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Concentration of *B-CG* and *sFlt-1* in Rattus Norvegicus Model of Preeclampsia with Swimming Exercise Treatment

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Abstract

Preeclampsia (PE) is a life-threatening pregnancy complication for the mother and fetus. High concentrations of human chorionic gonadotrophin (hCG) and soluble fms-like tyrosine kinase-1 (sFLt-1) during pregnancy may have a role in the pathophysiology of PE. Swimming Exercise (SE) is one of the physical activities recommended for pregnant women and carries a minimal risk. This study is aimed at analyzing the interaction between the conditions of rats (normal and PE), the onset of PE (early onset and late onset), and the time of SE (SE 0 minutes; SE 5 minutes; SE 10 minutes) on the concentrations of B-CG and sFlt-1 in the Rattus norvegicus (R. norvegicus) model of PE. 72 R. norvegicus were included in this study and divided into 12 experimental groups (each group n = 6 individuals). R. norvegicus PE was prepared by inducing L-Nitro-Arginine-Methyl Ester (L-NAME) at a 75 mg/kg BW/day dose. The determination of PE was supported by the observation of differences in the values of urine protein (PU), urine glucose (GU), and urine leukocytes (LU) in R. norvegicus before and after injection of L-NAME. The three-factorial statistical test showed a significant interaction between the concentration of B-CG and the condition of R. norvegicus, the onset of PE, and the time of SE, with a p-value <0.001. The three-factorial statistical test also showed a significant interaction between the sFLt-1 concentration and the condition of R. norvegicus, the onset of PE, and the time of SE with p<0.05. The difference in the concentration of B-CG and sFLt-1 R. norvegicus in each treatment group was influenced by the condition of the rats (normal and PE), the onset of PE (early onset and late onset), and the time of SE (SE 0 minutes; SE 5 minutes; SE 10 minutes). Research related to SE on PE still needs to be continued to decide on recommendations on whether SE can be used as a preventive measure in complementary midwifery care for preventing and reducing symptoms of PE in pregnancy.

Keywords:
Preeclampsia;
Early Onset;
Late Onset;
L-NAME;
B-CG;
sFlt-1;
Swimming Exercise.

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1- Introduction

Preeclampsia (PE) is a very serious complication of pregnancy, affecting 5–7% of all pregnancies and causing around 70,000 maternal deaths and 500,000 fetal deaths worldwide each year [1]. Various risk factors for PE have been suggested, including age, obesity, chronic hypertension, kidney disease, and diabetes mellitus. Despite the involvement of several risk factors in PE, the specific etiology is still unknown [2]. PE is clinically characterized by hypertension and

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proteinuria, which occur in 3–4% of pregnancies [3]. Historically, PE was characterized by early-onset development before 34 weeks of gestation and late-onset development after 34 weeks [4].

Recently, many studies have focused on the use of biomarkers related to the etiology of PE, and a number of potential biomarkers have been identified with various clinical uses [5]. Evidence suggests that Human Chorionic Gonadotrophin (hCG) appears to be involved in many aspects of angiogenesis and immune tolerance. Human Chorionic Gonadotrophin dysregulation can lead to pregnancy complications such as PE. Studies have found that the levels of hCG were found to be higher in PE full-term pregnancy serums compared to normal pregnancy serums [6]. High hCG concentrations in early pregnancy are associated with the risk of PE. The effect of high hCG concentrations on the balance between pro- and antiangiogenic factors during pregnancy may play a role in the pathophysiology of PE [7]. Pregnancy success depends on quiescence in the myometrium, so uterine contractions are delayed until the fetus has completed its full-term growth and maturation. A number of factors are essential in keeping the quiescence of the uterus quiet. First, circulating hCG levels continue to increase gradually in the second and third trimesters of pregnancy, where the hCG acts on receptors in the myometrium to maintain myometrial relaxation by inhibiting the production of gap junctions necessary for labor [8]. Apart from hCG, various clinical studies have shown a strong correlation between PE and levels of soluble fms-like tyrosine kinase-1 (sFlt-1) [9]. The ratio of sFlt-1 to placental growth factor (PlGF) increases substantially both before and at the clinical onset of PE [10]. Fms-like tyrosine kinase-1 is an antagonist of vascular endothelial growth factor (VEGF) and PlGF, which is considered a factor that causes endothelial damage in PE [11]. The resulting imbalance between molecular-vascular factors (PlGF, VEGF, and sFlt-1) during PE results in a higher susceptibility to oxidative cell damage of the placenta [12].

In response to many previous studies that have revealed the involvement of several PE predictive markers, including hCG and sFlt-1, research on physical activity as a complementary therapeutic opportunity in PE cases is still limited. The mother's lifestyle during pregnancy affects the mother's health and the fetus's development, one of which is physical activity such as swimming exercise (SE). Many researchers have revealed the health benefits derived from physical activity. Regular physical activity is highly recommended in all phases of life, including pregnancy [13–15]. One study showed that SE is one of the health system's most important activities and is considered a practical non-pharmacological approach to managing type 2 diabetes and hypertension [16]. A meta-analysis study showed that SE in pregnant women has a positive effect on preventing maternal weight gain and macrosomia (above the normal birth weight of the baby) [17]. Swimming exercise is a recommended physical activity for pregnant women and has minimal risks. Swimming exercise is one method to prevent many different risks during pregnancy, one of which is PE [18].

The purpose of this study was to analyze the interaction between the rat condition groups (Normal and PE), onset (early onset and late onset), and SE time (SE 0 minutes; SE 5 minutes; SE 10 minutes) on the concentration of chorionic gonadotropin beta (B-CG) and sFlt-1 in the R. norvegicus model injected with L-Nitro-Arginine Methyl Ester (L-NAME). Here, R. norvegicus c was injected with L-NAME, which is closely related to the pathogenesis of PE, at a dose of 75 mg/kg body weight/day, which was determined in the preliminary study based on modifications from previous researchers [19–22]. Before and after the experimental animals were injected with L-NAME, changes in the values of urine protein (PU), urine glucose (GU), and urine leukocytes (LU) were observed and used as supporting data for PE markers [23–25] using urinalysis reagent strips. The blood pressure of the rats was not checked and thus was not used as a marker for PE because of the limited research tools available in the research laboratory.

2- Materials and Methods

2-1- Materials

L-NAME was obtained from Sigma-Aldrich Pte Ltd. (Singapore). L-NAME was used to induce PE in the R. norvegicus model. Urinalysis reagent strips (ACON Laboratories, Inc. San Diego, USA) were used to measure PU, GU, and LU values. The enzyme-linked immunosorbent assay (ELISA) kit for rat B-CG was obtained from Bioenzy (Jakarta, Indonesia) catalog number BZ-08188250-EB, and the ELISA rat sFLt-1 kit was obtained from Bioenzy (Jakarta, Indonesia) catalog number BZ-08183321-EB.

2-2- Study Design

The study was conducted on pregnant R. norvegicus of the Wistar strain obtained from the Technical Implementation Unit (UPT) of Experimental Animals, Faculty of Veterinary Medicine, Universitas Syiah Kuala, Banda Aceh. This was a purely experimental study conducted in vivo in the laboratory with a three-factorial design. The first factor was the condition of R. norvegicus, namely the normal group (N) and the PE group; the second factor was the onset of PE, namely early onset (EO) and late-onset (LO); and the third factor was the time of SE, which is 0 minutes, 5 minutes, and 10 minutes [26]. The procedure used in this study can be seen in Figure 1.

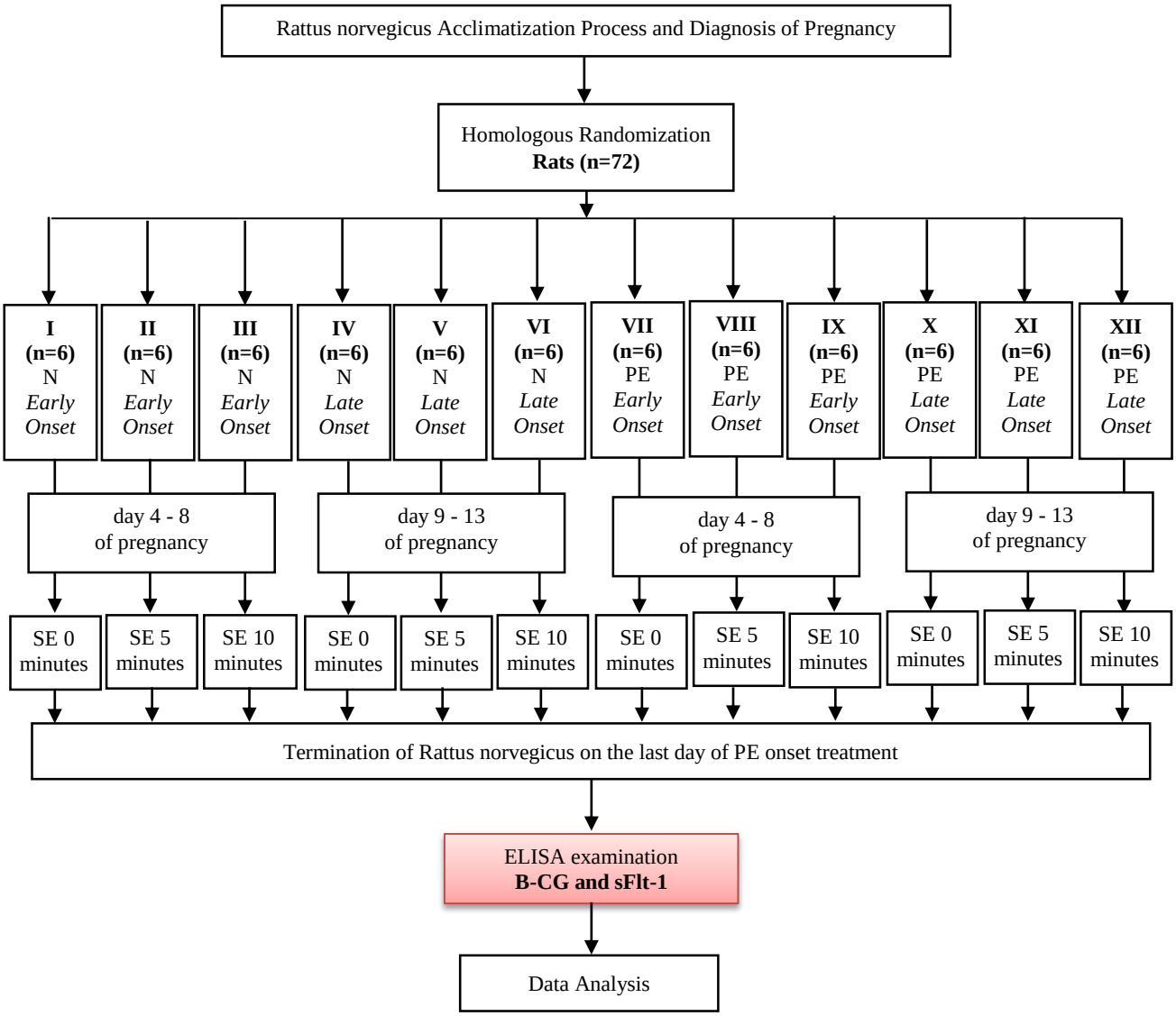

Figure 1. Flowchart of research procedures. R. norvegicus, by homologous randomization, were divided equally into 12 groups. The process of L-NAME injection in the early onset group of pregnant R. norvegicus was carried out on the fourth to the eighth day of pregnancy. In contrast, in the late-onset group of pregnant R. norvegicus, it was carried out on the ninth to the thirteenth day of pregnancy. Termination of R. norvegicus was carried out on the last day of treatment according to the PE onset group, plasma collection for analysis of B-CG and sFlt-1 concentrations.

2-3- Experimental Animal Treatment

Seventy-two R. norvegicus of Wistar virgin strains weighing 200–250 grams were acclimatized before mating. The acclimatization process for R. norvegicus was carried out for one week, during which R. norvegicus were allowed to swim for 15 minutes at the same time (at 09.00 a.m.) every day so that they became accustomed to the water environment. R. norvegicus was kept in a cage under controlled conditions and was given food and water ad libitum. Furthermore, female R. norvegicus were confined for 24 hours with male R. norvegicus, with a ratio of male and female R. norvegicus of 1:3. The occurrence of mating, which was marked with a vaginal plug and supported by the help of CCTV Robot IP Camera C. Robot PH A-33, was categorized as day 0 of pregnancy in female R. norvegicus. By homologous randomization, 72 pregnant rat samples were divided into 12 groups (n = 6 for each group).

Group I is the normal early onset R. norvegicus group with 0-minute SE (NEOSE0), Group II is the normal early onset R. norvegicus group with 5-minute SE (NEOSE5), Group III is the normal early onset R. norvegicus group with 10-minute SE (NEOSE10), Group IV is the R. norvegicus normal late onset with 0-minute SE (NLOSE0) group, Group V is the late onset normal R. norvegicus group with 5-minutes SE (NLOSE5), Group VI is the normal late onset R. norvegicus group with 10 minutes SE (NLOSE10), Group VII is the early onset R. norvegicus PE group without SE (PEEOSE0), Group VIII is a group of R. norvegicus PE early onset rats with 5 minutes SE (PEEOSE5, Group IX is the early onset R. norvegicus PE group with 10 minutes SE (PEEOSE10), Group X is the late onset R. norvegicus PE group without SE (PELOSE0), Group XI is a late onset R. norvegicus PE group with 5-minute SE (PELOSE5), and Group XII is a late onset R. norvegicus PE group with SE 10-minute SE (PELOSE10). The L-NAME injection process at a dose of

75 mg/kg body weight/day in pregnant R. norvegicus in the EO group was carried out on the fourth to the eighth day of pregnancy. In contrast, the pregnant R. norvegicus in the LO group was carried out on the ninth to the thirteenth day of pregnancy. One to two hours before and after R. norvegicus was injected with L-NAME. PU, GU, and LU values were checked as supporting data for PE markers using urinalysis reagent strips (Figure 2-a).

(a) (b)

Figure 2. Process of collecting R. norvegicus fresh urine before and after injection of L-NAME for examination of urine protein, urine glucose, and urine leukocytes by urinalysis reagent strips

The R. norvegicus group that received SE treatment was allowed to swim based on the time group. Swimming exercise was carried out in a glass pond measuring 30 cm wide × 30 cm long × 50 cm high filled with 30 cm of water, available at the Technical Implementation Unit (UPT) of Experimental Animals, Faculty of Veterinary Medicine, University Syiah Kuala, Banda Aceh. Swimming exercise was carried out at the same time (at 09.00 a.m.) every day (Figure 2-b). After swimming, R. norvegicus was dried using a hair dryer and put back into the cage. The treatment of SE R. norvegicus was a modification of previous studies [13]. Termination of R. norvegicus was carried out by euthanasia using inhalation of chloroform on the last day of treatment, according to the PE onset group. 2 ml of blood was taken through the heart for further plasma collection for analysis of B-CG and sFlt-1 concentrations.

2-4- Biochemical Analysis

An enzyme-linked immunosorbent assay (ELISA) was performed to determine the concentrations of B-CG and sFlt-1. The ELISA for rat B-CG and sFlt-1 was performed according to the manufacturer's instructions. Reagents, standard solutions, and samples were brought to room temperature according to the instructions before use. The test was carried out at room temperature. The number of strips required for the test was determined and loaded into the frame. Unused strips should be stored at 2–8 °C. Fifty microliters of the standard solution were added to the standard well. 40 microliters of the sample were added to the sample well, and then 10µl anti-COR antibody was added to the sample well. Next, 50µl streptavidin-HRP was added to the sample well and standard well (not the empty control well), then mixed thoroughly. The well plate was covered with sealer and incubated for 60 minutes at 37°C. The next step was to remove the sealer and wash the plate five times with a wash buffer. The wells were immersed in at least 0.35 ml of wash buffer for 30 seconds to 1 minute for each wash. All wells were aspirated with a volumetric pipette for automatic washing and washed five times with a wash buffer. The wells were then filled with wash buffer. The wheel plate was dried using a paper towel or other absorbent material. Fifty microliters of substrate A solution were added to each well, and then 50µl of substrate B solution were added to each well. The plate was incubated and covered with new sealer for 10 minutes at 37°C in a dark place. The reaction was stopped by adding 50µl Stop Solution to each well. The optical density was read at a wavelength of 450 nm using a microplate reader set within 10 minutes. Standard curves were generated using computer software. After subtracting the blanks, the value was obtained by comparing it with the standard curve.

2-5- Statistical Analysis

The PU, GU, and GU data collected were tested by repeated ANOVA statistical tests followed by post hoc paired-wise comparison tests. For data on concentrations of B-CG and sFlt-1, a three-factorial statistical test (ANOVA) was carried out with Duncan's post hoc follow-up test. A three-factorial test was carried out to analyze the interaction between groups of rat conditions and the onset and time of SE. Statistical analysis was conducted using SPSS 24.

3- Results

3-1- Urine Protein, Urinary Glucose, and Urinary Leukocytes

The mean levels of PU, GU, and LU are presented in Figures 3 to 5, respectively. Differences in PU, GU, and LU levels in the N and PE groups before and after L-NAME injection are presented in Table 1. PU levels on day 1 showed no difference before L-NAME injection between the N and PE groups ($p>0.05$), but there was a difference in PU levels after L-NAME injection between the N and PE groups ($p<0.001$). PU levels on day 2 showed differences between the N and PE groups before and after the injection of L-NAME ($p<0.001$). PU levels on day 3 showed a difference before L-NAME injection between the N and PE groups ($p<0.05$). There was a difference in PU levels after L-NAME injection between the N and PE groups ($p<0.001$). PU levels on day 4 showed differences before L-NAME injection between the N and PE groups ($p<0.05$). There were differences in PU levels after L-NAME injection between the N and PE groups ($p<0.001$). PU levels on day 5 showed that there was a difference before L-NAME injection between the N and PE groups ($p<0.05$), and there was a difference in PU levels after L-NAME injection between the N and PE groups ($p<0.001$). Post hoc paired-wise comparison results showed differences in PU levels before and after L-NAME injection from the first to the fifth day of observation ($p<0.001$).

Table 1. Differences in urine protein, urine glucose, and urine leukocyte levels in the normal and PE groups before and after injection of L-NAME in R. norvegicus were observed every day (days 1 to 5) in the experiment

Parameter	Treatment Group	Examination Day									
		Day 1		Day 2		Day 3		Day 4		Day 5	
		Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Urine Proteins (mg/dL)	Normal	45.56 ± 29.51	45.56 ± 29.51	49.44± 31.80	49.44± 31.80	45.56± 29.51	45.56± 29.51	41.67± 26.46	41.67± 26.46	41.67± 26.46	41.67± 26.46
	PE	37.78 ± 22.31	850.00± 826.52	147.78± 98.19	1102.78± 860.73	294.72± 530.75	1002.78± 855.40	387.22± 663.31	1002.78± 855.40	387.22± 663.31	1002.78± 855.40
	P-Value	>0.05	<0.001	<0.001	<0.001	<0.05	<0.001	<0.05	<0.001	<0.05	<0.001
	Post hoc	<0.001									
Urine Glucose (mg/dL)	Normal	120.83± 52.61	131.94± 82.07	104.17± 25.00	115.28± 70.53	100.00± 0.00	104.17± 25.00	108.33± 34.85	104.17± 25.00	104.17± 25.00	108.33± 34.85
	PE	150.00± 71.71	280.56± 131.08	131.94± 82.07	198.61± 129.00	108.33± 34.85	156.94± 91.15	100.00± 0.00	136.11± 84.19	116.67± 47.81	143.06± 102.23
	P-Value	>0.05	<0.001	>0.05	<0.05	>0.05	<0.05	>0.05	>0.05	>0.05	>0.05
	Post hoc	<0.001									
Urine Leukocytes (Leu/µL)	Normal	31.67± 42.41	28.75± 37.84	38.06± 40.73	26.11± 31.22	40.42± 86.23	30.83± 42.89	47.50± 85.29	43.61± 85.78	18.06± 28.69	17.78± 26.82
	PE	20.00± 29.78	12.78± 21.66	44.03± 33.25	31.81± 34.10	55.28± 28.11	49.58± 37.35	77.22± 42.60	74.44± 47.63	43.47± 88.45	19.58± 29.96
	P-Value	>0.05	<0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05
	Post hoc	>0.05									

Data is presented as mean±SD (n=6). Significant differences were indicated by p values <0.05 and p values <0.001 through repeated ANOVA tests followed by post hoc paired-wise comparison.

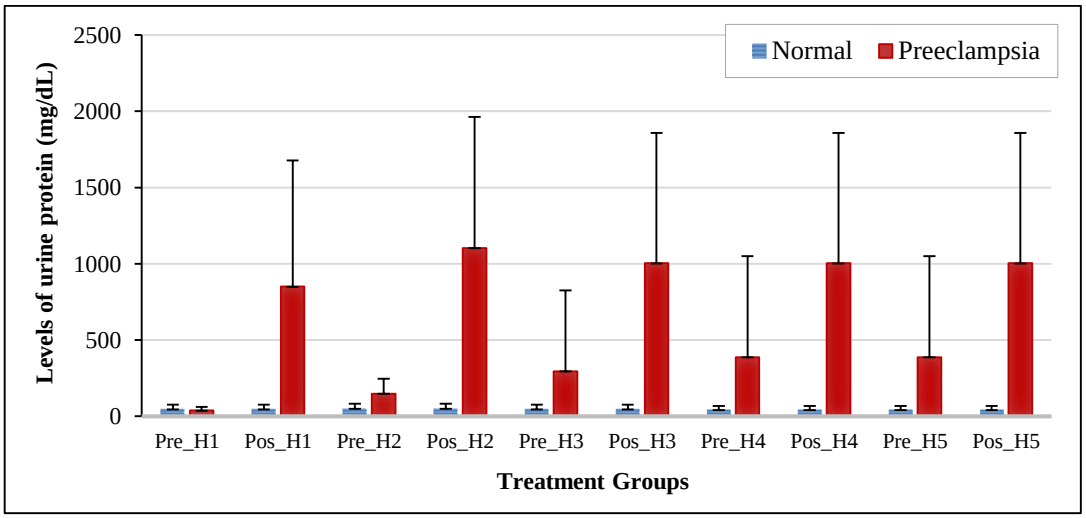

Figure 3. Differences in urine protein levels in the N and PE groups before and after injection of L-NAME in R. norvegicus were observed every day (days 1 to 5) of the experiment

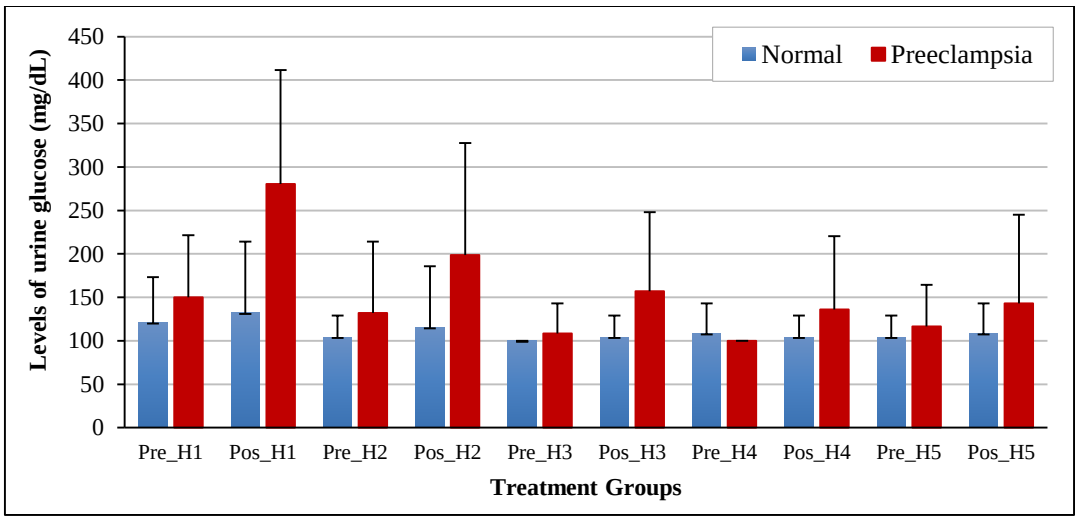

Figure 4. Differences in urine glucose levels in the N and PE groups before and after injection of L-NAME in R. norvegicus were observed every day (days 1 to 5) of the experiment

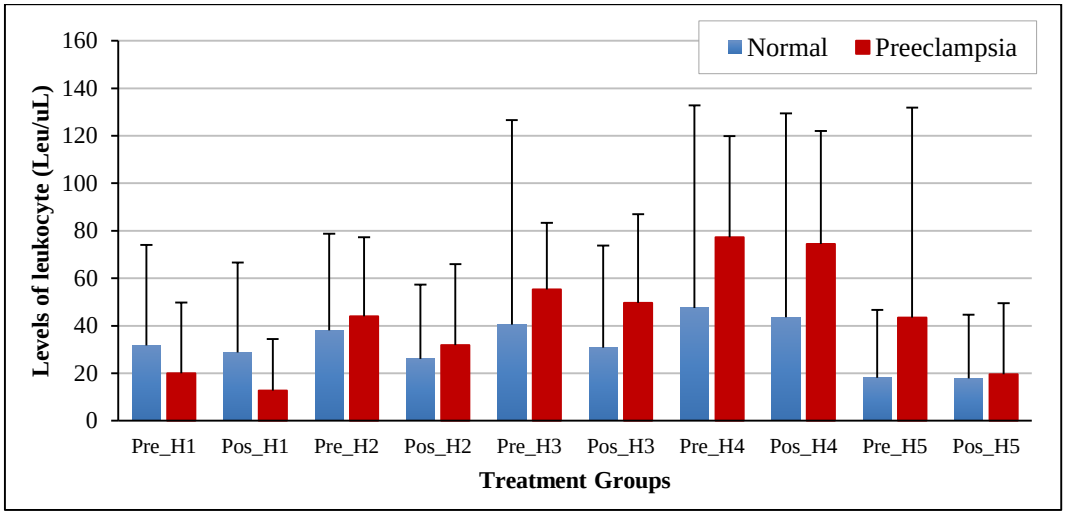

Figure 5. Differences in urinary leukocyte levels in the N and PE groups before and after injection of L-NAME in R. norvegicus were observed every day (days 1 to 5) of the experiment

GU levels on day 1 showed no difference in GU levels before L-NAME injection between the N and PE groups ($p>0.05$), but there were differences in GU levels after L-NAME injection between the N and PE groups ($p<0.001$). GU levels on day 2 showed no difference before L-NAME injection between the N and PE groups ($p>0.05$), but there was a difference in GU levels after L-NAME injection between the N and PE groups ($p<0.05$). GU levels on day 3 showed no difference before L-NAME injection between the N and PE groups ($P>0.05$), but there was a difference in GU levels after L-NAME injection between the N and PE groups ($p<0.05$). GU levels on day 4 showed no difference before and after L-NAME injection between the N and PE groups ($p>0.05$). Likewise, GU levels on day 5 showed no difference before or after L-NAME injection between the N and PE groups ($p>0.05$). A post hoc paired-wise comparison showed differences in GU levels before and after L-NAME injection from the first to the fifth day of observation ($p<0.001$).

LU levels on day 1 showed no difference in LU levels before L-NAME injection between the N and PE groups ($p>0.05$), but there were differences in LU levels after L-NAME injection between the N and PE groups ($p<0.05$). LU levels on day 2, day 3, day 4, and day 5 showed no difference between groups N and PE before and after L-NAME injection ($p>0.05$). A post hoc paired-wise comparison showed no difference in LU levels before and after L-NAME injection from the first to the fifth day of observation ($p>0.05$).

3-2- Concentration of B-CG and sFlt-1 in Rattus Norvegicus Model of Preeclampsia with Swimming Exercise (SE)

The average concentration of B-CG is presented in Figure 6. The three-factorial statistical test showed a significant interaction between the B-CG concentration and the condition of the rats, the onset of PE, and the time of SE with $p<0.001$. Through Duncan's post hoc, the highest B-CG concentration was significantly highest in the NEOSE0 rat group (mean 22.05 ± 3.35 mIU/mL). The next highest B-CG concentration was significantly found in the PEEOSE10 rat group (mean 20.98 ± 1.84 mIU/mL), and the lowest B-CG concentration was significantly found in the PELOSE10 group (mean 16.15±1.02 mIU/mL) (Table 2).

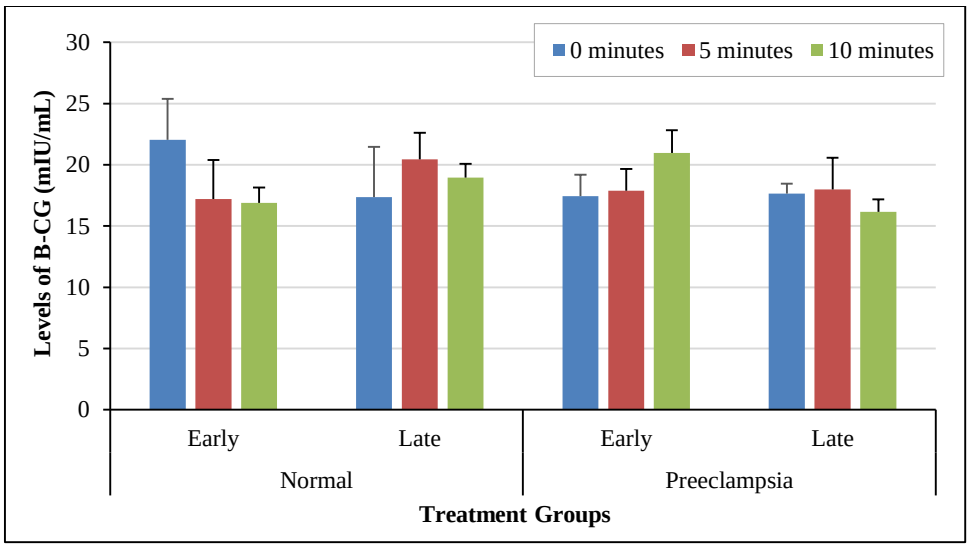

Figure 6. Differences in the concentration of B-CG R. norvegicus in each experimental group

Table 2. B-CG and sFlt-1 concentrations in R. norvegicus model of PE with SE

Parameter	SE (minute)	Treatment Group			
		N		PE	
		EO	LO	EO	LO
B-CG (mIU/mL)	SE0	22.05±3.35^{a}	17.37±4.08^{c}	17.44±1.75^{b}	17.64±0.83^{b}
	SE5	17.21±3.18^{c}	20.43±2.17^{a}	17.88±1.77^{b}	17.99±2.59^{b}
	SE10	16.90±1.24^{c}	18.95±1.13^{b}	20.98±1.84^{a}	16.15±1.02^{c}
sFlt-1 (ng/mL)	SE0	3.00±0.92^{a}	1.97±0.48^{c}	2.43±0.75^{b}	3.76±0.60^{a}
	SE5	3.32±1.06^{a}	3.43±0.79^{a}	2.48±0.61^{b}	2.49±0.57^{b}
	SE10	3.31±0.94^{a}	2.64±0.86^{b}	2.79±0.93^{b}	1.99±0.21^{c}

Data are presented as mean±SD (n=6). Using Duncan's test, different subscript letters in one column showed significant differences ($p<0.05$ and $p<0.001$)

The average concentration of sFlt-1 is presented in Figure 7. The three-factorial statistical test showed a significant interaction between the sFLt-1 concentration and the condition of the mice, the onset of PE, and the time of SE with $p<0.05$. Duncan's post hoc test showed that the highest concentration of sFlt-1 was significantly highest in the PELOSE0 rat group (mean 3.76 ± 0.60 ng/mL). The next highest concentration was significantly found in the NLOSE5 rat group (mean 3.43 ± 0.79 ng/mL), and the lowest concentration of sFLT-1 was significantly lower in the NLOSE0 rat group (mean 1.97±0.48 ng/mL) (Table 2).

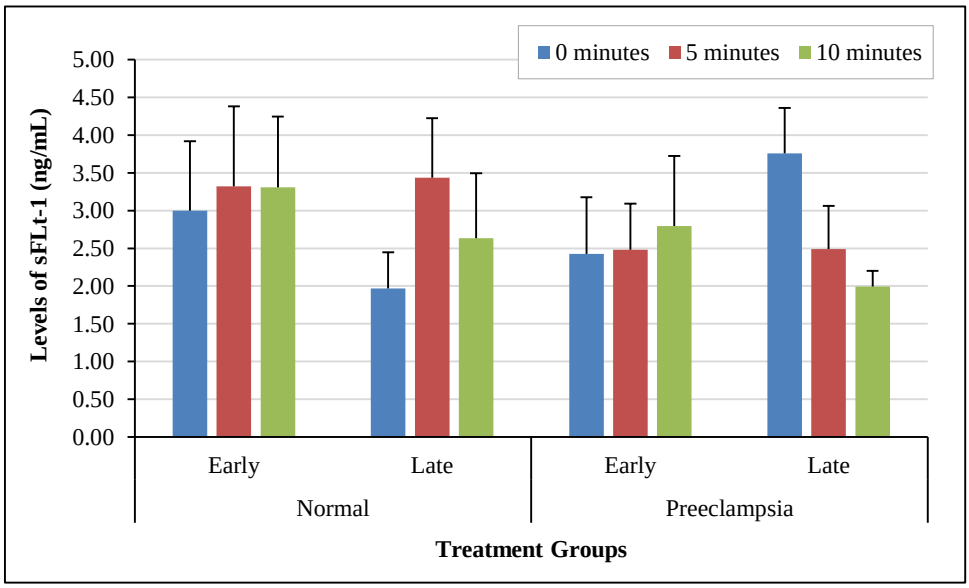

Figure 7. Differences in the concentration of sFlt-1 in R. norvegicus in each experimental group

4- Discussion

In this study, PE was observed in R. norvegicus after injection of L-NAME based on PU, GU, and LU levels. Previously, studies have measured proteinuria in experimental animals in semi-quantitative management using urinalysis reagent strips on fresh urine samples. This colorimetric assay is relatively specific for albumin, rated from 1+ to 4+, which corresponds to the following approximate protein concentrations: 1+: 30 mg/dL, 2+: 100 mg/dL, 3+: 300 mg/dL, 4+: more than 1000 mg/dL. A urine protein level greater than 100 mg/dL is considered positive evidence of proteinuria. Severe proteinuria was defined as ≥3+ on the urine dipstick, and mild proteinuria was defined as <3+ [21]. Here we try to observe whether urinalysis reagent strips can also be applied to GU and LU examinations in R. norvegicus to obtain PE markers.

This study showed significant differences in PU levels in the R. norvegicus group before and after the injection of L-NAME, which were observed during the five days of the experiment. A study showed that giving L-NAME to pregnant R. norvegicus can inhibit nitric oxide (NO), which causes long-term hypertension, hypovolemia, intrauterine growth retardation, proteinuria, thrombocytopenia, and abnormal kidney morphology, similar to the symptoms of hypertensive disorders in pregnancy [19]. In contrast, a recent review of studies stated that repeated measurement of proteinuria for women with PE is not recommended. Proteinuria does not appear to be associated with poor maternal and neonatal outcomes. The diagnosis of PE in women with chronic kidney disease may be based on changes in protein excretion. Evaluation of basic protein excretion is essential in certain conditions such as chronic hypertension, diabetes, and autoimmune or other renal disorders [25].

In observing GU, this study also showed significant differences in GU levels in the R. norvegicus group before and after injection of L-NAME, which were observed for five days of the experiment. One study showed that GU patients had higher proteinuria, serum creatinine, and a lower estimated glomerular filtration rate (eGFR) [27]. Other studies have shown that a history of PE may serve as an additional risk factor for gestational diabetes mellitus (GDM) in subsequent pregnancies, as GDM is independently associated with PE in singletons. Optimizing the treatment and management of GDM can reduce the incidence of PE, which helps improve pregnancy outcomes [24, 28]. The levels of LU were also observed, where the results showed no significant difference in LU levels in the group of mice before and after injection of L-NAME, which were observed during the five days of the experiment. These results align with a study that showed increased levels of adhesion molecules in leukocytes or decreased levels of soluble adhesion molecules in plasma that were not associated with prematurity or any degree of PE [29].

Next, the R. norvegicus group was observed, which was given the SE treatment, and analyzed the concentrations of B-CG and sFlt-1. The results of this study showed a significant interaction between the R. norvegicus condition, the onset of PE, and the time of SE on B-CG concentration and sFlt-1 concentration as a predictive protein marker for PE. Chorionic gonadotrophin in humans operates as the main regulator of human pregnancy, which functions in blastocyst development, implantation, vascular remodeling, placental invasion, maternal immunosuppression in early pregnancy, and fetal development [30]. One study showed that high and/or low hCG concentrations were associated with PE risk and increased sFlt-1 concentrations, which are common in PE women and may be a marker of impaired placentation [7]. In one study, measurements of pre- and post-exercise plasma sFlt-1 levels in 63 white and African American female volunteers aged between 18 and 44 years showed that moderate exercise significantly increased plasma sFlt-1 levels. The data supports the hypothesis that exercise-induced plasma sFlt-1 levels could be an important clinical biomarker to explore the mechanism of exercise training in reducing breast cancer development [31].

There are no studies of the exact same nature to this study so far, as other research was traced and used for the references in this study. However, many studies have expressed opinions about the benefits of SE and other physical activities during pregnancy. Exercise during pregnancy has a potential therapeutic effect on reducing fetal stress [32]. Swimming administered to small-for-gestational-age mice when they were mature and pregnant reduced DNA damage and lipid peroxidation. In addition, maternal physical exercise results in less genotoxicity in the offspring, reducing/preventing DNA damage and macrosomia [33]. Other studies have revealed that involuntary swimming exercise can have an undesirable effect on the neurodevelopment of the fetus, in which there is an increase in cortisol levels in the amniotic fluid [34]. This gives confidence that good physical exercise for pregnant women is a physical exercise that is done regularly, not physical exercise that is forced. A prospective study examining the association between swimming in pregnancy and preterm and post-term birth, small fetal growth size for gestational age, and congenital malformations did not show that swimming in the pool was associated with adverse reproductive outcomes [35].

As far as our observations in this study, SE is a physical activity that is safe to do in PE, EO, and LO cases. Swimming exercise with regular time duration and under expert supervision affects healthy and sick pregnant women, such as those experiencing PE. The limitation of this study was that it only examined two of the many marker proteins predictive of PE. In the future, it will be essential to investigate the association of SE with changes in other markers, accompanied by histological analysis of R. norvegicus PE tissue.

5- Conclusion

Giving L-NAME 75 mg/kg body weight/day in pregnant rats can induce Rattus norvegicus to become PE, characterized by increased urine protein and glucose. In the 5-day observation, there was a significant difference in the urine protein and glucose levels in the group of mice before and after the L-NAME injection. However, there was no significant difference in the urinary leukocyte levels in the group of mice before and after the L-NAME injection. Observation of SE activity in Rattus norvegicus showed that there was a significant interaction between the condition of the rats (N and PE), the onset of PE (EO and LO), and the time of SE (SE 0 minutes, SE 5 minutes, and SE 10 minutes) on the concentration of B- CG and sFlt-1 concentration as a PE predictive protein marker. In further tests, it was observed that the average concentrations of B-CG and sFlt-1 in R. norvegicus PE can change with SE activity, both in EO and LO PE conditions, with a duration of SE of 5 minutes or SE of 10 minutes. These findings prove that SE is safe in PE cases during the EOPE and LOPE periods. SE can prevent or reduce PE symptoms as long as it is carried out under expert supervision and with a regulated time duration. Further research is needed on the extent to which SE can be used as complementary midwifery care, especially for preventing or treating PE cases. There are still many ways to prevent and treat PE that is still unknown. It is expected that the research conducted in this study will become of interest to academics and research funding providers who are continuously working to solve this problem in order to reduce maternal and infant mortality.

6- Declarations

6-1- Author Contributions

Conceptualization, O.S., and M.A.; methodology, G.G.; software, O.S. and G.G.; validation, S.W., K.K., and O.S.; formal analysis, O.S.; investigation, O.S. and G.G.; resources, O.S.; data curation, O.S.; writing—original draft preparation, O.S., M.A., and S.W.; writing—review and editing, O.S., M.A., S.W., K.K., and G.G.; visualization, O.S.; supervision, M.A., S.W., K.K., and G.G.; project administration, O.S.; funding acquisition, O.S. All authors have read and agreed to the published version of the manuscript.

6-2- Data Availability Statement

The data presented in this study is available on request from the corresponding author.

6-3- Funding

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6-5- Institutional Review Board Statement

Ethical approval for experimental animals was issued by the Veterinary Ethics Committee, Faculty of Veterinary Medicine, Syiah Kuala University, Banda Aceh, Ref: 61/KEPH/VIII/2020, on August 15, 2020.

6-6- Informed Consent Statement

Not applicable.

6-7- Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancies have been completely observed by the authors.

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