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Research Article:

# Sonographic Assessment of Submandibular Salivary Glands in Hypothyroid Rat's Pups Before and After Ashwagandha Root Extract Treatment

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## Abstract

**Background:** One of the most common chronic diseases globally is hypothyroidism (insufficient thyroid hormone secretion and release by the thyroid gland). Thyroid hormones serve a significant role in maintaining the proper function and shape of the salivary gland.

**Aim:** Investigate the function of Ashwagandha roots extract in providing a protection role for salivary glands in hypothyroid rats following postnatal exposure to Propylthiouracil.

**Materials and Methods:** Ten pregnant albino rats were obtained. Until the rat pups were detected, each pregnant rat was kept individually in clean rodent plastic. At postnatal day 3 (PND3), forty male rats were randomly assigned to one of two groups: group A, which served as a control group, and group B, which received orally (1mg/kg) Propylthiouracil (PTU) for three weeks. At PND22, group B was divided up into three subgroups: B1, the hypothyroid group that got no therapy; B2, the hypothyroid group that received an aqueous extract of Ashwagandha roots (200 mg/kg) for 21 days; and B3, the hypothyroid group that received Levothyroxine (4g/100g/day) for 21 days. At the end of the trial, the submandibular gland was assessed using sonographic instruments in all groups.

**Results:** the sonographic assessment of hypothyroid group's submandibular gland showed an increase in the overall size of the gland, a heterogeneous gland with a honeycomb appearance and hypoechoic regions in group remains without treatment, while the results in hypothyroid pups fed with Ashwagandha roots extract indicated a small improvement in gland size and echotexture when compared to pups who received levothyroxine, owing to its antioxidant properties.



## 1. Introduction

One of the most important endocrine glands is the thyroid gland (1). It is designed to produce, store, and release the thyroid hormones thyroxin (T4) and triiodothyronine (T3) (T3). Thyroid hormones are tropic hormones that are essential for the growth and development of bodily organs such as the circulatory, digestive, salivary, and neuromuscular systems (2). Hypothyroidism is an endocrine disorder characterized by inadequate thyroid hormone production by the thyroid gland (3). The thyroid hormones' target organs include the submandibular salivary glands, which are the second biggest gland in rats (4). Scientists are interested in morphological research on these glands since there is a direct relationship between mouth health and thyroid function (5).

The submandibular gland of rats is an excellent model for demonstrating the many features of hypothyroidism's influence on various tissues(4). Rodent submandibular glands include granular convoluted tubules, which contain a high variety of physiologically active peptides such as nerve growth factor (NGF), hepatic growth factor (HGF), epidermal growth factor (EGF), and transforming growth factor-alpha (TGF-a) (6). These growth factors are critical for salivary gland development, proliferation, differentiation, and

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function (7). Thyroid hormone fluctuations may have an impact on salivary gland growth because they finished growing during the postnatal period (8) As a result, there is a need to focus on this issue and its therapy, which may include thyroxin and other potential medications.

Ashwagandha roots extract is one of the traditional medicinal herbs. According to a number of researches, Ashwagandha roots extract contains a tonic organic protection, and neurological stimulation due to its broad range of activities like antioxidant, and anticarcinogenic activity (9). The purpose of this study is to compare the effects of ashwagandha roots extract versus thyroxin hormone on the morphological consequences of hypothyroidism in rats following postnatal exposure to Propylthiouracil.

## 2. Materials and Methods

### 2.1. Animals

Ten healthy pregnant albino rats were obtained from the University of Mosul's College of Veterinary Medicine's animal house. Each pregnant rat was kept in a clean rodent plastic cage with a wire mesh top (28×22×18) cm. Until the babies were delivered, they were given bedding made of homogenized wood shavings. The rats were housed under optimal conditions of temperature (22 + 0.5 0C) and humidity (about 50 %), with a twelve-hour cycle of light/dark and ad libitum feeding of chow and tap water (5). Throughout the inquiry, the health of all rats was examined every morning by a veterinarian. The newborn rats were discovered on post-natal day zero (PND 0), and to prevent hormonal influences in females, male rat pups were chosen for experimental testing at PND3, rather than females (10).

Classification of pups (at PND3) into two main groups: control (group A=10 pups) and hypothyroid groups(group B=30 pups ).

### 2.2. Hypothyroidism induction

The control group (n=10) received distilled water only as a placebo from PND3 to PND42 to encounter the same conditions as the experimental group.

While Propylthiouracil (PTU) Propycil® (recordati ilac group, Turkey). Prepared as fresh solution (1mg/kg) and administered orally every day, for 21 days to pups of hypothyroid groups(n=30) (11).

Then on day 22 the hypothyroid-induced group divided into 3 subgroups as:

**B1**: The hypothyroid group remains without treatment (n=10): received distilled water only as a placebo, to encounter the same conditions as the other groups.

**B2**: The Hypothyroid group + Ashwagandha roots extract (n=10): Ashwagandha roots extract powder was obtained from (naturalYA Kimya,Turkey) .Rats treated orally by oral gavage needle with 0.5 ml aqueous of Ashwagandha root extract (100 ml water +5000mg plant) at dose of 200 mg \ kg body weight (50 mg/rats) for 21 days) from PND22 to PND43.(12)

**B3**: The Hypothyroid group + Levothyroxine (n=10): includes pups which were received orally fresh suspension of Levothyroxine (anthrax25µg ®, Mark, Germany) (4µg/100g/day) orally for 21 days from PND22 to PND43.(11)

### 2.3. Sonographic Assessment of Submandibular Salivary Glands

Experiments were conducted on day 43 at the internal medicine lab / College of Veterinary Medicine / University of Mosul / Mosul, Iraq. All ultrasound tests were done using a Keebomed k 5100 portable ultrasound equipment (USA). For animal research, use a 3.5MHz linear transducer with a prob. On the PND43 after being sedated intraperitoneally with (100mg/kg) Ketamine hydrochloride (anesthetic agent) in conjunction with xylazine (8m/kg) (sedative, muscle relaxant, and analgesic). Five minutes later, the rat's reflexes were checked to confirm that anesthetic had been administered (13), the mandibular gonial area was shaved (14), and the Submandibular salivary gland was evaluated using ultrasound devices (Figure 1).

The rats' submandibular salivary glands were evaluated for parameters such as size (Length and width were measured for each submandibular gland to evaluate the length, the probe was placed parallel to the inferior edge of the mandible and the maximum value was measured, while the width was measured by placing the probe vertically in the middle of the ramus of the mandible and vertically to the body of the mandible, and the maximum values were measured), echo (15).

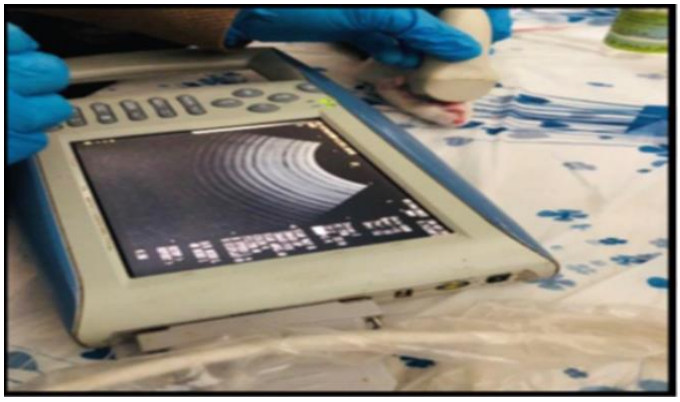

Figure 1: An ultrasound photo of the rat's Submandibular salivary gland.

### 2.4. Statistical Analysis

The statistical analysis was carried out with the help of IBM SPSS statistics for Windows, Version 26.0, Armonk, NY: IBM Corp., 2019. The information was given as a mean±SD. An independent sample t-test was used to analyze the differences between groups in this test. The statistical significance level for comparisons was set at ($p< 0.05$). (16).

## 3. Results

PND43 sonographic examination of submandibular salivary glands acquired, the submandibular salivary gland was encapsulated and of homogenous echotexture, intraglandular ducts are observed as linear, hyperechoic structures (Figure 2).

While the B1 group (hypothyroid group continues untreated), the expansion of the submandibular salivary glands was bigger than in the other groups (control group illustration found in Figure 3), which might be explained by an inflammation in the gland, with evident hetero echotexture, hypoechoic, and atrophy found in many parts of the gland (Figure 4).

In contrast, submandibular salivary gland ultrasound imaging in group B2 (hypothyroid treatment with Levothyroxine) revealed a considerable improvement in gland size, virtually matching that of pups compared to the control group (A) with hypoechoic in tiny glandular regions (Figure 5).

Finally, there are amelioration effects in the size, echotexture, and echogenicity of the submandibular salivary gland in the hypothyroid group treated with Ashwagandha roots extract (B3) compared to the B1 group (hypothyroid group with no treatment), but these effects are less pronounced than those with levothyroxine treatment (Figure 6).Finally, there are amelioration effects in the size, echotexture, and echogenicity of the submandibular salivary gland in the hypothyroid group treated with Ashwagandha roots extract (B3) compared to the B1 group (hypothyroid group with no treatment), but these effects are less pronounced than those with levothyroxine treatment (Figure6).

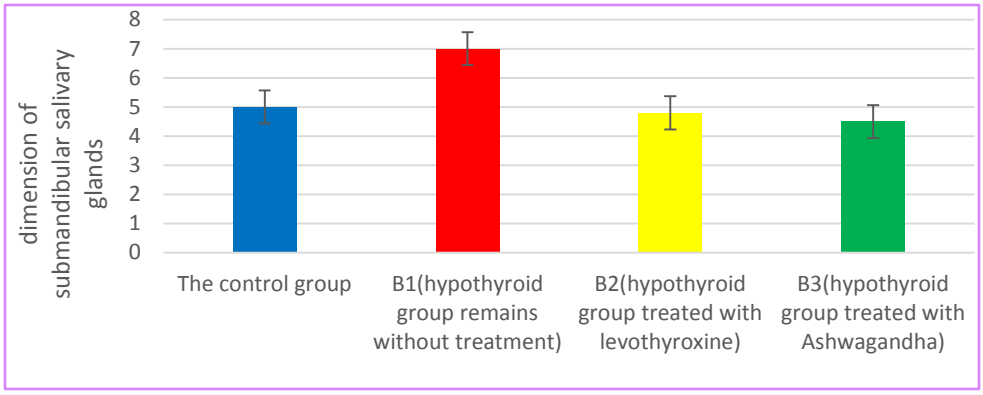


Figure 2 : A histogram shows the dimensions of the submandibular salivary glands.

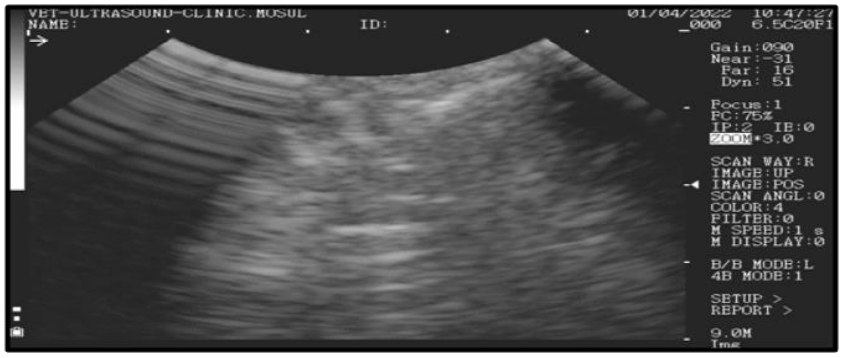


Figure 3: A photograph of ultrasound image of rat submandibular salivary gland of group A (control group).

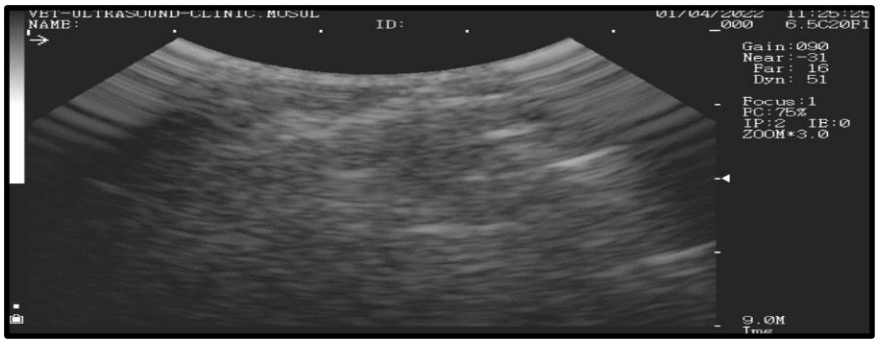


Figure 4: A photograph of ultrasound image of rat submandibular salivary gland of group B1 (hypothyroid group with no treatment).

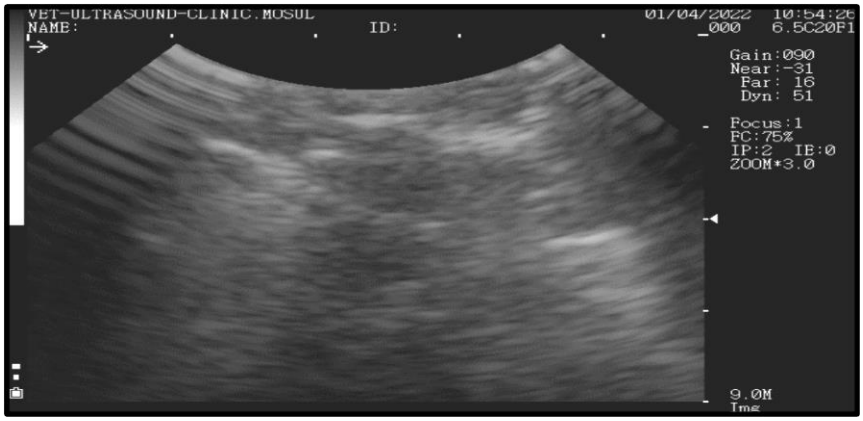


Figure 5: A photograph of ultrasound image of rat submandibular salivary gland of group B2 (hypothyroid group treated with levothyroxine).

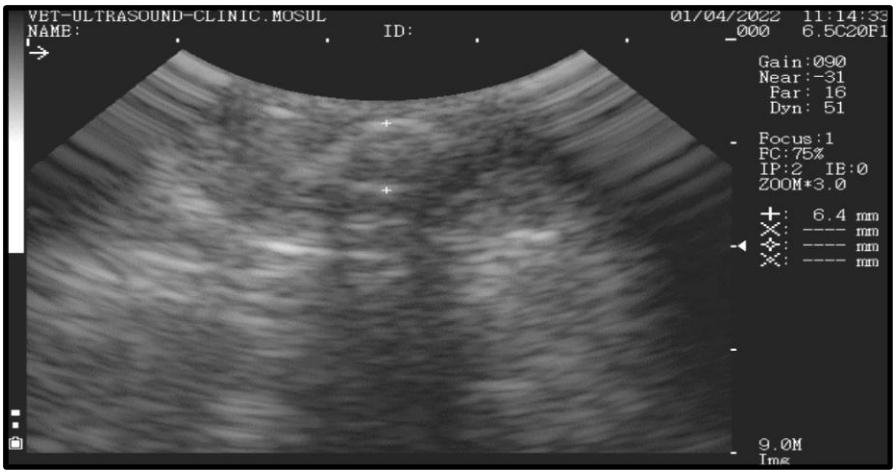


Figure 6: A photograph of ultrasound image of rat submandibular salivary gland of group B3 (hypothyroid group treated with Ashwagandha roots extract).

## 4. Discussion

Sonography is a low-cost, safe, and easily accessible tool for evaluating patients (17). It is also particularly useful for tracking changes in salivary glands induced by a variety of clinical disorders, and it gives crucial data. In terms of the morphologic alterations in the glands (18).The current study assessed these alterations using sonography as an assessment tool. Several alterations were detected, including an increase in the overall size of the submandibular salivary glands. This increase was statistically significant in the hypothyroid group that did not get therapy (group B1), a heterogeneous gland with a honeycomb appearance and hypoechoic regions. There were also evidence of inflammation and edema, which caused the gland to enlarge. Bradley (2006) proposed that the size of salivary glands only varies in acute settings, whereas it decreases in chronic situations due to atrophy (19).

The pathogenic consequences of hypothyroidism provide a substantial danger of antioxidant imbalance. Thyroid hormones increase cellular responses and increase oxidative metabolism. When enzymes that govern active transport pumps are excited, demand for cellular oxygen increases, and as ATP synthesis increases, heat is produced. Hypothyroidism weakens the immune system, which can lead to oxidative stress (20). greater concentrations of TSH may trigger the production of inflammatory cytokines and reduce antioxidant status (21). Others have studied hypothyroidism-related oxidative stress, which is caused by an increase in free radical formation as well as a decrease in antioxidative defense capabilities. Thyroid hormone levels may be one of the most important physiological modulators of in vivo cellular oxidative stress due to their known effects on mitochondrial respiration. It has been proposed that an increase in reactive oxygen species generated by thyroid hormone deficiency may result in oxidative stress in the brain, heart, glands, liver, and different skeletal muscles, with an increase in inflammation as a result (22). Furthermore, the results of the hypothyroid pups treated with levothyroxine (group B2) demonstrated a considerable improvement in the size of the gland which returned to normal (reduction in size) and in the gland parenchyma. Since this study recently evaluated the role of thyroid in the control of antioxidant systems, oxidative stress appears to be an essential mechanism behind the progression of

inflammation. Thyroid hormone replacement treatment can protect hypothyroid rats by regulating antioxidant levels (23).

Finally, the results in hypothyroid pups fed with Ashwagandha roots extract (group B3) indicated a small improvement in gland size and echotexture when compared to group B2 who received levothyroxine, owing to its antioxidant, immunomodulatory, adaptogenic, and immunostimulant qualities (24). Antioxidants protect endothelial cells by reducing mitochondrial dysfunction which will refer to a normal structure of the gland , since administration of Ashwagandha roots extract will preserve the tissue and is an excellent source of enzymes and non-enzyme antioxidant components (25).

## 5. Conclusion

The submandibular salivary gland had significant morphological alterations as a result of hypothyroidism. The extract of Ashwagandha roots was quite effective in counteracting these alterations. In addition, ultrasound imaging can be utilized to evaluate these alterations in hypothyroidism conditions.

## 6. Conflict of Interest

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

## 7. Acknowledments

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## التقييم بالموجات فوق الصوتية للغدد اللعابية تحت الفك السفلي في صغار الفئران المصابة بقصور الغدة الدرقية قبل وبعد استخدام مستخلص جذور الأشواغاندا

زهراء قصي الحلاوجي و غادة عبد الرحمن طاقة

**الملخص :** قصور الغدة الدرقية هو أحد أكثر الأمراض المزمنة شيوعًا على مستوى العالم ( ويعرف على انه عدم كفاية إنتاج هرمون الغدة الدرقية وإفرازه). تلعب هرمونات الغدة الدرقية دورًا مهمًا في الحفاظ على الوظيفة والشكل المناسبين للغدة اللعابية .الهدف من الدراسة: التحقيق في وظيفة مستخلص جذور الأشواغاندا في حماية الغدد اللعابية للصغار الجرذان المصابة بقصور الغدة الدرقية بعد التعرض لبروبيل ثيوراسيل مباشرة بعد الولادة. المواد وطرائق العمل: تم اخذ عشرة جرذان البينو اناث حوامل و الاحتفاظ بكل جرذ حامل على حدة في قفص بلاستيك نظيف ومراقبتها بشكل يومي حتى موعد الولادة حيث تم اعتباره يوم صفر . في يوم 3بعد الولادة ، تم اخذ أربعين جرذ حديث الولادة بشكل عشوائي وتقسيمهم الى مجموعتين: المجموعة أ ، والتي كانت بمثابة مجموعة ضابطة التحكم ، والمجموعة ب ، التي تلقت عن طريق الفم (1 مجم / كجم)من عقار البروبيل ثايويوراسيل لمدة ثلاثة أسابيع. في يوم22 بعد الولادة ، تم تقسيم المجموعة ب إلى ثلاث مجموعات فرعية: ب1 ، مجموعة قصور الغدة الدرقية التي لم تحصل على علاج. ب2 ، مجموعة الغدة الدرقية التي تلقت مستخلصًا مائيًا من جذور الأشواغاندا (200 مجم / كجم) لمدة 21 يومًا ؛ و ب3 ، مجموعة الغدة الدرقية التي تلقت ليفوثيروكسين (4 جم / 100 جم / يوم) لمدة 21 يومًا. في نهاية التجربة ، تم تقييم الغدة تحت الفك السفلي باستخدام أدوات التصوير بالموجات فوق الصوتية في جميع المجموعات. .النتيجة: أظهر التقييم بالموجات فوق الصوتية للغدة تحت الفك السفلي لمجموعة الغدة الدرقية زيادة في الحجم الكلي للغدد اللعابية تحت الفك السفلي ، وظهورها بشكل غير متجانس ذا مظهر قرص العسل ووظهور مناطق ناقصة الصدى في المجموعة التي لم تتلقى علاج ، في حين أن النتائج للصغار الجرذان التي تعاني من قصور الغدة الدرقية التي تم اعطاءها بمستخلص جذور الأشواغاندا أظهرت إلى وجود تحسن طفيف في حجم الغدة وصدى النسيج عند مقارنته بصغار الجرذان التي تلقت عقار الليفوثيروكسين ، بسبب خصائصها المضادة للأكسدة.

**الكلمات المفتاحية:** قصور الغدة الدرقية، جذورالاشواغاندا، التقييم بالموجات فوق الصوتية، الغدد اللعابية