



# The effect of swimming exercise on thyroid function, spatial memory and anxiety in normal and propylthiouracil-induced hypothyroidism in Wistar rats

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## ABSTRACT

**Introduction:** Swimming exercises improve various nerve growth factors and angiogenesis that encouraged the researchers to investigate the effect of swimming exercises on thyroid function (cyclic adenosine monophosphate [cAMP] and dual oxidase 2 [DUOX<sub>2</sub>] in free T<sub>4</sub> [FT<sub>4</sub>] secretion), spatial memory, behavior and anxiety on normal Wistar pups and those given with propylthiouracil (PTU).

**Methods:** The subjects of this research were normal Wistar pups and those given 25 ppm PTU from the 1<sup>st</sup> week until the 12<sup>th</sup> week of age. Swimming activity was started in 4-week-old pups after acclimatization for 1 week. Swimming exercises were conducted with a load of 1–2% body weight and 30min duration per day, 5 times a week for 8 weeks. The levels of FT<sub>4</sub> serum, cAMP and DUOX<sub>2</sub> of the thyroid gland homogenate were measured using enzyme-linked immunosorbent assay. The rats' ability to maintain spatial memory was measured using the Morris water maze and anxiety using the open field maze.

**Results:** The FT<sub>4</sub> levels significantly decreased after the administration of 25 ppm PTU for 3 weeks and it was much more decreased after 12 weeks administration. The administration of 25 ppm PTU for 12 weeks reduced the cAMP levels, increased DUOX<sub>2</sub> and reduced the spatial memory skills and exploration behavior of rats. Swimming exercise increased and normalized these parameters.

**Conclusion:** Swimming exercises for 8 weeks improve thyroid function, learning ability and spatial memory of normal and PTU-induced hypothyroid Wistar rats.

## Keywords:

Anxiety

cAMP

FT<sub>4</sub>

Propylthiouracil

Spatial memory

## Introduction

Adequate levels of thyroid hormones are vital to fetal nervous system development during the growth period. Thyroid function disorders caused by various antithy-

roid substances consumed for therapy or obtained from food have been observed. The use of antithyroid drugs needs to be approved by a doctor (Taylor and Vaidya, 2012). However, the presence of antithyroid or goitro-

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gens substances found in food and drinks is often not realized, and it disturbs thyroid function nonetheless. Consumption of whole grains containing maximum concentration of glycosyl flavone exerts maximum antithyroid effect and causes a significant increase in thyroid size, accompanied by the inhibition of thyroid peroxidase (TPO) activity (Gaitan et al., 1989). The presence of perchlorate as food contamination interferes with iodine absorption (Pleus and Corey, 2018), reduces thyroid hormone levels and increases thyroid size (Braverman, 2009; Steinmaus et al., 2016). The level of consumption of goitrogen-containing foods in school-aged children is quite high, for examples processed soy foods (tempeh, tofu, milk and soybean sauce), onions, tomatoes, cassava leaves, cassava, cabbage, broccoli, turnip, mustard greens, radishes, bamboo shoots and legumes (Dewi, 2013; Bajaj et al., 2016). Green tea is a strong goitrogen (Chandra and De, 2010; Sakamoto et al., 2001), which is now widely consumed as a beverage or used as a food flavoring.

Various disorders caused by antithyroid substances, which decreases the free  $T_4$  ( $FT_4$ ) levels, increases the secretion of thyrotropin-releasing hormone (TRH) and thyroid-stimulating hormone (TSH) (Bajaj et al., 2016). The increase in TSH secretion enables the thyroid follicle cell glands to generate cyclic adenosine monophosphate (cAMP), which is an intracellular messenger that synthesizes the activity of the thyroid hormones (Kronenberg, 2007; Carvalho and Dupuy, 2017). In previous studies, the administration of levothyroxine, which is used in hormone replacement therapy, to improve nerve function by measuring the spatial memory skills of rats with propylthiouracil (PTU)-induced hypothyroidism was tested (Taheri et al., 2018). In some exercises of subjects (experimental animals and humans) with hypothyroidism, an increase in  $T_3$  and  $T_4$  levels and a decrease in TSH levels were reported (Ciloglu et al., 2005; Shin et al., 2013).

Changes in the levels of cAMP and dual oxidase 2 ( $DUOX_2$ ) as a result of exercise have not been studied much by previous researchers. Increased cAMP demonstrates the sensitivity of thyroid follicular cells to TSH stimulation because cAMP is the second messenger formed by the TSH stimulation. The  $DUOX_2$  molecule is an enzyme that provides  $H_2O_2$  during organification. Changes in the  $DUOX_2$  levels due to exercise indicate that exercise's oxidative stress can affect  $H_2O_2$  levels.

Therefore, this research is necessary to determine the effects of physical activity or exercise on the action mechanism of thyroid hormone synthesis. The cAMP levels indicate cellular response to TSH stimulation, whereas the  $DUOX_2$  levels indicate interference with PTU antithyroid substances in the process of organification by TPO in collaboration with  $DUOX_2$  as the producer of  $H_2O_2$ .

Swimming was chosen as the treatment indicator because it is a beneficial training that causes minimal muscle damage (Veskoukis et al., 2018). The swimming training program was found to significantly ameliorate the depression parameters, stress, cognitive flexibility, selective attention and motor coordination in children with attention deficit hyperactivity disorder (Silva et al., 2020). Forced swimming for 10min increased blood  $T_4$ ,  $T_3$  and TSH levels, pituitary TSH $\beta$  mRNA expression and TRH PVN in rats that underwent adrenalectomy. However, forced swimming for 10min in normal mice did not make a significant difference (Sun et al., 2016). Acute swimming causes anxiety, but after the acclimatization process, swimming for more than 15 days does not cause anxiety anymore. Swimming for 30min increases serotonin levels (Higgins, 2019). A 30-min swimming exercise for 4 weeks increases serotonin expression and suppresses apoptosis to reduce anxiety and memory disorders after old age (Park et al., 2020). In addition, swimming treatment in rats does not require expensive tools. This study aimed to examine the effect of swimming exercises on the cAMP and  $DUOX_2$  levels of the homogeneous thyroid gland and on the synthesis of  $FT_4$  in rats that had thyroid dysfunction due to PTU administration.

## Material and methods

This study uses experimental and pretest-posttest control group designs. The initial and final data were measured to analyze the changes and differences in the thyroid function of  $FT_4$  synthesis and secretion. The cAMP and  $DUOX_2$  levels of the thyroid gland homogenate, spatial memory skills and anxiety were measured at the end of the research.

### *Research and subject's maintenance*

This study was approved by the Ethics Committee of Research at Gadjah Mada University with certificate No. KE/FK/1372/EC on 28 December 2018 and

amendment approval was given on 4 July 2019. The subjects of this study were six Wistar rat mothers and their newborn pups that were obtained from the Faculty of Pharmacy at Gadjah Mada University. The animals were kept in 40×30×15cm cages during breastfeeding and 25×17×12cm cages after being grouped according to the treatment at the UGM PAU Laboratory. The food compositions were water: maximum of 12%, crude protein: minimum of 18%, crude fat: 3–7%, crude fiber: 6%, calcium: 0.9%–1.1%, phosphor: minimum of 0.5% and distilled water for drinking. The food and drink were given ad libitum. The 12:12 h light/dark cycle was used and the room was equipped with blowers and an air conditioner with 21–24°C air temperature. After 5 weeks of treatment, the rats were transferred to the UGM Physiology Laboratory for spatial memory, behavior and anxiety measurements. The Physiology Laboratory used blowers but did not use an air conditioner, so the room temperature ranged from 25 to 28°C.

#### *The administration of PTU*

After the six Wistar rat mothers and their pups underwent the adaptation period, three breastfeeding mothers were given 25 ppm of antithyroid PTU (Behzadi et al., 2018; Goldey et al., 1995) mixed in the drinking water, which started on the 7<sup>th</sup> day after giving birth (Gilbert, 2011) and the rest was remained normal without any PTU administration. The number of pups per mother varied from 7 to 11. The Wistar pups and their mothers were not separated because the pups continued to suckle for 28 days. After weaning, the pups were continuously given antithyroid PTU mixed in their drinking water until the end of the study (Shin et al., 2013). Rats were grouped according to the treatment, gender and weight, with each group having 10 rats (7 male and 3 female). The treatment groups in this research were the non-PTU (normal) group, normal+ swimming group, PTU group and PTU+ swimming group.

#### *Swimming exercises*

The pups were acclimatized to swimming at 3–5 weeks of age. The swimming protocol in rat pups followed a study on rat pups in the growth period (Simkin et al., 1989) with modifications. A rectangle swimming pool (50×100cm) was used with a water depth of 20–30cm adjusted for large rats. Surface blocks were used to separate two rats that were simultaneously swimming. The

water temperature was between 31–34°C, slightly lower than rats' core temperature to prevent rats become hyperthermic or hypothermic and maintaining the normal hemodynamic system (Kregel, et al., 2006; Stone, et al., 2015). Swimming exercises were performed five times per week for 8 weeks (Nermin et al., 2018). Swimming training in rats started at the age of 5 weeks. The time for swimming was between 9:00 and 15:00 and the swimming duration was 30min for each training (Stone et al., 2015). Bodyweight of 1–2% was used during the load administration when swimming, which started in week 5. The load was in the form of metal tied to the tail with a rope.

#### *Measurement of FT<sub>4</sub> serum hormones, cAMP and DUOX<sub>2</sub> levels of the thyroid gland homogenates*

The serum collection for the measurement of FT<sub>4</sub> levels was performed at the beginning (4-week-old pups) and the end of the research. At the end of the research, rats were anesthetized with ketamine and their blood was drawn to collect serum by centrifuging at a speed of 5000 rpm for 10min. Serum FT<sub>4</sub> levels were measured using enzyme-linked immunosorbent assay (ELISA) (Biotech Laboratories, Shanghai, China). Then, the thyroid gland was removed and separated from the surrounding tissue, washed with phosphate-buffered saline (PBS) and stored at –20°C to be measured immediately the next day. The selection of temperature was based on general ELISA sample preparation protocol for less than 1-month stored samples (Xia, 2018). When measuring the DUOX<sub>2</sub> and cAMP levels, the thyroid gland was defrosted at room temperature, then a 10% (m/v) homogenate was made in sterile PBS and DUOX<sub>2</sub>, and cAMP levels were measured using ELISA, following the procedure from the Bioassay Technology Laboratory.

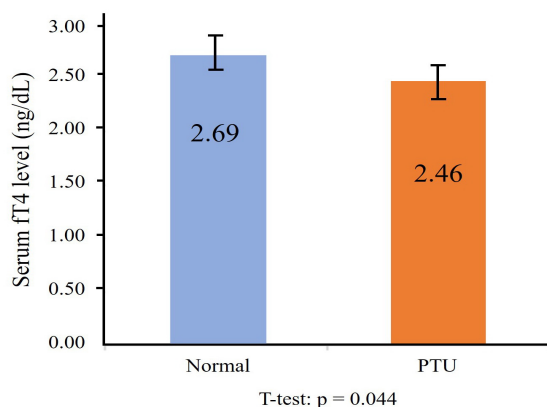
#### *Spatial memory*

The ability to maintain spatial memory were measured using the Morris water maze (MWM) to evaluate memory associated with space, fields, shape recognition, distance, area and knowledge on direction and position. Each rat was given two trials per day for 8 consecutive days. In each experiment, the rats swam for 60s to find the hidden platforms. The time when the rats found the platform was recorded (escape latency). If it did not work in 60s, the rat was given a 60-s latency score. When successful, rats were allowed a 30-s rest

on the platform. Memory retention tests (probe trials) were conducted to examine animals' abilities to maintain spatial memory against the platform location. Rats underwent memory persistence tests twice the following day (24h after the trial completion). Conducting probe test without the platform in the pool aimed to measure rats' memory of the previous platform location. This was done by measuring the percentage of time spent and swimming distance in the previous target quadrant and the number of crossings above the previous platform location. This assessment provided a second estimation of the rats' memory strength and accuracy of the previous platform location (Terry, 2009). Videos were recorded to measure the time and distance of the rats swimming.

### Anxiety

Rats' anxiety and behavior was measured using the open field maze (OFM). The OFM can be used to measure behavior, locomotor and anxiety (Lecorps et al., 2016; Seibenhener and Wooten, 2015). At the end of the research, the measurement was performed once. Rats were placed in an open box (120×120×50cm) for 10min. The rats' behaviors were recorded using a camera. The parameters measured in the OFM were the number of crossing, standing (rearing), and the number of rat defecations. Rats that were crossing exhibited courage, whereas rats that were standing (rearing) exhibited an exploratory behavior. Also, the increasing number of rat defecations exhibited a high level of anxiety (Seibenhener and Wooten, 2015).



**FIGURE 1.** The differences in serum free T<sub>4</sub> (FT<sub>4</sub>) levels of normal rats and the rats that received 25 ppm propylthiouracil (PTU) for three weeks.

### Statistical analysis

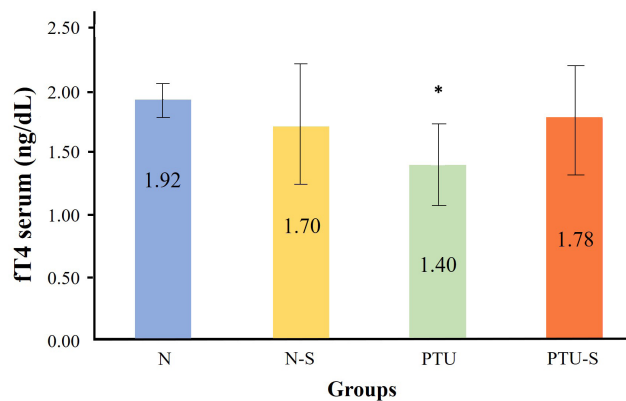
Data analysis performed in this study was paired t-test, independent t-test, Pearson correlation, and one-way ANOVA continued by LSD post hoc.

## Results

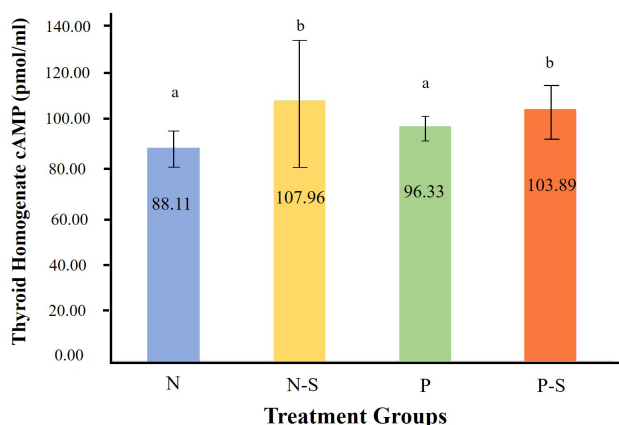
The serum FT<sub>4</sub> levels measurement at the beginning of the study, before the pups started swimming, is presented in Figure 1. The FT<sub>4</sub> levels in 25 ppm PTU administration for 3 weeks group experienced a statistically significant decrease (P=0.044), even though these levels were still within the normal limits. The differences in the FT<sub>4</sub> levels of rat serum based on the treatment group after 8 weeks of swimming exercise are presented in Figure 1.

The schematic shows the differences in the FT<sub>4</sub> levels of rat serum based on the treatment group after 8 weeks of swimming exercise (Fig. 2). There was no significant difference in FT<sub>4</sub> serum levels between the normal and normal+ swimming groups (P= 0.460), and the FT<sub>4</sub> levels in the PTU group were significantly lower than those in the normal group (P=0.007) and PTU+ swimming group (P= 0.0141). Figure 3 presents the average cAMP level of the thyroid gland homogenate. The highest cAMP level after treatment was found in the normal+ swimming group (P= 0.015). The cAMP levels in the PTU+ swimming group were higher than the PTU group, but not significant (P=0.232).

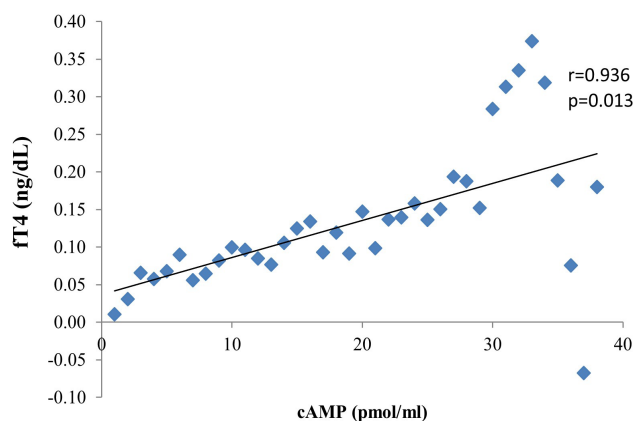
A schematic of the thyroid gland homogenates cAMP levels positively and strongly correlated with serum FT<sub>4</sub> (r=0.936, P=0.013, Fig. 4). This shows that an increase



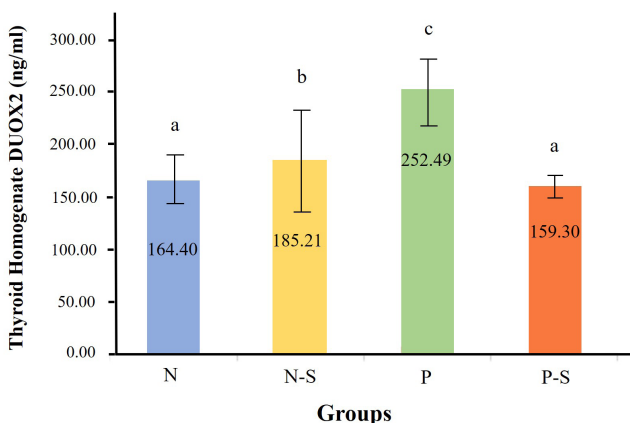
**FIGURE 2.** A schematic showing the differences in the free T<sub>4</sub> (FT<sub>4</sub>) levels of rat serum based on the treatment group after 8 weeks of swimming exercise. N= normal; N-S= normal swimming; P= propylthiouracil; P-S= propylthiouracil swimming.



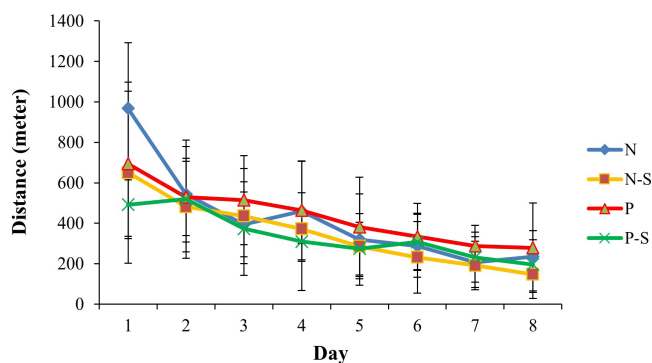
**FIGURE 3.** The Differences in cAMP levels of rat thyroid gland homogenate between treatment groups. N= normal; N-S= normal swimming; P= propylthiouracil; P-S= propylthiouracil swimming. Different letter notation (a and b) above the bar is considered significant after an LSD posthoc test ( $P < 0.05$ ).



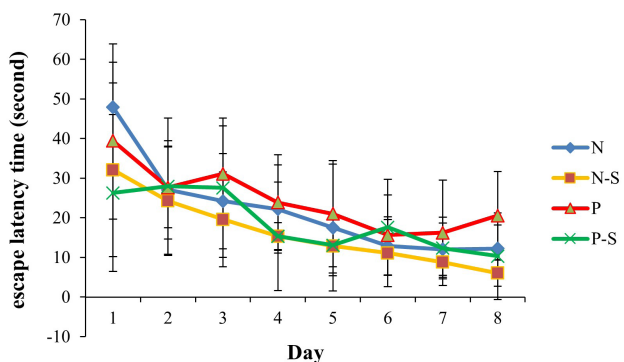
**FIGURE 4.** The Correlation graft between the cAMP levels of the thyroid gland homogenates with serum FT<sub>4</sub>.



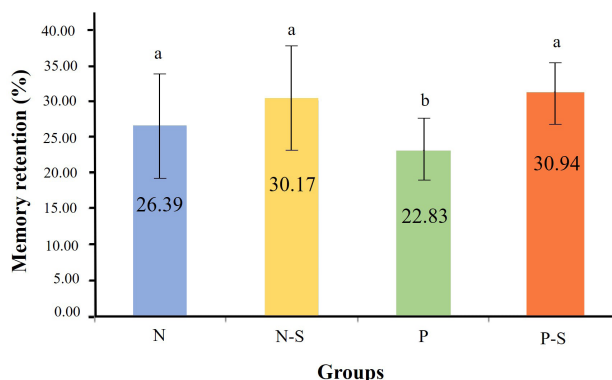
**FIGURE 5.** The Differences in dual oxidase 2 (DUOX<sub>2</sub>) levels in rat thyroid gland homogenate between treatment groups. N= normal; N-S= normal swimming; P= propylthiouracil; P-S= propylthiouracil swimming. Different letter (a, b and c) notation above the bar is considered significant after a Mann-Whitney test ( $P < 0.05$ ).



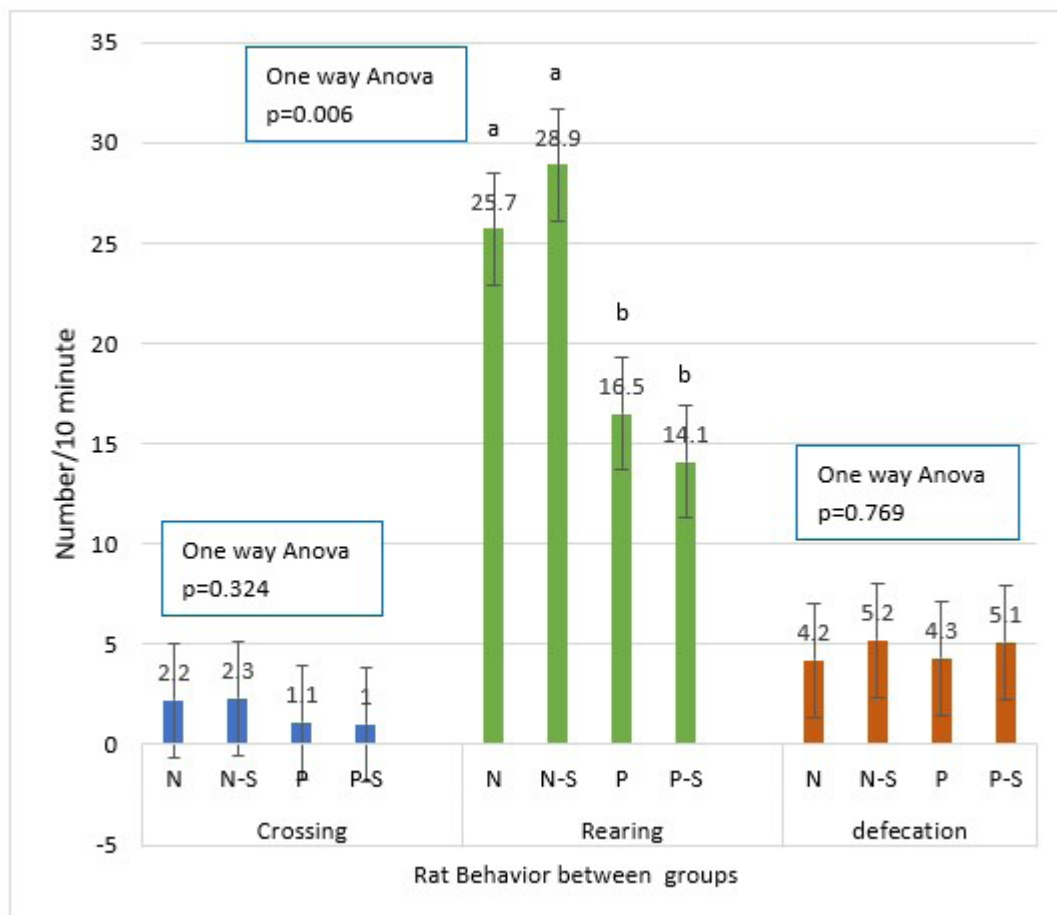
**FIGURE 6.** The average path length of the Morris water maze. N= normal; N-S= normal swimming; P= propylthiouracil; P-S= propylthiouracil swimming. The error bar represents standard error.



**FIGURE 7.** The average latency time of the Morris water maze. N= normal; N-S= normal swimming; P= propylthiouracil; P-S= propylthiouracil swimming. The error bar represents standard error.



**FIGURE 8.** Differences in spatial memory retention on platform locations in the Morris Water Maze test among treatment groups. N= normal; N-S= normal swimming; P= propylthiouracil; P-S= propylthiouracil swimming. Different letter notation (a and b) above the bar is considered significant after an LSD posthoc test ( $P < 0.05$ ).



**FIGURE 9.** The difference between the number of crossing, rearing, and defecation rats in the open field maze test among treatment groups. N= normal; N-S= normal swimming; P= propylthiouracil; P-S= propylthiouracil swimming. Different letter notation (a and b) above the bar is considered significant after an LSD posthoc test ( $P < 0.05$ ).

in intracellular cAMP accumulation will increase the serum  $FT_4$  levels. The highest level of  $DUOX_2$  in the thyroid gland homogenate was in the PTU group and the lowest was in the PTU+ swimming group, yet the level of  $DUOX_2$  in the PTU+ swimming group was not different from the normal  $DUOX_2$  level (Figure 5). The results of subsequent research are parameters of memory and behavior. Rats' ability to maintain spatial memory was measured using the MWM and the behavior using the OFM.

The graphs of the distances and the latency time of rats in finding the platform in the MWM as shown in Figures 6 and 7. It can be concluded that all groups learned well because, in the last days, all rats were able to find the platform before 60s. Although not significant, the graph line of the PTU group is at the longest time position. This indicates that the PTU group traveled a long distance ( $P=0.058$ ) and spent a long latency time ( $P=0.181$ ) in finding the platform. Meanwhile, the data of the PTU+ swimming group was parallel to the normal

group. The normal+ swimming group was the best in learning to find the platform of MWM. This is explained in the memory retention test results in Figure 8.

The measurement of rat behavior using the OFM (Figure 9) shows that there is no difference in terms of the courage to cross, even though it can be seen that the normal group was better than the PTU group from the observed numbers. The normal+ swimming group exhibited the best exploration behavior in rearing; however, the difference was not significant compared to the normal group. The exploration behaviors of the PTU and PTU+ swimming groups were significantly lower than those of the normal and normal+ swimming groups. In the number of defecations, no significant differences were observed among the treatment groups.

## Discussion

The  $FT_4$  level of the group receiving 25 ppm PTU for 3 weeks experienced a statistically significant decrease, even though the levels were still within the normal lim-

its. These results are consistent with the results of previous studies in Sprague Dawley rats that the administration of 30 ppm PTU dose decreases FT<sub>4</sub> and FT<sub>3</sub> serum levels equivalent to the doses of 100 and 300 ppm (Hood, 1999). Prenatal administration of 25 ppm PTU can dramatically reduce the T<sub>4</sub> serum levels of Long Evans pups at all ages and induce various developmental disorders (Goldey et al., 1995). In previous research, 25 ppm PTU was administered to eight pregnant dams continuously after birth to postnatal day 60, which induced congenital hypothyroidism in rats, with the FT<sub>4</sub> level of the half from normal (Behzadi et al., 2018). The administration of PTU 0.025% to Wistar rats mixed in drinking water, from the 1st day of pregnancy to the 21st day postnatal, insignificantly reduced the FT<sub>4</sub> and FT<sub>3</sub> levels in pups on day 60. However, the administration of PTU 0.025% postnatal starting from the 1st day of lactation to the age of 21 days significantly decreased the FT<sub>4</sub> and FT<sub>3</sub> levels on day 60 (Hamouli-Said et al., 2007). The dose is 10 times greater than the dose used in this study. Research on Wistar rats administered with lower PTU doses (PTU doses 1.5, 3 and 6 ppm starting on the first day after birth until 12 weeks) was conducted by Taheri et al. (2018). The T<sub>4</sub> serum levels decreased significantly in the 6-ppm dose group, yet the decrease was not significant. The T<sub>4</sub> serum level decrease in response to the administration of 6 ppm PTU was lower in male rats than female rats.

PTU is one of the antithyroid substances that inhibit TPO activity. Aside from PTU, TPO activity is also inhibited by competitive substances from the carbamazepine group, namely thiouracil, methimazole and carbimazole (Bajaj et al., 2016; Kronenberg, 2007). TPO cannot oxidize its substrate without being oxidized by H<sub>2</sub>O<sub>2</sub> molecules first. Nowadays, it is known that the enzyme that produces H<sub>2</sub>O<sub>2</sub> and is associated with oogenesis hormone is the NADPH oxidase, which depends on calcium production and is related to DUOX<sub>2</sub>.

Swimming activity in rats that received PTU 25 ppm until the end of the study (12 weeks) can maintain high levels of FT<sub>4</sub> equivalent to normal rats. This is proven by the measurements of cAMP level in thyroid gland homogenates. The highest cAMP levels after treatment were seen in the normal-swimming group and the next sequence was the PTU-swimming group. The increase in cAMP level indicates a good response of the thyroid gland cells to TSH stimulation. The cAMP is a second

messenger that forms intracellular thyroid follicular cells after stimulated by TSH. It relaxes various intracellular responses in the synthesis of thyroid hormones. The function of thyroid cells stimulated by TSH is an increase in I<sup>-</sup> in the follicular lumen mediated by phospholipase C (PL-C), a gradual increase in NIS expression through the cAMP mechanism and an increase in I<sup>-</sup> efflux from thyroid cells into colloids mediated by pendrin molecules (Pesce et al., 2012); and increased thyroid bloodstream through the mechanism of nitric oxide synthesis; increased thyroid blood flow through the mechanism of nitric oxide synthesis; the formation of hydrogen peroxide in the synthesis of thyroid hormones is mediated by PL-C; the formation of thyroglobulin and TPO is mediated by cAMP; pinocytosis of thyroglobulin, the release of thyroglobulin into the plasma is mediated by cAMP. Mitogenesis of thyroid cells chained by cAMP. While the mechanism of NADPH formation via the pentose-phosphate pathway is not yet clearly known (Kronenberg, 2007; Carvalho and Dupuy, 2017;).

The highest level of DUOX<sub>2</sub> was observed in the PTU group. Meanwhile, the lowest DUOX<sub>2</sub> level was observed in the PTU+ swimming group and this level was not different from the normal DUOX<sub>2</sub> level. PTU-induced hypothyroidism in rats will experience organification inhibition by TPO (Furman, 2016; Taylor and Vaidya, 2012). The inhibition of TPO activity activates various components, one of which is DUOX<sub>2</sub>, which plays a role in the synthesis of H<sub>2</sub>O<sub>2</sub>. DUOX and TPO are in very close locations and work together to maintain optimal levels of H<sub>2</sub>O<sub>2</sub>. Also, the regulation of the relationship between DUOX and TPO is observed in the Gq-phospholipase C-Ca<sup>2+</sup>/protein kinase C pathway and down-regulated through the Gs-cAMP/protein kinase A pathway (Song et al., 2010)

The DUOX<sub>2</sub> levels in the PTU+ swimming group were not different from the normal group. This phenomenon happens because swimming and other exercises induce oxidative stress and an increase in the reactive oxygen species family (anion superoxide, hydroxyl radical, hydrogen peroxide and hypochlorous acid), which can compensate for the effects of PTU, inhibit TPO activity and decreasing H<sub>2</sub>O<sub>2</sub>. There is evidence that antioxidant supplementation combined with antithyroid methimazole can be useful in reducing oxidative stress (Duntas, 2005).

The role of DUOX in the thyroid gland is to produce

hydrogen peroxide, which is then used in iodide oxidation mediated by thyroid peroxidase to form a reactive compound (De Deken et al., 2014). DUOX protein forms  $H_2O_2$ , which is needed for thyroid peroxidase/TPO activity. TPO plays a role in the synthesis of thyroid hormones. The catalytic activity by  $DUOX_2$  is observed in the reaction of  $NAD(P)H + O_2 = NAD(P) + H_2O_2$ . The activity of the peroxidase enzyme is inhibited by amino benzohydrazide because of its similarity. While, the activity of NADPH oxidase depends on Ca ions (Ameziane-El-Hassani et al., 2005). The  $DUOX_2$  gene mutation was found to be associated with cases of congenital hypothyroidism caused by defects in iodine organification. It is suspected that the presence of  $DUOX_1$  in thyroid cells can compensate for the presence of  $DUOX_2$  defects, although not completely (Carvalho and Dupuy, 2017).

The rats were able to learn about space, as shown by the graphs of their path length and latency time to find the platform in the MWM. Our study showed that the normal+ swimming group was the best in learning to find the platform in the MWM test. A mild decrease in the ability of the rat to study and remember space is thought to be due to a mild decrease in  $FT_4$  levels. In the learning process, normal nerve structure and adequate energy metabolism are needed for outstanding nerve performance. During this time, it was not realized that a mild decrease in thyroxine levels had the same effect as the decrease in nerve performance. Various thyroid function disorders have a direct impact on the working mechanism of the nerves (Ahmed, 2015; Lazarus, 2012). Our research provided evidence that swimming exercises improved thyroid gland function by maintaining the  $FT_4$  levels within normal limits. Besides, the far-reaching effects of normal  $FT_4$  levels and the direct exercise on the nervous system improve learning and memorization skills. The effect of swimming exercises on nerve function in spatial memory is in line with the therapeutic effect of levothyroxine on rats with PTU-induced hypothyroidism (Taheri et al., 2018).

It has been shown that voluntary and forced exercise in rats induces angiogenesis in the brain (Bloor, 2005; Al-Jarrah et al., 2010), adipose tissue (Lee, 2018) and heart (Hajje et al., 2014), and increases blood flow and density of the hippocampal blood vessels (Nishijima and Soya, 2006). Even 3 days of training initiated angiogenesis by up-regulating vascular endothelial growth factor

(VEGF) of the heart muscle (Wu et al., 2009). Exercise stimulates the increase in VEGF and BDNF in nerve cell proliferation (Ahmed, 2015). Smooth bloodstream and neural plasticity play a significant role in the integrity of the central nervous system function, brain plasticity and cognitive improvement in kittens (Shafiee et al., 2016) and rats (Kerr et al., 1999).

The rat behavior measured by the OFM exhibited no difference in the courage to cross among all treatment groups, even though it can be seen that the normal groups were better than the PTU group from the presented numbers. The exploration behavior exhibited by standing rats (rearing) was the best in the normal+ swimming group yet the difference was not significant compared with the normal group. The exploration behaviors of PTU and PTU+ swimming groups were significantly lower than those of the normal and normal+ swimming groups. Rearing behavior indicates exploratory behavior. Rearing behavior indicates curiosity about environmental conditions. In mice that practiced swimming, this behavior showed more curiosity about the new environment. The number of rat defecations during the OFM test exhibited no difference among the treatment groups. This indicates that the stress levels of rats in all groups are equivalent.

A mild decrease in the  $FT_4$  levels has less impact on stress levels and the courage of rats to take risks by crossing open spaces or in the middle of open spaces. However, the impact is more visible in the exploration behaviors of rats, which indicates learning behavior to know the surrounding conditions. This research shows that swimming exercises tend to improve the behaviors of rats. This is consistent with the evidence that swimming exercises significantly stimulates the expression of several growth factors (BDNF, GDNF, NGF, NT-3, FGF<sub>2</sub>, VEGF, and IGF-1) and peptides (VGF and NPY), increases anti-apoptotic Bcl-xL expression and normalizes the down-regulation that occurs in chronic mild stress-induced rats (Jiang et al., 2014).

## Conclusion

Swimming exercises for 8 weeks can restore decreased thyroid function caused by PTU and improve thyroid function, learning ability and spatial memory of normal Wistar rats and rats with PTU-induced hypothyroidism. We suggest conducting further research on the effects of swimming training on the thyroid function of subjects



who consume goitrogens with strong effects, such as green tea and raw vegetables. Also, it is necessary to research the effects of swimming on hypothyroid subjects or subjects undergoing thyroid dysfunction therapy.

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## Conflict of interest

The authors declare there is no conflict of interest.

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