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Valproic Acid Administration in Pregnant Rats Affects Litter Size, Birth Weight, and Postnatal Growth of the Offspring

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Abstract

An experiment was conducted to study the effects of valproic acid, a well-known teratogen compound during the process of organogenesis, on fetal survival, prenatal and postnatal growths of the offspring. Thirty pregnant female Sprague-Dawley rats were divided into 4 groups i.e., pregnant rats without valproic acid administration as a control and pregnant rats administered with 250 mg valproic acid on days 10, 13, 16 of pregnancy. Litter size at parturition and individual birth weight of the offspring were measured. A total of 84 offspring rats born were used to measure the growth performances until the age of 32 weeks postpartum. The results showed that the administration of valproic acid on days 10 and 13 of pregnancy reduced the survival of fetus during pregnancy that was reflected in the decreased number of offspring born or litter size at parturition.

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The litter size and birth weights of the offspring were significantly different in maternal rats administered with valproic acid on days 10 and 13 of pregnancy. The litter size of the maternal experimental rats administered valproic acid on days 10 and 13 of pregnancy decreased by 64.76 and 41.62%, respectively, compared to control. However, the prenatal growth and development of the offspring were improved due to the decreased number of litter size during pregnancy. The average birth weight of the offspring rats born to the maternal rats administered with valproic acid on days 10 and 13 of pregnancy increased by 57.86 and 33.21%, respectively, compared to control. The improved prenatal growth as indicated by the increased birth weights of offspring rats born to maternal rats administered with valproic acid on days 10 and 13 of pregnancy significantly increased post natal growth as indicated by the higher growth rate from birth to the mature age of 32 weeks. The reduced litter size in maternal rats administered valproic acid on days 10 and 13 of pregnancy did not decrease the postnatal growth of the offspring. Administration of valproic acid on day 16 of pregnancy did not affect litter size as well as prenatal and postnatal growth performances of the offspring.

Keywords: Litter Size; Birth Weight; Postnatal Growth; Valproic Acid.

1. Introduction

Administration of drugs or certain chemicals during pregnancy, especially during the stage of organogenesis, will affect embryonic development [1] that can affect the structure and functions of organs, embryonic and fetal survivals [2], as well as prenatal growth and development [3] that eventually affect quality of post natal life [4]. Valproic acid (VPA, 2-propylpentanoic acid) is a simple 8-carbon branched chain carboxylic acid that has been used extensively in the clinic for over three decades for the management of epilepsy and other seizure disorders [5]. VPA is a drug that affects the modification of histones during the process of proliferation and cells differentiation [6] by influencing the activity of histone deacetylase (HDAC) [7] which plays an important role in gene expression during the process of organogenesis [8].

Valproic acid has toxic effects than can affect the pregnant maternal rats and the growing embryos and fetus in the uterus and placenta. Study in Sprague-Dawley rats showed that oral administration of 200-800 mg/kg VPA (5-20x human therapeutic dose) from gestational days 8 to 17 resulted in increasing maternal toxicity at the higher doses with 100% maternal lethality at 800 mg/kg [9]. Administration of valproic acid in various animals on variable gestational days during organogenesis resulted in dose-dependent developmental toxicity manifested as increased embryo/fetal mortality, intrauterine growth retardation, and craniofacial and skeletal defects [10].

Valproic acid is well known to have teratogenic effects in human and animals and exposure of this compound during pregnancy resulted in the increased rate of major anomalies such as cardiac, craniofacial, skeletal, and limb defects and a possible set of dysmorphic features and the decreased intrauterine growth [11]. It was shown that exposure to valproic acid during pregnancy resulted in developmental defects of the digits and, especially, the axial skeleton, both in mice and rats [12].

Studies in Sprague-Dawley rats revealed dose-dependent fetal growth retardation as evidenced by the decreased fetal weight and length and the incidence of skeletal defects, including abnormal vertebrae, ribs, and craniofacial

dysmorphia in addition to the under-ossification of both the axial and appendicular skeleton [9]. In addition, administration of valproic acid in high doses was reported to produce intrauterine growth retardation, craniofacial, and skeletal anomalies in rhesus monkeys [10]. Further study in mice indicated that multiple administrations of VPA on day 9 of gestation result in a low incidence of spina bifida aperta and a high incidence of spina bifida occulta, and provides a relevant model for the study of human spina bifida defects. [13]. Injection of VPA (350 mg VPA-Na/kg body weight) subcutaneously three times on d 9 of gestation at 0, 6, and 12 h induced spina bifida in mice [14].

Since most effects of valproic acid are on histone deacetylase (HDAC) which plays an important role in gene expression during the process of organogenesis, this study was designed to evaluate the effects of single dose of valproic acid administrations on days 10, 13, and 16 of pregnancy on the survival of fetus as was reflected in the number of offspring born at parturition by using Spraque-Dawley rats as a model for polytocus animal. In addition, to evaluate prenatal growth and development, birth weight of the experimental offspring rats were measured. To study the effect of valproic acid administration during pregnancy on the postnatal performance of the offspring, body weights and growth rates of the offspring experimental rats were measured at the ages of 4, 8, 12, 16, 20, 24 and 32 weeks. The limitation of this study was the mortality of fetus during pregnancy was not measured. For further study, measurement of fetal mortality at certain ages of pregnancy will be required to explain the effect of single dose injection of valproic acid on fetal survival and mortality. In addition, it is important to study the structures and functions of organs in offspring born to maternal rats injected with valproic acid on days 10, 13, and 16 of pregnancy.

2. Materials and Methods

Thirty mature female Sprague-Dawley rats with the ranges of body weights of 200-250 g and age of 3-4 months were used in this study. The experimental rats were adapted to the experimental conditions before treatment. Prior to mating, the experimental rats were injected with PGF2α (Noroprost®) intramuscularly to synchronize estrous cycle. The experimental female rats showing estrous signs were mixed with male rats for mating. The presence of the sperm in the vagina was determined as day 0 of pregnancy.

During pregnancy, all pregnant experimental rats were maintained with the same management and feeding condition. The treatment was administration of 250 mg valproic acid on days 10, 13, or 16 of pregnancy. The days of administration were chosen based on the effects of valproic acid on the proliferation and differentiation of the pancreas, which starts from the day 8.75 to the day 12.5. The pregnant experimental rats were assigned into a completely randomized design with 4 treatments i.e., 1) pregnant rats without valproic acid administration as a control (T1); 2) pregnant rats administered with valproic acid (Depakote® manufactured by Abbott Laboratories) at a dose of 250 mg orally on day 10th of pregnancy (T2), at the same time with the expression of Pdx1 gene; 3) pregnant rats administered with valproic acid at a dose of 250 mg on day 13th of pregnancy (T3), at the same time with the expression of Nkx6.1 gene; and 4) pregnant rats administered with valproic acid at a dose of 250 mg on day 16th of pregnancy (T4), at the same time with the expression of Ngn3 gene.

The experimental pregnant rats were maintained until parturition. At parturition, the number of pups born to each maternal rats were measured and birth weights of the offspring rats were measured and the offspring rats were maintained with their maternal rats until weaning at the age of 1 month. At the age of 1 month, the offspring rats were separated from their maternal rats. A total of 84 of offspring rats, each group with 21 experimental offspring rats, were maintained until the age of 32 weeks or 8 months postpartum. The experimental offspring rats were sacrificed at the ages of 4, 8, 12, 16, 20, 24, and 32 weeks (3 experimental offspring rats from each treatment group) and body weights were measured to evaluate the growth performance of the offspring experimental rats born to the maternal experimental rats administered with valproic acid on days 10, 13, and 16 of pregnancy. The experiment was conducted according the Animal Ethics issued by the Department of Pathology of Bogor Agricultural University with the registration number SKEH Number: 031/KEH/SKE/IV/2015.

The collected data on litter size of experimental maternal rats, birth weight, and growth rate of the offspring rats born to the experimental maternal rats were analyzed using analysis of variance (ANOVA). All of the data analysis was done by the general linear models procedure in SAS version 9.4 program. Results were expressed as mean ± SD. If there is a specific difference ($P < 0.05$) in the mean of each group, the Duncan post-hoc test [15] was conducted.

3. Results

3.1. Litter size and birth weight of the experimental offspring rats

The observation on litter size or the number of offspring rats born in the maternal rats showed that maternal rats without valproic acid administration had normal and the highest litter size among the groups of experimental rats. Administration of 250 mg valproic acid on days 10 (T2) and 13 (T3) of pregnancy significantly decreased litter size ($P<0.05$). Maternal rats administered with 250 mg valproic acid on days 10 of pregnancy had the lowest litter size and decreased by 64.76% compared to control without valproic acid administration. However, administration of valproic acid on later stage on day 13 of pregnancy only reduced litter size by 41.62% compared to control maternal rats without valproic administration. Extension of valproic acid administration from day 10 to day 13 of pregnancy increased litter size by 65.68% (Table 1).

Table 1: The average litter size and birth weight of the experimental offspring rats born to control maternal rats (T1) and those born to maternal rats administered with 250 mg valproic acid on days 10 (T2), 13 (T3), and days 16 (T4) of pregnancy.

Group	Number of Maternal rats	Number of Offspring	Litter Size	Birth Weight (g)
T1	6	63	10.50 ± 1.38^{a}	5.42 ± 0.90^{c}
T2	10	37	3.70 ± 0.82^{c}	8.54 ± 1.06^{a}
T3	8	49	6.13 ± 1.13^{b}	7.22 ± 0.97^{b}
T4	6	59	9.83 ± 1.60^{a}	5.14 ± 0.60^{c}

Different superscripts in the same row indicate a significant difference (P <0.05)

Administration of valproic acid on later stage of pregnancy on day 16 of pregnancy (T4) did not affect litters size (P>0.05) and this group of experimental rats had similar litter size with the control maternal rats without valproic administration. The experimental maternal rats administered with 250 mg valproic acid on day 10 of pregnancy had 62.36% lower litter size compared to those administered with valproic acid on day 16 of pregnancy and experimental maternal rats administered with valproic acid on day 16 of pregnancy had 165.68% higher litter size compared to those administered with valproic acid on day 10 of pregnancy. The experimental maternal rats administered with 250 mg valproic acid on day 13 of pregnancy had 37.64% lower litter size compared to those administered with valproic acid on day 16 of pregnancy and experimental maternal rats administered with valproic acid on day 16 of pregnancy had 60.36% higher litter size compared to those administered with valproic acid on day 13 of pregnancy

3.2. Birth weights (g) of experimental offspring rats (regardless of slaughtering age)

The higher the litter size in the control maternal rats without valproic acid administration and maternal rats administered with 250 mg valproic acid on day 16 of pregnancy will increase the competition in obtaining nutrients and compounds required by the developing fetuses that eventually affected the growth and development of the fetuses that will be realized in the lower birth weight. The observation in this experiment showed that the average birth weight of offspring born to maternal rats administered with 250 mg valproic acid on days 10 and 13 of pregnancy were significantly higher (P<0.05) (due to the lower litter size) compared to those of born to control maternal rats without valproic acid administration and those born to maternal rats administered with 250 mg valproic acid on day 16 of pregnancy. Similar to the pattern of litter size, the average birth weights of experimental offspring rats born to control maternal rats and maternal rats administered with 250 mg valproic acid on day 16 of pregnancy were similar (P>0.05) and were significantly lower (P<0.05) than those born to maternal rats administered with 250 mg valproic acid on days 10 and 13 of pregnancy (Table 1).

The average birth weight of offspring rats born to experimental maternal rats administered with 250 mg valproic acid on day 10 of pregnancy were 57.56% higher (P<0.05) compared to those born to control maternal rats without valproic administration. The average birth weight of offspring rats born to experimental maternal rats administered with 250 mg valproic acid on day 13 of pregnancy increased by 33.21% (P<0.05) compared to those born to control maternal rats without valproic administration (Table 1).

The average birth weight of offspring rats born to experimental maternal rats administered with 250 mg valproic acid on day 10 of pregnancy increased by 18.28% (P<0.05) compared to those born to maternal rats administered with 250 mg valproic acid on day 13 of pregnancy. The average birth weights of offspring rats born to maternal rats administered with 250 mg valproic acid on day 10 of pregnancy were 66.15% higher (P<0.05) than those of born to maternal rats administered with 250 mg valproic acid on day 16 of pregnancy. The average birth weights of offspring rats born to maternal rats administered with 250 mg valproic acid on day 13 of pregnancy were 40.47% higher (P<0.05) than those of born to maternal rats administered with 250 mg valproic acid on day 16 of pregnancy. The higher birth weights of offspring rats born to maternal rats administered with valproic acid on days 10 and 13 of pregnancy will affect the growth and development after

birth and affects organ (pancreas) growth during postnatal life

3.3. Birth weight of experimental offspring rats (g) grouped according to slaughtering ages

Since birth weight will affect the growth and development after birth, the birth weight of experimental offspring rats born to control maternal rats and maternal rats administered with 250 mg valproic acid on days 10, 13, and 16 of pregnancy were grouped according to the ages of the offspring sacrificed i.e., 4, 8, 12, 16, 20, 24, and 32 weeks are presented in Table 2. There was no change in the pattern of birth weights of the experimental offspring rats when grouped into the ages of observations. In all groups of age of observations, offspring rats born to maternal control rats without valproic acid administration and maternal rats administered with 250 mg valproic acid on days 10, 13, and 16 of pregnancy the patterns of birth weights were similar to those presented in Table 1. In general, offspring rats born to maternal rats administered with 250 mg valproic acid on days 10 and 13 of pregnancy consistently had higher birth weights compared to those born to control maternal rats without valproic acid administration and those born to maternal rats administered with 250 mg valproic acid on day 16 of pregnancy.

Table 2: The average birth weights grouped according to slaughtering age of the experimental offspring rats born to control maternal rats (T1) and those born to maternal rats administered with 250 mg valproic acid on days 10 (T2), 13 (T3), and days 16 (T4) of pregnancy.

Parameter	Time (week) (n=3)	Group			
		T1	T2	T3	T4
Birth weight grouped according to slaughtering age (g)	4	5.80 ± 0.26^{c}	9.22 ± 0.23^{a}	8.15 ± 0.20^{b}	5.94 ± 0.03^{c}
	8	5.96 ± 0.22^{c}	8.74 ± 0.17^{a}	8.28 ± 0.25^{b}	5.87 ± 0.11^{c}
	12	5.69 ± 0.20^{b}	8.58 ± 0.28^{a}	8.63 ± 0.16^{a}	5.89 ± 0.06^{b}
	16	6.66 ± 0.23^{b}	8.80 ± 0.17^{a}	8.77 ± 0.21^{a}	6.94 ± 0.11^{b}
	20	7.21 ± 0.32^{b}	8.95 ± 0.35^{a}	8.73 ± 0.20^{a}	7.42 ± 0.14^{b}
	24	7.46 ± 0.38^{c}	9.92 ± 0.06^{a}	9.43 ± 0.18^{b}	7.59 ± 0.31^{c}
	32	8.57 ± 0.12^{c}	9.99 ± 0.20^{a}	9.41 ± 0.11^{b}	8.16 ± 0.13^{d}

Different superscripts in the same row indicate a significant difference ($P < 0.05$)

In the experimental offspring rats slaughtered at the age of 4, 8, 24 weeks, the experimental offspring rats born to control maternal rats and maternal rats administered with 250 mg valproic acid on days 16 of pregnancy had similar ($P>0.05$) birth weights that were lower than those of offspring born to maternal rats administered 250 mg valproic acid on days 10 and 13 of pregnancy. However, in this group of slaughtering ages, offspring rats born to maternal rats administered with 250 mg valproic acid on days 10 of pregnancy had higher birth weight compared to those born to maternal rats administered with valproic acid on days 13 of pregnancy ($P<0.05$). In the experimental offspring slaughtered at the age of 12, 16, 20 weeks, the experimental offspring rats born to control maternal rats and maternal rats administered with 250 mg valproic acid on days 16 of pregnancy had similar ($P>0.05$) birth weights that were lower than those of offspring born to maternal rats administered 250 mg valproic acid on days 10 and 13 of pregnancy. However, in this group of slaughtering ages, offspring rats born to maternal rats administered with 250 mg valproic acid on days 10 and 13 of pregnancy had similar birth

weights (P>0.05). In the experimental offspring rats slaughtered at the age of 32 weeks, the experimental offspring rats born to maternal rats administered with 250 mg valproic acid on days 10 of pregnancy had the highest (P<0.05) birth weights compared to those born to control maternal rats and maternal rats administered with valproic acid on day 13 and 16 of pregnancy. Offspring rats born to maternal rats administered with 250 mg valproic cid on days 13 of pregnancy had higher birth weights (P<0.05) compared to those born to control maternal rats and those born to maternal rats administered valproic acid on days 16 of pregnancy(P<0.05). However, in this group of slaughtering, offspring rats born to maternal rats administered with 250 mg valproic acid on days 16 of pregnancy had lower birth weight compared to those born to control maternal rats without valproic acid administration (P<0.05).

3.4. Body weight (g) at slaughtering

The body weights of the experimental offspring rats observed at different ages are presented in Table 3. In the experimental offspring rats slaughtered at the age of 4 weeks postpartum, offspring rats born to maternal rats administered with 250 mg valproic acid on days 10 and 13 of pregnancy had similar body weights (P>0.05) that were significantly higher than the body weights of offspring rats born to control maternal rats and maternal rats administered with 250 mg valproic acid on days 16 of pregnancy. However, offspring rats born to maternal rats administered with 250 mg valproic acid on days 16 of pregnancy had higher (P<0.05) body weights compared to those born to control maternal rats without valproic acid administration, and this pattern was different from the pattern of birth weight.

Table 3: The average body weight at slaughtering of the experimental offspring rats born to control maternal rats (T1) and those born to maternal rats administered with 250 mg valproic acid on days 10 (T2), 13 (T3), and 16 (T4) of pregnancy.

Parameter	Time (week) (n=3)	Group			
		T1	T2	T3	T4
Body weights (g)	4	70.11 ± 1.635^{c}	92.10 ± 1.731^{a}	89.02 ± 1.934^{a}	74.04 ± 1.589^{b}
	8	126.7 ± 1.927^{b}	151.5 ± 1.518^{a}	151.3 ± 1.445^{a}	125.7 ± 1.057^{b}
	12	146.5 ± 2.402^{d}	217.4 ± 2.580^{b}	239.8 ± 1.151^{a}	163.0 ± 2.473^{c}
	16	174.5 ± 2.239^{d}	277.9 ± 1.841^{a}	259.5 ± 2.354^{b}	201.0 ± 2.596^{c}
	20	194.0 ± 2.980^{d}	288.2 ± 2.166^{a}	263.0 ± 2.528^{b}	232.4 ± 1.612^{c}
	24	234.6 ± 1.237^{d}	304.3 ± 3.513^{a}	289.5 ± 2.943^{b}	241.2 ± 1.140^{c}
	32	287.1 ± 2.618^{c}	369.7 ± 2.568^{a}	302.2 ± 2.676^{b}	273.7 ± 1.680^{d}

Different superscripts in the same row indicate a significant difference (P <0.05)

In the experimental offspring rats observed (slaughtered) at the age of 8 weeks postpartum, offspring rats born to maternal rats administered with 250 mg valproic acid on days 10 and 13 of pregnancy had similar body weights (P>0.05) that were significantly higher than the body weights of offspring rats born to control maternal rats and maternal rats administered with 250 mg valproic acid on days 16 of pregnancy. However, in this group of slaughtering, offspring rats born to control maternal rats without valproic acid administration had similar body weights to offspring rats born to maternal rats administered with 250 mg valproic acid on days 16 of

pregnancy, similar to the pattern of birth weight.

In the experimental offspring rats observed (slaughtered) at the age of 12 weeks postpartum, offspring rats born to maternal rats administered with 250 mg valproic acid on days 10 had lower body weights than those offspring rats born to maternal rats administered with valproic acid on day 13 of pregnancy ($P<0.05$). However, in this group of slaughtering, offspring rats born to control maternal rats without valproic acid administration had lower body weights ($P<0.05$) compared to offspring rats born to maternal rats administered with 250 mg valproic acid on days 16 of pregnancy. Similar to the pattern of birth weight, the body weights of offspring rats born to control maternal rats and maternal rats administered with 250 mg valproic acid on days 16 of pregnancy were lower ($P<0.05$) than those born to maternal rats administered valproic acid on days 10 and 13 of pregnancy.

Different from those observed at the age of 12 weeks, in the experimental offspring rats observed (slaughtered) at the age of 16, 20, and 24 weeks postpartum, offspring rats born to maternal rats administered with 250 mg valproic acid on day 10 had higher body weights than those offspring rats born to maternal rats administered with valproic acid on day 13 of pregnancy ($P<0.05$). However, in these groups of slaughtering, offspring rats born to control maternal rats without valproic acid administration had lower body weights ($P<0.05$) compared to offspring rats born to maternal rats administered with 250 mg valproic acid on days 16 of pregnancy. However, similar to the general pattern of birth weights, the body weights of offspring rats born to control maternal rats and maternal rats administered with 250 mg valproic acid on day 16 of pregnancy were lower ($P<0.05$) than those born to maternal rats administered with valproic acid on days 10 and 13 of pregnancy.

In the experimental offspring rats observed (slaughtered) at the age of 32 weeks postpartum, offspring rats born to maternal rats administered with 250 mg valproic acid on day 10 of pregnancy had the highest birth weight ($P<0.05$) compared to those born to maternal rats administered with 250 mg valproic acid on days 13 and 16 of pregnancy and offspring rats born to control maternal rats without valproic acid administration. Offspring rats born to maternal rats administered with 250 mg valproic acid on day 13 of pregnancy had higher body weights ($P<0.05$) compared to those born to maternal rats administered with 250 mg valproic acid on day 16 of pregnancy and those born to control maternal rats without valproic administration. Offspring rats born to control maternal rats without valproic acid administration had higher body weight ($P<0.05$) that those born to maternal rats administered with 250 mg valproic acid on day 16 of pregnancy. In this group of slaughtering age, similar to the pattern of birth weights, offspring rats born to control maternal rats without valproic acid administration and those born to maternal rats administered with valproic acid on day 16 of pregnancy had lower body weight compared to offspring rats born to maternal experimental rats administered valproic acid on days 10 and 13 of pregnancy.

3.5. Growth rate (g/d)

The growth rate of experimental offspring rats during 32 weeks of age postpartum are presented in Table 4. In general, regardless of age of the experimental offspring rats, offspring born to maternal rats administered with 250 mg valproic acid on day 10 of pregnancy had the highest growth rate ($P<0.05$) compared to those born to control maternal rats without valproic acid administration and those born to maternal rats administered with

valproic acid on days 13 and 16 of pregnancy. Offspring experimental rats born to maternal experimental rats administered with valproic acid on day 13 of pregnancy had the lowest (P<0.05) growth rate compared to the other treatments. Offspring experimental rats born to maternal rats administered with 250 mg valproic acid on day 10 of pregnancy had 3.71% higher (P<0.05) growth rate compared those born to control maternal rats without valproic administration. However, offspring experimental rats born to maternal rats administered with 250 mg valproic acid on day 13 of pregnancy had 8,91% lower growth rate (P<0.05) compared to those born to control maternal rats without valproic acid administration (Table 4).

Offspring experimental rats born to maternal rats administered with valproic acid on day 16 of pregnancy had 2.35 % lower growth rate even though it was not statistically significant (P>0.05) compared to those born to control maternal rats without valproic acid administration. Offspring experimental rats born to maternal experimental rats administered with valproic acid on day 13 of pregnancy had 12.17% lower (P<0.05) growth rate compared to those born to maternal rats administered with valproic acid on day 10 of pregnancy. Offspring born to maternal experimental rats administered with valproic acid on day 16 of pregnancy had 5.81% (P<0.05) lower growth rate compared to those born to maternal rats administered with valproic acid on day 10 of pregnancy. Offspring experimental rats born to maternal rats administered with valproic acid on day 16 of pregnancy had 7.20% higher (P<0.05) growth rate compared to those born to maternal rats administered valproic acid on day 13 of pregnancy (Table 4).

Table 4: The average growth rate at slaughtering of the experimental offspring rats born to control maternal rats (T1) and those born to maternal rats administered with 250 mg valproic acid on days 10 (T2), 13 (T3), and days 16 (T4) of pregnancy.

Parameter	Time (week) (n=3)	Group			
		T1	T2	T3	T4
Growth rate (g/day)	4	2.30 ± 0.07^{c}	2.96 ± 0.05^{a}	2.89 ± 0.07^{a}	2.43 ± 0.06^{b}
	8	2.16 ± 0.03^{b}	2.55 ± 0.03^{a}	2.56 ± 0.02^{a}	2.14 ± 0.02^{b}
	12	1.68 ± 0.03^{d}	2.49 ± 0.03^{b}	2.75 ± 0.02^{a}	1.87 ± 0.03^{c}
	16	1.50 ± 0.02^{d}	2.40 ± 0.02^{a}	2.24 ± 0.02^{b}	1.73 ± 0.02^{c}
	20	1.33 ± 0.02^{d}	1.99 ± 0.01^{a}	1.82 ± 0.02^{b}	1.61 ± 0.01^{c}
	24	1.35 ± 0.01^{d}	1.75 ± 0.02^{a}	1.67 ± 0.02^{b}	1.39 ± 0.01^{c}
	32	1.24 ± 0.01^{c}	1.61 ± 0.01^{a}	1.31 ± 0.01^{b}	1.19 ± 0.01^{d}

Different superscripts in the same row indicate a significant difference (P <0.05)

Regardless of treatment, the experimental offspring rats had positive growth rates until the age of 32 weeks. However, the growth rate of the experimental offspring rats decreased with the constant lower degree up to the age of 16 weeks and then decreased dramatically on age 20 weeks. However, at the age of 20 weeks until 32 weeks, the experimental offspring rats showed greater growth rates (P<0.05). Regardless of treatment, growth rate of the experimental offspring rats were high at the age of 4 and 8 weeks and the growth rate decreased by 5.79% (P<0.05) compared to that at the age of 4 weeks. At the age of 12 weeks, the experimental offspring rats had 5.81% lower growth rate (P<0.05) compared to at the age of 8 weeks. At the age of 16 weeks, the experimental offspring rats had 7.94% lower growth rate (P<0.05) compared to that at the age of 12 weeks. At

the age of 20 weeks, the experimental offspring rats had 30.22% lower growth rate ($P<0.05$) compared to that at the age of 16 weeks. However, at the age of 24 weeks, the experimental offspring rats had 21.43% higher growth rate ($P<0.05$) compared to that at the age of 20 weeks. Further, at the end of experiment at the age of 32 weeks, the experimental offspring rats had 18.86% higher growth rate ($P<0.05$) compared to at the age of 24 weeks.

The profiles of growth rates of the experimental offspring rats at respective age showed that offspring rats born to experimental maternal rats administered with 250 mg valproic acid on days 10 and 13 of pregnancy constantly and consistently higher ($P<0.05$) compared to those born to control maternal experimental rats without valproic acid administration and those born to maternal experimental rats administered with valproic acid on day 16 of pregnancy measured at the ages of 4, 8, 12, 16, 20, 24 and 32 weeks of pregnancy. Regardless of the degree of growth rates changes during this period of age, the consistence and constant higher growth rate in the experimental offspring rats born to maternal experimental rats administered with 250 mg valproic acid on days 10 and 13 of pregnancy indicated the supports of a better function of pancreas during this postnatal growth period.

From this observation it was clear that administration of 250 mg valproic acid on day 10 of pregnancy in addition to the effect on decreasing fetal survival that finally determine litter size that eventually increased birth weight and growth rates, it did not affect the function of the pancreas. In contrast, administration of 250 mg valproic acid on day 13 of pregnancy also decreased fetal survival and litter size and increased birth weight to a lower degree, but decreased the growth rates dramatically to the degree that could be associated with the decreased function of the pancreas due to the valproic acid administration. The increased rate of decrease in growth rates of the experimental offspring rats postpartum and reached the highest decreased on ages 16 to 20 weeks could be related to the function of the pancreas at this age. Offspring rats born to maternal rats administered with 250 mg valproic acid on day 13 of pregnancy had 18.43% ($P<0.05$) lower weight gain compared to those born to maternal rats administered with valproic acid on day 10 of pregnancy.

4. Discussion

The improved prenatal growth and development of survived fetus until parturition indicated that the toxic developmental effects of valproic acid administration during early pregnancy observed in rhesus monkey [10], did not reduce the growth and development of survival fetuses. However, prenatal and postnatal growth rates of the survival fetuses were far better than normal offspring rats born to control maternal rats without valproic acid administration and those born to maternal rats administered with valproic acid on day 16 of pregnancy. In addition, administration of single dose of 250 mg valproic acid on day 16 of pregnancy did not affect prenatal growth of the survival fetuses.

These results indicated that the single dose of valproic acid administration during early and middle of pregnancy significantly decreased litter size that can be associated with embryonic and fetal survival. This result indicated that administration of valproic acid during early pregnancy reduced the embryonic and fetal survival with the final result the reduction of the total number of born offspring. Administration of valproic acid during late

pregnancy (days 16 of pregnancy) did not affect the survival of embryo and fetus as was indicated by the non-significant change in the number of litter size as compared to control maternal rats without valproic administration. Observation in rhesus monkey reported the increased embryonic and fetal mortalities in dose-dependent pattern [10].

The observation in this experiment clearly showed that the administration of valproic acid during early pregnancy decreased the survival of the embryos and fetus as indicated by the decreased litter size. Since the earliest administration of valproic acid was conducted on day 10 of pregnancy, the stage of placental, the reduction of litter size observed in this experiment was not associated with the inhibition of fertilization and implantation, but related to the inhibition or reduction of growth and development of the fetus. In addition, the reduction of litter size was not related to the toxic effect of the valproic acid to the fetus since administration of valproic acid on day 16 of pregnancy did not affect the survival of the fetus as was indicated by the normal litter size. The reduction of litter size in maternal rats administered with 250 mg valproic acid on days 10 and 13 of pregnancy will affect the degree of fetal competition in obtaining space and nutrients as well as compounds required for growth and development that eventually affected birth weight, growth of offspring rats and organs during postnatal life. The survived fetuses in the maternal rats administered with valproic acid on days 10 and 13 of pregnancy did not show any negative effect of valproic acid exposure during fetal stage of development. In contrast, the survived fetuses grew better during prenatal and postnatal lives.

Even though it was observed that the weight of pancreas, the number and diameter of islet of Langerhans, the number of cells of the islet of Langerhans, and the immunoreaction of beta cells to insulin were lower in the offspring rats born to maternal rats administered with valproic acid on days 10 and 13 of pregnancy [16], the decrease in pancreatic functions did not affect the normal growth and development of the offspring during prenatal and postnatal lives. These results imply that single administration of 250 mg valproic acid on day 10 or 13 of pregnancy could be used to reduced litter size in polytocus animals to improve prenatal and postnatal growth of the offspring. The results of this experiment clearly showed the reduction of litter size would increase prenatal and postnatal growth of the offspring.

5. Conclusion and Recommendation

Administration of single dose of 250 mg valproic acid on day 10 or 13 of pregnancy significantly decreased litter size. The decreased litter size in maternal rats administered with 250 mg valproic cid on day 10 or 13 of pregnancy did not inhibit the prenatal and postnatal growths of the survived offspring rats. Instead, the survived fetuses grew better during prenatal growth that eventually had higher birth weight and better growth rate until the age of 32 weeks. The results of this experiment recommend the injection of valproic acid at the middle age of pregnancy can be used to reduce litter size in polytocus animals to improve prenatal growth and postnatal growth and performances.

Conflict of interest

There is no conflict of interest in this research.

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