

Pengaruh Vitamin E terhadap Kadar *Malondialdehyde* Jaringan Testis Tikus (*Rattus novergicus*) Strain Wistar dengan Diabetes Mellitus Tipe I

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Abstrak

Latar belakang. Diabetes mellitus (DM) merupakan kelainan endokrin yang menyebabkan kerusakan sistemik dan memicu stres oksidatif di tingkat seluler. Malondialdehyde (MDA) adalah produk stres oksidatif berupa lipid peroksidase yang berhubungan dengan kondisi anomali dan asthenozoospermia. Upaya menekan stres oksidatif adalah dengan vitamin E yang telah lama menjadi antioksidan melawan stres oksidatif.

Tujuan Penelitian. Mengetahui pengaruh pemberian vitamin E terhadap kadar malondialdehyde (MDA) serta pengaruh pemberian vitamin E dengan berbagai dosis terhadap kadar MDA.

Metode penelitian. Penelitian eksperimental menggunakan 30 ekor tikus putih *strain Wistar* jantan yang dibagi menjadi 5 kelompok perlakuan yaitu kelompok tikus tidak DM (KN), kelompok tikus DM (KP), kelompok tikus DM yang mendapat terapi vitamin E dosis 50 iu/kgbb/hr (KP1), dosis 100 iu/kgbb/hr (KP2) dan dosis 150 iu/kgbb/hr (KP3). Pasca perlakuan dilakukan pengambilan organ testis pada semua kelompok dan dianalisis kadar MDA jaringan testis dengan metode *Thiobarbituric Acid Reactive Substances* (TBARS). Analisis data kadar MDA jaringan testis menggunakan *one way* ANOVA $\alpha=0,05$ dengan uji lanjutan LSD.

Hasil penelitian. Pemberian vitamin E dapat menurunkan secara bermakna ($P<0,05$) kadar MDA jaringan testis tikus putih DM. Pemberian vitamin E dosis 150 iu/kgbb/hr tidak berbeda secara nyata ($P>0,05$) dibandingkan dengan dosis 100 iu/kgbb/hr, namun keduanya berbeda secara nyata ($P<0,05$) dibandingkan dosis 50 iu/kgbb/hr.

Kesimpulan. Pemberian vitamin E dapat menurunkan kadar MDA testis tikus dengan kondisi diabetes mellitus tipe 1. Pemberian vitamin E dosis 150 iu/kgbb/hr lebih baik dibandingkan dengan dosis 100 iu/kgbb/hr dan 50 iu/kgbb/hari.

Kata kunci: vitamin E, diabetes mellitus, malondialdehyde

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Effect of Vitamin E on Malondialdehyde Level in Testicular Tissues of Wistar Strain White Mice (*Rattus norvegicus*) with Diabetes Mellitus Type 1

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Abstract

Background. Diabetes mellitus (DM) is the abnormality of endocrine that cause systemic damage and triggers oxidative stress in cellular level. Malondialdehyde (MDA) is a product from oxidative stress, consist lipid, peroxide, and is related to anomaly condition and asthenozoospermia. The effort to suppress the oxidative stress is by using vitamin E that is known well for its function as antioxidane to oppose the oxidative stress.

Aims. To find the effect of vitamin E usage to the level of Malondialdehyde and its effect with different dosage

Methods. Experimental study using 30 white male strain wistar mice divided into 5 treatment groups: non-DM group (KN), DM group (KP), rats with DM that was given Vitamin E 50 IU/kgbw/day (KP1), 100 IU/kg/day (KP2) and 150 IU/kgbw/day (KP3). After intervention, mice testicles in all group were collected. The MDA level of the testicle tissue was analyzed with TBARS method. The data was analyzed with one way ANOVA =0,05 and continues with LSD test.

Results. Vitamin E usage can reduce ($p=0,05$) MDA level in testicle tissue of diabetic white rats. Vitamin E with dosage 150 iu/kgbw/day is not statistically different ($p=0,05$) comparing to 100 iu/kgbw/day dosage, but both dosage is statistically different ($p=0,05$) comparing to dosage 50 iu/kgbw/day

Conclusions. Vitamin E administration can reduce testicular MDA levels in mice with diabetes mellitus type 1. Dosage of vitamin E up to 150 IU/kg/day is better than the lower dosages of 100 IU/kgbw/day and 50 IU/kgbw/day.

Keywords. vitamin E, diabetes mellitus, malondialdehyde

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Introduction

Diabetes Mellitus (DM) is an endocrine disorder caused by increased of insulin resistance and decreased of insulin secretion due to the dysfunction of β cell that lead to hyperglycemia. In 2014, WHO estimated global prevalence of diabetes has risen to 9% in adults over 18 years of age and caused sexual in male due to spermatogenesis impairment and erectile dysfunction. In worldwide, infertility occurred in 15-20% sexually active couples that hopes to have offspring but not pregnant within 1 year. Idiopathic male infertility considered as an multifactorial disorder that is influenced by genetic and environment. Another reported factor is the presence of varicocele, urogenital tract infection, endocrine metabolic disorder, obesity, and abnormal congenital blockage of semen duct.

Related to oxidative stress and destruction of spermatozoa in diabetes mellitus, malondialdehyde, a molecular marker, which is an index of lipid peroxidation and a marker of oxidative stress. MDA, a product of lipid peroxidation, that is generated in constant proportion from the destruction of polyunsaturated lipid.

MDA is a good indicator for lipid peroxidation. MDA level in plasma has been reported to correlate with the survival, motility, morphology and low concentration of sperm.

Oxidative stress occurs in infertility induced by diabetes mellitus, which requires molecular management in term of improving sperm quality. One of the treatment is the use of vitamin E as an antioxidant. This primary antioxidant has properties as a free radical scavenger and breaks the peroxy chain radical reaction by giving one hydrogen atom.

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These also occur with vitamin E, especially α -tocopherol that acts as a radical reaction chain breaker antioxidant and also has potential as singlet oxygen scavenger and other reactive species via hydrogen atom transfer from 6-hydroxyl in chromanol ring together with superoxide dismutase (SOD) enzyme and GPx enroll in decreasing and prevention of lipid peroxidation reaction. There for, this study aimed to assess effect of vitamin E on malondialdehyde (MDA) in white mice testicular tissues with diabetes mellitus. Hopefully the result of this study can become a scientific based of vitamin E as antioxidant to prevent infertility in diabetes mellitus patient, so that it can become an alternative prevention and further management of the condition.

Study Design

This study was a laboratory experimental study using post-test only control group design. Each unit of population was homogen and has the same characteristic. The group selection used random assignment. The experimental group was given stimulus and observed the outcome, meanwhile the control group act as a comparison. The study was conducted in Laboratorium Terpadu Fakultas Kedokteran

Hewan Universitas Syiah Kuala Banda Aceh, from May to June 2017.

Subject

The subject of the study were male white mice *rattus wistar*, adults, aged 3-4 months, weight 200-220 grams, supplied by Fakultas Kedokteran Hewan Universitas Syiah Kuala. The subjects was randomly divided into 5 group, which was: KN: negative control group, mice without diabetes mellitus. KP: positive control group, mice with diabetes mellitus and received aquabidest as placebo. KP1: sample group of diabetes mellitus mice received a dosed of 50 iu/kg/day vitamin E. KP2: sample group of diabetes mellitus mice received a dosed of 100 iu/kg/day vitamin E. KP3: sample group of diabetes mellitus mice received a dosed of 150 iu/kg/day vitamin E.

Inclusion criteria was white mice (*rattus norvegicus*) strain Wistar, in perfect health condition, aged 3-4 months, weight 200-220 grams, with fasting glucose blood level post induced >126 mg/dl in experimental group. Exclusion criteria was mice with different genus and species, different strain. Drop out criteria was ill mice within adaptive period (inactive period), decreased of weight > 10% and death

within study. The minimum samples for each group was 5 mice and with drop out consideration of 10%, the samples of each group was 6 mice. Independent variable in this study was to received vitamin E in different doses, started from 50 iu/kg/day, 100 iu/kg/day to 150 iu/kg/day. The dependent variable was malondialdehyde in term of malondialdehyde level in testicular tissue. Outer variable includes foods, water, genetics, sex, age, weight and temperature. Uncontrolled variable was the mice psychological.

Samples of 30 *Rattus wistar* mice, male, adult, aged 3-4 months. Received homogenization with monitoring the mice weight before received treatment, the rectal temperature and has underwent adaptive period for 2 weeks in cage. The cage temperature was set to room temperature. Every day the mice was feed with 20 grams pellet and water ad libitum. 24 mice was induced with 125 mg/kg alloxan intraperitoneal. After 4-7 days, the mice was fasted for 24 hours and was drawn 1 ml of blood using 1 ml syringe from the mice tail. Then it was examined using glucoDR glucose monitoring stick. Fasting glucose level >126mg/dl was considered as DM and was set to the experimental group. Vitamin E dosage used pure

vitamin E powder of 50 iu, 100 iu and 150 iu diluted with 2 ml olive oil via sonde feeding. Glucose blood test was re-examined on day 28.

Surgery was conducted in all five groups on day 31. The samples was sedated with chloroform. Surgery was attempted on the abdomen. The testis was rinsed using 0.9% NaCl cold solution. It was then soaked in Phosphate Buffer Saline (PBS) with pH 7.4 for Malondialdehyde (MDA) analyzation using Thiobarbituric Acid Reactive Substance (TBARS) methods. TBA/TCA/HCl reagent solution was solute with 4 times water.

The solution was stir using a magnetic stirrer and added BHT until reached a 0.03% of final concentration, testis lysate was centrifuged for 20 minutes with 8000 rpm. 100 µl supernatant, that was produced by centrifugation, added with 550 µl, 100 µl TCA and underwent vortex, then added with 250 µl HCl 1N and underwent vortex. Added again with 100 µl NaThio 1% and underwent vortex again. After these, the solution was centrifugated for 15 minutes with 500 rpm. Supernatan that was produced by centrifugation was separated and moved to a new microtube. Afterward the solution underwent heating in 100°C water bath for 30 minutes. Sample was then measured with spectrophotometry in maximum length wave (532 nm) for assessing

the level of MDA from testicular lysate solution. After absorbance rate had been obtained, level of MDA was calculated by linear regression equation of a standard curve MDA solution.

Data analysis consist of descriptive criteria (body weight, vital sign, glucose blood level before and after treatment) and normality was analyzed with ShapiroWilk $p>0.05$, homogeneity was analyzed with Levene test and one way ANOVA. Then the data was analyzed with LSD test.

Result

The observation of white mice display by weight gain seen that the average weight of white mice during the study increased in all treatment groups. The longer the study, the heavier weight white mice were obtained. The average body weight of mice before treatment ranged between 189-194 grams, increased to 197,67-205,00 gram after the administration of alloxan treatment that induced DM in mice and administration of vitamin E in group trial 1 was 50 iu/kgbw/day, group trial 2 was 100 iu/kgbw/day group trial 3 was 150 iu/kgbw/day. The highest weight gain of mice was found in the

group without treatment (KN) which was $10,83\pm 3,87$ grams and the lowest weight gain of mice was found in group administer with alloxan (KP) which was $5,00\pm 4,15$ grams.

Statistical analysis of variance (ANOVA) with one way obtained significant value $0.00 < 0.05$, administration of vitamin E has an effect on reducing blood glucose levels in white mice with diabetes mellitus. The results of Post Hoc analysis with LSD test showed that the average vitamin E dose of 150 iu/kgbw/day had a significant difference to the positive control treatment group (KP) but does not have a difference with the administration dosage vitamin E of 50 iu/kgbw/day and dosage vitamin E of 100 iu/kgbw/day. These results indicate that the best dosage of vitamin E was 150 iu/kgbw/day.

Testicular tissue MDA levels *Rattus norvegicus* mice in various treatment groups are presented in Table 2.

Statistical analysis of variance (ANOVA) with one way obtained significant value $0.044 < 0.05$, administration of vitamin E has an effect on reducing blood glucose levels in white mice with diabetes mellitus. The results of Post Hoc

analysis with LSD test showed that the average vitamin E dose of 150 iu/kgbw/day had a significant difference to the positive control treatment group (KP) but does not have a difference with the administration dosage vitamin E of 50 iu/kgbw/day and dosage vitamin E of 100 iu/kgbw/day. These results indicate that the best dosage of vitamin E was 150 iu/kgbw/day.

Discussion

Diabetic condition occurred after the mice had been injected with alloxan. The diabetes condition induced oxidative stress that were related with an increase of MDA level in testicular tissue. In baseline characteristic, an increase of body weight was in accordance with length of study. The longer length study resulted to a heavier white mice. The average weight before treatment was between 189-194 grams. There were an increase of body weight to 197.67-205.00 grams after injected with alloxan that induced diabetic in the samples. Vitamin E dosage for treatment 1 was 50 iu/kg/day, treatment 2 was 100 iu/kg/day and treatment 3 was 150 iu/kg/day.

Glucose blood level

The average glucose blood level in control group was 99.40 ± 12.20 mg/dl. As reported previously, the normal glucose blood level of mice is 62.8-175 mg/dl or the average of 143.7 mg/dl. The average glucose blood level of injected mice with alloxan group was increased to 240.20 ± 16.89 mg/dl. Meanwhile, the glucose blood level was decreased in the diabetic mice group that underwent treatment.

The blood level with dosage of vitamin E 50 iu/kgbw/day, 100 iu/kgbw/day and 150 mg/kg/day were $271,20 \pm 271,20$ mg/dl, $196,20 \pm 75,38$ mg/dl and $154,60 \pm 53,05$ mg/dl.

The above condition has proven that administration of alloxan can increased blood glucose level, and induced diabetic in mice. The same was reported by Nurdiana et al. in 1998, where the blood glucose level of a diabetic mouse was above 150 mg/dl. Injection of alloxan induced the increased of glucose blood level by causing necrotizing of β pancreatic cell so that it decreased the insulin secretion. The effects of necrotizing of β pancreatic cell and decreased of insulin secretion are causing cell body unable to utilized the glucose in blood, hence the blood

glucose will rise. Necrotizing of β pancreatic cell after administration of alloxan was caused by increased production of oxygen reactive oxygen and caused in-homeostasis disturbance. Increased reactive oxygen formation is a major factor in pancreatic Langerhans β -island cell damage. Another study reported an increase in blood sugar levels due to alloxan, works directly on the pancreatic Langerhans β -island cells, stimulates the formation of hydrogen peroxide, damages the cell lysosomes and can cause degeneration and resorption of pancreatic cells which can cause insulin deficiency. Similarly, the vital function of cells as a provider of insulin hormone is also disrupted, because lipid peroxidation causes an increase in cell membrane permeability.

The production of insulin hormone becomes reduced, and also its function, so it is unable to use glucose into the tissue. This condition causes the blood glucose blood level to risen.

The results of measurement of blood glucose levels in diabetes mellitus mice induced by alloxan and administration of vitamin E at a dose of 50-150 iu /kgbw/ day showed a marked decrease compared to alloxan-induced diabetes mellitus mice (KP). These results prove that vitamin E in mice with diabetes mellitus has an

effect on reducing fasting blood glucose levels in rats with diabetes mellitus. The decrease in blood glucose levels in diabetic mice after administration of vitamin E may be caused by the repair β cells of the islets of Langerhans of the pancreas through its function as antioxidants to prevent or terminate the chain reaction of free radicals. As reported by Kusuma in 2005 that chemicals that contain antioxidants can reduce the activity of free radicals and protect the langerhans islet from damage or oppose the cytotoxic effects. Furthermore, Watkins in 2008 reported substance that contain antioxidants have been shown to reduce free radicals and protect pancreatic cells from the effects of diabetogenic agents such as streptozizine (STZ).

The effect of vitamin E on testis MDA level on diabetes mellitus mice *Rattus novergicus*

Malondialdehyde was formed from lipid peroxidation on the cell membrane in the cell membrane of reactions with free radicals (hydroxyl radical) with *Poly Unsaturated Fatty Acid* (PUFA). The reaction occurs in a chain, with the final product of the chain reaction was hydrogen peroxide. Hydrogen peroxide can cause decomposition of some aldehyde products

which were toxic to cells and affect the increased risk of infertility and testicular fertility disorders.

The results of the evaluation of testicular MDA levels in each treatment group, was found that the mean MDA testis showed the highest value in the control positive group (KP) $24,286 \pm 10,969 \mu\text{g}/\text{gr}$. Meanwhile in the group given several doses of vitamin E, showed a decrease compared to the group KP. The mean value of MDA testis in each vitamin E administration (50, 100, 150 iu/kgbw/day) were $13,931 \pm 9,146 \mu\text{g}/\text{gram}$, $12,995 \pm 6,103 \mu\text{g}/\text{gram}$ and $10,995 \pm 4,680 \mu\text{g}/\text{gram}$.

The lowest testicular MDA was shown in the group given dosage vitamin E of 150 iu/kgbw/day with value of $10,995 \pm 4,680 \mu\text{g}/\text{gram}$. The difference in mean values of MDA levels in each group also showed significant values ($p \text{ value} = 0,044$) so it can be explained that the administration of vitamin E affects MDA. The results of Post Hoc analysis with LSD test showed that the average vitamin E dose of 150 iu/kgbw/day had a significant difference to the positive control treatment group (KP) but does not have a difference with the administration dosage vitamin E of 50 iu/kgbw/day and dosage vitamin E of 100

iu/kgbw/day. These results indicate that the best dosage of vitamin E was 150 iu/kgbw/day in suppressing MDA in testicular tissue of mice with diabetes mellitus.

The results of this study confirm that the diabetes mellitus can lead to an increase in MDA. MDA systemically been reported to have relation with metabolic parameters in subjects with diabetes type I and II. It has been reported that patients with poor metabolic control will show the highest plasma MDA concentration, significantly different from the group with better control. This phenomenon may occur either due to increased glycosylation and aggregation of platelets, or damage to cellular antioxidative protective system. Increased production of free radicals can play a role in the pathogenesis of metabolic vasculopathy and various cell damage due to DM that induces MDA.³³ Nakhjavani et al. also reported that MDA levels were significantly increased in DM patients compared with controls ($p < 0,001$), and to those who suffering from diabetes over 120 months compared to DM for 120 months or less ($P < 0,001$). Levels of MDA are significantly correlated with the duration of suffering from DM.

In the mechanism of oxidative stress accompanied by an increase in MDA, the condition of infertility in men may occur as a result of damage to the testes and spermatozoa. The study of Colagar et al. also supports that MDA can damage testicular tissue, reported MDA concentrations in infertile male semen plasma ($0,94 \pm 0,28$ nmol/ml) are significantly higher than fertile male ($0,65 \pm 0,17$ nmol/ml) (p value $<0,001$), and have a negative relation with sperm quantity and morphology, and concluded that the increase in lipid peroxidation was associated with the sperm membrane damage and high levels of MDA. These findings suggest that excess ROS production from MDA may play a role in the mechanism of testicular degeneration which was related to infertility.

In this study, vitamin E was administered as an antioxidant in experimental animals with diabetes mellitus, evaluation of an increase in testicular MDA was performed as an indicator of oxidative stress in the testis and its relationship with infertility. by administering several doses of vitamin E showed that MDA has a significant decrease. This is thought to occur due to the role of vitamin E which is able to improve oxidative stress conditions due to DM through a decrease in the results of lipid peroxidation. The

mechanism of vitamin E suppressed the MDA is thought to occur through this primary antioxidant effect as a free radical scavenger and break the chain reaction with peroxy radicals by donating one atom and together with the SOD enzyme, so that it can slow down and prevent the onset of lipid peroxidation reactions in which finally suppress the production of MDA.^{7,8} Another study by Jain *et al.* 1997, also confirm the benefit of vitamin E to suppress MDA in diabetes mellitus condition and also the evaluation of major protein glycosylation that was present on the diabetes mellitus conditions decreased by administering vitamin E. In these experimental animals there was a decrease in glycosylation through inhibition of MDA. In the study MDA and all activities of oxidative stress that cause cell injury was also decreased and inhibited.⁴² The MDA inhibition process that occurs will create better testicular and spermatozoa conditions so that infertility can be prevented. The results of this study also prove that vitamin E helps improve oxidative stress and testicular destruction that was evaluated through MDA biomarkers.

Conclusion

Vitamin E can significantly decreased MDA level in testicular tissue of mice with diabetes mellitus ($p=0.004$). Vitamin E dosage also has an effect to reduce the MDA level in the testicular tissue of mice with diabetes mellitus. Dose of 150 iu/kgbw/day vitamin had a higher reduce effect of MDA level than 100 iu/kgbw/day and 50 iu/kgbw/day vitamin E.

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Table 1. The average blood glucose levels of Wistar diabetes white mice induced by alloxan after treatment with various levels of doses of vitamin E for 28 days

Trial	Glucose blood level (mg/dl)	NB
KN	99,40 ± 12,20 ^a	:
KP	294,20 ± 16,89 ^c	
P1	271,20 ± 94,84 ^c	
P2	196,20 ± 75,38 ^b	
P3	154,60 ± 53,05 ^b	

superscript different letter in the same column show significant difference (p<0,05)

KN = Negative groups who do not get any treatment

KP = Positive group induced by alloxan

KP1 = Group Trial 1 administered with alloxan and dosage vitamin E 50 iu/kgbw/day

KP2 = Group Trial 2 administered with alloxan and dosage vitamin E 100 iu/kgbw/day

KP3 = Group Trial 3 administered with alloxan and dosage vitamin E 150 iu/kgbw/day

Table 2. The average levels of MDA testis in Wistar diabetes white mice induced by alloxan after treatment with various levels of vitamin E for 28 days.

NB : superscript different letter in the same column show significant difference (p<0,05)

Treatment	MDA testicular level (µg/gr)
KN	10,46 ± 4,74 ^a
KP	23,29 ± 11,96 ^b
P1	14,04 ± 6,20 ^a
P2	13,24 ± 10,05 ^a
P3	11,00 ± 5,23 ^a

KN = Negative groups who do not get any treatment

KP = Positive group induced by alloxan

KP1 = Group Trial 1 administered with alloxan and dosage vitamin E 50 iu/kgbw/day

KP2 = Group Trial 2 administered with alloxan and dosage vitamin E 100 iu/kgbw/day

KP3 = Group Trial 3 administered with alloxan and dosage vitamin E 150 iu/kgbw/day