

Research Article

Ulva lactuca Linnaeus Potentially Promotes Reproductive Indices and Depressive-like Behavior of Hypertriglyceridemia Male Wistar Rats (*Rattus norvegicus* Berkenhout, 1769)

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ABSTRACT

Excessive consumption of fatty foods can lead to hyperlipidemia, which is often coupled with hypertriglyceridemia (HTG), a condition where blood plasma triglyceride (TG) levels elevated beyond normal levels. This condition may disturb physiological functions of the body, such as reproductive functions, and other physiological imbalances leading to chronic stress and depression. *Ulva lactuca* is a potential natural treatment for HTG, as it contains various nutrients to aid physiological functions. This seaweed also has high levels of Cd, which can increase depression. Therefore, research on the potential benefits of *U. lactuca* should be followed by an investigation of its health risks. This research aimed to examine the effects of HTG and treatment with *U. lactuca* on reproduction and depressive-like behavior of male Wistar rats (*Rattus norvegicus* Berkenhout, 1769). The data collected in this research include body weight, serum TG concentration, gonadosomatic index (GSI), serum testosterone concentration using competitive ELISA, and depressive-like behaviors assessed using the Forced Swim Test (FST) and Open Field Test (OFT). Data were analyzed using One-Way ANOVA followed by DMRT, independent- and paired-samples t-test, and Kruskal-Wallis H test with a significance value of $\alpha=0.05$. Body weight, serum TG and testosterone concentration, GSI, and depressive-like behaviors were increased by the HTG condition. *Ulva lactuca* at the 1500 mg/kg BW/day did not significantly affect body weight, testosterone concentration, and depressive-like behaviors of HTG rats. Meanwhile, this treatment significantly increased the GSI and depressive-like behaviors of healthy rats. These results suggest that *Ulva lactuca* treatment not only enhances gonad growth and development but also increases depressive-like behaviors.

Keywords: depression, hypertriglyceridemia, male reproduction, triglycerides, *Ulva lactuca*

INTRODUCTION

The definition of an unhealthy lifestyle often involves the habit of smoking, lack of physical exercise, excessive consumption of alcohol and artificial sweeteners, as well as consumption of foods with a high-fat content (Ashakiran & Deepth, 2012). In developing countries, cooking oil is often used repeatedly, therefore it goes through repeated heating, which may cause physical and chemical alterations of the oil. These physical changes include the darkening of color, turning the yellow-tinted oil

to black, and increasing viscosity. Chemical changes include the breaking of double bonds on the carbon chain of fatty acids, transforming unsaturated fatty acids to saturated fatty acids, and the increase of free fatty acids (Suroso, 2013).

Consuming high saturated fatty acids and free fatty acids foods increase health risks such as hyperlipidemia, hypertriglyceridemia (HTG), atherosclerosis, obesity, coronary heart disease, and other cardiovascular diseases (Blesso & Fernandez, 2018). One of the most common conditions arising from excessive consumption of foods that were cooked in overused frying oil is hyperlipidemia. This condition is often paired with HTG, an elevation of blood plasma triglyceride (TG) levels beyond the

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normal level. The Endocrine Society 2010 defines this as more than 150 mg/dL (Berglund *et al.*, 2012). Hypertriglyceridemia may have a negative effect on the functions of certain organs, such as the liver, heart, kidneys, and reproductive organs (Lockman *et al.*, 2012; Minguez-Alarcon *et al.*, 2017). High concentrations of TG also increase its chances of crossing the brain-blood barrier, inducing central resistance of leptin (Banks *et al.*, 2018), thus leading to low leptin levels in the brain, a condition found in depressive patients (Ge *et al.*, 2018).

Natural substances are often preferred for the prevention and treatment of health conditions, are usually considered to be safer, causing minimum side effects. *Ulva lactuca*, commonly called the “sea lettuce”, is a species of seaweed that has been used as food in Vietnam (White & Wilson, 2015) as well as a traditional medicine in China (Tseng & Chang, 1984). This seaweed contains high amounts of antioxidants, proteins at 10-21 g/100 g dry weight, as well as antibacterial, antifungal, and antitumor properties (Erniati *et al.*, 2010). The antihyperlipidemic and antioxidant properties of *U. lactuca* are found in its polysaccharides (Sathivel *et al.*, 2008; Hassan *et al.*, 2011) and ethanolic extract (Widyaningsih *et al.*, 2016). Mulyati *et al.* (2019) found that *U. lactuca* is quite abundant in nutrients, comprised of macronutrients, micronutrients, secondary metabolites, and antioxidants, although cadmium (Cd) levels were also identified to be higher than the recommended limit regulated by *Badan Pengawas Obat dan Makanan* (BPOM) (2018) for plant-based foods. Cadmium is a heavy metal that potentially increases depressive symptoms (Lamtai *et al.*, 2018; Scinicariello & Buser, 2015), amongst other health risks. This research aims to examine the effects of HTG and treatment with *U. lactuca* on reproduction and depressive-like behaviors of male Wistar rats (*Rattus norvegicus* Berkenhout, 1769).

MATERIALS AND METHODS

Materials

Twenty 12-weeks old male Wistar rats (*Rattus norvegicus* Berkenhout, 1769) with a body weight range of 200-300 grams obtained from LPPT UGM unit 4 were used as animal models. Materials administered into the animals included: saturated fat comprised of overused cooking oil and beef fat (1:1) for HTG induction; *U. lactuca* obtained from the south coast of Gunungkidul, D.I. Yogyakarta, which were air-dried and grounded at the Laboratory of Animal Physiology, Faculty of Biology, UGM, to create a powdery texture; and over-the-counter drug Gemfibrozil for treatment of HTG. AD II pellets

and reverse osmosis water (RO) were both given *ad libitum*. Materials for data collection included: Testosterone ELISA Kit TE373S (Calbiotech), 20x20x40 cm³ glass chambers, a 40x40x40 cm³ opaque black box, YI Action Camera (XiaoYi), semi-analytical and analytical balances, centrifuge, dissecting set, ketamine-xylazine/ cocktail (1:1), distilled water, NaCl 0.9% solution, neutral buffered formalin (NBF) 10%.

Methods

This research was conducted under the Ethical Clearance certificate number 00046/04/LPPT/VIII/2019, issued by the Institutional Committee of Animal Use and Care (ICAUC) of Universitas Gadjah Mada.

Animal Treatment

This experiment was conducted for 54 days. The first 14 days was the induction period, and the next 40 days was the treatment period. All treatments were given orally. Twenty rats were divided into 5 groups: H, HTG-induced only; H.O, HTG-induced and treated by Gemfibrozil (10 mL/kg BW/day); H.U, HTG-induced and treated by *U. lactuca* (1500 mg/kg BW/day); S.U, not HTG-induced and treated by *U. lactuca* (1500 mg/kg BW/day); K.S, healthy control, not HTG-induced and not treated. Saturated fat was used for HTG induction at 15 mL/kg BW/day during the induction period and 7.5 mL/kg BW/day during the treatment period. Rats in S.U and K.S groups were given distilled water at the same dose as the saturated fat.

Body Weight

Body weight was measured using a semi-analytical balance. This data was used to calculate the amount of saturated fat, Gemfibrozil, *U. lactuca* (Eq. 1), and ketamine-xylazine cocktail (Eq. 2-4) needed for administration. Body weight was also used to calculate the gonadosomatic index (GSI).

$$\text{daily intake} = \text{daily dose} \times \text{body weight} \quad (1)$$

Daily dose of saturated fat is 15 mL/kg BW or 7.5 mL/kg BW, Gemfibrozil is 10 mg/kg BW, and *U. lactuca* is 1500 mg/kg BW.

$$\text{volume of ketamine (mL)} = 2 \times \text{volume of ketamine (mL)} \quad (2)$$

$$\text{volume of ketamine (mL)} = \text{weight of ketamine (mg)} \div \text{ketamine concentration} \quad (3)$$

$$\text{weight of ketamine (mg)} = \text{dose} \times \text{body weight} \quad (4)$$

Ketamine dosage for anesthesia is 50 mg/kg BW. For euthanasia, the dosage is doubled. The concentration of ketamine solution used was 100 mg/mL.

Serum TG Concentration

Blood serum was obtained from centrifugation (10,000 rpm, 10 min) of blood samples collected from the retro-orbital plexus of rats on D-0, D-14, D-34, and D-54. Triglyceride concentrations in the blood serum were determined using Triglycerides FS (DiaSys) based on its protocol (DiaSys, 2015) at LPPT UGM unit 2.

Serum Testosterone Concentration

Testosterone concentrations of blood serum collected on D-0 and D-54 were determined by competitive ELISA using Testosterone ELISA Kit TE373S (Calbiotech).

Forced Swim Test

This procedure was done based on the protocol by Yankelevitch-Yahav *et al.* (2015) using glass chambers sized 20 x 20 x 40 cm³. The chambers were filled with clean water set at room temperature up to 30 cm in depth. Video recording using YI Action Camera (XiaoYi) was done throughout the testing. The rat behavior recorded in this test was immobility, the rat floats, only performing movements to keep its nose above the water.

Open Field Test

The procedure for this test was modified from Sestakova *et al.* (2013). Each rat was placed in the center of a 40 x 40 x 40 cm³ opaque black wooden chamber. The video camera was placed directly above the chamber to record the rat's behavior, 5 min for each rat. After 5 min, the rat was removed and the chamber was sterilized with 70% ethanol, before placement of the next rat. The number of crossings between the central area (20 x 20 cm²) and outer area, frequency of rearing, and duration of freezing were recorded.

Gonadosomatic Index (of testes soaked in NBF for 24 hours)

Upon necropsy, the testes were collected, washed in NaCl 0.9% solution, then stored in NBF 10%. An incision was made on each testis' capsule, 2 hours after collection. After 24 hours of collection, the testes were weighed using an analytical balance. Gonadosomatic Index is calculated using Eq. (5) based on Nurhidayat *et al.* (2017).

$$GSI = \frac{\text{testes weight}}{(\text{body weight} - \text{testes weight})} \times 100\% \quad (5)$$

Data Analysis

Quantitative data were statistically analyzed using SPSS v.16 with the One-Way ANOVA followed by DMRT for body weight, TG D-0, D-20, and D-40 treatment, depressive-like behaviors in OFT, and GSI; paired-samples T-test for D-0 of HTG-induction and testosterone concentration, or Kruskal-Wallis H Test for depressive-like behavior in FST. A value of $p \leq 0.05$ was considered significant statistically. Video recordings from OFT were analyzed using idTracker (Cajal Institute, Spain).

RESULTS AND DISCUSSION

Body Weight

Body weight was measured on day-0 (D-0) to record the initial body weight, day-14 (D-14) to examine the effect of HTG induction, day-34 (D-34) to examine the effects of 20 days of HTG treatment, and day-54 (D-54) to determine the GSI. On D-0 and D-14, the mean body weight of H is the lowest and it is significantly different amongst the other groups, but on D-34, it is no longer significantly different from the other groups (Table 1). According to Jung and Yoo (2018), HTG is related to obesity and

Table 1. Body weight of HTG male Wistar Rats (*Rattus norvegicus* Berkenhout, 1769) on D-0, D-14, D-34, and D-54.

Group	Body weight (g)			
	D-0	D-14	D-34	D-54
H	262.25±7.69 ^{a,w}	276.25±8.33 ^{a,w}	322.25±12.05 ^{a,x}	338.00±16.21 ^{a,x}
H.O	301.25±10.36 ^{b,w}	308.25±13.22 ^{ab,w}	337.50±11.87 ^{a,wx}	358.75±14.38 ^{a,x}
H.U	321.75±13.06 ^{b,w}	330.00±15.93 ^{b,w}	358.75±16.13 ^{a,w}	372.75±20.42 ^{a,w}
S.U	305.25±3.20 ^{b,w}	323.50±9.18 ^{b,wx}	346.50±9.73 ^{a,xy}	370.25±9.92 ^{a,y}
K.S	294.25±14.30 ^{b,w}	311.50±15.12 ^{ab,wx}	342.75±12.74 ^{a,xy}	360.25±12.27 ^{a,y}

Data were presented as mean±SE. The different superscript letters note a significant difference at $p < 0.05$: **ab** compares values between groups within the same day, **wxy** compares values between days within the same group. H: HTG-induced, H.O: HTG-induced with Gemfibrozil treatment, H.U: HTG-induced with *U. lactuca* treatment, S.U: not HTG-induced with *U. lactuca* treatment, K.S: healthy control, not HTG-induced nor given treatment. D-0 – D-14: HTG induction period; D15 D-15-D-54: treatment period with maintained HTG induction.

cardiovascular disorders. Obesity is defined as a condition of excessive fat build up in the body, with a high body mass index (Ofei, 2005). Consumption of foods with high Fe content such as *U. lactuca*, which has Fe content of 873.72 mg/kg (Mulyati *et al.*, 2019), has potency to increase body weight by approximately 7 grams/day (Aukett *et al.*, 1986). Yokus and Gedik (2016) reported that iron therapy can be used to increase Hb, body weight, and ferritin.

Serum TG Concentration

The three groups induced with HTG for 14 days showed 24.3% of serum TG concentration elevation (Table 2), but not statistically significant ($p > 0.05$). Table 3 showed the increasing and decreasing serum TG concentration in all groups during the treatment periods. Until D-20 of the treatment period, all groups showed a reduction of serum TG concentration, as the daily dose of saturated fat was reduced by 50%. This was seen in the H group, whose TG concentrations decreased from 293.40 mg/dL to 170.38 mg/dL. This design implicates a person with HTG who limits their fat intake to reduce their blood TG concentration.

From D-0 to D-20, treatment with Gemfibrozil and *U. lactuca* also showed a significant decrease in TG concentrations ($p < 0.05$). This is because Gemfibrozil is intended as a hypolipidemic or antihyperlipidemic drug, especially targeting TGs. Frick *et al.* (1987) stated that Gemfibrozil could reduce TG in the blood by 35%, while Vinik and Colwell (1993) found the decrease to be 26.4%. These findings confirm that Gemfibrozil may treat HTG.

U. lactuca's main polysaccharide, ulvan, has potency as a lipid antiperoxidative and antihyperlipidemic (Kidgell *et al.*, 2019). In addition, the content of secondary metabolites in *U. lactuca* such as terpenoids, sterols, carotenoids, and polyphenols can also act as antihyperlipidemic properties (Silva *et al.*, 2013). The content of omega-3 (0.1% relative), omega-6 (0.1% relative), omega-9 (0.1% relative), and several unsaturated fatty acids such as pentadecenoic acid (11.60% relative), heptadecanoic acid (7.58% relative), and linoleic acid (18.54% relative) (Mulyati *et al.*, 2019) could reduce TG concentration in blood plasma approximately 25-50% if consumed regularly (Shearer *et al.*, 2012). Therefore, the TG

Table 2. Serum TG concentration of male Wistar Rats (*Rattus norvegicus* Berkenhout, 1769) during the 14 day-HTG induction period.

Group	Mean TG concentration (mg/dL)		Δ Mean TG concentration		Sig. (2-tailed)
	D-0	D-14	mg/dL	%	
H					
H.O	207.08±25.63	273.63±33.49	66.55	24.32	0.129
H.U					

H: HTG-induced, H.O: HTG-induced with Gemfibrozil treatment, H.U: HTG-induced with *U. lactuca* treatment, S.U: not HTG-induced with *U. lactuca* treatment, K.S: healthy control, not HTG-induced nor given treatment. Sig. (2-tailed) < 0.05 shows significant difference.

Table 3. Serum TG concentration of HTG male Wistar Rats (*Rattus norvegicus* Berkenhout, 1769) during the 40 day treatment period.

Group	Serum TG concentration (mg/dL)		
	D-0*	D-20*	D-40*
H	293.40±72.50 ^{a.w}	170.38±15.61 ^{c.w}	206.72±29.30 ^{ab.w}
H.O	286.38±48.84 ^{a.x}	122.50±5.44 ^{ab.w}	130.83±14.40 ^{a.w}
H.U	199.41±23.25 ^{a.x}	103.80±8.19 ^{a.w}	146.70±15.17 ^{ab.w}
S.U	196.05±44.28 ^{a.w}	131.65±7.15 ^{ab.w}	182.20±16.74 ^{ab.w}
K.S	233.80±30.68 ^{a.w}	151.02±17.60 ^{bc.w}	227.17±34.06 ^{b.w}

Data are presented as mean±SE. Different superscript letters note significant difference at $p < 0.05$: **abc** compares values between groups within the same day, **wx** compares values between days within the same group. H: HTG-induced, H.O: HTG-induced with Gemfibrozil treatment, H.U: HTG-induced with *U. lactuca* treatment, S.U: not HTG-induced with *U. lactuca* treatment, K.S: healthy control, not HTG-induced nor given treatment. *D-0, D-20, and D-40 of the treatment period.

concentration decrease in the H.U group was significant between D-0 and D-20 ($p < 0.05$).

However, at D-40 all groups have different TG concentrations. This might be caused by sensitization towards the administered fat. Sensitization is the opposite of tolerance, where there will be an increase in response when treated by a substance with the same dose continuously or repeated dose (Tomek & Olive, 2018). In addition, individual differences in TG concentrations can be influenced by differences in physiological sensitivity to the treatment or even the environment (André *et al.*, 2018; Nistiar *et al.*, 2012).

Gonadosomatic Index (of testes soaked in NBF for 24 hours)

This index shows the growth and development of the reproductive system, which correlates with sexual maturity (Barber and Black, 2006). In this research, an increase of testes weight was followed by an increase of GSI (Table 4). Based on Table 4, GSI of H and H.U groups were not significantly different ($p > 0.05$), although GSI of H group was greater than H.U group. This may be caused by the presence of the cadmium (Cd) contaminant in *U. lactuca*, which is 11-12 times higher than the recommended limit by BPOM 2018 (Mulyati *et al.*, 2019). Cadmium can inhibit the performance of bioactive nutrients in *U. lactuca* such as selenium (Se) and zinc (Zn) to enhance the male reproductive profile (Dewantari, 2013). According to Elhafeez *et al.* (2019), chronic exposure of Cd to Wistar rats caused a decrease of GSI because it can interfere with the HPG axis regulation by increasing the occurrence of lipid peroxidation, which further causes atrophy. In addition, according to El-Shahat *et al.* (2009), the presence of Cd in the body increases the risk of degenerative cell death (necrosis). Furthermore, it causes a decreasing number of Sertoli cells in the seminiferous tubules. A low GSI also can be caused by abnormal hormone levels, which might be influenced by the use of anti-lipid drugs such as Gemfibrozil. Lee *et al.* (2019) and Semet *et al.* (2017) found that Gemfibrozil may cause

hormonal imbalance in the body, therefore, inhibiting the growth and development of organs in the reproductive system.

Gonadosomatic index of S.U group was the highest amongst the other groups, suggesting that *U. lactuca* increases the growth and development of the reproductive system. *Ula lactuca* contains nutrients including Zn and Se. Zinc maintains sexual function and increases spermatogenesis. In fact, lack of Zn causes a reduction in testosterone production and shrinking of the testes, while Se serves as an antioxidant and increases fertility in males (Dewantari, 2013). Our results suggest that there is a dual effect by *U. lactuca*, where it increases GSI in healthy rats, but reduces GSI in HTG rats.

Serum Testosterone Concentration

The highest rise in testosterone concentration was shown in H group (Figure 1). An increase in testosterone concentration happens as a result of age maturity (Alvarado *et al.*, 2019; Stanworth & Jones, 2008). An increase in testosterone is followed by testes development. Cholesterol is a precursor of testosterone. Sources of cholesterol include de novo cholesterol synthesis, plasma membrane cholesterol, LDL, and HDL cholesterol, as well as lipid droplets (Hu *et al.*, 2010). Triglycerides are different from cholesterol, although Freeman and Ontko (1992) state that TG is stored in cells as lipid droplets as cholesteryl ester. Indirectly, these lipid droplets can also be used in steroidogenesis.

An increase of testosterone concentration was also found in H.U group, although only at 1.90% ($p > 0.05$). The presence of Cd in *U. lactuca* can prevent the bioactive nutrients found in *U. lactuca* such as Se and Zn, which play roles in increasing fertility, spermatogenesis, as antioxidants that can prevent the oxidation of sperm cells, and able to increase the production of testosterone hormone (Dewantari, 2013). According to Zeng *et al.* (2004), the presence of contaminants such as Cd in the human body can cause a decrease in the hormone testosterone, but not significant.

A significant decrease ($p < 0.05$) in testosterone

Table 4. Gonadosomatic Index (GSI) of male Wistar Rats (*Rattus norvegicus* Berkenhout, 1769).

Group	Total testes weight (gram)	GSI* (%)
H	2.16±0.36	0.655±0.122 ^{ab}
H.O	1.34±0.14	0.375±0.033^a
H.U	1.92±0.39	0.518±0.094 ^{ab}
S.U	2.82±0.24	0.773±0.141^b
K.S	1.62±0.31	0.455±0.184 ^a

Different superscript letters note a significant difference at $p < 0.05$. H: HTG-induced control, H.O: HTG-induced with Gemfibrozil treatment, H.U: HTG-induced with *U. lactuca* treatment, S.U: not HTG-induced with *U. lactuca* treatment, K.S: healthy control, not HTG-induced nor given treatment. *GSI of testes soaked in NBF 10% for 24 hours.

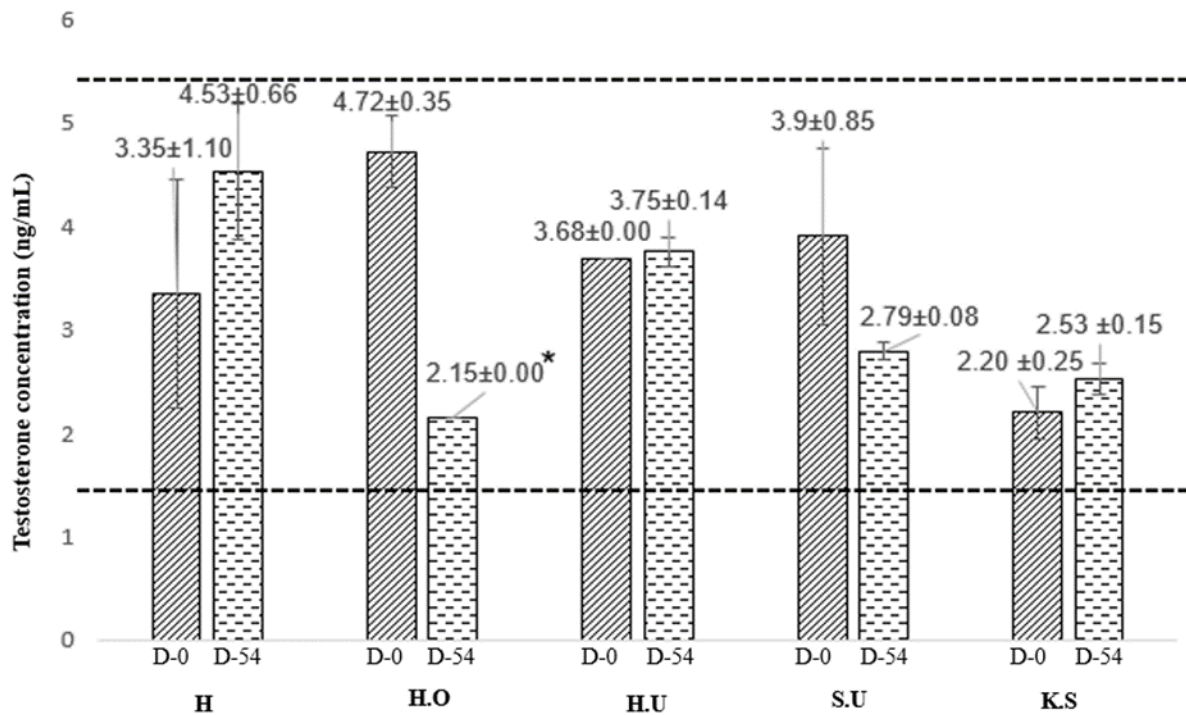


Figure 1. Serum testosterone concentration of male Wistar rats (*Rattus norvegicus* Berkenhout, 1769) on D-0 and D-54 of induction (D-40 of treatment).

Data are presented as mean ± SE. H: HTG-induced control, H.O: HTG-induced with Gemfibrozil treatment, H.U: HTG-induced with *U. lactuca* treatment, S.U: not HTG-induced with *U. lactuca* treatment, K.S: healthy control, not HTG-induced nor given treatment. *significant difference with D-0.

concentration was shown in H.O group. This is caused by the effect of Gemfibrozil as an antilipid, that can decrease its precursor, cholesterol, about 11 % (Frick *et al.*, 1987). Gemfibrozil affects exocrine function in spermatogenesis by altering spermatogenic cells and/or Sertoli cells. The effect of this drug on the endocrine system of the testes is by transforming or damaging Leydig cells and the hormone regulation of the Hypothalamus-Pituitary-Gonad axis (Semet *et al.*, 2017). Lee *et al.* (2019) found that Gemfibrozil given to *Oryzias latipes* significantly reduces testosterone concentrations of this freshwater fish. De Keyser *et al.* (2015) also states that the consumption of other cholesterol-lowering drugs such as statin significantly drops cholesterol concentration in humans and rats. Carrier *et al.* (2018) link male reproduction to depression by anxiolytic and antidepressant-like properties of testosterone.

Anxiety-like and Depressive-like Behaviors

Planchez *et al.* (2019) state that behavioral despair is a symptom of major depressive disorder that can be assessed as a depressive-like behavior in animal models through FST. Duration of immobility in FST reflects behavioral despair, as the administration of antidepressants reduces the duration of immobility (Cryan & Holmes, 2005). The highest duration of immobility is shown in S.U group, suggesting that

treatment with *U. lactuca* increases behavioral despair in this group, although this pattern is not shown in H.U group, which is not significantly different from, and in fact slightly lower than, H group (Figure 2a). *Ulva lactuca* might increase depressive-like behaviors because of its high Cd content (Mulyati *et al.*, 2019). Lamtai *et al.* (2018) has found that injection of Cd into rats at 0.25 – 1 mg/kg BW for 8 weeks increases depressive-like behaviors, while Scinicariello and Buser (2015) found that increased blood Cd levels increased possibilities of depressive symptoms in 20 – 39 years old men and women. Lamtai *et al.* (2018) discussed in their paper that chronic exposure to Cd could lead to Cd accumulation in the brain, where Cd inhibits enzymes of the serotonergic system, reducing serotonin levels. Low serotonin levels are more likely to be found in individuals with depression (Kamel *et al.*, 2011).

Thigmotaxis, the preference to be near the chamber's walls, is a sign of anxiety (Seibenhener & Wooten, 2015). Thigmotaxis can be visually observed in Figure 3 and supported by crossing frequency (Figure 2b). Fewer crossings to the central area mean that the rat tends to stay in the outer area, closer to the walls. Both H.U and H groups have the lowest values for crossing and rearing frequency (Figure 2b-c), and the highest values for the freezing duration (Figure 2d). These parameters illustrate

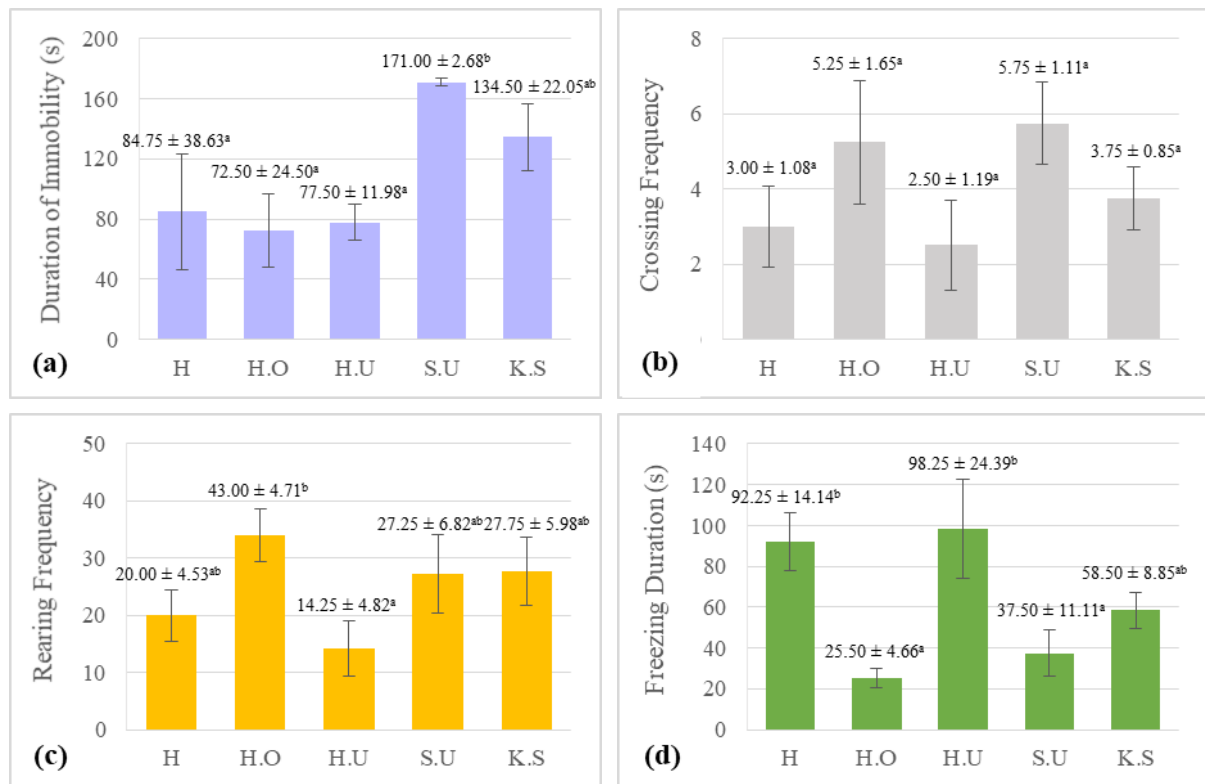


Figure 2. Behavioral data recorded from FST is the duration of immobility (a). Behavioral data recorded from OFT: number of movements crossing over between the central area and the outer area (b), frequency of rearing (c), and freezing duration (d). Data are presented as mean ± SE. Different superscript letters note a significant difference at $p < 0.05$. H: HTG-induced control, H.O: HTG-induced with Gemfibrozil treatment, H.U: HTG-induced with *U. lactuca* treatment, S.U: not HTG-induced with *U. lactuca* treatment, K.S: healthy control, not HTG-induced nor given treatment.

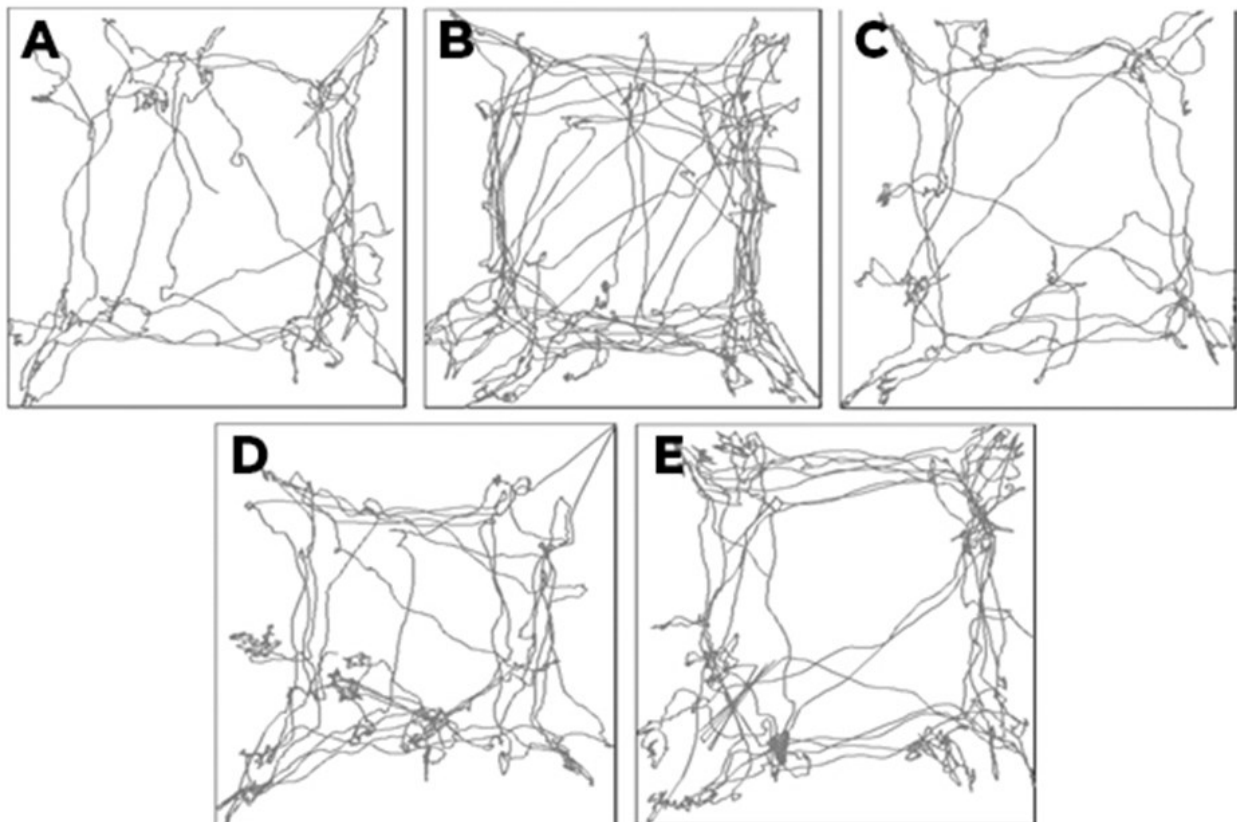


Figure 3. Trajectory pattern in OFT of rats from group H (a), H.O (b), H.U (c), S.U (d), and K.S (e) obtained from idTracker. Lines in each box represent the paths made by the animal on the chamber floor. H: HTG-induced control, H.O: HTG-induced with Gemfibrozil treatment, H.U: HTG-induced with *U. lactuca* treatment, S.U: not HTG-induced with *U. lactuca* treatment, K.S: healthy control, not HTG-induced nor given treatment.

exploratory activities in a novel environment (Choleris *et al.*, 2001; Ennaceur, 2014; Sestakova *et al.*, 2013). Furthermore, Magara *et al.* (2015) found that a rat model of depression showed less exploratory activities and more reactive coping (freezing, motionless behavior) when first introduced to a new cage, compared to healthy Sprague-Dawley rats, suggesting that exploratory behaviors in OFT can indicate depressive-like symptoms. High levels of TG in blood could cause TG to cross the blood-brain barrier (BBB), where it creates leptin resistance in the brain, through what might be an allosteric or post-receptor mechanism (Banks *et al.*, 2018). Leptin is a peptide hormone that has been found to have antidepressant properties (Lawson *et al.*, 2012; Lu, 2007). This might explain the increased depressive-like behaviors in the HTG-induced rats. These behaviors were lowered in H.O group, possibly because Gemfibrozil was able to lower and maintain serum TG.

In analyzing these behaviors, it must be noted that rearing has been interpreted as a result of both an anxiogenic and anxiolytic treatment in different studies (Ennaceur, 2014). A decrease in thigmotaxis of H.O suggests that Gemfibrozil has anxiolytic properties, further suggesting that in this research, rearing behavior is not an anxiety-like behavior, because H.O has the highest rearing frequency (Figure 2c). Freezing is considered a defensive behavior in avoiding danger (Choleris *et al.*, 2001), as well as a physiological sign of fear (Sestakova *et al.*, 2013). A decrease in the number of crossing and rearing, added with an increase of freezing in H.U group (Figure 2b-d), insignificant to H group, suggests that the *U. lactuca* treatment did not aid in reducing anxiety-like behaviors in HTG rats. Results from OFT also did not show that the *U. lactuca* treatment was able to reduce anxiety-like behaviors in healthy rats, except in crossing frequency, which is highest in S.U group, although insignificant to K.S group. The high level of Cd in *U. lactuca* (Mulyati *et al.*, 2019) might contribute to these results, canceling out the potential antihypertriglyceridemic effect in *U. lactuca*.

CONCLUSION

Hypertriglyceridemia increases body weight, serum TG and testosterone concentration, GSI, and depressive-like behaviors in FST and OFT. Treatment of HTG using *U. lactuca* at 1500 mg/kg BW/day did not show significantly different results compared to HTG controls. Meanwhile, an increase in GSI, testosterone concentration, and depressive-like behaviors can be observed compared to healthy controls. These results suggest that this treatment not only enhances gonad growth and development

but also increases depressive-like behavior.

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