



Effect of *Withania Somnifera* Supplement on Thyroid Gland Hormones, Body Weight, Body Temperature and Thyroglobulin in Males Rabbits Induced with Hypothyroidism

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Abstract | The aim of current study was conducted to evaluate the prevention role of *Withania somnifera* supplement on thyroid hormones of males rabbits induced hypothyroidism by propylthiouracil (PTU). Twenty-four rabbits were divided into four groups: the first group received tap water only (control group), the second group received *Withania somnifera* supplement at a dose of 500 mg/kg B.W., while the third group received PTU at a dose of 100 mg/kg B.W. and finally the forth group T2 was received both PTU and *Withania somnifera* supplement. Blood samples were collected at the start (zero time) and at the end of the study after 30 days for analysis. The animals were euthanized to obtain the thyroid gland for histopathological study. The results showed the thyroid hormones (T4 and T3) levels were significantly increased ($P < 0.05$) and the thyroid stimulating hormone (TSH) level was decreased in group treated with both PTU and *Withania somnifera* supplement compared with control and other treated groups. In contrast, the PTU treated group showed a significant reduction ($P < 0.05$) in T4 and T3 hormones levels with an increase in TSH levels compared with the other treated groups. The results for body weight, body temperature and thyroglobulin (TG) showed no significant differences between all treated groups. The histopathological changes of *Withania somnifera* supplement group showed a marked increase in variably-sized thyroid follicles with varying amounts of intraluminal pink colloid. In contrast, the thyroid of PTU-treated animals showed hypothyroid changes and architectural distortion, characterized by a decrease in the size of most follicles, while the thyroids section tissues of the group treated with both PTU and *Withania somnifera* supplement showed a significant reversal of histological changes with most follicles restored. The current study concluded the using of *Withania somnifera* supplement is improved the production of thyroid hormones by stimulating the thyroid gland lead to increase the biosynthesis of T4 and T3 hormones.

Keywords | Hypothyroidism, Ashwagandha, Propylthiouracil, Histopathology, Thyroxine, Triiodothyronine, Thyroglobulin, Thyroid-stimulating hormone, Body weight, Body temperature

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The thyroid gland is an important component of the endocrine system. It is synthesized and released two essential hormones including triiodothyronine (T3) and tetraiodothyronine (T4) (Al-Shaibani *et al.*, 2014). Thyroid hormones had an important role in regulating the basal energy levels by direct effect on protein, lipid and carbohydrate metabolism (Al-Zubaidi and Al-Issa, 2023). As well as, it had an effect on glucose metabolic pathways (Ibrahim, 2021). Hypothyroidism is a thyroid disorder that happened when the thyroid gland fails to synthesis sufficient T3 and T4. It is one of the most common endocrine dysfunction globally and it was recognized in a wide range of animals species such as dogs, cats and horses (Sabea and Al-Qaiym, 2024). Herbal medicine products and supplements had been become very prevalent worldwide in recent decades to maintenance the animal health (Faleh *et al.*, 2024). Herbal plants are commonly used in different treatments because they are often contained a potent pharmaceutical active compounds. So, there was a biggest interesting for using of medicinal plants in different fields such as pharmaceuticals, herbal remedies, spices, perfumes and cosmetics (Saad *et al.*, 2012; Fahd and Saliem, 2024). Ashwagandha is a natural plant commonly used in traditional medicine and it was known scientifically as *Withania somnifera* and commonly referred to as Indian Ginseng or Winter Cherry, this herb is significant in Indian traditional medicine (Sharma *et al.*, 2017; Srivastava *et al.*, 2018). It grows in semi-arid regions of India, Congo, South Africa, Egypt, the southeastern hemisphere, and Morocco (Hamood *et al.*, 2017). The safety and effectiveness of Ashwagandha supplementation suggested that Ashwagandha could be helpful in improving thyroid hormone levels in hypothyroidism (Mikulska *et al.*, 2023). The positive effects of Ashwagandha on thyroid function are attributed to its direct influence on thyroid activity and metabolism (Elgar, 2021).

The Ashwagandha plant was consisted of distinct parts—root, stem, leaves, flowers and fruit—each containing important medicinal properties. Ashwagandha also contained two active compounds (withanolides and withaferin A), which they were contributed to its medicinal properties such as antioxidant, anti-inflammatory, hepatoprotective, anti-stress and endocrine-protective effects (Verma and Kumar, 2011). Ashwagandha had a high therapeutic and nutraceutical potential due to its pharmacologically active steroidal lactones called withanolides. Additionally, withanolide D and withaferin A were exhibited antitumor and cytotoxic properties, which they were helped in preventing the growth of cancer cells (Ahmed *et al.*, 2018; Sari *et al.*, 2020). The plant was also contained alkaloids, steroids, phenolics, saponins, phytophenols, flavonoids and glycosides, which they were a widely used in traditional medicine as

antipyretic, analgesic, anti-inflammatory and adaptogenic agents (Alam *et al.*, 2011; Kalra and Kaushik, 2017). The increasing interest among researchers in *Withania somnifera* plant was due to its traditional therapeutic applications, pharmaceutical action and nutraceutical potential (Saleem *et al.*, 2020). Propylthiouracil (six-n-propyl-two -thiouracil, or PTU) is a medication commonly used to induce hypothyroidism and it acted as an anti-thyroid drug, it was used in treatment of hyperthyroidism by inhibiting thyroid peroxidase and deiodinase (EL-Tantawi and Abozeid, 2019). So, this project was conducted to study the prevention role of *Withania somnifera* supplement on thyroid hormones of males rabbits induced hypothyroidism by propylthiouracil (PTU).

MATERIALS AND METHODS

EXPERIMENTAL DESIGN

Twenty-four male's rabbits were obtained from the animal house at the College of Veterinary Medicine, University of Baghdad. The rabbits were between 6 months to 1 year of age and weighed between 1 kg to 1.5 kg. The animals were equally distributed into four groups as following : the first group received tap water only (control group), the second group received *Withania somnifera* supplement at a dose of 500 mg/kg B.W., (Giri *et al.*, 2022) while the third group received PTU at a dose of 100 mg/kg B.W. (Hussein *et al.*, 2022) and finally the forth group T2 was received both PTU and *Withania somnifera* supplement. The period of treatment was 30 days for each group.

CHEMICALS

Propylthiouracil (six-n-propyl-two-thiouracil; PTU) is an antithyroid (goitrogen) agent in tablet form (50 mg/kg B.W.) obtained from a local pharmacy in Baghdad. Ashwagandha plant supplement capsules (250 mg/kg B.W.) were obtained from a local health shop in Baghdad.

BLOOD SAMPLES

Blood samples were collected from the heart before the experiment (zero time) and at the end of the experiment (day 30) for hormones analysis. After blood collection, the animals were euthanized using an overdose of ketamine-xyzazine anesthesia to obtain the thyroid gland for histopathology.

THYROID HORMONE MEASUREMENTS

Thyroxine (T4) kits (Cloud-Clone Corp, USCN, China), Triiodothyronine (T3) kits (Cloud-Clone Corp, USCN, China), and Thyroid-Stimulating Hormone (TSH) kits (FineTest, China) were used. Serum free triiodothyronine (FT3) and free thyroxine (FT4) levels were determined by ELISA technique using rabbit ELISA kits. Thyroglobulin was measured using the Elecsys Tg-cobas kit (Roche, Ger-

many).

PHYSICAL EXAMINATION

Body weight and body temperature were measured along the period of experiment (every week). Body weight was measured using a weight balance and body temperature was measured using a thermometer by inserting the tip of thermometer gently to depth 4 cm into the rectum.

HISTOPATHOLOGICAL CHANGES EXAMINATION

The thyroid glands were dissected out and immediately placed in 10% buffered formalin saline solution. Five micrometer thick paraffin sections were stained with hematoxylin and eosin. The tissue sections were then examined under a light microscope (Luna, 1968).

STATISTICAL ANALYSIS

Statistical analysis of data was performed using SAS (Statistical Analysis System - version 9.1). Two-way ANOVA with interaction and least significant differences (LSD) post hoc test were performed to assess significant differences among means. $P < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

The results of T4 and T3 levels are shown in (Table 1 and 2) respectively. The T4 and T3 levels of *Withania somnifera* supplement and PTU with *Withania somnifera* supplement treated groups were significantly ($P < 0.05$) higher after 30 days compared with control group and other treated groups. While, the T4 and T3 levels in the PTU treated group showed a significant ($P < 0.05$) decrease after 30 days compared with all other treated groups. As well as, the *Withania somnifera* supplement and PTU with *Withania somnifera* supplement treated groups showed significantly ($P < 0.05$) lower TSH levels at 30 days compared with control group. The mean of TSH level in the PTU treated group was significantly increased ($P < 0.05$) after 30 days compared with all other groups as in Table 3. These results were agreed with findings obtained by Hosny *et al.* (2021), who found that *Withania somnifera* had a therapeutic efficacy in treating hypothyroidism and elevating thyroid hormone levels. The effects of *Withania somnifera* on the thyroid gland may be due to increased intra-thyroidal biosynthesis of T4 (Panda and Kar, 1998). Additionally, other study recorded by Sharma *et al.* (2018) noted activation of the hypothalamic-pituitary-adrenal axis and elevation of the cortisol levels lead to inhibition in the hypothalamic-pituitary-thyroid axis and this effect perhaps due to the role of *Withania somnifera* by regulating thyroid function and its anti-inflammatory, anti-stress and anti-dopamine properties. This study also correlated with findings reported by Abdel-Wahhab *et al.* (2019), they also discovered the elevating thyroid hormones in rats with induced hypothyroidism after treatment with *Withania somnifera* and they

were attributed the improvement in thyroid function to antioxidant effect of *Withania somnifera*. Propylthiouracil (PTU) was inhibited the production of thyroid hormones by blocking the thyroid peroxidase enzyme, which it is considered a crucial for converting iodide to iodine and incorporating iodine into the amino acid tyrosine (Moftah *et al.*, 2022). The exact mechanism by which supplement of *Withania somnifera* was stimulated the thyroid gland to biosynthesize thyroid hormones is not fully understood, but the bioactive compounds in the herb might be responsible for stimulating the thyroid gland and increasing its activity. Other study recorded by Ibrahim *et al.* (2023) they also mentioned that *Withania somnifera* had the ability in ameliorating hypothyroidism by increasing T3 and T4 levels and restoring them to normal, furthermore, they were mentioned *Withania somnifera* might be a safe and effective for restoring thyroid hormone levels in hypothyroid conditions.

Table 1: Effect of *Withania somnifera* on level of T4 ($\mu\text{g}/\text{dl}$) in induced hypothyroidism by Propylthiouracil (PTU) in male's rabbits.

Groups /T4	Zero time	30 day
Control	8.41 $\pm$ 0.94 A a	7.50 $\pm$ 0.84 A c
Withania somnifera	8.22 $\pm$ 1.06 B a	24.50 $\pm$ 0.93 A b
PTU	8.32 $\pm$ 1.12 A a	5.49 $\pm$ 0.40 B c
PTU and <i>Withania somnifera</i>	8.24 $\pm$ 1.11 B a	27.65 $\pm$ 1.65 A a
LSD	2.34	

*Means with a different small letter in the same column are significantly different ($P < 0.05$); *Means with a different capital letter in the same row are significantly different ($P < 0.05$).

Table 2: Effect of *Withania somnifera* supplement on level of T3 (ng/dl) serum in induced hypothyroidism by Propylthiouracil (PTU) in male's rabbits.

Groups /T3	Zero time	30 day
Control	477.92 $\pm$ 38.94 A a	498.66 $\pm$ 38.51 A c
Withania somnifera	482.85 $\pm$ 35.74 B a	3018.44 $\pm$ 392.25 A a
PTU	489.68 $\pm$ 24.70 A a	316.41 $\pm$ 69.26 B d
PTU and <i>Withania somnifera</i>	430.74 $\pm$ 27.77 B a	787.04 $\pm$ 15.09 A b
LSD	113.13	

*Means with a different small letter in the same column are significantly different ($P < 0.05$); *Means with a different capital letter in the same row are significantly different ($P < 0.05$).

The role of *Withania somnifera* on body weight of induced hypothyroidism in male rabbits was shown in Table 4, there were a slight increasing in body weight with no significant differences between the groups. Body weight of PTU treat-

ed group was appeared a significant ($P<0.05$) decrease than other group in 1week, while PTU with *Withania somnifera* supplement treated group showed no a significant ($P<0.05$) difference compared with other group in all weeks. While the body temperature results are shown in Table 5. This results might be attributed to hypothyroidism typically leads to an increase in body weight (Umezu *et al.*, 1998). The results of current study were consistent with the findings of Purohit and Purohit (2018), who is mentioned no significant differences between hypothyroid and treated groups. Furthermore, *Withania somnifera* had the ability to manage and reduce body weight (Choudhary *et al.*, 2017). In addition, the anti-stress activity of *Withania somnifera* was played a role in reducing food cravings and improving eating behaviors by alleviating stress. In other study recorded by Namdev *et al.* (2023), who was seen *Withania somnifera* effects on weight loss and obesity control might be due to the bioactive compounds it contains, such as triterpenoids, alkaloids, flavonoids, carotene, phenolics, saponins, and tannins, which are responsible for the positive effects on body weight (Yousif *et al.*, 2023). In addition to previous studies (Broeders *et al.*, 2016; Gavrilu *et al.*, 2017; Maushart *et al.*, 2019) they were reported that hypothyroidism affects body temperature by impairing thermogenesis leading to cold intolerance. The current study results were agreed with results reported by of Choudhary *et al.* (2017), who found that *Withania somnifera* did not produced a significant changes in body temperature. *Withania somnifera* might be controlled the body weight and energy expenditure by enhancing thermogenesis, a process of energy generation through heat. Our results were in contrast with findings recorded by Lee *et al.* (2020) and Nowak *et al.* (2023), when they found *Withania somnifera* lead to increasing thermo-

genesis through stimulation the activity of mitochondrial in brown adipose tissue.

Table 3: Effect of *Withania somnifera* supplement on serum level of TSH ($\mu\text{U/ml}$) in induced hypothyroidism by Propylthiouracil (PTU) in male rabbits.

Groups /TSH	Zero time	30 day
Control	11.22 $\pm$ 0.23 A a	10.93 $\pm$ 0.11 A b
Withania somnifera	11.08 $\pm$ 0.15 A a	3.05 $\pm$ 0.17 B d
PTU	11.16 $\pm$ 0.20 B a	12.4 $\pm$ 0.17 A a
PTU and <i>Withania somnifera</i>	11.31 $\pm$ 0.39 A a	7.03 $\pm$ 0.21 B c
LSD	0.68	

*Means with a different small letter in the same column are significantly different ($P<0.05$); *Means with a different capital letter in the same row are significantly different ($P<0.05$).

In current study, the histopathological examination of the thyroid glands were shown in (Figures 1, 2, 3 and 4). The *Withania somnifera* treated group showed a marked increase in variably-sized thyroid follicles with varying amounts of intraluminal pink colloid. Some follicles exhibited significant dilation with large amounts of colloid in their lumens. In contrast, the thyroid of PTU-treated animals (T1) showed hypothyroid changes and architectural distortion, they were characterized by a decrease in the size of most follicles, a drop in or lack of colloid and an increase in the thickness of the follicular lining epithelium. However, the thyroids tissues of the PTU with *Withania somnifera* supplement treated group showed a significant reversal of histological changes, with most follicles restored and

Table 4: Effect of *Withania somnifera* supplement on body weight (gm) in induced hypothyroidism in male rabbits.

Groups/ Weeks	Control	Withania somnifera	PTU	PTU and Withania somnifera
1Week	1.35 $\pm$ 0.10AB a	1.35 $\pm$ 0.09AB b	1.22 $\pm$ 0.09 B a	1.48 $\pm$ 0.03 A a
2Week	B1.20 $\pm$ 0.12 a	1.48 $\pm$ 0.02 A a	1.35 $\pm$ 0.07 AB a	1.33 $\pm$ 0.12 AB a
3Week	A1.28 $\pm$ 0.11 a	1.40 $\pm$ 0.10 A ab	1.31 $\pm$ 0.02 A a	1.41 $\pm$ 0.10 A a
4Week	A1.10 $\pm$ 0.09 a	1.17 $\pm$ 0.10 A b	1.26 $\pm$ 0.04 A a	1.31 $\pm$ 0.06 A a
LSD	0.25			

*Means with a different small letter in the same column are significantly different ($P<0.05$); *Means with a different capital letter in the same row are significantly different ($P<0.05$).

Table 5: Effect of *Withania somnifera* supplement on body temperature ($^{\circ}\text{C}$) in induced hypothyroidism in male rabbits.

Groups/Weeks	Control	Withania somnifera	PTU	PTU and Withania somnifera
1Week	37.52 $\pm$ 0.27 A a	37.57 $\pm$ 0.38 A a	37.14 $\pm$ 0.22 A a	37.00 $\pm$ 0.31 A a
2Week	37.06 $\pm$ 0.30 A a	37.44 $\pm$ 0.23 AB a	37.58 $\pm$ 0.19 A a	37.80 $\pm$ 0.27 A a
3Week	37.26 $\pm$ 0.27 A a	37.04 $\pm$ 0.26 A a	37.70 $\pm$ 0.25 A a	37.30 $\pm$ 0.18 A a
4Week	36.80 $\pm$ 0.35 B a	36.84 $\pm$ 0.18 A a	37.28 $\pm$ 0.20 AB a	37.08 $\pm$ 0.13 AB a
LSD	0.73			

*Means with a different small letter in the same column are significantly different ($P<0.05$); *Means with a different capital letter in the same row are significantly different ($P<0.05$).

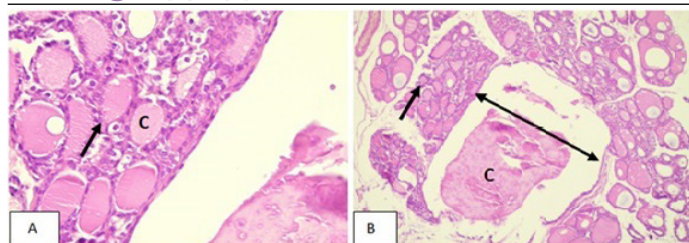


Figure 1: Photomicrographs of the thyroid of rabbits of negative control group (C1) showed normal architecture of the thyroid gland. It comprised of different-sized thyroid follicles (black arrow) filled with acidophilic homogenous colloid (C), and individual follicle was lined with a simple layer of cubical epithelial cell (Blue arrow). (A)H&E 10X. (B) H&E 40X.

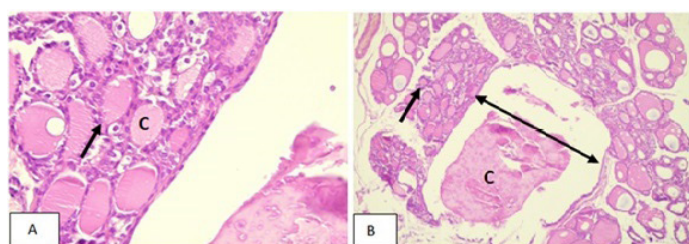


Figure 2: Photomicrographs of the thyroid of rabbits of Ashwaganda treated group (C2) showed markedly packed variably-sized thyroid follicles with various amount of intraluminal acidophilic homogenous colloid (C). Also, section showed a huge dilation of one follicle (Double-headed arrow) with accumulation of large amount of colloid in its lumen (C). (A)H&E 10X (B) H&E 40X.

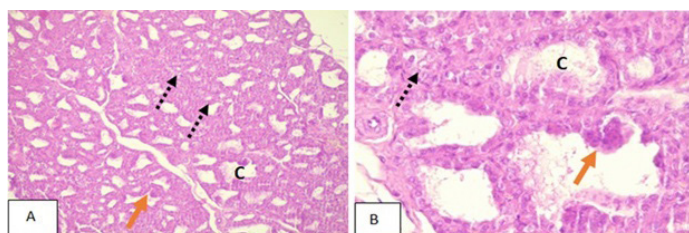


Figure 3: Photomicrographs of the thyroid of rabbits of PTU-treated group (T1) showed hypothyroid changes and distortion in its architecture that characterized by decrease in the size of most follicles (Dashed black arrow) with drop in or lack in the amount of colloid, and increased thickness of follicular lining epithelium (Red arrow). (A) H&E 10X. (B)H&E 40X.

increased colloid deposition within follicular lumens compared with the alterations seen in the PTU-treated animals. This result might be attributed to *Withania somnifera* contained two active compounds (withanolides and withaferin A), which they were contributed to antioxidant, anti-inflammatory and endocrine-protective effects (Verma and kumar , 2011). Our findings agreed with results recorded by Abdel-Wahhab *et al.* (2019), who is discovered the role of *Withania somnifera* in enhancement thyroid follicles of a hypothyroid group. Furthermore, the histopathological

result of current study was agreed with other study mentioned by Ibrahim *et al.* (2023) who is also reported the *Withania somnifera* treated group played an important role in restoration of thyroid follicles to normal size and filling it with colloid.

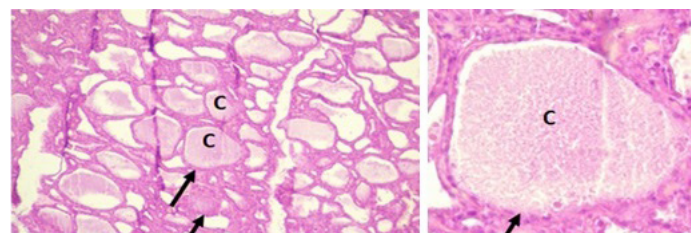


Figure 4: Photomicrographs of the thyroid of rabbits of PTU-Ashwaganda treated group (T2) showed significant reversed in histological changes with restored most of follicles (Black arrow) and increase colloid deposition within follicular lumens (C).(A) H&E 10X (B)H&E 40X.

CONCLUSIONS AND RECOMMENDATIONS

The current investigation of this study concluded that *Withania somnifera* supplement played an important role in increasing thyroid hormone in induced hypothyroidism by stimulating the thyroid gland and the study recommended using the supplement of *Withania somnifera* in the future as an alternative medication due to its positive effect on thyroid hormones.

FUTURE PROSPECTIVE

Further studies are needed to explore the mechanism of action of *Withania somnifera* in the thyroid gland, also is still unknown how *Withania somnifera* has increased the production of thyroid hormone and study the effect of *Withania somnifera* on the adrenal gland due to the anti-stress properties of Ashwagandha and the interference between the adrenal gland and thyroid gland.

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NOVELTY STATEMENT

The novelty of this study lies in highlighting the physiological role of Ashwagandha (*Withania somnifera*) supplementation on the thyroid gland in hypothyroidism. Multiple side effects were associated with conventional thyroid drugs, it is imperative to explore alternative sources of thyroidal medication.

AUTHORS' CONTRIBUTIONS

All authors contributed equally to this study.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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