



## Ashwagandha Roots Extract Attenuate Some Anatomical and Behavioral Outcomes in Rat Pups After Early Postnatal Induction of Hypothyroidism



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**BACKGROUND :** Thyroid hormones are essential for the growth and maintenance of the central nervous system. Those in charge how quickly brain and neural cells grow and change during development. **Aims of the study:** To explore the ameliorating effect of Ashwagandha Roots extract (*Withania somnifera*) in male rats after early postnatal hypothyroidism induced by propylthiouracil. **Material and methods:** Forty healthy Albino male rat pups were at postnatal day 3 (PND3) divided into four groups (10 pups per group): Group 1: Control group, pups received distilled water (D.W.) for 21 days orally then continuous administration for further 21 days. The groups 2, 3, 4 were received Propylthiouracil (1mg/kg) from PND3 for 21 day to induce hypothyroidism state. Then group 2 received D.W. for 21 days from PND24 to PND45 to encounter the same conditions as other groups, while group 3 were received Levothyroxine (4µg/100g/day) orally for further 21 days, finally group 4 received Ashwagandha Roots extract (200 mg/kg) orally for 21 day. Hormonal assay, developmental tests, and behavioral tests were done. **Result:** The present study showed that PTU cause significant decrease in total serum T4 hormone level in the hypothyroid pups in comparison to the control group, these deficiencies during development period in rat's life causes severely impairment in growth and development of the rat's brain as well impairing behavior. **Conclusion:** The oral administration of Ashwagandha Roots extract showed ameliorating effects compared to levothyroxine effects on Hypothyroidism and related damage in brain maturation.

**Keywords:** Ashwagandha, Propylthiouracil, Hypothyroidism, Nest seeking, Open field test, Novel rejection test.

### Introduction

L-triiodothyronine (T3) and thyroxine (T4), two thyroid hormones (THs), are essential for the growth and maintenance of the central nervous system. They control the morphogenesis and proliferation of brain and neural cells during development [1]. Severe cognitive impairment comes from congenital hypothyroidism [2]. Moreover, there is evidence that neurocognitive impairments may continue even when early detection and therapy have been found to improve

many of these problems [3]. Numerous studies show that thyroid hormones influence a variety of behaviors throughout life by acting in multiple brain areas both during development and in maturity [4]. Thyroid hormones, for instance, have an impact on the cerebellum's granule cell proliferation and migration, synaptogenesis, and dendritic development of cerebellar Purkinje cells [1,3]. Thyroid hormone deficiency throughout development therefore causes disturbed motor coordination in adults. Hypothyroidism also

interferes with granule cell migration and pyramidal cell dendritic development in the hippocampus [5], inducing aberrant synaptic function and learning. Propylthiouracil (PTU) was administered to rat pups until PND24 to reversibly block thyroid hormone production in the pups to induce hypothyroidism in this developmental stage in rat [2], which reflects the time period between the third trimester of gestation to the second postnatal year in the human [6]. The current investigation focused on the impact of postnatal hypothyroidism and recovery on areas with continuing postnatal neurogenesis since some brain regions undergo considerable development and neuronal creation after birth and well into adulthood, consequences of acute hypothyroidism in the first three weeks of rat life, specifically [7]. Ashwagandha Roots extract (Indian Ginseng) attenuates hepatic and cognitive deficits in the hypothyroid – induced rat model via induction of Nrf2/HO-1 and mitigation of NF- $\kappa$ B/MAPK signaling pathways. It has anti-oxidative, anti-stress, anti-genotoxic, and immunomodulatory properties [8]. Additionally, Ashwagandha Roots Extract is used to treat insomnia, memory-related illnesses, nerve weariness, and to enhance learning and memory. It has also been shown in preclinical studies to be effective in hepatoprotection and alleviating cognitive impairment [9]. To our knowledge, there was no previous report on the attenuating effect of Ashwagandha Roots extract on the rat behavior after postnatal induction of hypothyroidism using PTU. So, the aims of the present study to identify the effect of hypothyroidism through using PTU on the behavioral outcomes in rat's pups and to assess the ability of Ashwagandha Roots extract to attenuate these actions (if present) in comparison with levothyroxine which considered as a replacement therapy.

## Material and Methods

### Chemicals

1. Propylthiouracil (PTU): Propycil® (Recordati ilac group, Turkey) was available as white discoid tablets containing 50 mg of the active ingredient.
2. Ashwagandha Roots extract powder: it was obtained from (Natural YA Kimya, Turkey). The drug is administered orally in the form of a suspension in distilled water.
3. Levothyroxine sodium (LTH4): anthrax®,

Mark, Germany) (25 $\mu$ g). The drug is ground and administered orally in the form of a suspension in distilled water.

### Laboratory Animals

Ten healthy pregnant albino rats were obtained from animal house at a College of Veterinary Medicine, University of Mosul, Iraq. Each pregnant rat kept singly in clean rodent plastic cages (28 $\times$ 22 $\times$ 18) cm with wire mesh covers. Homogenized wood shavings were employed as bedding until birth. At ambient temperature (22 $\pm$ 2 °C) and humidity (55 $\pm$ 5%). Food and water *ad libitum*, were supplied in standard light condition (12- hour light / 12- hour dark cycle) [10]. All efforts were made to minimize animal suffering and to use only the number of animals necessary to produce reliable scientific data. All animal experiments comply with the institutional animal research ethics committee's requirements (UoM. Dent/A.L.20/22). Every morning, the pregnant rats were examined by a Veterinarian, to evaluate their general health and delivery of animals. When the pups were first noticed, that day was designated as PND 0, At PND3, male rat pups were selected for experimental study and not the females to avoid hormonal effects in female [11].

### Experimental Design and Procedures

At PND3, 40 male rat pups were divided randomly into four groups as follows:

- The Control group (1): (n=10): includes pups that received water orally once daily from PND3 to PND24, to encounter the same conditions as other groups. Then, they received water orally for 21 days PND24 to PND45 to encounter the same conditions as other groups.
- The Hypothyroid group with no treatment (2): (n=10): to induce postnatal hypothyroidism, Propylthiouracil (that was diluted in drinking water) was administered as (1mg/kg) dissolved in 5 ml water from PND3 until PND24. They received water for 21 days from PND24 to PND45 to encounter the same conditions as other groups [4,14].
- The Hypothyroid group + Levothyroxine (3): (n=10): includes pups which were received orally PTU (1 mg/kg/once /day from PND3 to PND24 to induce hypothyroidism and then they were treated with Levothyroxine (4 $\mu$ g/100g/day) [10] orally for 21 days from PND24 to PND45.

- The Hypothyroid group+ Ashwagandha Roots extract (4): (n=10) includes pups which were received orally PTU once daily from PND3 to PND23 as (1 mg/ kg/ once/day) and then they were treated with Ashwagandha Roots extract (200 mg / kg) [12] orally for 21 days from PND23 to PND45.

The gavage needle was properly attached to the 1ml syringe. It was introduced out of the side of the mouth with the observation of the mouth roof. Then PTU, LTH4 and Ashwagandha Roots extract were administered into the esophagus toward the stomach once the needle passed to the right length to ensure that the pups receive the all dose required [13].

#### *Hormonal level Assay*

On PND 23, blood samples of all groups were collected from a Retro Orbital vein. The tubes were immediately placed on ice, the blood was allowed to clot for up to a half hr. The tubes were centrifuged at 3000 round per minutes for 15 min. Serum free thyroxin T4 concentration was determined by radioimmunoassay (kit from Roche products) to ensure that hypothyroidism induced, and then these tests were repeated on PND45 to see the progress after treatment administration [14].

#### *Developmental Tests*

##### a. Estimation of the Change in the Body Weight:

Weighing the pups at the start of the experiment (PND3) was done, which was then tracked weekly until the end of the study (PND45), and the difference between groups was calculated [15].

##### b. Observation for the Day of Eye Opening

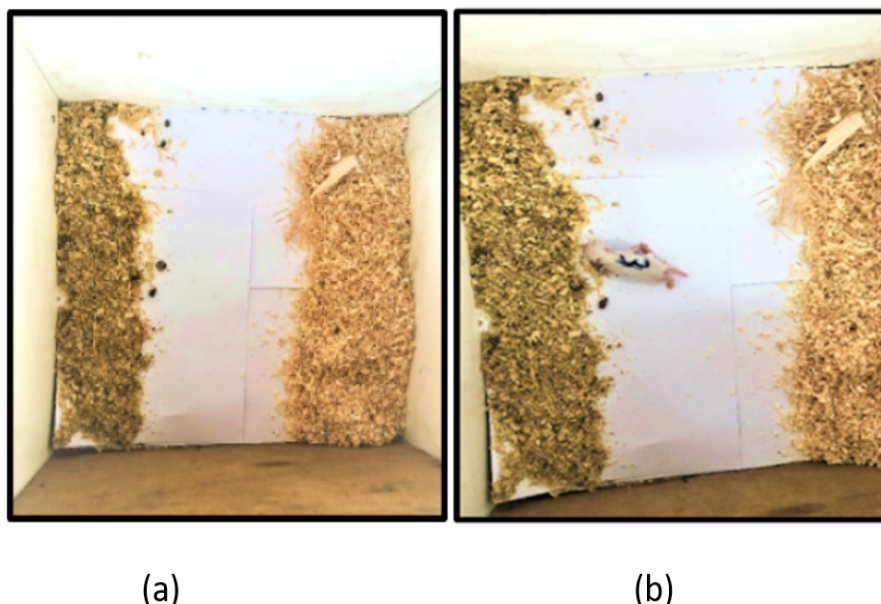
Eye opening was observed once daily, the expected day was from PND 13 to PND 15 [16].

#### *Behavioral Tests*

Male pups were used in this work to assess treatment effectiveness with levothyroxine and Ashwagandha Roots extract on some behavioral outcomes in hypothyroid pups. Behavioral tests were performed during the daytime at PND23 and PND45 [17]. Animals were acclimated to the experiment site for a minimum of an hour before to the behavioral experiments, which were conducted. These tests including:

##### *Nest-Seeking Test*

On PND9, the latency to approach maternal bedding was assessed .Bedding from the maternal home cage was used with another and clean bedding and were placed on paper at either corner of the testing cage (31.7x17.2x14.2 cm), and the observer recorded the latency to approach bedding using a mobile camera for 60 seconds[18]. Fig. (1 a , b)



**Fig. 1-a: A photograph showed nest seeking test. 1-b: A photograph showed pup during test emerged toward mother's bedding.**

### *The Open Field Test*

All rats were undergoing open field testing at two times (i.e. at PND20 and PND42). The purpose of this test is to measure the level of anxiety and locomotor activity. The open-field test arena is a square box (50×50 cm) divided into 25 squares of identical size (10×10 cm). The box is virtually demarcated into a central zone and peripheral zones. Pup was brought to the center of the arena and given three minutes to examine the arena [19]. The video camera of the mobile measured the activity of a tested pup in the box. The total distance that moved (total bar crossing in units) in the whole arena (outer + inner) and the entire distance traveled in the inner zone (measured centrally at the bar crossing) was recorded. The number of squares that each pup moved is considered an indicator of locomotor activity, while total distance moved in the inner zone is considered an indicator for anxiety level [20] Fig. 2.

### *Novel object recognition test (NOR)*

It is used to test the learning and memory in rats. The test was done at PND 21 and then repeated on PND 43 in three sessions: one habituation session, one training session, and one test session [21]. The time expended for exploration was recorded throughout this exploring duration and was manifested by sniffing from 2cm of each object or by direct touch of the objects. Excluding of the time expended on the object's top or climbing. Random assignment of both novel and familiar objects in the area of placement was done, Preference index calculated by the following equation [22]:

Preference index = The amount of time spent exploring Novel object / Total time spent exploring both objects (familiar + novel) \*100%

The preference index above 50% indicates a novel object preference, below 50% familiar object preference, and 50% no preference [23]. Fig. (3)

### *Statistical analysis*

The statistical analysis was performed using IBM SPSS statistics for windows, Version 26.0, Armonk, NY: IBM Corp. Released 2019. The data were presented as a mean ± SD. In this test, the differences between group were examined using ANOVA test. The significance of statistical analysis, comparisons was set at ( $p < 0.05$ ).

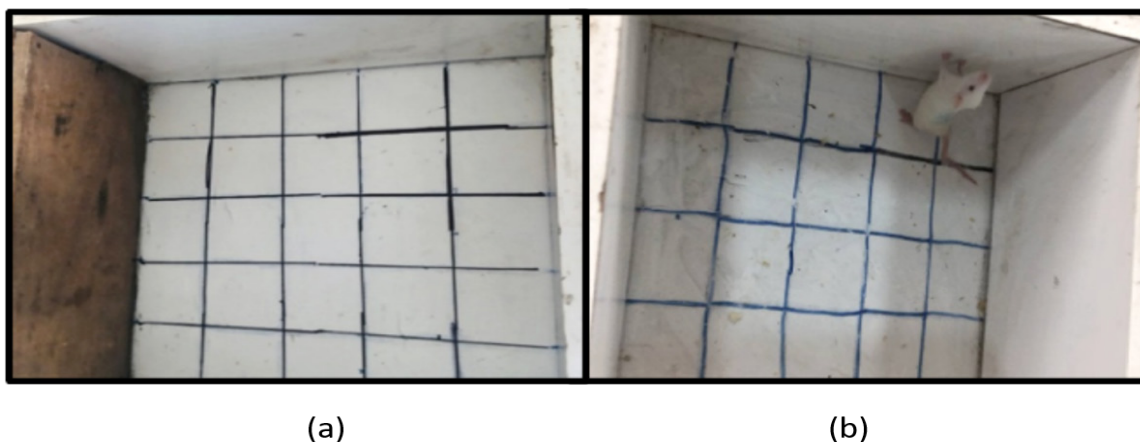
## **Results**

*All rats survived throughout the experimental period. Data were represented as the following*

### *Thyroid Hormonal concentration level*

In the current work, all animals received PTU become hypothyroid as there was a significant decrease in T4 serum levels (Table 1). At the end of experiment (at PND45), there is still significant differences between the total serum T4 concentrations of control group (1) and hypothyroid groups (2) which receives no treatment, while there is a significant improvement in the T4 level in the group treated with levothyroxine (3) as well as in the group treated with Ashwagandha Roots extract (4) in compared with control group.

On the other hand, the improvement in the T4 concentration level in pups of the hypothyroid group treated with Ashwagandha Roots extract was not significant in comparison with the hypothyroid group (Table 2).



**Fig. 2. A photographs illustrates a pup during the open field test.**



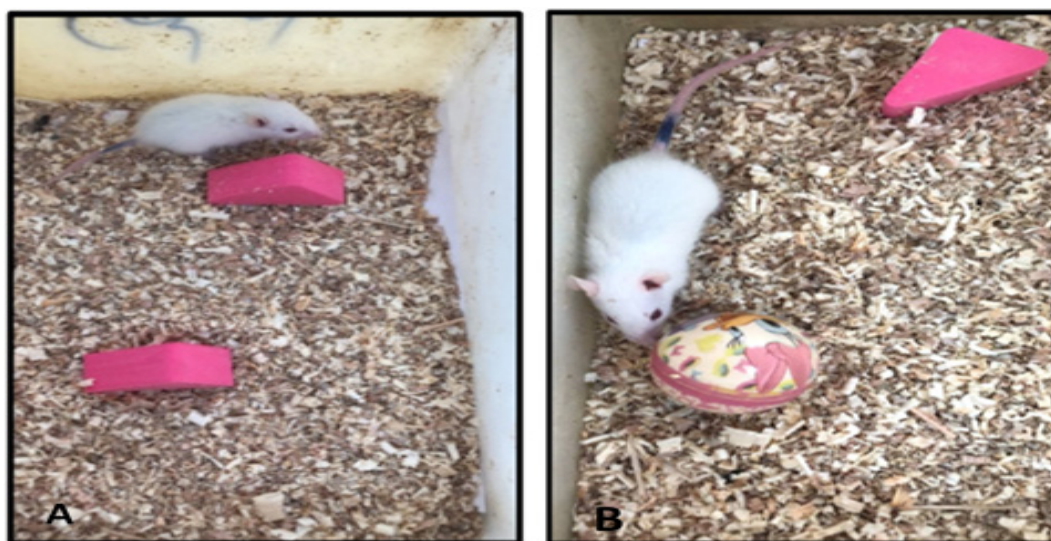


Fig. 3. A photographs of the Novel object recognition test sessions.  
(A): Training session. (B): Testing session.

TABLE 1. The mean values of T4 hormone level of all pups at PND21.

Groups	Control Group (mean $\pm$ SD)	Hypothyroid Group without treatment (mean $\pm$ SD)	Hypothyroid Group+ levothyroxine (mean $\pm$ SD)	Hypothyroid Group+ Ashwagandha (mean $\pm$ SD)
Serum T4 Mmol/l	9.537 $\pm$ 0. 314 <sup>a</sup>	8.473 $\pm$ 0. 369 <sup>b</sup>	8.490 $\pm$ 0. 315 <sup>b</sup>	8.533 $\pm$ 0. 300 <sup>b</sup>

Different letter mean significant difference between group at  $p \leq 0.05$ .

TABLE 2. The mean value of T4 hormone level of all pups at PND43.

Groups	Control Group (mean $\pm$ SD)	Hypothyroid Group without treatment (mean $\pm$ SD)	Hypothyroid Group+ Levothyroxine (mean $\pm$ SD)	Hypothyroid Group+ Ashwagandha (mean $\pm$ SD)
T4 nmol/l	97.12 $\pm$ 12.89 <sup>a</sup>	78.193 $\pm$ 7.343 <sup>b</sup>	95.007 $\pm$ .731 <sup>a</sup>	83.913 $\pm$ 9.414 <sup>a</sup>

-The similar letter among groups means there is no significant difference at  $p \leq 0.05$ .

#### Developmental Tests

##### Body Weight

A statically analysis of the body weight via ANOVA test from PND3 to PND23 shows marked difference in the mean body weight between all groups in the study started at PND15, which revealed that the mean body weight of rats were belonged to a hypothyroid groups(with no treatment, treated with Levothyroxine and treated with Ashwagandha Roots extract) was significantly lower than what the control group experienced. (Table 3, Fig. 4).

Furthermore, At PND 24 with the administration levothyroxine and Ashwagandha Roots extract, the weight measured weekly to the end of experiment at day 45, the result showed the therapeutic effects of levothyroxine, and ameliorating effects of Ashwagandha Roots extract by retrained the weight to the normal level of development to the hypothyroid pups , to be almost tike the weight of control group ,when compared to the pups of hypothyroid group with no treatment, at  $p \leq 0.05$  Fig. (5).

### Eye Opening

The results of the current work showed that the mean scores  $\pm$  SD of the eye opening day of the pups in all the study groups were ( $14.50 \pm 0.674$ ,  $15.77 \pm 0.833$ ,  $15.77 \pm 0.971$  and  $15.70 \pm 0.823$ ) in the control group (1), a hypothyroid group with no treatment (2), hypothyroid group + levothyroxine (3) and hypothyroid group + Ashwagandha Roots extract (4), respectively. Fig. ( 6).

### Behavioral Tests

#### Nest- Seeking Results

In PND 9, the statistical analysis by one – way

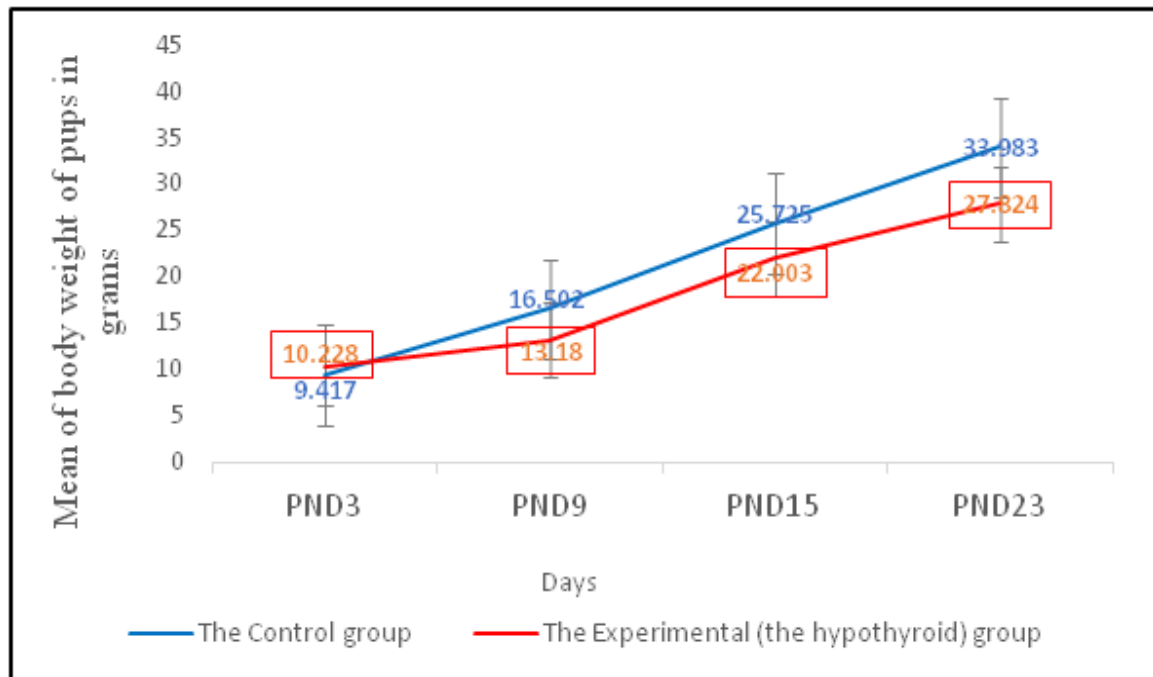
ANOVA at  $p \leq 0.05$  showed that the mean time that pups were needed to reach the home bedding was ( $10.33 \pm 3.02$ ) sec in pups of control group, while those pups of hypothyroid groups(hypothyroid without treatment , hypothyroid treated with levothyroxine and hypothyroid treated with Ashwagandha Roots extract) were ( $40.83 \pm 14.428$ ), ( $38.13 \pm 14.397$ ), and ( $41.6 \pm 11.415$ ) sec., respectively. These results showed that the time required for the pups of hypothyroid groups (2, 3, 4) to reach maternal bedding was much longer than time of the control group pups (1). Fig. (7).

**TABLE 3.** The values of mean body weight of all pups in different groups from PND3 to PND23.

Groups	The control groups (mean $\pm$ SD)	The hypothyroid group with no treatment (mean $\pm$ SD)	The hypothyroid group + Levothyroxine (mean $\pm$ SD)	The hypothyroid group + Ashwagandha (mean $\pm$ SD)
Body weight at PND3	9.417 $\pm$ 1.565 <sup>a</sup>	10.228 $\pm$ 1.459 <sup>a</sup>	9.070 $\pm$ 1.176 <sup>a</sup>	10.271 $\pm$ 1.460 <sup>a</sup>
Body weight at PND9	16.502 $\pm$ 2.087 <sup>a</sup>	13.180 $\pm$ 2.763 <sup>a</sup>	15.658 $\pm$ 1.420 <sup>a</sup>	16.026 $\pm$ 3.360 <sup>a</sup>
Body weight at PND15	25.725 $\pm$ 2.701 <sup>a</sup>	22.003 $\pm$ 4.766 <sup>b</sup>	19.376 $\pm$ 3.345 <sup>b</sup>	21.656 $\pm$ 4.789 <sup>b</sup>
Body weight at PND23	33.983 $\pm$ 5.275 <sup>a</sup>	27.824 $\pm$ 8.470 <sup>b</sup>	25.406 $\pm$ 2.609 <sup>b</sup>	28.086 $\pm$ 3.923 <sup>b</sup>

- The similar letter among groups means there is no significant difference at  $p < 0.05$ .

- Different letter mean significant difference between group at  $p \leq 0.05$ .



**Fig. 4.** A histogram of the values of mean body weight of all pups in different groups from PND3 to PND23.

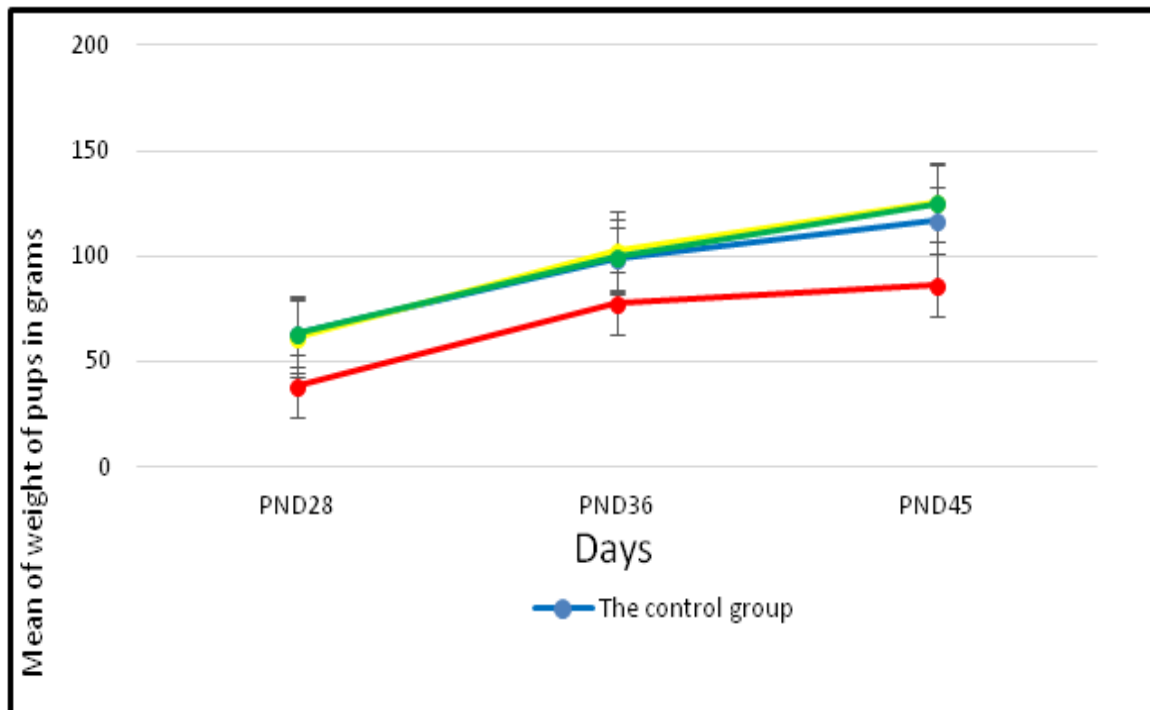


Fig. 5. A histogram of the values of mean body weight in grams of all pups in different groups at PND28, 36 and 45.

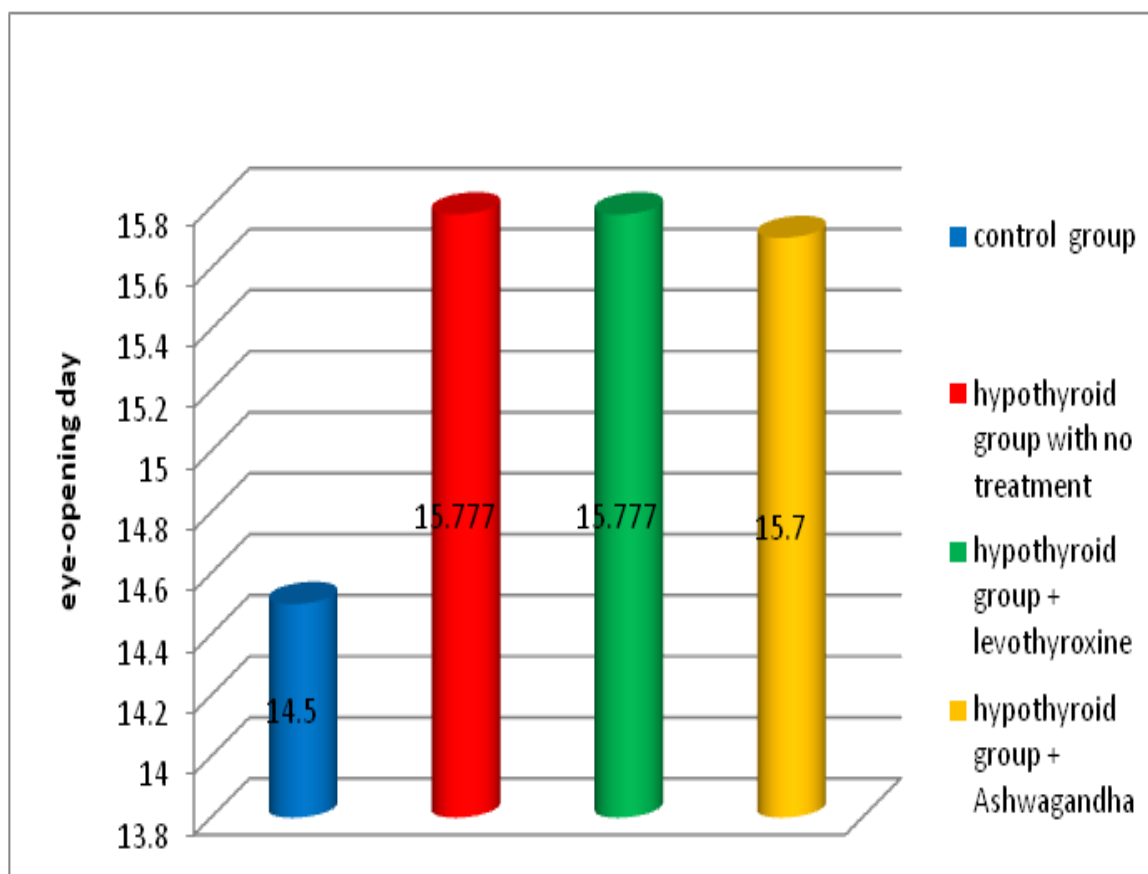


Fig. 6. A histogram of the values of eye-opening day of the pups in all study groups.

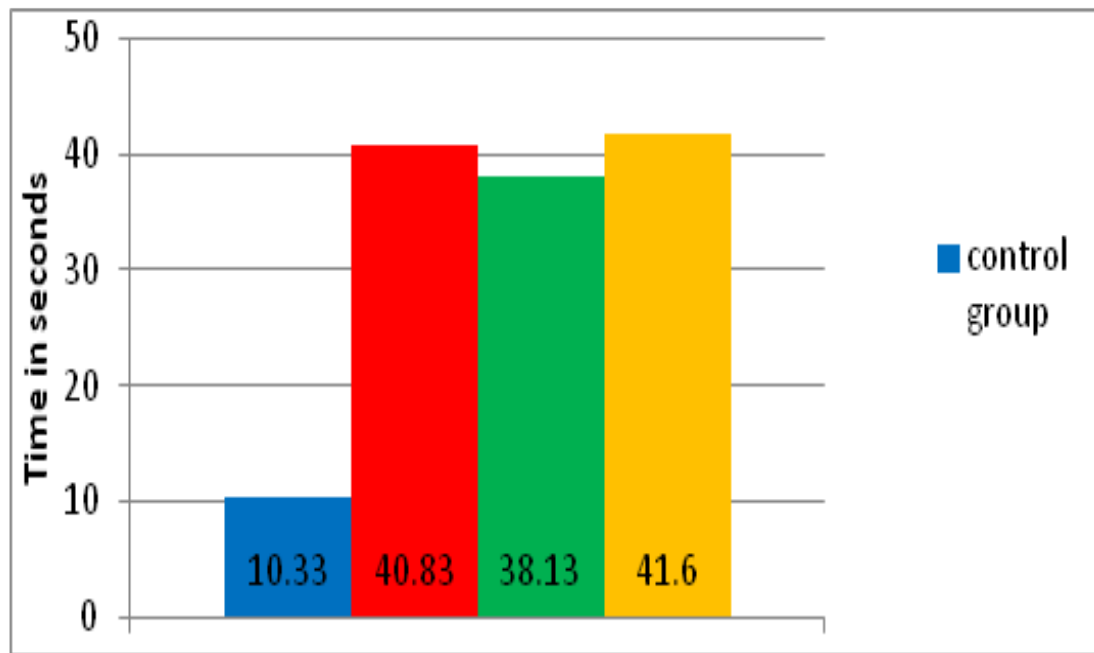


Fig. 7. A histogram of the Nest-seeking results of the pups in all study groups at PND9.

#### Open field measurements

A statistical analysis by one-way ANOVA test at  $p \leq 0.05$  on day 23 showed that the mean total distance that pups travelled of hypothyroid groups (hypothyroid without treatment, hypothyroid treated with levothyroxine and hypothyroid treated with Ashwagandha Roots extract) were ( $86.33 \pm 20.723$ ,  $79.66 \pm 23.82$ ,  $97.33 \pm 23.65$ , respectively) as the hypothyroid pups went noticeably greater distance overall (number of squares traversed) than did the pups in the control group ( $50.00 \pm 30.00$ ). On the other hand, the difference in the average number of central squares traveled was not statistically significant between hypothyroid groups (hypothyroid without treatment, hypothyroid treated with levothyroxine and hypothyroid treated with Ashwagandha Roots extract) which was ( $6.50 \pm 2.73$ ,  $4.14 \pm 2.54$ ,  $7.25 \pm 3.50$ , respectively) and control group ( $4.25 \pm 2.05$ ). While, there was significant variations in the mean value of the pups' peripheral distance traveled between the control group and each of the hypothyroid groups which indicate that locomotor activity of hypothyroid groups as they were significantly higher than the control group. Fig. (8).

At day 45, open field test repeated, the data of the mean total distance was  $63.00 \pm 24.20$  in Hypothyroid group treated with levothyroxine, and was  $69.00 \pm 2.64$  in Hypothyroid group which

treated with Ashwagandha Roots extract. There is significant improving in the locomotion activity in comparison with pups of hypothyroid group which remain without treatment ( $77.17 \pm 19.95$ ). Fig. (9).

#### Novel object recognition test observations

On day 22, in the training session, all pups from the all groups spent an equal amount of time exploring either of the two objects. However, during the retention performance test (test session) 24 hr. after the training session, the result showed the preference Index value for control group was ( $67.76 \% \pm 10.44$ ) with a novel object preference, while for hypothyroid groups (hypothyroid without treatment, hypothyroid treated with levothyroxine and hypothyroid treated with Ashwagandha Roots extract) were ( $25.73 \% \pm 28.94$ ,  $30.00 \% \pm .00$ ,  $26.10 \% \pm 19.94$ ), respectively. Fig. (10).

The test repeated again at PND44 to show the whether there is improving effects of treatment on cognitive function through the Preference Index. In fact, there was an ameliorating effect on retention ability with the group that treated with Ashwagandha Roots extract ( $53.60 \pm 12.818\%$ ), while a significant improvement in pups' ability in those treated with levothyroxine ( $80.14 \pm 13.696\%$ ) in comparing to hypothyroid group that remains with no treatment ( $45.83 \% \pm 51.03$ ), Fig. (11).



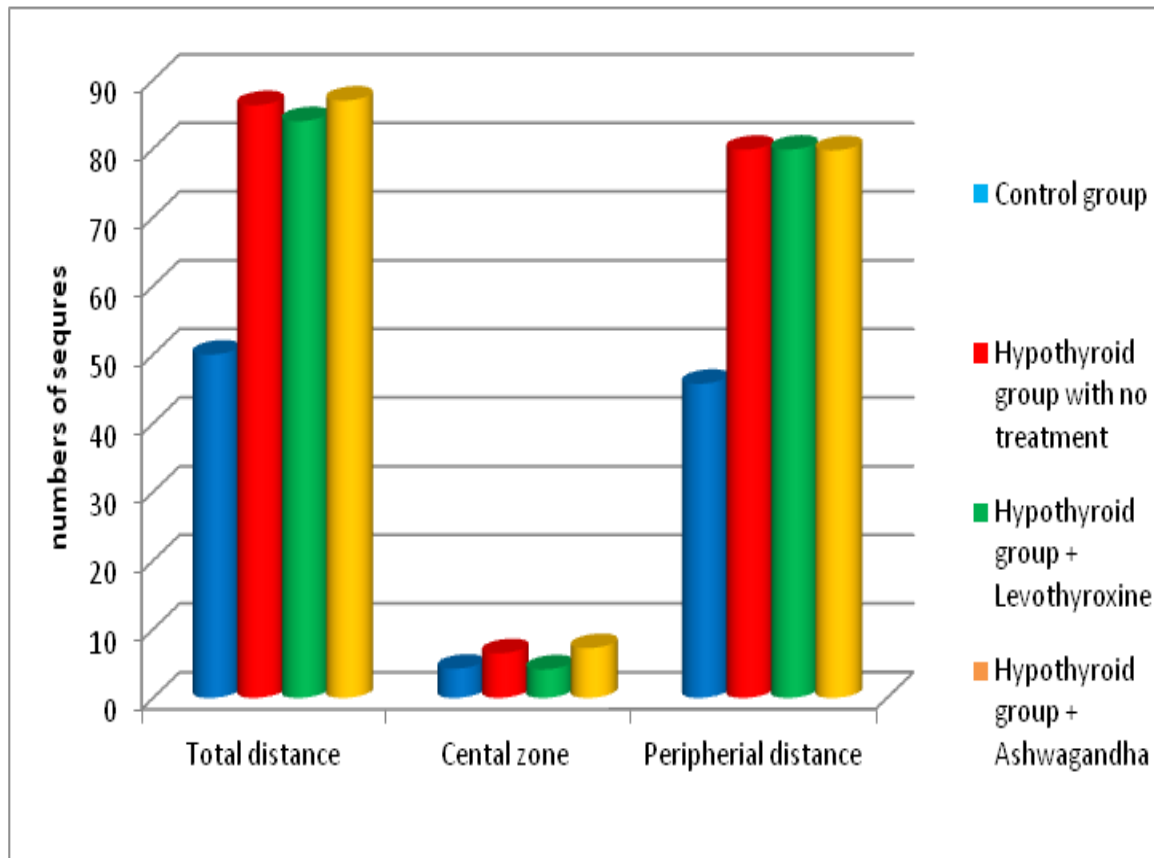


Fig. 8. A histogram of the open field test data of all pups at day 23.

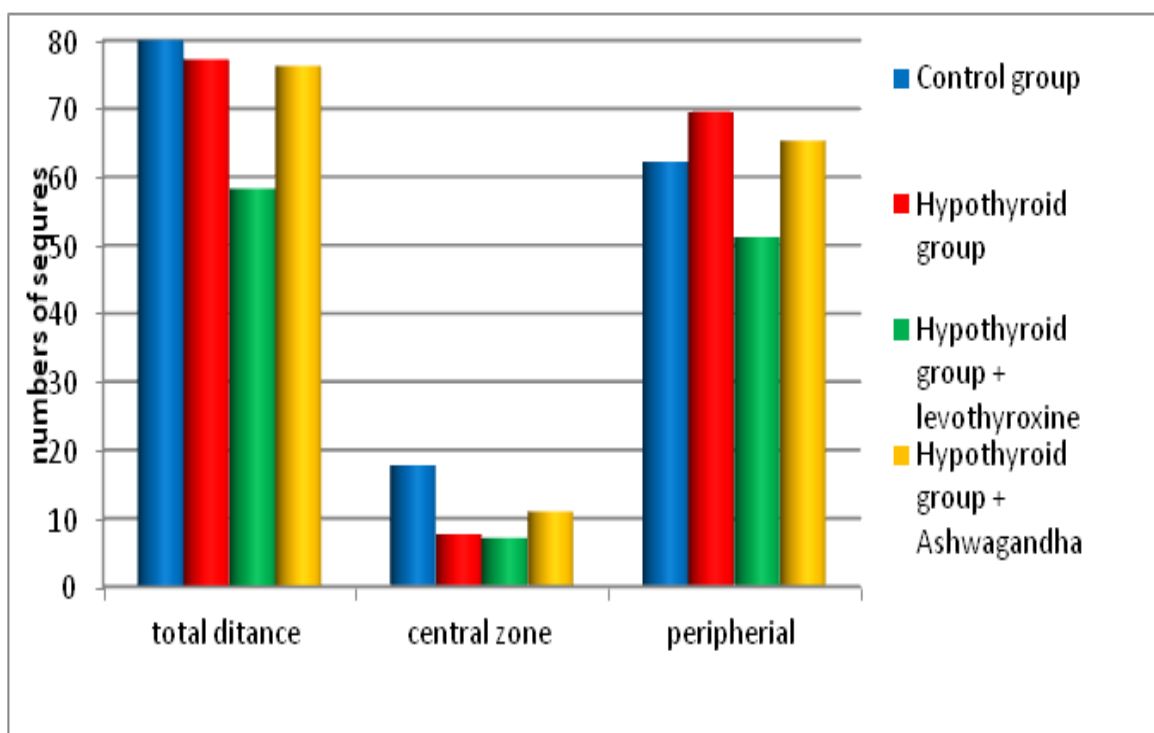


Fig. 9. A histogram of the open field test data of all pups at day 45.

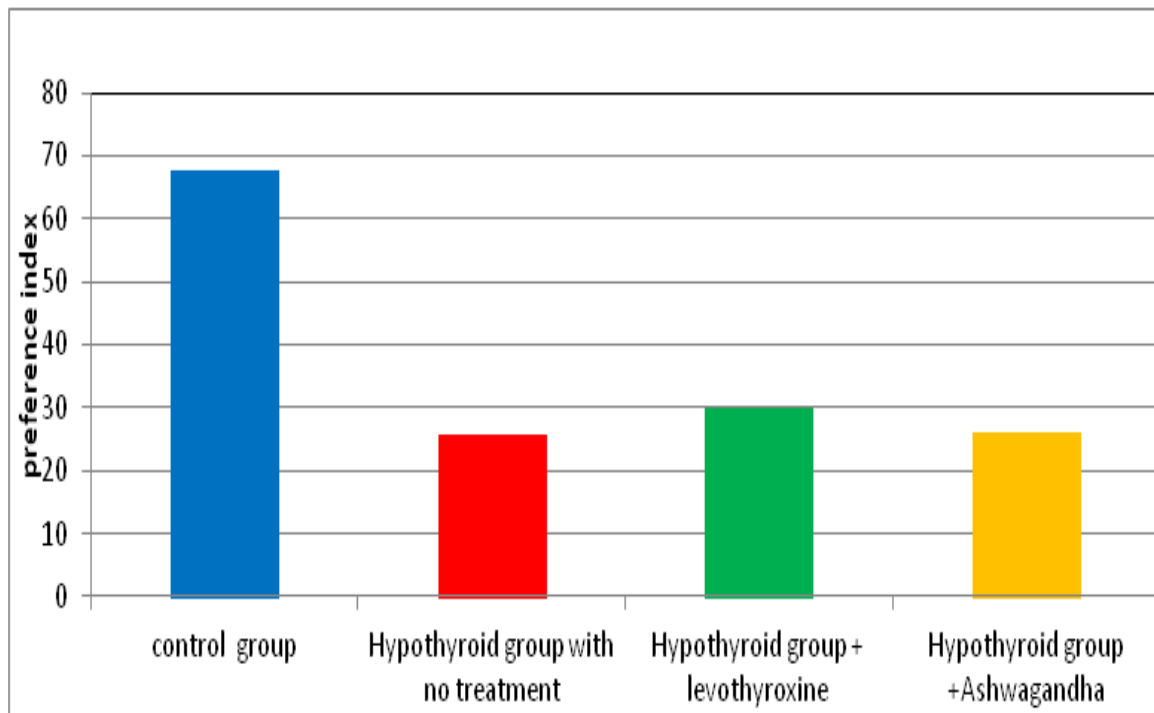


Fig. 10. A histogram of Preference Index at PND22 during the test phase.

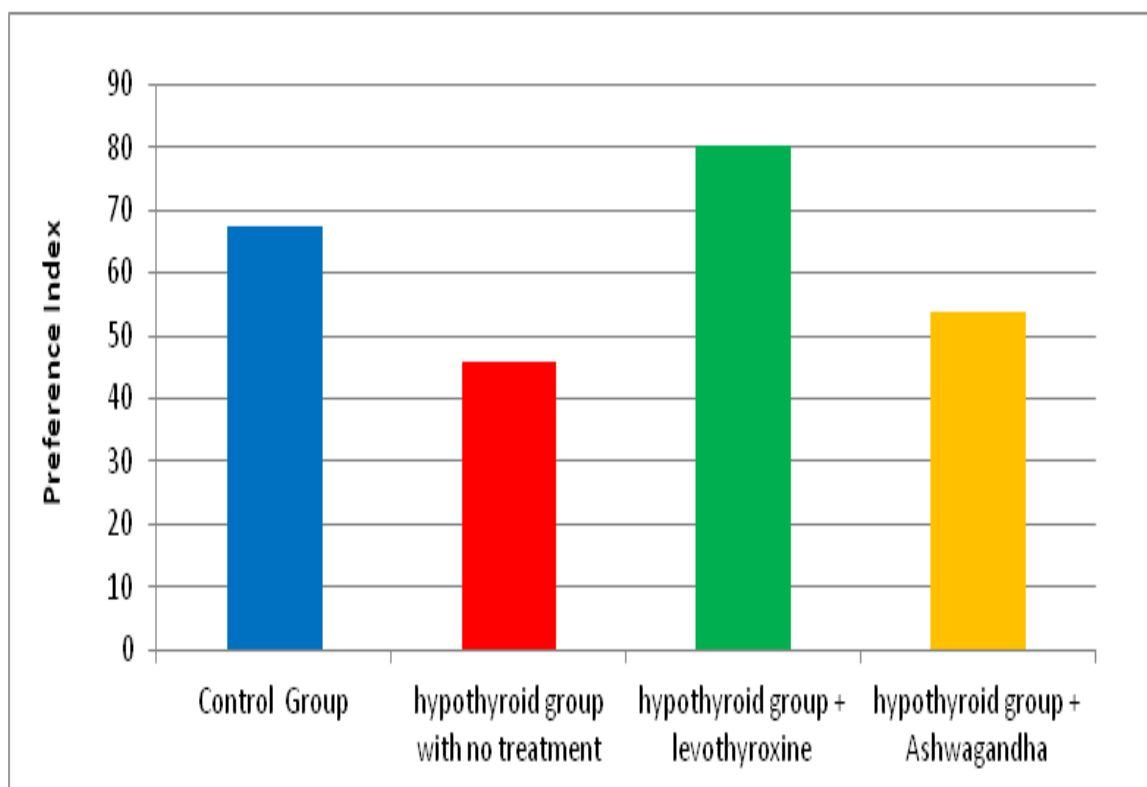


Fig. 11. A histogram of Preference Index at PND44 during the test phase.

## Discussion

Thyroxine (T4) and triiodothyronine (T3), which make up the majority of the thyroid hormones, have a significant impact on how the mammalian brain develops. They play a vital function in mammals throughout the early stages of nervous system development and have an impact on growth-related processes such RNA production and neural cell formation [24]. In order to investigate the growth and differentiation of neurons, rats have shown to be a good model. Thyroid hormones may have an impact on growing neurons in early postnatal animals since the majority of mammals produce their neurons during pregnancy and the early postnatal period [19]. Particularly, it has been demonstrated that thyroid hormone excess or deficit affects cell differentiation, migration, and gene expression. Therefore, low thyroid hormone levels throughout key stages of neurodevelopment can result in long-term cognitive and behavioral deficits [20].

In this work, hypothyroidism was induced in rats by PTU. Because thyrotropin release from the pituitary gland is increased in hypothyroidism, the thyroid gland becomes hyperplastic more quickly and T4 levels drop as a result [25]. In this work, comparing the hypothyroid rats to the controls, their serum T4 levels drastically dropped, demonstrating that propylthiouracil treatment successfully induced hypothyroidism in the experimental rats at the end of the induction period on PND23. At day 45, The thyroid function test assessed again to evaluate the effect of administration of Ashwagandha Roots extract to (Hypothyroid group + Ashwagandha), that appeared to increase the levels of T4 comparison with (Hypothyroid group with no treatment) and an ameliorating effect on T4 level in comparing with Levothyroxine (standard replacement therapy).

Furthermore, according to the physiological parameters results, one of the most important observations was the decrease in body weight gain in rats treated with PTU compared to the control group, as previously reported in various studies [26]. This decrease could be attributed to the decline in levels of T3 and T4 that have a potential effect on the metabolism and homeostasis in animal body. Also, certain study referred to the previous result of impairment in energy metabolic processes along with a decrease in the basal metabolic rate [27]. On another hand, it would seem that the drug's more direct impact on the

adrenal cortex is what is causing these alterations, stating that thiouracil induces adrenal atrophy, which is a decrease in organ weight relative to control animal organs, whether represented as an absolute weight or as a ratio of organ weight to total weight. Who showed that thiouracil causes overall development delay and organ weight loss [28]. After administration of levothyroxine and Ashwagandha Roots extract, the body weight significantly improvement to be the growth of the treated pups almost like pups in the control group, since levothyroxine showed a significant increase in food and water consumption compared with the hypothyroid with no treatment group that due to levothyroxine regulates hypothyroidism-induced metabolism abnormality [26]. Also, this study showed that Ashwagandha Roots extract inhibited PTU-induced loss of body weight by increases the amount of thyroid hormones in the blood while controlling oxidative stress and glucose metabolism. These findings were consistent with research showing that giving mice an Ashwagandha root extract boosts thyroid function and improves liver tissue's resistance to oxidation [29].

Another physiological parameter for rat's pup's maturation was day of eye opening, which was there significant delay in the eye-opening day by 1-2 days in the hypothyroid groups in comparison with that of pups which belonged to the control group. However. In fact, the exposure to propylthiouracil during the period of neurological development induces nervous system damage, that will lead to mental retardation and delay in eye opening due to histopathological changes in neonatal cerebellar cortex that proved by other researchers [30].

Different behavioral parameters were done in this work. First was nest seeking test (olfactory discrimination) done at PND9, the hypothyroid Pups showed significantly increased the mean time required to reach their maternal bed in comparison with pups of the control group. The impairment of the olfactory discrimination test may be due decrease thyroid hormones, which are crucial for olfactory receptor neurons to mature [31]. Olfactory receptor neurons are distinct in that they are exposed to external environments. Under such conditions, these neurons are prone to injury, and they are known to be the only believed to be the only neuronal cells with the ability to undergo regeneration throughout their lifetime [19]. Many variables influence neuronal growth

and differentiation in the olfactory system, the consequences of which are yet unknown. The dentate gyrus and the olfactory bulb are both prone to neurogenesis, and both the hippocampus and the olfactory bulb contain nuclear receptors for triiodothyronine, making both regions more sensitive to hypothyroidism [18].

Defects in the sense of smell are a typical clinical concern in persons suffering from primary hypothyroidism [32]. The number of olfactory receptor neurons in the olfactory epithelium is reduced in hypothyroidism. However, the effects of hypothyroidism on the olfactory epithelium were observed only in immature rats that were turned hypothyroid by the administration of propylthiouracil (PTU) from birth, not in adult rats [33]. Hypothyroidism may cause altered axonal development as a result of decreased microtubular synthesis. Thyroid hormones' impact on olfactory receptor neuron development may be mediated via neurotrophins [21].

Regarding data of Open Field Test which was performed to assess the locomotor activity and anxiety – like behavior, the observations of the current work indicated that Hypothyroid groups showed alterations in locomotor activity (hyper-activity) and reduced in anxiety-like behavior compared to those of control rats, these findings are consistent with those of other researchers [34]. On the other hand, we observed higher locomotor activity during the first minute of the open field test, this might be due to greater anxiety level and, as a result, prominent escape behavior during the initial minutes of the test [24]. These findings significantly support the concept that appropriate thyroid hormones in early postnatal life are vital to the development of the central nervous system, which is required for emotion and motor control [27]. The relationship between thyroid hormone abnormalities, particularly early hypothyroidism, and alterations in anxiety-related behaviors is widely documented but not entirely understood [35]. The amygdala is an important area for anxiety-like behavior. The medial prefrontal cortex, controls anxiety-like behavior through its reciprocal connections with the amygdala. Furthermore, the medial prefrontal cortex plays an important role in the contextual control of fear memory following extinction, a behavioral area associated with worry [26].

In addition, although the exact mechanisms involved in the learning, memory, and cognition impairments induced by hypothyroidism is

still unknown. It appears that hypothyroidism alters the oxidative stress state in several brain areas, triggering subsequent processes. It is also suggested that there are relationships between oxidative stress and other hypothyroidism-affected biochemical events including  $\text{Na}^+/\text{K}^+$ -ATPase activity, polyunsaturated fatty acid, nNOS, uptake of the neurotransmitter glutamate, acetylcholinesterase activity, and intracellular  $\text{Ca}^{2+}$  concentration which creates a multivariate condition with the outcome of brain tissues oxidative damage [36]. It is also believed that in hypothyroidism, a deficit of the antioxidant system has a function in the regulation of signaling pathways connected to cell growth and cell death. Changes in active oxygen metabolism have been shown to directly regulate transcription and translation, which in turn controls the thyroid hormones [28]. Propylthiouracil has been shown to affect the newborn neuroendocrine system by producing free radicals, which may induce severe neurological impairment in the cerebral cortex due to oxidative stress [37], by its disruptive effect on endocrine function by prevent conversion of T4 to T3, that thyroid hormones have an impact on the brain development. Levothyroxine, as a replacement treatment is frequently effective in avoiding developmental abnormalities. Furthermore, while early detection and therapy has been found to improve many of these deficiencies. There is evidence that neurocognitive deficits may continue [4].

Regarding Novel Object Recognition (NOR) test, Hypothyroid groups showed reduced in the preference index in compare with control group (that spend more time exploring the novel object during the first A few minutes of the test phase), specifically, the capacity to identify a previously seen item as familiar from the training phase [38]. The subject's cognitive abilities are required for novelty recognition [39]. On other hand the results of the NOR paradigm may be influenced by both hippocampal and cortical lesions due to deficiency in the thyroid hormones during the development period [23]. It is commonly acknowledged that the perirhinal cortex plays a significant part in object recognition memory in both the monkey and rat brains. This brain region is vital in the creation of recognition memories, and when it is damaged, performance in recognition memory tests is impaired [40].

The interesting finding in Open Field Test and NOR test, is that there is an significant

improvement in the locomotion activity, anxiety-like behaviors and cognitive ability in the Hypothyroid group treated with levothyroxine, and a good improvement in the hypothyroid pups treated with Ashwagandha Roots extract in compared with control group, and Hypothyroid group without treatment, by restored it to be more close to normal condition. This finding supports the usefulness of Ashwagandha Roots extract as a neuroprotective agent in a variety of disease states due to its powerful antioxidant and free radical quenching characteristics [29]. It has been shown to affect brain oxidative stress indicators such as superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx), lipid peroxidation (LPO), and non-enzymatic antioxidants such as glutathione (GSH). Oxidative stress in the brain causes an increase in the production of reactive oxygen species (ROS) such as superoxide anion, hydrogen peroxide, and hydroxyl radicals. These ROS work together to promote LPO of cell membrane lipids. LPO, a hazardous peroxide product, promotes cellular and molecular damage and has higher amounts of LPO in various areas of the brain in a neuropathological environment. In most studies, several data that explain Ashwagandha Roots action on brain LPO levels revealed a significant decrease and reversion to normal [37]. Furthermore, Ashwagandha Roots extract might promote axon and dendritic expansion, indicating a possible role in neural regeneration. The newly generated neurons are capable of integrating into neural networks as fully functional neurons, these new neurons are thought to play an important role in hippocampal-dependent spatial learning and memory [4]. This process may aid in recognizing the impact of thyroid hormone replacement treatment (Levothyroxine) and Ashwagandha Roots extract, which has an ameliorative effect on T4 levels, resulting in improvements in several behavioral indices. These include difficulties with movement, stress, and cognitive and memory impairment. The generation of new neurons allows for compensation or healing of some damage. Thyroid hormones are responsible for the speed of growth and change of brain and nerve cells during development. In hypothyroidism, these hormones are produced less and cause neurological complications [41, 42]. Today, medicinal plants are a valuable source for treatment [43-45], especially in hypothyroidism.

## Conclusion

Ashwagandha Roots extract has ameliorating effects on development and behavioral alterations caused by postnatal induction of hypothyroidism in rat's pups which may be used in future for clinical practice.

## Acknowledgement

To all staff in the department of dental basic sciences

## Conflicts of interest

The authors declared no competing interests.

## Funding Statements

None.

## Ethical Approval

All procedures involving animals in this study followed the National Institutional Health Principles of Laboratory Animal Care guidelines. The authors disclosed that this work received institutional ethical approval REC reference no. (UoM.Dent/A.L. 20/22).

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### مستخلص جذور الأشواغاندا بأمكانه تخفيف بعض النتائج التشريحية والسلوكية في صغار الجرذان حديثي الولادة بعد الاستحداث المبكر لقصور الغدة الدرقية

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الخلفية: هرمونات الغدة الدرقية ضرورية لنمو والحفاظ على الجهاز العصبي المركزي. إنها تتحكم في سرعة نمو الدماغ والخلايا العصبية وتغيرها أثناء التطور. طرائق العمل: تم تقسيم أربعين جرّاء من ذكور الجرذان البيضاء السليمة في اليوم الثالث بعد الولادة (PND3) إلى أربع مجموعات (١٠ صغار لكل مجموعة): المجموعة (١): المجموعة الضابطة، تلقت الجراء الماء المقطر لمدة ٢١ يوماً عن طريق الفم ثم أعطيت الماء المقطر لمدة ٢١ يوماً أخرى. تم تلقي المجموعة (٢، ٣، ٤) البروبيل ثيورايسيل (١ مجم / كجم) من (PND3) لمدة ٢١ يوماً للحث على حالة قصور الغدة الدرقية. ثم تلقت المجموعة (٢) لمدة ٢١ يوماً من PND24 إلى PND45 لمواجهة نفس الظروف مثل المجموعات الأخرى، بينما تم تلقي المجموعة (٣) عقار الليفوثيروكسين (٤ ميكروغرام / ١٠٠ جم / يوم) عن طريق الفم لمدة ٢١ يوماً آخر، وأخيراً تلقت المجموعة (٤) مستخلص جذور الأشواغاندا (٢٠٠ مجم / كجم) عن طريق الفم لمدة ٢١ يوماً. تم عمل فحص الهرمونات والاختبارات التشريحية والسلوكية. أظهرت الدراسة الحالية أن عقار البروبيل ثايوراسيل يسبب انخفاضاً كبيراً في إجمالي مستوى هرمون T4 في مصل الدم لدى صغار الجرذان المصابون بقصور الغدة الدرقية المستحدث مقارنة بالمجموعة الضابطة، وهذا النقص خلال فترة النمو في حياة الجرذان يسبب ضعفاً شديداً في نمو وتطور الدماغ بالإضافة إلى إعاقات واضحة في سلوكيات الجرذان. لقد استنتجنا من هذه الدراسة أن تناول مستخلص جذور الأشواغاندا عن طريق الفم له تأثيرات محسنة مقارنة بتأثير الليفوثيروكسين في حالة قصور الغدة الدرقية والأضرار المرتبطة بنمو وعمل الدماغ.

**الكلمات المفتاحية:** أشواغاندا، بروبييل ثيورايسيل، قصور الغدة الدرقية، البحث عن العش، اختبار المجال المفتوح، اختبار الجسم الجديد