 23±2 °C for a duration of 12 hours, with light and

dark cycles. They were given abundant water and food that was typical of a rodent's baseline diet.

Propylthiouracil was purchased from Amoun pharmaceutical CO. S.A.E. Egypt., while, levothyroxine was purchased from Aspen Pharma Trading Limited, Ireland. Ashwagandha root extract was obtained from NOW Foods, USA.

Experimental design and grouping of rats

At the onset of the research, six equal groups of animals were randomly assigned. The CON group was maintained as the standard control group and was given 0.5 ml/kg b.w orally of saline 0.9% NaCl solution for 8 weeks. ARE group was given 500 mg/kg of ashwagandha root extract by gavage, and the LEV group was given 20 µg/kg of levothyroxine by gavage for the same amount of time. For 8 weeks, the PTU group was given 0.05% (w/v) propylthiouracil (PTU) in drinking water. PTU+ARE group induced to hypothyroidism by PTU for 4 weeks and treated with ARE for the following 4 weeks. PTU+LEV group induced to hypothyroidism by PTU and treated with LEV for the following 4 weeks.

Sampling

Two blood samples were drawn: one at the end of 6 weeks trial and the other after 8 weeks. Every time, five rats were sacrificed in each group. Rats that had been starved for ten hours while under the influence of tetrahydrofuran inhalation anesthesia had samples of their

retroorbital veins taken. The blood was taken for hematological investigations involving a full blood count and placed into a tube containing K2EDTA as an anticoagulant. Hematological parameters estimation

Hematological parameters estimation

The estimated parameters were Hb, RBCs, HCT, erythrocytic indices (MCV, MCH and MCHC), total and differential leukocytes count, as well as platelet count.

Statistical Analysis

For every tested group, One Way Analysis of Variance (ANOVA) was used to analyze the study's data. Using Duncan's Multiple Range test, the means were separated. Windows SPSS version 22 was used to evaluate the current data. At the probability level of 0.05 ($P \leq 0.05$), the results are deemed significant. Using an analysis of variance, the impact of therapies on hematological parameters was evaluated. The means and standard errors of the values are displayed.

Results

At the 6th and the 8th weeks of the experimental period, Hb concentration, RBCs count and HCT value exhibited non-significant variation in ARE group normal control group. While LEV group exhibited a substantial ($P \leq 0.05$) increase in comparison to normal control group. On the other hand, there was a substantial ($P \leq 0.05$) decrease in their values in PTU group in comparison to normal

control group. Both PTU+ARE and PTU+LEV groups showed a substantial ($P \leq 0.05$) increase in their values in comparison to PTU group. Also, **Table (1)** showed that at the 6th and the 8th weeks of the experimental period, MCV and MCH exhibited non-significant variation in ARE group when compared with normal control group. While, LEV group exhibited a substantial

($P \leq 0.05$) increase in comparison to normal control group. On the other hand, PTU group exhibited non-significant variation in MCV and MCH when compared with normal control group. There was non-significant change in both PTU+ARE and PTU+LEV groups in comparison to PTU group.

Table (1) showed that at the 6th and the 8th weeks of the experimental period, MCHC % exhibited non-significant changes in between groups in comparison to normal control group.

At the 6th and the 8th weeks of the experiment, platelets count exhibited non-significant variation in ARE group in comparison to normal control group. While, LEV group exhibited a substantial ($P \leq 0.05$) decrease in comparison to

normal control group. Also, PLT showed a significant ($P \leq 0.05$) decrease in PTU group in comparison to normal control group. Both PTU+ARE and PTU+LEV groups showed a substantial ($P \leq 0.05$) increase in comparison to PTU group. Notably, PTU+ARE group showed a substantial ($P \leq 0.05$) increase in comparison to PTU+LEV group.

Table (2) showed that at the 6th and the 8th weeks of the experiment, WBCs count, lymphocytes and monocytes exhibited non-significant variation in ARE group when compared with normal control group. While, LEV group and PTU group exhibited a substantial ($P \leq 0.05$) decrease in their values when compared with normal control group. But, both PTU+ARE and PTU+LEV groups showed a substantial ($P \leq 0.05$) increase in comparison to PTU group.

Table (2) showed that at the 6th and the 8th weeks of the experiment, neutrophils and eosinophil count exhibited non-significant changes in between groups in comparison to normal control group.

Table (1): The effects of ARE and LEV on erythrogram of all experimental groups at the 6th and the 8th weeks of the experiment.

Groups Parameters	CON	ARE	LEV	PTU	PTU+ARE	PTU+LEV
At the 6 th week						
Hb (g/dl)	12.57±0.19 ^b	12.53±0.03 ^b	14.46±0.20 ^a	10.30±0.25 ^d	11.93±0.12 ^{bc}	11.57±0.32 ^c
RBCs (x10 ⁶ /µl)	7.22±0.12 ^b	7.38±0.15 ^b	7.90±0.10 ^a	6.00±0.07 ^d	7.10±0.18 ^b	6.81±0.13 ^c
HCT (%)	40.25±0.37 ^b	40.65±0.63 ^b	45.30±1.00 ^a	33.50±0.46 ^d	39.27±0.37 ^{bc}	38.11±0.56 ^c
MCV (fl)	55.75±0.21 ^b	55.08±0.38 ^b	57.34±0.55 ^a	55.83±0.55 ^b	55.31±0.24 ^b	55.96±0.70 ^b
MCH (pg)	17.41±0.15 ^b	16.98±0.34 ^b	18.30±0.11 ^a	17.17±0.42 ^b	16.80±0.11 ^b	16.99±0.14 ^b
MCHC (%)	31.22±0.27 ^a	30.83±0.47 ^a	31.92±0.34 ^a	30.75±0.74 ^a	30.38±0.32 ^a	30.36±0.97 ^a
At the 8 th week						
Hb (g/dl)	12.63±0.22 ^b	12.67±0.29 ^b	15.43±0.46 ^a	9.83±0.09 ^c	12.20±0.26 ^b	11.67±0.52 ^b
RBCs (x10 ⁶ /µl)	7.30±0.09 ^b	7.31±0.14 ^b	8.50±0.06 ^a	6.10±0.27 ^c	7.16±0.09 ^b	7.09±0.13 ^b
HCT (%)	40.11±0.69 ^b	40.34±1.97 ^b	50.21±0.13 ^a	34.00±1.36 ^c	39.43±0.49 ^b	39.00±0.61 ^b
MCV (fl)	54.95±0.31 ^b	55.18±0.86 ^b	59.07±0.31 ^a	55.74±0.21 ^b	55.07±0.51 ^b	55.01±0.28 ^b
MCH (pg)	17.30±0.25 ^b	17.33±0.73 ^b	18.15±0.39 ^a	16.11±0.58 ^b	17.04±0.55 ^b	16.46±0.68 ^b
MCHC (%)	31.49±0.50 ^a	31.41±1.47 ^a	30.73±0.84 ^a	28.91±0.26 ^a	30.94±0.78 ^a	29.92±1.13 ^a

Mean ± SE is used to express values.

In every raw, means with different superscripts are considered significant at (P≤0.05).

Table (2): The effects of ARE and LEV on platelets and leukogram count of all experimental groups at the 6th and the 8th weeks of the experiment.

Groups Parameters	CON	ARE	LEV	PTU	PTU+ARE	PTU+LEV
At the 6 th week						
PLT (x10 ³ /µl)	1008.33±4.41 ^a	1011.00±4.40 ^a	923.33±3.33 ^d	865.33±2.73 ^c	965.33±2.96 ^b	951.33±0.67 ^c
WBCs (x10 ³ /µl)	10.15±0.35 ^a	10.27±0.04 ^a	8.64±0.16 ^b	8.36±0.38 ^b	10.23±0.23 ^a	9.66±0.14 ^a
Lymphocytes (x10 ³ /µl)	6.16±0.17 ^a	6.23±0.13 ^a	4.94±0.03 ^b	4.70±0.21 ^b	6.28±0.16 ^a	5.83±0.02 ^a
Neutrophils (x10 ³ /µl)	2.44±0.06 ^a	2.61±0.07 ^a	2.55±0.12 ^a	2.56±0.12 ^a	2.48±0.03 ^a	2.44±0.12 ^a
Monocytes (x10 ³ /µl)	1.33±0.18 ^a	1.23±0.03 ^a	0.93±0.06 ^{bc}	0.87±0.06 ^c	1.27±0.03 ^a	1.18± 0.06 ^{ab}
Eosinophils (x10 ³ /µl)	0.22±0.01 ^a	0.20±0.01 ^a	0.22±0.01 ^a	0.23±0.01 ^a	0.20±0.01 ^a	0.21±0.01 ^a
At the 8 th week						
PLT (x10 ³ /µl)	1278.00±1.73 ^a	1266.33±1.86 ^a	946.00±2.64 ^d	877.00±1.15 ^c	1213.33±8.09 ^b	1146.00±3.48 ^c
WBCs (x10 ³ /µl)	10.62±0.10 ^{ab}	10.89±0.12 ^a	9.30±0.07 ^d	8.83±0.02 ^c	10.36±0.20 ^b	9.87±0.06 ^c
Lymphocytes (x10 ³ /µl)	6.59±0.13 ^a	6.72±0.05 ^a	5.67±0.15 ^c	5.48±0.11 ^c	6.27±0.02 ^b	6.02±0.09 ^b
Neutrophil (x10 ³ /µl)	2.50±0.06 ^a	2.65±0.05 ^a	2.51±0.01 ^a	2.49±0.03 ^a	2.55±0.05 ^a	2.54±0.06 ^a
Monocytes (x10 ³ /µl)	1.31±0.17 ^a	1.31± 0.02 ^a	0.91±0.02 ^b	0.65±0.08 ^c	1.34±0.03 ^a	1.10±0.05 ^{ab}
Eosinophils (x10 ³ /µl)	0.22±0.01 ^a	0.21±0.01 ^a	0.21± 0.01 ^a	0.21±0.01 ^a	0.20±0.01 ^a	0.21±0.01 ^a

Mean ± SE is used to express values.

In every raw, means with different superscripts are considered significant at (P≤0.05).

Discussion

Insufficient thyroid hormone production or insufficient thyroid hormone activity in target tissues is known as hypothyroidism. Numerous organ systems' metabolism and functioning are impacted by hypothyroidism (*Almandoz and Gharib, 2012*). The purpose of this study was to assess the effectiveness of ashwagandha root extract (ARE) and levothyroxine (LEV) and look into the impact of PTU-induced hypothyroidism in rat models as anti-hypothyroidic agents and testing their influence on hematological parameters.

Our hematological findings revealed that at the 6th and the 8th weeks of the experiment, Administration of ARE to normal rats had no significant effect on the hematological parameters when compared with normal control group during the experimental period. These results were similar to *Raut et al. (2012)*.

At the 6th and the 8th weeks of the experiment, LEV group rats showed a significant alteration in hematological parameters than normal control. There were increase in RBCs count, Hb concentration, HCT % and increase in other blood indices; MCV, MCH, MCHC, while there was a significant decrease in PLT count. These findings coincided with *Araujo et al. (2011)* and *Mokhbatly and Farid (2021)*. Moreover, has a suppressive effect on thrombocytopoiesis; yet, the best accurate techniques for measuring

platelet production are those described by *Sullivan and McDonald (1992)*. The literature's contradictory findings can be explained by increased basal metabolic rate throughout hyperthyroidism, which leads to accelerated lipid peroxidation (*Guerrero et al., 1999; Messarah et al., 2010*). According to *Dönmez and Keskin (2009)*, The rise in platelet counts may be a healing reaction to a tendency for bleeding. WBCs count and its DLC including lymphocytes and monocytes were significantly decreased in LEV rats than normal control while there were no changes in neutrophils and eosinophils (*Mokhbatly and Farid (2021)*). Thyroid hormones play a vital role in enhancing the process of erythropoiesis, which can explain the alteration in hematological parameters in hypothyroidism and hyperthyroidism, as concluded by *Zahediasl et al. (2010)*.

PTU-induced hypothyroidic rats showed a significant alteration in hematological parameters than normal control. There were decreases in Hb concentration, RBCs count, HCT % and PLT count but there were no changes in blood indices; MCV, MCH and MCHC that resulted in normocytic normochromic anemia. These results were in agreement with *Purohit and Purohit (2018)* and *Akane et al. (2022)*.

PTU is one of the thionamide-based antithyroid medications that is known to have negative bone

marrow suppressive effects, leading to aplastic anemia (*Tajiri et al., 1993; Bartalena et al., 1996*). This could account for the decrease in RBCs, Hb, and HCT. Rats under stress from reduced food intake frequently exhibit lower bone marrow cellularity and platelet counts (*Everds et al., 2013*). WBCs count and DLC including lymphocytes and monocytes were significantly decreased in PTU-induced hypothyroidic rats than normal control while there was no change in neutrophils and eosinophils. These results were in accordance to *Purohit and Purohit (2018)* and *Akane et al. (2022)*. According to *Bendyug et al. (2003)* experimental hypothyroid animals suffered from disorders in the immune system, such as, cellularity of lymphoid organs and decreased count of lymphocytes.

At the 6th and the 8th weeks of the experiment, PTU-induced hypothyroidic rats treated with ARE seemed to significantly ameliorate the altered hematological parameters when compared with untreated hypothyroidic rats. These findings coincided with *Patel et al. (2016)* and *Abdel-Wahhab et al. (2019)*.

According to *Ziauddin et al. (1996)*, When hypothyroid rats were treated with ashwagandha methanolic extract, their hemoglobin levels tended to return to normal. It was discovered that ashwagandha raised the concentration of Hb and the count of red blood cells. This, in

turn, boosts the blood's ability to carry oxygen directly to all tissues, offering definitive proof of the ergogenic impact of ashwagandha. When compared to untreated control mice, *Withania sominifera*-treated mice showed significantly higher hemoglobin concentration, red blood cell count, white blood cell count, platelet count, and body weight (*Namdev et al., 2023*). Similar study also observed that ashwagandha showed marked increases in the WBCs and platelets count after bone marrow suppression induced by cyclophosphamide (*Ali et al., 2015*). At the 6th and the 8th weeks of the experiment, PTU-induced hypothyroidic rats treated with LEV seemed to significantly ameliorate the altered hematological parameters when compared with untreated hypothyroidic rats. These findings coincided with *Bashir et al. (2012)* and *Abdel-Wahhab et al. (2019)*.

According to *Golde et al. (1977)*, two methods exist by which LEV increases erythropoiesis: a direct mechanism mediated by adrenergic receptor stimulation of red cell precursors and an indirect mechanism mediated by erythropoietin.

Christ-Crain et al. (2003) reported that LEV treatment affects the leukocytes, lymphocytes and monocytes distribution of peripheral blood cells especially in autoimmune thyroiditis. *Krysiak and Okopien (2011)* stated that LEV

increased count of monocytes and lymphocytes as LEV affects monocyte and lymphocyte cytokine release and also, LEV has a systemic anti-inflammatory effect.

Conclusion

We recommended using ashwagandha root extract as a supplement to alleviate the hematological changes caused by hypothyroidism.

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دراسات دموية على تأثير بعض المواد المستخدمة في علاج اضطرابات الغدة الدرقية في الجرذان

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الملخص العربي

أجريت الدراسة الحالية لمعرفة تأثير مستخلص جذور الأشواغندا (العُعبب المنوم) والليفوثيروكسين كعوامل مضادة لقصور الغدة الدرقية في الجرذان. تم استخدام ستين جرذاً أبيضاً ذكراً عادياً في الدراسة الحالية ، تم تقسيمهم إلى ست مجموعات متساوية. أظهرت النتائج أن الجرذان التي تم إعطاؤها الدروبابل ثيوبراثيل في مياه الشرب لإحداث قصور الغدة الدرقية أصيبت بفقر الدم السوي الكرات والصبغة مع نقصان في الصفائح الدموية وكرات الدم البيضاء وهذا مقارنة بالمجموعة الضابطة. بينما أظهرت المجموعات المعالجة باستخدام مستخلص جذور الأشواغندا والليفوثيروكسين تحسن ملحوظ في صورة الدم.