Toxicity effects of 2-methoxyethanol on the nitrite level and damage in tissue of pancreas as a cause of diabetes in mice (Mus musculus) Balb/C

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Toxicity Effects of 2-Methoxyethanol on the Nitrite Level and Damage in Tissue of Pancreas as a Cause of Diabetes in Mice (*Mus musculus*) Balb/C

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Abstract. 2-Methoxyethanol (2-ME) is one of the plasticizer able to induce hormonal system disorders, such insulin resistance. This research was designed to determine the effect of 2-methoxyethanolon blood glucose, levels of nitrite and damage Langerhans island of mice (*Mus musculus* L.). This research was experimental research with Completely Randomized Design (CRD). Female mice strain Balb/C was used as an animal model. Samples were divided into 5 groups; Negative control (NC), KP Positive control (PC) injected intraperitoneally with Streptozotocin (STZ) dose of 30 mg/kg Body Weight (BW) daily for five consecutive days; Treated mice (T1, T2, T3 were injected intraperitoneally with 2-ME daily for ten consecutive days, at dose of 200 mmol/kg BW, 250 mmol/kg and 300 mmol/kg BW respectively). On 16th day and 21thday, mice were sacrificed using chloroform. Fasting blood glucose and nitric oxide (NO) levels were observed in this study. Liver tissue was processed histologically by paraffin method stained with hematoxylin eosin. NO in serum was observed using spectrophotometer with a wavelength of 540. The diameter of Langerhans islands was measured with light microscope. The result of this research was analyzed using the One Way Anova test ($\alpha = 0.05$) was performed to determine the effect of the treatment and Duncan test to find out the difference between the treatment groups. The result showed 2-methoxyethanol was able to increase blood glucose level, while NO was found to be decreased especially in a group treated with 200 mmol/kg BW 2-ME.

Keywords: 2-Methoxyethanol, blood glucose, nitric oxide (NO), Langerhans island,mice.

INTRODUCTION

The prevalence of the incidence of diabetes mellitus (DM) in the world reached more than 350 million people in 2013 [1-3]. International Diabetic Federation (IDF) projecting a 55% increase in the prevalence of diabetes in the world in 2035. This shows that there may be an increase in larger, especially when considering that 80% of people with diabetes live in countries with a standard of living low and middle income [1]. Indonesia is a country that was ranked fourth in the world by the number of people with DM 8.4 million people in 2000 and this figure is expected to rise to 21.3 million people by 2020 [4].

2-Methoxyethanol (2-ME) is one of the metabolites result from dimethoxy ethilphatalate (DMEP). Dimethoxy ethilphatalate is one group of phthalic acid esters is widely used as a plasticizer in the manufacture of plastics. 2-ME compound is highly flammable, colorless and volatile [4]. The use of 2-ME can also be found in companies that manufacture semiconductors, textiles, leather finishing and plastic food boxes, widely used as a solvent, especially used in paint, ink, paint thinner, smear, and coatings [5].

Alonso-Magdalena estimated that the widespread use of plastic materials for household use associated with the food packaging can explain the possibility of an increase in the epidemic of diabetes and obesity are more common

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in industrialized nations [6]. The data shows an increase in diabetes, the incidence of obesity, atherosclerosis, coronary heart disease, infectious disease and renal disease [7].

Some research suggests that the plasticizer compounds capable of causing obesity and cause hormonal system disorders, such as insulin resistance. Thus, 2-ME was suspected to be the cause of diabetes emergence through insulin resistance. Oxidative stress occurred in patients with diabetes is caused by an imbalance of redox reactions due to changes in the metabolism of carbohydrates and lipids, in addition to decrease in antioxidant capacity. Increased concentrations of free fatty acids occurs with increased superoxide production by the mitochondria and an increased risk of exposure of the cells by ROS.

NO had an extremely short half-life of about 3-5 seconds, since NO will quickly react with O_2 to form nitrite (NO_2) and nitrate (NO_3), which is ultimately excreted through the kidneys [8]. Therefore, in this study was designed to know the effect of the induction of 2-ME is toxic in the body organs, blood glucose levels, tissue damage in the pancreas, liver and the amount of nitric oxide in experimental animals of mice.

METHODS

This research was conducted at the Laboratory of Molecular Biology, Department of Biology, Faculty of Science and Technology, Airlangga University, Surabaya. We used glucometer Accu-Check Active to observed blood glucose. To measure levels of NO were used a spectrophotometer (λ=540). Histopathology of the pancreas and liver was observed using paraffin method and stained with hematoxylin eosin. 2-Methoxyethanol used from Wako Pure Chemical Industries, Ltd., Japan with doses of 200, 250, and 300 mmol/ kg BW, while the streptozotocin/2-deoksidasil-(3-(metil-3-nitrosourea)-1-D-glukopiranosa (STZ) S0130-1G, from sigma, injected with dose of multiple low-dose. This research was a laboratory experiment with a completely randomized design (CRD). Female mice Balb/C, from the Faculty of Pharmacy, University of Airlangga were used.

Before treated, fasting blood glucose of all mice was recorded. Positive control and treatment groups were given lard for 21 days to induce obesity. After lard induction, positive control was injected with STZ for 5 days, while treatment group was divided into 3 groups based on 2-ME doses given; P1 (200 mmol /kg BW 2-ME), P2 (250 mmol/kg BW 2-ME), and P3 (300 mmol/kg BW 2-ME) for 10 consecutive days. The negative control was injected with distilled water. On the 2^{nd}, 7^{th}, and 14^{th} day of treatment, fasting blood glucose all mice were observed. Blood and serum were collected after treatment. NO was observed by adding to a mixture of Griess reagent 1 and 2 to the serum. Spectrophometer at λ 540 nm is used to measure the nitric oxide level. All data were analyzed statistically using SPSS 15 (α=0.05).

RESULTS AND DISCUSSION

To determine the effects of 2-ME compounds on the incidence of diabetes mellitus, blood glucose level was recorded before and after treatment. From these results show that fasting blood glucose levels were changed in various groups of treatment. Fasting blood glucose levels were changed in various treatment groups ranging from fasting blood glucose levels in mice one day after administration of lard, the second day after the induction of 2-ME, the seventh day after the induction of 2-ME and day 14 after induction 2-ME shows the results are diverse (Fig. 1).

The result showed that on the second day after the induction of 2-ME, blood glucose of treatment group was significantly increase compared to both control groups, while on 7^{th} day 2-ME injection, P1 and P3 did not differ significantly to both controls. Lard was injected to induce obesity, as obesity is associated with insulin resistance that would lead to diabetes mellitus. Free fatty acids due to obesity could affect insulin signaling through stimulation of isoform protein kinase (CCP). Free fatty acids can also disrupt the release of glucose from the liver [9,10].

In the second day post injected of 2-ME was the most significant to induce high blood glucose or hyperglycemia. We suggested that when 2-ME entered the body, oxidation was promptly occurred, especially in the cytoplasm of liver mitochondria. Thus the main target of 2-ME in the body was the liver. In the body, 2-ME would circulate within the bloodstream and into the cells, then 2-ME to be transformed metabolically to produce primary and secondary metabolites. On the other hand, streptozotocin (STZ) as a diabetic agent selectively destroys the β cells in the islets of Langerhans in the pancreas [11]. 2-ME whose main goals are liver mitochondria, which then would

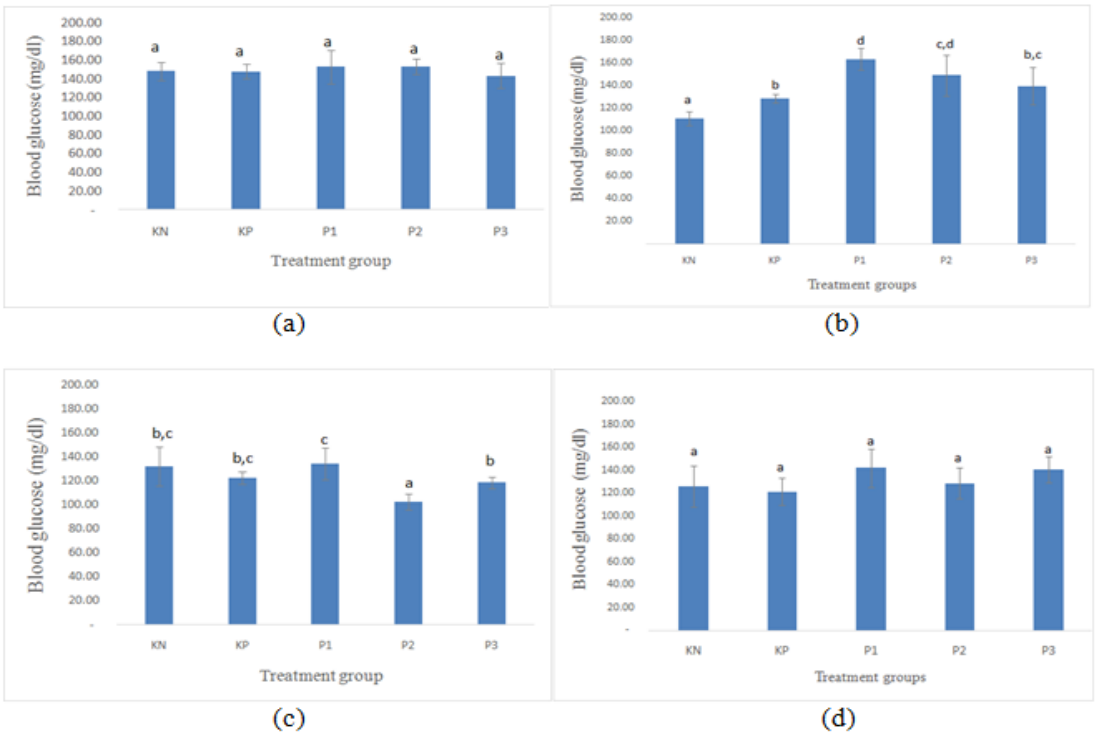

FIGURE 1. Fasting blood glucose level from each group at (a) after lard administration, (b) second day, (c) seventh day, (d) 14th day after 2-ME induction. KN: normal control, KP: positive control, P1: 200 mmol/kg BW 2-ME, P2: 250 mmol/kg BW 2-ME, P3: 300 mmol/kg BW 2-ME. Same letters indicate no significant difference between groups based on Duncan test (α=0.05).

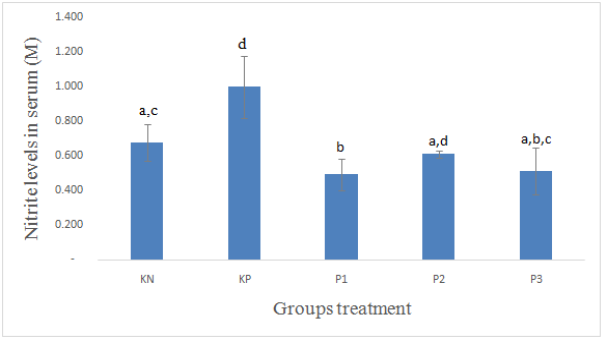

FIGURE 2. Resulting nitrite (NO) level from each treatment group. KN: normal control, KP: positive control, P1: 200 mmol/kg BW 2-ME, P2: 250 mmol/kg BW 2-ME, P3: 300 mmol/kg BW 2-ME. Different letters indicate the statistical difference.

produce secondary metabolites methoxyacetic acid (MAA) and MALD after being oxidized in the liver. Both MAA and MALD in cell body could induce necrotic to cells [12], while MAA had teratogenic and toxic effects [5].

Due to the toxic effect of MAA and MALD, onset of ROS could be triggered, as 2-ME has been shown to induce radical H_2O_2 in the process of oxidation, reduce molecular oxygen during cell respiration in the mitochondria, and also trigger the formation of radicals by producing superoxide (O_2), hydroxyl (HO) and hydrogen peroxide (H_2O_2) [13]. The increasing number of free radicals contained in the body will lead to increase the likelihood of damage to other organs, and in this case, if the organ damage pancreatic β cell damage then it will affect the stability of blood glucose levels themselves.

Oxidation of 2-ME into MAA in serum and plasma occurred quickly, at half-life of about 6 hours in rats, but the excretion of MAA was slow, with a half-life of about 20 hours in monkeys and half-life of MAA in human urine is 77 hours. MAA is excreted in human urine about 86% of all 2-ME inhaled [14,15].

As previously shown in Fig. 2, STZ injection could elevate nitrite level, but 2-ME on the other hand decreased nitrite level compared to control. It was possible that three weeks after induction of 2-ME, NO would decrease because of NO immediately oxidized to nitrite and the remainder in the form of nitrate spread throughout the body [16, 8]. Half-life of NO in blood is very short, less than 5 seconds and 13 minutes for nitrite oxide [16].

The effect of 2-ME in the pancreas gland was shown in Fig. 3. It was especially visible on the size of the island of Langerhans. The diameter of Langerhans Islets was measured using to determine the alteration to it after 2-ME exposure. There are several types of cell comprising langerhans islets, such as alpha cell, beta cell, delta cell, and F cell, but the largest component of langerhans islets is beta cell with 70% parts of the langerhans islets, while the alpha cell is 15%, delta cell only 10%, and the smallest part is F cell. Based on that previous research, the diameter of langerhans islets is measured to represent the beta cells [17].

Increased blood glucose levels in the blood for a long time induced in insulin secretion and other signaling mechanisms. Abnormalities in insulin secretion mechanism would cause reduced intake of glucose into the cells and increase in of blood glucose levels, in the other word hyperglycemia [18]. Hyperglycemia could result in the formation of reactive oxygen species (ROS). Excessive ROS induced oxidative stress, leading to damage β cells [19].

2-Methoxyethanol in the body is metabolized in hepatocytes cells by alcohol dehydrogenase into 2-methoxyacetaldehyde and then again by aldehyde dehydrogenase metabolized into toxic MAA. MAA can increase cell membrane permeability that results in an influx of $Ca2^{+}$ ions. The abundance of $Ca2^{+}$ in the cell will inhibit oxidative phosphorylation, so the acquisition of ATP is reduced because the energy used to pump Ca^{2+} excess. In addition, the abundance of Ca^{2+} also activates the enzyme protease and phospholipase that degrade the proteins of the cytoskeleton that are required in building the structure of the cell. $Ca2^{+}$ is also the as a mediator for apoptosis [5,20,21]. The presence of apoptotic phase will result in reduced β cell mass in the islets of Langerhans of the pancreas and insulin synthesis will decline.

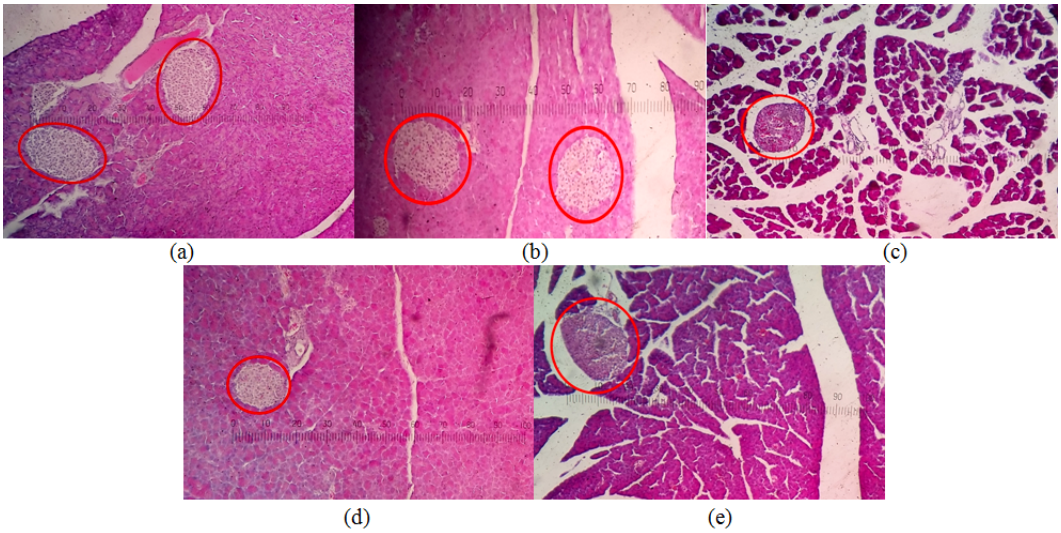

FIGURE 3. The diameter of the islets of Langerhans from (a) normal control, (b) positive control, (c) P1(200 mmol/kg BW 2-ME), (d) P2 (250 mmol/kg BW 2-ME), (e) P3 (300 mmol/kg BW 2-ME). Red circles indicate the Langerhans islets.

TABLE 1. Statistical analysis of the diameter of the islets of Langerhans as a result of administration of 2-ME. The figure followed different letters indicate significant differences (α = 0.05)

Groups of treatment	Averages of islet Langerhans diameter (µm)
Control (KN)	132,78 ±22,58^{b}
Injected STZ (KP)	108,28±22,07^{ab}
200 mmol/kg BW 2-ME (P1)	89,78±21,23^{a}
250 mmol/kg BW 2-ME (P2)	97,75±17,34^{a}
300 mmol/kg BW 2-ME (P3)	116,42±17,99^{ab}

From histological observation of pancreas, KP group had lower average diameter compared to the normal control (KN). This indicated that there was a significant effect of lard and STZ to average diameter of the islets of Langerhans. On the other hand, P1 (200 mmol/kg BW 2-ME) had the lowest diameter of the islets of Langerhans compared to other treatment groups. The results of statistical analysis using Duncan test $\alpha = 0.05$ in Table 1 showed a significant difference between the P1 and P2 to KN. However, P3 showed no significant difference to KN.

The diameter of the islets of Langerhans in a group P1 and P2 was lower compared to KP caused by cellular apoptosis due to blood glucose level has exceeded certain critical threshold [22]. Administration of 2-ME with a higher dose of 300 mmol/kg BW in the P3 group, showed higher diameter of the islets of Langerhans compared to P1 and P2. This could be associated with compensatory mechanisms of cells β islets of Langerhans, due to the increase in blood glucose levels the cells would increase its efforts to produce more insulin [23].

PPAR-α, PPAR-γ, c-Myc were the type of transcription factors required for regulation of insulin secretion. Their level would increase before cell hypertrophy occurred. If the β cells have entered adaptation stage to high blood glucose level, β cells in the islet will proliferate to balance demand for more insulin secretion. However, before able to adapt and proliferate, β cells in the islands of Langerhans undergo apoptosis due to loss of response of acute glucose-stimulated insulin secretion (acute GSIS). Thus, P3 had higher diameter of Langerhans Islets compared to other treatment groups which were given a lower dose of 2-ME.

The previous study had demonstrated that 2-ME in the animal's body would be metabolized in the liver. 2-ME would undergo metabolism and converted into Methoxyacetic acid (MAA). Both 2-ME and MAA induced necrotic and apoptotic in the embryonal and adult cells of mice, and also congenital malformation in mice and rat [24-29], but in adult animals, effect on other organ had not yet clear, especially in the mechanism of tissue damage that is capable of supporting the emergence of diabetes mellitus.

CONCLUSIONS

From these results, it can be concluded that 2-ME cause increased blood glucose and nitric levels. Increased blood glucose may be related to by damage to Langerhans islet cells of the pancreas characterized by a decrease in the diameter of the island of Langerhans.

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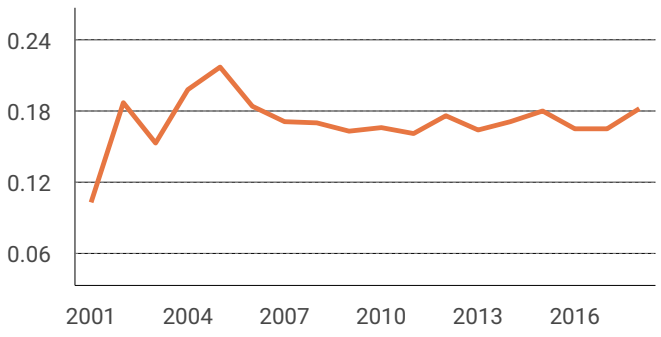

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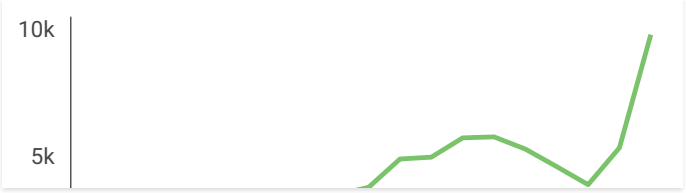

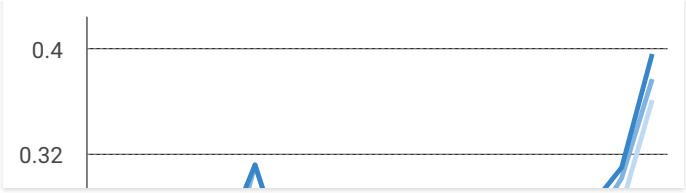

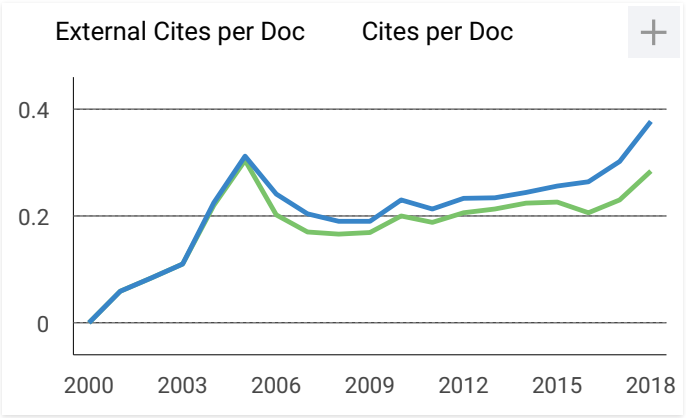

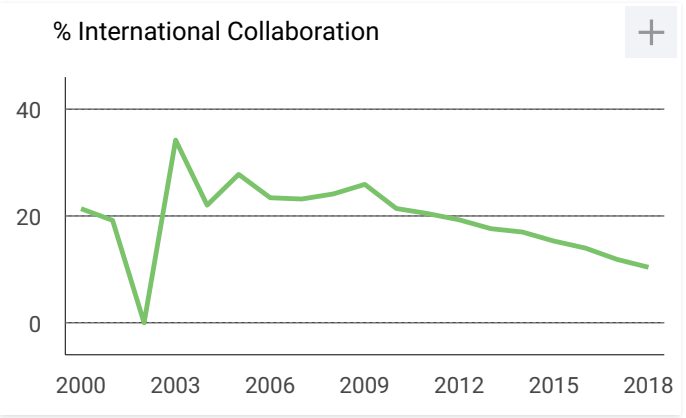

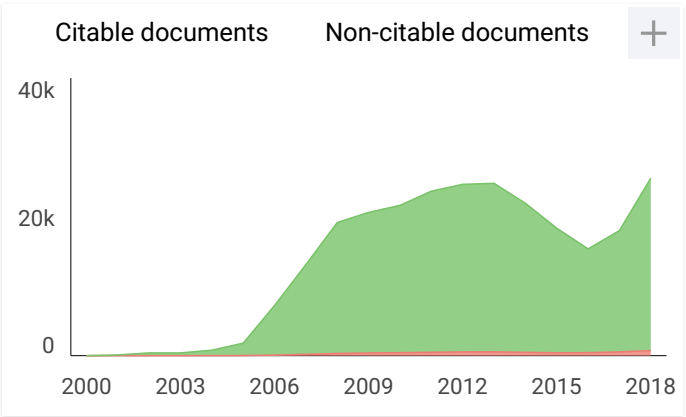

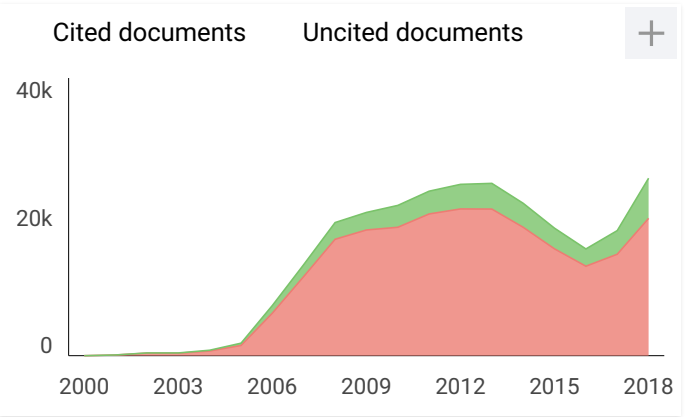

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Purification and characterization of polyclonal antibody against acrylamide

L. D. Assaat, T. A. Ivandini and E. Saepudin

AIP Conference Proceedings **2023**, 020106 (2018);
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Synthesis and photocatalytic activity of nanocomposite based on sodium alginate from brown algae with ZnO impregnation

H. Helmiyati and K. D. Wahyuningrum

AIP Conference Proceedings **2023**, 020107 (2018);
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Modification of nitrogen-terminated boron-doped diamond electrodes with gold nanoparticles and hemoglobin for acrylamide biosensors

T. N. Annisa, E. Saepudin and T. A. Ivandini

AIP Conference Proceedings **2023**, 020108 (2018);
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Preparation and characterization of nitrogen-doped highly ordered titanium dioxide nanotubes (N-doped-HOTN): How far it will improve toward the visible light response and why?

A. D. Pangestuti and J. Gunlazuardi

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The study on *in vitro* formation of DNA adduct 8-hydroxy-2-deoxyduanosine (8-OHdG) from benzo[a]pyrene and Fenton-like reaction

B. Budiawan, D. O. Putri, S. Handayani, I. C. Dani and R. Bakri

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Enzymatic synthesis of glycerol ester hydrolyzed coconut oil fatty acid and lauric acid as emulsifier and antimicrobial compound

K. Sangadah, S. Handayani, S. Setiasih and S. Hudiyono

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Interspecific competition between *Callyspongia aerizusa* sponge and scleractinian coral at Pramuka Island, Kepulauan Seribu National Park Jakarta

N. Widya and Y. Yasman

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Molecular identification of the medicinal herb plant *Ruta angustifolia* from Lembang Bandung using chloroplast DNA markers

S. Noer, A. Abinawanto and A. Basith

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Three new species of Aquilaria (Thymelaeaceae) from Borneo Indonesia

T. Mulyaningsih and I. Yamada

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The relationship between copper (Cu) concentration on phytoplankton and *Nitzschia* dominance at Blanakan fish ponds, Subang, West Java

T. Siswantining, N. D. Takarina and A. Wiriawan

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Toxicity effects of 2-methoxyethanol on the nitrite level and damage in tissue of pancreas as a cause of diabetes in mice (*Mus musculus*) Balb/C

W. Darmanto, J. A. Claudia, B. A. Turnip, S. P. A. Wahyuningsih, S. A. Husen, N. S. Aminah and E. S. Sajidah

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Plants diversity on postpartum recovery medical used in sundanese community forecourts in Ciburial village, Banten

S. D. Rosadi, N. Nisyawati and A. Putrika

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DNA barcoding to identify the genetic diversity of gabus Sentani fish (*Oxyeleotris heterodon*, Weber 1907) at Putali Gulf Sentani Lake

A. Abinawanto and E. D. Sriyani

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Isolation and screening antibacterial activity of actinomycetes from mangrove ecosystem, Pramuka Island, Kepulauan Seribu, Jakarta, Indonesia

Q. G. Fadhilah, I. Santoso and Y. Yasman

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Anatomical and sound intensity comparison of bamboo culms that used as Angklung Gubrag in Cipining Village

S. Maulidyawati, N. Nisyawati and A. Putrika

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The heavy metal analysis of mercury (Hg) and chromium (Cr) on frozen escolar *Lepidocybium flavobrunneum* collected from fisheries management area 573

D. Ratnasari, N. D. Takarina and T. Siswantining

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Molecular identification of fungal species from deteriorated old Chinese manuscripts in Central Library Universitas Indonesia

A. Oetari, M. Rahmadewi, M. K. Rachmania and W. Sjamsuridzal

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Implementation DNA barcoding for genetic identification of cuscus from Ambon Island

E. N. Kusumaningrum and A. Abinawanto

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Molecular phylogenetic analyses of

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filamentous fungi from deteriorated old Chinese manuscripts in Central Library Universitas Indonesia

M. K. Rachmania, A. Oetari, M. Rahmadewi and W. Sjamsuridzal

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The growth differences between *Leptolyngbya* HS-16 isolated from Gunung Pancar hot spring (69 °C) and *Leptolyngbya* HS-36 isolated from Maribaya hot spring (42 °C) incubated in 20 °C and 50 °C

Z. D. Pertiwi and N. B. Prihantini

AIP Conference Proceedings **2023**, 020125 (2018);
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Isolation and screening antimicrobial activity of actinomycetes from sediment's coastal Pramuka Island, Kepulauan Seribu, Jakarta, Indonesia

R. Alfisyahri, I. Santoso and Y. Yasman

AIP Conference Proceedings **2023**, 020126 (2018);
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Application of immunohistochemistry methods for detecting of betanodavirus in tiger grouper fish (*Epinephelus fuscoguttatus*)

S. Andriyanto, A. Abinawanto and A. Salamah

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Impact of batik wastewater pollution on macrobenthic community in Pekalongan River

S. Naqsyabandi, E. Riani and S. Suprihatin

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Phytochemical contents and antioxidant activities of mangrove (*Avicennia marina*) leaves extract

N. D. Takarina, G. A. F. Arif and S. A. Juhriah

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Bioactivity of *Thalassia hemprichii* (hydrocharitaceae) methanolic extract from West Bali National Park as *Aedes aegypti* larvacide

Y. Yusniawati, Y. Yasman and W. Handayani

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Characteristic of semisolid isinglass from catfish (*Pangasius* sp.) swimbladder based on different concentration in acetic and citric acid solution

W. N. Asty, Y. Yasman and W. Wardhana

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Gonad maturity level of mackerel from fishing ground of Pandeglang area

A. Sutiana, N. D. Takarina and M. Nurhudah

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Sperm analysis of Lukas fish (*Puntius*

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A. Abinawanto, S. Yimastria and P. Pertiwi

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Morphological and microscopical characterization of fungi from deteriorated old Chinese manuscripts from Central Library Universitas Indonesia

M. Rahmadewi, A. Oetari, R. Fitri, T. Susetyo-Salim and W. Sjamsuridzal

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Characterization of culturable cyanobacteria isolated from geyser of Cisolok in West Java, Indonesia

N. B. Prihantini, W. Sjamsuridzal and A. Yokota

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Comparative morphology of single and double flowers in *Hibiscus rosa-sinensis* L.

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(Malvaceae): A homeosis study

A. Salamah, R. Prihatiningsih, I. Rostina and A. Dwiranti

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The bioacoustics analysis and the morphometrics study of the gaga's chicken (Ayam Ketawa) from Pinrang and Kebayoran Lama

A. Abinawanto and P. S. Effendi

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The profile of *HSP90* gene expression of Bali cattle to heat stress in West Sumbawa, West Nusa Tenggara

S. A. Puteri, S. B. Aritonang, H. T. Nussa, A. F. Rahmani, A. Bowolaksono and R. Lestari

AIP Conference Proceedings **2023**, 020138 (2018);
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Growth of *Rhizopus delemar* UICC 27, UICC 67, and UICC 121 on the slurry and palm

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R. S. Putri, A. Oetari and I. Santoso

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Plant diversity of building materials in yard based on Sundanese community perspectives in Ciburial Village, Cimanggu Sub-district, Banten

S. S. Widianingsi, N. Nisyawati and A. Putrika

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Phylogenetic analyses based on ITS regions of rDNA identified five *Rhizopus* strains from tempeh as *R. delemar* and *R. oryzae*

M. Khasanah, W. Sjamsuridzal, A. Oetari, I. Santoso and I. G. Roosheroe

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Morphometric and bioacoustic analysis Gaga chicken (*Gallus gallus domesticus*) at Bangkalan, Kamal Madura

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T. Zulistiana and A. Abinawanto

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Fermentation of antimicrobial substances of *Streptomyces* sp. BCy isolated from seagrass *Cymodocearotundata* using two different media

V. Damayanti, R. N. Rachma, I. Santoso, Y. Yasman and A. E. Maryanto

AIP Conference Proceedings **2023**, 020143 (2018);
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Isolation and morphological characterization of fungi from deteriorated old Chinese manuscripts from Central Library Universitas Indonesia

M. K. Rachmania, A. Oetari, R. Fitri, T. Susetyo-Salim and W. Sjamsuridzal

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The growth curve of *Synechococcus* HS-7 and *Synechococcus* HS-9 isolated from Indonesia hot springs grown in CT medium

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with initial pH 9

R. Julia and N. B. Prihantini

AIP Conference Proceedings **2023**, 020145 (2018);
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Flowering and fruit set of improved population of *Jatropha curcas* L. under climate of Northern Lombok, Indonesia

B. B. Santoso and I. G. M. A. Parwata

AIP Conference Proceedings **2023**, 020146 (2018);
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Growth of *Rhizopus microsporus* UICC 500, UICC 531, and UICC 539 on the palm oil processing waste

F. Prameswari, A. Oetari and I. Santoso

AIP Conference Proceedings **2023**, 020147 (2018);
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Identification of genetic diversity freshwater crayfish (Cherax spp.) in Baliem River – Pike village (Jayawijaya District) based on DNA barcoding analysis

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