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Valproic Acid Administration in Pregnant Rats Affects Microscopic Features of Endocrine Cells of their Offspring

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Abstract: Valproic Acid (VA) affects the activity of Histone Deacetylase (HDAC) which plays an important role in gene expression during the process of pancreatic organogenesis. Pancreatic endocrine plays an important role in maintaining blood glucose homeostasis. Endocrine beta cells dysfunction may increase the incidence of type 2 Diabetes Mellitus (DM). The study used 30 pregnant female Sparague dawley rats. The experimental pregnant rats were divided into 4 groups. The 1st group (T0 control group) was given distilled water or zero concentration of VA. The 2nd group (T1) was administered 250 mg VA on day 10th of pregnancy (at the same time with the expression of Pdx1 gene. The 3rd group (T2) was administered VA on day 13th of pregnancy (Nkx 6.1 gene). The 4th group (T3) was administered VA on day 16th of pregnancy (Ngn3 gene). A total of 84 rats born to the treated experimental rats were selected to undergo microscopic examination of the pancreatic endocrine cells, in four weeks interval, starting from week 4th to week 32nd. The results showed that the weight of the pancreas in the rats born to maternal rats treated with VA were similar with those born to control rats without VA administration ($p>0.05$). However, treatment of the maternal rats during pregnancy with VA decreased the diameter and the number of cells in the islets of langerhans ($p<0.05$). Administrations of VA to pregnant rats on days 10th, 13th and 16th of pregnancy, affected the pancreas organogenesis of their offspring as indicated by the changes in histomorphology.

Key words: Beta cells, organogenesis, pancreas, valproic acid, maternal, diameter

INTRODUCTION

Administration of drugs or certain chemicals during pregnancy, especially during the stage of organogenesis will affect embryonic development (Ornoy and Ergaz, 2010). The negative effects can be the abnormality of structure and function of organs that can affect the quality of post natal life. Specific factors in the materials or drugs can affect the cells proliferation and differentiation during embryonic development (Mamashlic *et al*., 2011). Valproic acid is an antiepileptic drug that affects the modification of histones during the process of proliferation and cells differentiation (Kurihara *et al*., 2014) by influencing the activity of Histone Deacetylase (HDAC) which plays an important role in gene expression during the process of pancreatic organogenesis (Zhang and Pradhan, 2014).

The pancreas is a mixed gland composed of two different cell populations, i.e., exocrine and endocrine. The exocrine component makes up the majority of the pancreas and includes the acinar and the ductal cells that secrete and transport digestive enzymes, respectively into the small intestine. The endocrine cells are segregated into groups and are called islets of langerhans. Immunocytochemical methods have shown that the islets consist of α cells producing glucagon (15-20%), β cells producing insulin and amylin (65-80%), δ cells producing somatostatin (3-10%), PP cells producing pancreatic polypeptide (3-5%) and epsilon cells producing ghrelin, an anti-satiety hormone (<1%) (Jarral *et al*., 2013).

The organogenesis of pancreas during embryonic development depends on signaling interactions with the surrounding tissue (Shih *et al*., 2013). The development of beta cells of pancreatic gland can be disrupted during the onset of organogenesis, along with the development of neuronal tube at early development of nerve cells (Kuwabara *et al*., 2011). The development of the pancreas includes two transitional regulations that begin on days 8.75-12.5th of pregnancy with the formation of buds on the endoderm layer then the cells proliferate and increase in size quickly. In addition, there is a morphogenesis change of tubular structure called the primary transition. In this transition, the epithelial cells do not undergo a process of differentiation (Pan and Wright, 2011).

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Meanwhile, in the secondary transition, the pancreatic epithelial cells expand and branch that will be followed by the process of differentiation of endocrine, acinar and ductal cells that are occurred on day 12.5th to birth (Shih *et al*., 2013).

The use of valproic acid during certain stage of pregnancy may disturb the expression of genes that play roles in the development of the pancreas. The expression of the Pdx1 gene produce signal that plays a central role in the on set of a complex signaling and transcriptional tissue regulation that eventually regulates the pancreatic proliferation and differentiation (Chun *et al*., 2015). The expression of Nkx6.1 gene produce signals that regulate and control the branching of the tip and tunk parts of the pancreas. Tip part will develop into the pancreatic exocrine while tunk part will develop into pancreatic endocrine (Taylor *et al*., 2013). The expression of Ngn3 gene produce signal that play roles in the expression of progenitor cells during development of pancreatic beta cells and nerve cells in the part of the dentate gyrus of the hippocampus (Sostrup *et al*., 2014). The purpose of the present experiment is to study the growth and development of the islets of langerhans cells and their function in rats born to mother administered with valproic acid at the certain stages of pregnancy that are related to the expression of certain gene controlling the organogenesis of the pancreas.

MATERIALS AND METHODS

Programmatic and maintenance of rats: This study, used 30 female S.dawley rats with the ranges of body weights of 200-250 g and age of 3-4 months. Prior to mating, the estrus cycles of the experimental rats were synchronized by injection of PGF2α (Noroprost®) intramuscularly. The estrus female rats were mixed with male rats for mating. The presence of the sperm in the vagina was determined as day 0 of pregnancy (Jarral *et al*., 2013). During pregnancy, all pregnant experimental rats were maintained with the same management and feeding condition. The pregnant experimental rats were assigned into a completely randomized design with 4 treatments of time of valproic acid administration. The 1st group (T0) consisted of pregnant rats without or with zero concentration of VA administration as a control. The 2nd group (T1) consisted of pregnant rats administered with valproic acid (Depakote® manufactured by Abbott Laboratories) at a dose of 250 mg orally on day 10th of pregnancy at the same time with the expression of Pdx1 gene. The 3rd group (T2) consisted of pregnant rats administered with VA at a dose of 250 mg on day 13th of pregnancy at the same time with the expression of Nkx6.1 gene. The 4th group (T3) consisted of pregnant rats administered with VA at a dose of 250 mg on day 16th of pregnancy at the same time with the expression of Ngn3 gene.

The experimental pregnant rats were maintained until parturition. After parturition, 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 days (3 experimental offspring rats from each treatment group) to measure birth weights, body weights, growth rate, the weight of pancreas, the relative weight of pancreas, the number of islets of langerhans, the number of cell and the diameter of cell in the islets of langerhans in the pancreas, the experimental offspring rats. The experiment was conducted according the Animal Ethics issued by the Department of Pathology of Bogor Agricultural University with the registration No. SKEH Number: 031/KEH/SKE/IV/2015.

Rats organ tissue sampling: The microscopic features of the pancreas of the offspring rats born to control maternal rats and maternal rats administered with 250 m valproic acid on days 10, 13 and 16 of pregnancy were observed on the ages of 4, 8, 12, 16, 20, 24 and 32 weeks postpartum. Prior to organ harvesting, the experimental offspring rats were anesthetized by injection of a combination of ketamine (75-100 mg/kg) and xylaszin (5-10 mg/kg) intraperitoneally. Once the experimental rats were completely deep anesthetized, the pancreatic organs were harvested, isolated and cleaned using physiological saline solution and finally immersed in 10% Neutral Buffered Formalin (NBF) solution.

Hematoxylin-eosin staining: Preparations of histology, photographs and readings were conducted at the Pathology Laboratory and Histology Laboratory, Faculty of Veterinary Medicine, Bogor Agricultural University. Paraffinized pancreatic tissue samples were deparafinized by using xylol and rehydrated with graded alcohol and water. The preparations were stained using Hematoxylin-Eosin (HE).

Analysis: Data 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 and SPSS Version 3.2. 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 (Dahlan, 2014) was conducted. Quantitative analysis of the microscopic feature of HE staining was performed using imageJ version 1.45 for Windows 8 while the qualitative analysis was performed using a microscope Olympus with MD-130 electronic eyepiece.

RESULTS

Absolute weights of pancreas of the experimental offspring rats: Regardless of VA treatment, the experimental offspring rats showed increased pancreas weight during 32 weeks of age ($p<0.05$) similar to the growth the body weight except at the ages of 4 and 8 weeks and at the ages of 12 and 16 weeks. At these ages (4-8 and 12-16 weeks), the increase in absolute pancreas weight were not significant ($p>0.05$).

Regardless of the age of offspring experimental rats, experimental offspring rats born to maternal experiment rats administered with VA on day 10 of pregnancy had similar absolute pancreas weights ($p>0.05$) compared to those born to maternal experimental rats administered VA on day 13 of pregnancy. The experimental offspring rats born to maternal experiment rats administered with VA on day 13 of pregnancy had similar absolute pancreas weights ($p>0.05$) compared to those born to maternal experimental rats administered VA on day 16 of pregnancy. The experimental offspring rats born to maternal experiment rats administered with 250 mg VA on day 16 of pregnancy had similar absolute pancreas weights ($p>0.05$) compared to those born to control maternal experimental rats without VA administration. However, administration of the maternal experimental rats with VA on day 10 of pregnancy decreased absolute pancreas weights by 18.46% ($p<0.05$) compared to those born to control maternal rats without VA administration.

In general, at the age of 4 weeks, the absolute weights of the pancreas were different among group of treatments. The highest absolute weight of pancreas was found in the offspring experimental rats born to maternal experimental rats administered with VA on day 16 of pregnancy and consecutively are followed by those born to control maternal experimental rats without VA administration, those born to maternal experimental rats administered with VA on daya 10 and 13 of pregnancy. In this age group, experimental offspring rats born to control maternal rats and maternal rats administered with VA on day 16 of pregnancy had higher absolute pancreas weights compared to those born to maternal experiment rats administered with VA on days 10 and 13 of pregnancy.

At the age of 8 weeks, the absolute weights of the pancreas were not different among group of treatments. Even though, they were not different significantly, the highest absolute weight of pancreas was found in the offspring experimental rats born to maternal experimental rats administered with VA on day 13 of pregnancy and consecutively are followed by those born to maternal experimental rats administered with VA on day 10 of pregnancy, those born to control maternal experimental rats without VA administration and those born to maternal experimental rats administered with VA on day 16 of pregnancy.

At the age of 12 weeks, the absolute weights of the pancreas were different among group of treatments. The highest absolute weight of pancreas was found in the offspring experimental rats born to maternal experimental rats administered with VA on day 10 of pregnancy and consecutively are followed by those born to maternal experimental rats administered with VA on day 13 of pregnancy, those born to control maternal experimental rats without VA administration and those born to maternal experimental rats administered with VA on day 16 of pregnancy. In this age group, experimental offspring rats born to control maternal rats without VA administration and those born to maternal rats administered with VA on days 10 and 13 of pregnancy had similar absolute pancreas weights.

At the ages of 16, 20 and 24 weeks the absolute weights of pancreas were similar among the treatment groups. However, even though it is not statistically significant at the age of 16 weeks, the highest absolute weights of the pancreas was found in the offspring rats born to maternal rats administered with VA on day 13 of pregnancy and consecutively followed by those born to control maternal rats without VA administration, those born to maternal rats administered with VA on days 16 and 10 of pregnancy. At the age of 20 weeks, the highest absolute weights of the pancreas was found in the offspring rats born to maternal rats administered with VA on day 16 of pregnancy and consecutively, followed by those born to maternal rats administered with VA on days 10 and 13 of pregnancy and those born to control maternal rats without VA administration.

At the age of 24 weeks, the highest absolute weights of the pancreas was found in the offspring rats born to control maternal rats without VA administration, consecutively, followed by those born to maternal rats administered with VA on day 16 of pregnancy and those born to maternal rats administered with VA on days 13 and 10 of pregnancy. At the age of 32 weeks, the absolute weights of pancreas were significantly different among treatment groups ($p<0.05$). However, the absolute

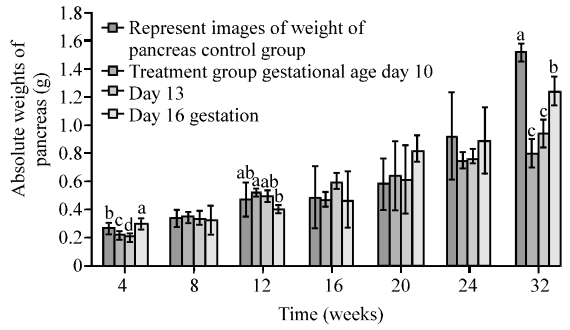

Fig. 1: Absolute weights of pancreas of the experimental offspring rats

pancreas weights in the offspring experimental rats born to maternal experimental rats administered with 250 mg VA on days 10 and 13 were not significantly different ($p>0.05$). The highest absolute weights of the pancreas was found in the offspring rats born to control maternal rats without VA administration, consecutively followed by those born to maternal rats administered with VA on day 16 of pregnancy and those born to maternal rats administered with VA on days 13 and 10 of pregnancy. Absolute pancreas weights is presented in Fig. 1.

In general, the absolute weights of pancreas increased from the ages of 1-4 months. At the age of 4 months, the offspring rats born to control maternal rats without VA administration had higher ($p<0.05$) absolute pancreas weight compared to those born to maternal rats administered with VA on days 10, 13 and 16 of pregnancy. The patterns of pancreas weights from the age of 1 month until 7 months were relatively similar ($p>0.05$). However, at the age of 8 months, the offspring rats born to maternal rats administered with VA on days 10, 13 and 16 of pregnancy had 47.68, 38.41 and 17.88% lower absolute pancreas weights compared to control offspring rats born to maternal rats without valproic acid administrations.

Relative weights of pancreas of the experimental offspring rats: Regardless of treatment of VA, the relative weight of pancreas increased with the increase of age. The highest relative pancreas weights of the offspring experimental rats (0.37) were found at the age of 32 weeks and the lowest (0.22) were found at the age of 16 weeks. The order of relative pancreas weights from the lowest to the highest were at the age of 16, 8, 12, 20, 4, 24 and 32 weeks.

Regardless of ages of experimental offspring rats, the order of relative pancreas weights in the from the lowest to the highest were found in the experimental offspring rats born to maternal experiment rats administered with VA on days 10, 13 and 16 of pregnancy followed by the experimental offspring rats born to control maternal rats without VA administration. So, the highest relative pancreas weights were found in the experimental offspring rats born to control maternal rats and those born to experimental maternal rats administered with VA on day 16 of pregnancy. The lowest relative pancreas weights were found in offspring rats born to maternal experiment rats administered with VA on days 10 and 13 of pregnancy.

The relative weights of pancreas in offspring rats born to maternal rats administered with VA on day 10 of pregnancy decreased relative pancreas weight by 37.14% ($p<0.05$) compared to those born to control maternal rats without VA administration. The relative weights of pancreas in offspring rats born to maternal rats administered with VA on day 13 of pregnancy decreased relative pancreas weight by 31.43% ($p<0.05$) compared to those born to control maternal rats without VA administration. However, offspring rats born to control maternal rats and those born to maternal rats administered VA on day 16 of pregnancy had similar relative pancreas weights ($p>0.05$). In addition, offspring rats born to maternal rats administered VA on days 10 and 13 of pregnancy had similar relative pancreas weights ($p>0.05$). Offspring experimental rats born to maternal experimental rats administered with VA on day 10 of pregnancy had 33.33% lower relative pancreas weight ($p<0.05$) compared to those born to maternal experimental rats administered with VA on day 16 of pregnancy. In addition, offspring experimental rats born to maternal experimental rats administered with 250 mg VA on day 13 of pregnancy had 27.27% lower relative pancreas weight ($p<0.05$) compared to those born to maternal experimental rats administered with VA on day 16 of pregnancy.

In general, observations during 32 weeks of age showed the similar patterns of relative pancreas weights, i.e., the highest were found in the offspring rats born to control maternal rat without VA administration and those born to maternal experimental rats administered with 250 mg VA on day 16 of pregnancy followed by the offspring rats born to maternal experimental rats administered with VA on days 10 and 13 of pregnancy. However, the significant differences in relative pancreas weights were only found in the offspring rats at the ages of 4, 12 and 32 weeks. In the other ages, the differences in relative pancreas weights were not statistically significant ($p>0.05$).

However, at the ages of 1, 6 and 8 months, the relative pancreas weight of the control offspring rats born to control maternal rats and those born to maternal rats

administered with VA on day 16 of pregnancy had similar patterns that were higher than those born to maternal rats administered with 250 mg VA on the age of 10 and 13 days of pregnancy ($p<0.05$). At the age of 8 months, offspring rats born to maternal rats administered VA on days 10 of pregnancy had 59.61 and 53.33% lower relative pancreas weight compared to control offspring rats and those born to maternal rats administered VA on days 16 of pregnancy ($p<0.05$). In addition at the same age of 8 months, offspring born to maternal rats administered 250 mg VA on days 13 of pregnancy had 40.38 and 31.11% lower relative pancreas weight compared to control offspring rats and those born to maternal rats administered with 250 mg VA on days 16 of pregnancy ($p<0.05$).

Results of statistical analysis on the weight of the pancreas offsprings showed a positive correlation ($p<0.05$) between the period of growth with the increased weight of the pancreas, both to the rat from controls parent group and rat from VA group. The weights of the pancreas of rat from the group whose given VA and control group showed significant differences ($p<0.05$) at 4, 12 and 32 weeks. The weight of pancreas in rats from the control group have greater than rat from the group given VA at 10, 13 and 16 days of gestation, increased weight of the pancreas rat from control group seen in the growth period of 32 weeks is 1.51±0.06, 0.796±0.05, 0.936±0.09 and 1.24±0.10 grams, respectively. The pancreatic relative weights rat from the group whose given VA and control group showed significant differences ($p<0.05$) at 4, 12 and 32 weeks. The pancreatic relative weights in rats from the control group have greater than rat from the group given VA at 10, 13 and 16 days of gestation. Increased relative weights of the rat seen in the growth period of 32 weeks is 0.0053±0.0002, 0.0022±0.0002, 0.0031±0.0003 and 0.0045±0.0004 g, respectively. Relative weights from offspring is presented in Table 1.

The absolute and relative weights of pancreas in the experimental offspring rats are presented in Fig. 1 and Table 1. At the age of 8, 16, 20 and 24 weeks, the absolute weights of pancreas in all groups of experimental offspring rats were similar ($p>0.05$). However, at the ages of 4, 12 and 32 week, there were significant differences in absolute pancreas weights among different group of experimental offspring rats.

The number of islet of langerhans: At the age of 4 weeks, the number of islet of langerhans in offspring rats born to control maternal rats without VA administration (T0) and those born to maternal rats administered with VA on day 13 of pregnancy (T2) were similar ($p>0.05$). At the age of 4 weeks, the number of islet of langerhans in offspring rats born to maternal rats administered with VA on days 10 and 16 of pregnancy were similar ($p>0.05$). However, the number of islet of langerhans in offspring rats born to control maternal rats without VA administration and those born to maternal rats administered with VA on day 13 of pregnany were statistically different from ($p<0.05$) those in offspring born to maternal rats administered with VA on days 10 and 16 of pregnancy.

At the age of 8 weeks, the offspring rats born to maternal rats administered with VA on day 10 of pregnancy had the highest number of islet of langerhans ($p<0.05$) compared to those born to maternal rats without VA administration and maternal rats administered with VA on days 13 and 16 of pregnancy. The experimental offspring rats born to control maternal rats without VA administration and offspring rats born to maternal rats administered with VA on days 10 of pregnancy had the same number of islet of langerhans ($p>0.05$). The experimental offspring rats born to control maternal rats administered with VA on days 13 and 16 of pregnancy had the same number of islet of langerhans ($p>0.05$). However, the experimental offspring rats born to control maternal rats without VA administration had higher ($p<0.05$) number of islet of langerhans compared to those born to maternal rats administered with VA on day 16 of pregnancy. The number of islets of langerhans offspring is presented in Table 2.

At the age of 12 weeks, the offspring rats born to control maternal rats had the highest number of islet of langerhans ($p<0.05$) compared to those born to maternal rats administered with VA on days 10, 13 and 16 of pregnancy. The number of islet of langerhans in the offspring rats born to maternal rats administered with VA on days 10, 13 and 16 of pregnancy were similar ($p>0.05$).

At the age of 16 weeks, the control offspring rats born to control maternal rats without VA administration had the highest number of is let of langerhans ($p<0.05$) compared to those born to maternal rats administered VA on days 10, 13 and 16 of pregnancy. Offspring rats born to maternal rats administered with VA on days 10 and 16 of pregnancy had the same number of islet of langerhans ($p>0.05$) that were significantly higher than those born to maternal rats administered with VA on day 13 of pregnanct ($p<0.05$).

At the age of 20 weeks, experimental offspring rats born to control maternal rats without VA administration and those born to maternal rats administered with VA on day 13 of pregnancy had similar number of islets of langerhans that were higher than those in offspring rats born to maternal rats administered with VA on days 10

Table 1: The relative weights of the experimental offspring rats born to control maternal rats (T0) and those born to maternal rats administered with 250 mg VA on days 10 (T1), days 13 (T2) and days 16 (T3) of pregnancy

		Groups				
Parameter	Time (week) (n = 3)	T0 (10^{-3})	T1 (10^{-3})	T2 (10^{-3})	T3 (10^{-3})	p-values
Relative weights (g)	4	3.9±0.2^{a}	2.5±0.2^{b}	2.1±0.1^{c}	4.0±0.2^{a}	0.000
	8	2.7±0.5	2.3±0.6	2.4±0.2	2.6±0.8	0.785
	12	3.2±0.8^{a}	2.4±0.1^{b}	2.1±0.1^{b}	2.4±0.1^{b}	0.049
	16	2.8±1.2	1.7±0.4	2.3±0.2	2.3±1.0	0.497
	20	3.0±0.9	2.2±0.1	2.3±0.9	3.5±0.4	0.136
	24	3.9±1.3	2.5±0.1	2.7±0.2	3.7±1.0	0.169
	32	5.3±0.2^{a}	2.2±0.2^{d}	3.1±0.3^{c}	4.5±0.4^{b}	0.000

Different superscript a-d in the same row showed the significantly different ($p<0.05$)

Table 2: The number of the islets of langerhans in the pancreas tissue of the experimental offspring rats born to control maternal rats (T0) and those born to maternal rats administered with 250 mg VA on days 10 (T1), days 13 (T2) and days 16 (T3) of pregnancy

		Groups				
Parameter	Time (week) (n = 3)	T0	T1	T2	T3	p-values
Number of the islets of langerhans	4	30.66±1.52^{a}	24.00±2^{b}	29.66±0.57^{a}	24.00±1^{b}	0.000
	8	25.00±3^{b}	34.00±2^{a}	21.00±2^{bc}	17.00±2^{c}	0.000
	12	35.00±4.35^{a}	22.00±3^{b}	19.67±2.51^{b}	19.00±4^{b}	0.000
	16	31.00±1^{a}	17.00±2^{b}	6.00±1^{c}	19.00±3^{b}	0.000
	20	32.00±2^{a}	22.33±1.15^{c}	33.00±1.73^{a}	27.67±2.31^{b}	0.000
	24	30.67±0.57^{a}	38.00±1.73^{b}	29.00±1^{a}	37.00±1.73^{b}	0.000
	32	26.00±1.73^{b}	24.00±1^{b}	11.00±1^{c}	30.00±2.64^{a}	0.000

Different superscript a-c in the same row showed the significantly different ($p<0.05$)

and 16 of pregnancy. However, the number of islet of langerhans in the offspring rats born to maternal rats administered with VA on day 16 of pregnancy were higher ($p<0.05$) than those born to maternal rats administered with VA on day 10 of pregnancy.

At the age of 24 weeks, the number of islet of langerhans in offspring rats born to control maternal rats without VA administration (T0) and those born to maternal rats administered with VA on day 13 of pregnancy (T2) were similar ($p>0.05$). At the age of 24 weeks, the number of islet of langerhans in offspring rats born to maternal rats administered with VA on days 10 and 16 of pregnancy were similar ($p>0.05$). However, the number of islet of langerhans in offspring rats born to control maternal rats without VA administration and those born to maternal rats administered with VA on day 13 of pregnany were statistically different from ($p<0.05$) those in offspring born to maternal rats administered with VA on days 10 and 16 of pregnancy.

At the age of 32 weeks, the offspring rats born to maternal rats administered with VA on day 16 of pregnancy had the highest number of islet of langerhans ($p<0.05$) compared to those born to control maternal rats and those born to maternal rats administered with VA on days 10 and 13 of pregnancy. Experimental offspring rats born to control maternal rats without VA administration had similar number of islet of langerhans ($p>0.05$) with those born to maternal rats administered with VA on day 10 of pregnancy that were higher ($p<0.05$) than those born to maternal rats administered with VA on day 13 of pregnancy.

The number of cells in the islet of Langehans: At the age of 4 weeks, the number of cells in the islet of langerhans in the offspring rats born to control maternal rats without VA administration and those born to maternal rats administered with VA on day 16 of pregnancy were similar but higher than ($p<0.05$) those in offspring rats born to maternal rats administered with VA on days 10 and 13 of pregnancy. However, the number cells in the islets of langerhans of offspring rats born to maternal rats administered with VA on days 10 and 13 of pregnancy were similar ($p>0.05$).

At the age of 8 weeks, offspring rats born to maternal rats administered with VA on days 10, 13 and 16 of pregnancy had lower number of cells in the islet of langerhans compared to offspring rats born to control maternal rats without VA administration. Different time of VA administration did not affect the number of cells in the islet of langerhans ($p>0.05$).

At the age of 12 weeks, offspring rats born to maternal rats administered VA on days 10, 13 and 16 of pregnancy had lower number of cells in the islet of langerhans compared to control offspring rats born to control maternal rats without VA administration. Offspring rats born to maternal rats administered with VA on day 10 of pregnancy had higher ($p<0.05$) number of cells in the islets of langerhans compared to those born to maternal rats administered with VA on days 13 and 16 of pregnancy. However, the number of cells in the islet of langerhans of offspring rats born to maternal rats administerd with VA on days 13 and 16 of pregnancy were similar ($p>0.05$).

Table 3: The number of cells in the islets of langerhans of the experimental offspring rats born to control maternal rats (T0) and those born to maternal rats administered with 250 mg VA on days 10 (T1), days 13 (T2) and days 16 (T3) of pregnancy

		Groups				
Parameters	Time (week) (n = 3)	T0	T1	T2	T3	p-values
Number of cells in the islets of langerhans	4	98.6±13.24^{a}	81.2±8.81^{b}	76.2±7.39^{b}	102.8±5.93^{a}	0.001
	8	113.6±1.67^{a}	84.2±16.75^{b}	84.8±4.86^{b}	86.4±18.36^{b}	0.005
	12	135.2±12.94^{a}	87.4±1.51^{b}	83.2±2.38^{c}	75.8±1.92^{c}	0.000
	16	109.4±9.18^{a}	57.2±5.16^{c}	74.6±3.84^{b}	70.8±1.09^{b}	0.000
	20	70.8±6.98^{b}	64.2±2.38^{b}	59.8±5.35^{b}	127.4±19.3^{a}	0.000
	24	108.4±5.50^{a}	87.2±1.64^{c}	63.8±4.38^{d}	101.0±1.41^{b}	0.000
	32	102.4±2.51^{a}	87.2±1.64^{c}	64.8±3.42^{d}	96.4±1.34^{b}	0.000

Different superscript ($^{a-d}$) in the same row showed the significantly different ($p<0.05$)

At the age of 16 weeks, offspring rats born to maternal rats administered with VA on days 10, 13 and 16 of pregnancy had lower number of cells in the islet of langerhans compared to control offspring rats born to control maternal rats without VA administration. Offspring rats born to maternal rats administered with VA on day 10 of pregnancy had lower ($p<0.05$) number of cells in the islets of langerhans compared to those born to maternal rats administered with VA on days 13 and 16 of pregnancy. However, the number of cells in the islet of langerhans of offspring rats born to maternal rats administerd with VA on days 13 and 16 of pregnancy were similar ($p>0.05$).

At the age of 20 weeks, offspring rats born to control maternal rats without VA administration and those born to maternal rats administered with VA on days 10 and 13 of pregnancy had the same number of cells in the islet of langerhans ($p>0.05$) that were significantly lower than those in offspring rats born to maternal rats administered with VA on day 16 of pregnancy. The highest number of cells in the islet of langerhans was found in the offspring rats born to maternal rats administered with VA on day 16 of pregnancy. The number of cells in the islet of langerhans is presented in Table 3.

At the age of 24 and 32 weeks, the patterns of number of cells in the islet of langerhans were similar. In these ages, the number of cells in the islet of langerhans in different groups of experimental offspring rats were different ($p<0.05$). At the ages of 24 and 32, the highest number of cells in the islet of langerhans was found in the control offspring rats born to control maternal rats without VA administration. Offspring rats born to maternal rats administered with VA on day 16 of pregnancy were higher than those in the offspring rats born to maternal rats administered with VA on days 10 and 13 of pregnancy. However, offspring rats born to maternal rats administered with VA on day 10 of pregnancy had higher number cells in the islet of langerhans ($p<0.05$) compared to those born to maternal rats administered with VA on day 13 of pregnancy. Histomorphology cells in the islet of langerhans is presented in Fig. 2.

Diameter of the islets of langerhans: At the age of 4 weeks, the diameter of the islet of langerhans were lower in the offspring rats born to maternal rats administered with VA on days 10, 13 and 16 of pregnancy compared to control offspring born to maternal rats without VA administration. Offspring rats born to maternal rats administered with VA on days 10 and 13 of pregnancy had similar diameters of islet of langerhans ($p>0.05$) that were lower than ($p<0.05$) that of offspring rats born to maternal rats administered with VA on day 16 of pregnancy.

At the age of 8 weeks, the diameter of the islet of langerhans were lower in the offspring rats born to maternal rats administered with VA on days 10, 13 and 16 of pregnancy compared to control offspring born to maternal rats without VA administration. Offspring rats born to maternal rats administered with VA on days 10, 13 and 16 of pregnancy had similar diameters of islet of langerhans ($p>0.05$). The diameter of the islet of langerhans is presented in Table 4.

At the age of 12 weeks, the diameter of the islet of langerhans were lower in the offspring rats born to maternal rats administered with VA on days 10, 13 and 16 of pregnancy compared to control offspring born to maternal rats without VA administration. Offspring rats born to maternal rats administered with VA on days 10 and 13 of pregnancy had similar diameters of islet of langerhans ($p>0.05$) that were lower than ($p<0.05$) that of offspring rats born to maternal rats administered with VA on day 16 of pregnancy.

At the age of 16 weeks, the diameter of the islet of langerhans were lower in the offspring rats born to maternal rats administered with VA on days 10, 13 and 16 of pregnancy compared to control offspring born to maternal rats without VA administration. Offspring rats born to maternal rats administered with VA on days 13 and 16 of pregnancy had similar diameters of islet of langerhans ($p>0.05$) that were higher ($p<0.05$) than that of offspring rats born to maternal rats administered with VA on day 10 of pregnancy.

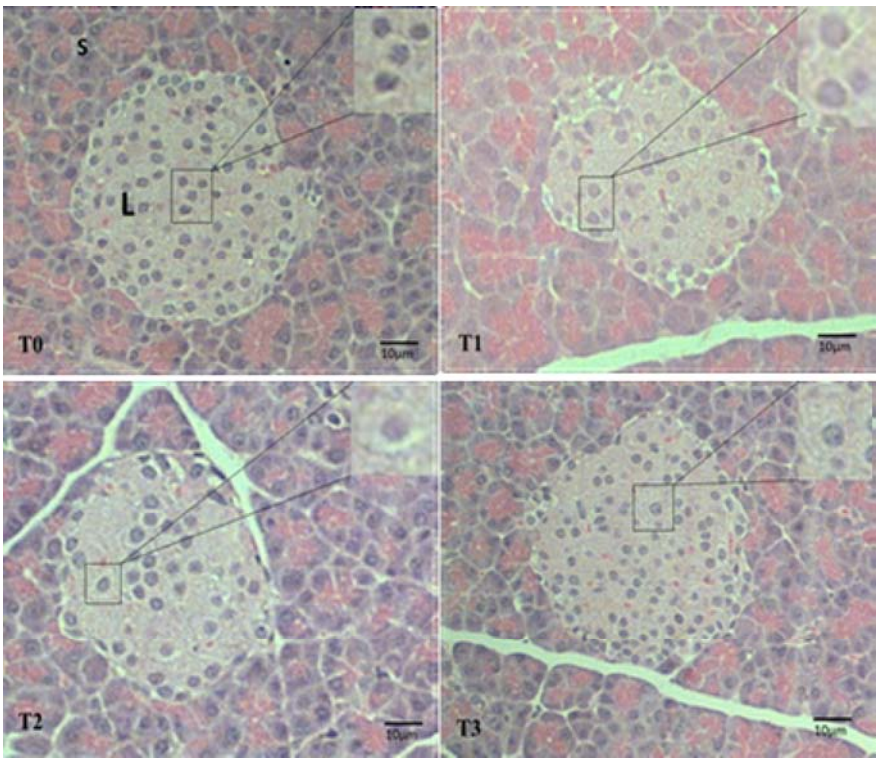

Fig. 2: Histomorphology the cells of islets of langerhans from the offspring rat control group (T0) and from treatment valproic acid at gestational age day 10 (T1), day 13 (T2), day 16 (T3), asini Serose (S) and islets Langerhans (L) (HE 40X)

Table 4: Diameter of the islets of langerhans in the pancreas tissue of the experimental offspring rats born to control maternal rats (T0) and those born to maternal rats administered with 250 mg VA on days 10 (T1), days 13 (T2) and days 16 (T3) of pregnancy

		Groups				
Parameters	Time (week) (n = 3)	T0	T1	T2	T3	p-values
Diameter of the islets of	4	107.20±4.83^{a}	68.30±10.88^{c}	64.36±10.54^{c}	86.91±7.62^{b}	0.000
langerhans (μm)	8	114.52±6.56^{a}	83.52±10.26^{b}	84.21±6.23^{b}	85.38±8.48^{b}	0.000
	12	120.27±4.29^{a}	86.10±5.50^{c}	87.90±5.49^{c}	98.52±6.82^{b}	0.000
	16	113.82±4.66^{a}	70.96±9.87^{c}	89.40±14.22^{b}	89.40±14.22^{b}	0.000
	20	72.84±26.15^{b}	65.78±12.07^{b}	64.52±12.22^{b}	95.53±3.83^{a}	0.026
	24	117.21±5.17^{a}	81.26±9.42^{b}	75.12±9.61^{b}	103.7±20.69^{a}	0.000
	32	119.21±3.79^{a}	83.26±5.34^{b}	75.32±9.82^{b}	105.7±17.24^{a}	0.000

Different superscript a-c in the same row showed the significantly different ($p<0.05$)

At the age of 20 weeks, the offspring rats born to maternal rats administered with VA on day 16 of pregnancy had the highest diameter of islet of langerhans ($p<0.05$) compared to those born to control maternal rats without VA administration and maternal rats administered with VA on days 10 and 13 of pregnancy. However, the diameters of islet of Langerhan in the offspring rats born to control maternal rats without VA administration and maternal rats administered with VA on days 10 and 13 of pregnancy. Histomorphology diameter of islet of langerhans is presented in Fig. 3.

At the age of 24 and 32 weeks, the diameter of the islet of langerhans in the offspring rats born to control maternal rats without VA administration and maternal rats administered with 250 mg VA on day 16 of pregnancy were similar ($p>0.05$). Offspring rats born to maternal rats administered with VA on days 10 and 13 of pregnancy were similar ($p>0.05$). At the age of 24 and 32 weeks, the diameter of the islet of langerhans in the offspring rats born to control maternal rats without VA administration and maternal rats administered with VA on day 16 of pregnancy were higher ($p<0.05$) than those in the offspring rats born to maternal rats administered with VA on days 10 and 13 of pregnancy were similar ($p>0.05$).

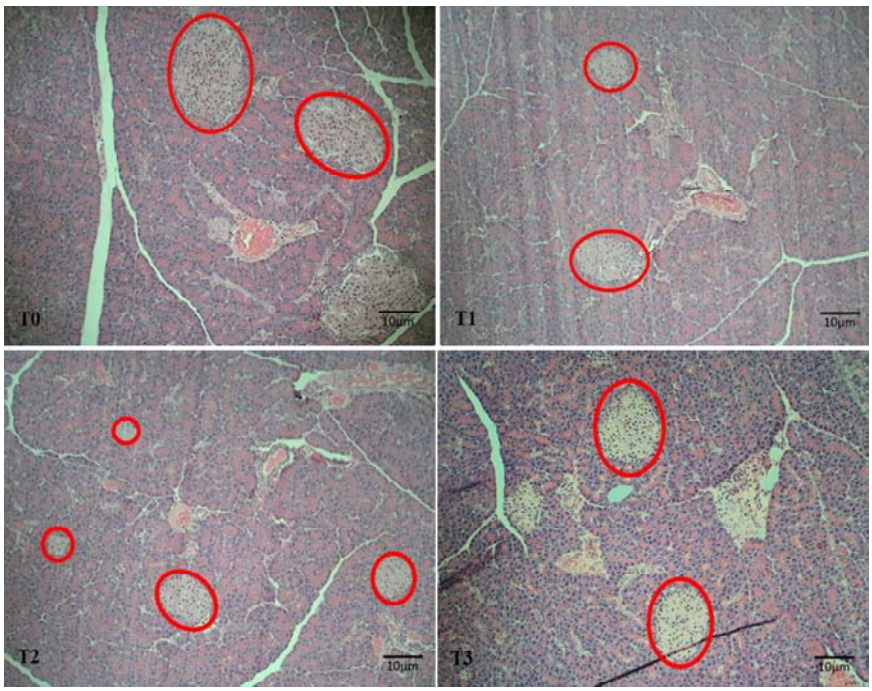

Fig. 3: Histomorphology the number of islets of langerhans from the offspring rat control group (T0) and from treatment valproic acid at gestational age day 10 (T1), day 13 (T2) and day 16 (T3) (HE 10X)

DISCUSSION

Islets of langerhans are formed from endoderm layers, transcription factors such as Pdx1, Ptfla and Sox9 as a signaling central point and primary transcriptional regulation effect on the proliferation and differentiation of the pancreas which starts from the day 8.75 (Shih *et al*., 2013) to the day 12.5 (Pan and Wright, 2011). The 3 gene expression that occur during the secondary transcription (12.5-born) as Nkx 6.1 expressed when branching tip and tunk occurs. Part tip through Ptf1a gene expression will develop into the exocrine pancreas while tunk through Nkx 6.1 gene expression will develop into pancreatic endocrine (Chun *et al*., 2015; Taylor *et al*., 2013). Gene expression Ngn3 play role in expressing progenitor cells during development, either in the β-pancreatic cell or nerve cells at the dentate gyrus of the hippocampus with neuro D1 gene expression (Fratticci *et al*., 2007; Machida *et al*., 2012; Sostrup *et al*., 2014).

VA administration to rats in gestational age day 10, 13 and 16 influence the the growth rate, the relative weights, microscopic feature of the islets of langerhans. Observation of the growth rate showed a decline in the offspring experimental rats born to maternal experimental rats administered with 250 mg VA on day 10, 13 and 16 of pregnancy, compared to control maternal rats without VA administration but the growth rate of the maternal experimental rats administered with 250 mg VA on day 10 of pregnancy, showing a higher growth rate with offspring experimental rats born from control maternal rats without VA administration, the high rate of growth in rat from group T1 because the number of children born from mother rats which given VA at gestational day 10 was fewer (3-5 individuals/birth) so that the weight initial body is greater than the Valproate acid treatment at gestational day 13 (5-8 individuals/birth) and day 16 (9-13 individual/birth) and parent from maternal rats control (9-13 individual/birth). Although, greater initial body weight of offspring rat from the maternal experimental rats administered with 250 mg VA on day 10 of pregnancy but the relative weights of offspring rat from maternal experimental rats administered with 250 mg VA on day 10 of pregnancy shows the lowest value, this is because offspring rat from maternal rats treatment days 10 have low pancreas weight when compared to other groups, thus affecting the value of the relative weights of the pancreas. According to Zafar and Naqvi (2010), a decrease in the relative weighting may occur as a result of weight loss associated with impaired pancreatic loss and destruction of cells that produce insulin. According to Singh and Gupta (2007), the loss of

islets of langerhans, previously followed by the shrinkage the diameter of the islets of langerhans as a result of their beta cell degranulation, hidropic degeneration, piknosis and necrosis. Shrinkage diameter of the islets of langerhans are shown in offspring rat from the maternal experimental rats administered with 250 mg VA when compared to offspring rat from the maternal rat which is not given VA. The average diameter of the offspring rat from the maternal rat control had 109.29μm, this diameter measurement results is supported by Wierup *et al.* (2014) suggested a islet of langerhans nomal diameter of 100-200 μm. The number development of islets of langerhans in normal rat will be increased for 90 days and will remain constant in number but the abnormal mice the decline can be seen before 90 days (Herbach *et al*., 2011). From the study result the number of islets of langerhans of offspring rat from the maternal experimental rats administered with 250 mg VA, we found the decrease in the number of islets of langerhans started at 8 weeks of growth or 56 days of growth.

Microscopic feature with HE staining showed a decrease in the number of cells due to beta cells damage. Endocrine cell degeneration appeared transformed into polymorph (non-uniform). The changes are described in changes endocrine cell nucleus into smaller form (piknosis) even began to disappear and remain only empty cytoplasm containing glycogen deposits and enlarged without the nucleus and cytoplasm shape that experiencing hyperchromatic. Thus, empty occur in the part of the endocrine pancreas (Rupnik, 2009) at a young diabetic, some beta cells showed complete degranulation and empty cytoplasm. Emptiness endocrine part of the pancreas due to a decrease in the number of beta cells, according to Rupnik (2009) was replaced with a hyperplasia non beta cell (Nugent *et al*., 2008), it is appeared in immunohistochemical staining pancreatic tissues of offspring rat from the maternal experimental rats administered with 250 mg VA on the day 16 of pregnancy, showing the greater number of alpha cells in peripheral part compared with controls (Komariah, 2017). According to Nugent *et al*. (2008) changes in cells caused by substances that have a cytotoxic effect, the downsizing of the islet of langerhans and decrease the number of pancreatic beta cells.

Detection of beta cells by immuno histochemistry may indicate the presence of beta cell destruction. In the pancreatic tissues of offspring rat from the maternal experimental rats administered with 250 mg valproic acid, have low immuno reactive insulin compared to offspring rat from maternal rats without valproic acid administration. Immuno reactive decrease to insulin that occurs in the beta cells of offspring rat from the maternal experimental rats administered with 250 mg valproic acid, due to the low capacity of beta cells in secrete insulin. Low insulin secretion resulting from the compensation of beta cell secretion or resistance decreased due to an increase in blood glucose levels, thereby lowering beta-cell function (Cantley and Ashcroft, 2015).

CONCLUSION

Valproic acid administration in maternal experimental rats on day 10, 13 and 16 of pregnancy can inhibit the process of organogenesis pancreas of offspring rat with the decrease in growth rate, the relative weights as well as the number and diameter of islets of langerhans, also a decrease in the number of cells in the islets of langerhans, while in HE staining microscopic observation, we found the damage in the islets of langerhans cells compared to offspring from maternal rats without valproic acid administration.

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