Effect of 2-methoxyethanol induction on mice (Mus musculus) liver, kidney and ovary

W Darmanto¹*, S P A Wahyuningsih¹, S A Husein¹, N S Aminah², A N Firdaus¹, E S Sajidah¹, M Izzatin¹ and F Khaleyla¹

1 Department of Biology, Faculty of Science and Technology, Airlangga University, Surabaya, 60115, Indonesia.
2 Department of Chemistry, Faculty of Science and Technology, Airlangga University, Surabaya, 60115, Indonesia.

*E-mail: Win Darmanto (windarmanto@fst.unair.ac.id; darmanto2000@yahoo.com)

Abstract. These research was designed to evaluate the effect of 2-methoxyethanol (2-ME) injection to the number of damaged hepatocyte, renal tubule, and follicle. This study was designed as Completely Randomized Design experimental study. Animal model used was 2-3 months old 20 female mice strain Balb/C mice weighed at 25-30 g. Mice were divided into 4 treatment groups; N (normal control), G1 (given 200 mg/kg BW 2-ME), G2 (given 250 mg/kg BW 2-ME), and G3 (given 300 mg/kg BW 2-ME). 2-ME was administrated intraperitoneally daily for 5 consecutive days. Liver, kidney, and ovarium were removed at the end of treatment period then processed into histological slides. Damage on hepatocyte, nephron, and follicle was evaluated from tissue sections. Data collected was analyzed statistically. Result showed that level of damage increased along with 2-ME dose given to mice. KP3 was found to have highest number of damaged cell in the three organs, followed by KP2, and KP1 had the lowest number of damaged cells compared to KN. Injection of 2-ME at various doses could induce damage on hepatocyte, renal tubule, and ovarian follicle.

1. Introduction

2-Methoxyethanol (2-ME) is one of dimethoxyethylphatalate (DMEP) derivates. Dimethoxyethylphatale is a group of phthalic acid esters (PAEs) widely used as plasticizer in plastics manufacturing. A lot of plastic material is now used for everyday life purpose, such as household appliances, packaging materials, water pipes, children's toys, and various medical equipments. 2-Methoxyethanol is also widely used as organic solvent for various industrial products manufacture such as paints, acetate cellulose, resins, varnish, nail polish, and wood coloring [1].

2-Methoxyethanol is formed as result of DMEP metabolism inside a mammalian body. DMEP compound in the body will be hydrolyzed into 2-ME, in which in turn will be oxydyzed by alcohol dehydrogenase into 2-methoxycetaldehyde (2-MALD). Further metabolism of 2-MALD will result in methoxyacetic acid (MAA) which have toxic and teratogenic effect [2]. Thus, 2-ME by itself is not teratogenic and toxic, but its metabolite result, MAA, is both teratogenic and toxic [3].

2-Methoxycetaldehyde (MALD) was found to be toxic for both hepar and testes [4]. If MALD were accumulated in the hepatocytes, it would trigger the formation of free radicals that induced...
oxidization of lipids, reducing membrane fluidity, and increasing permeability of cell membranes. In addition to liver, kidney and ovary were also risked to be exposed to free radicals formed by MALD. Kidney is main path for toxic substance excretion, in where blood circulate at high volume, toxic substance is concentrated in filtrate, and also certain toxic substances is activated, making it a main target for toxic substances [5]. In the other hand, damage in ovarian follicle can be induced either by cytotoxic effect or hormonal disturbance it caused due to enzymatic anti-oxidative activity as response to elevating free radicals level.

Liver and kidney were previously found to be able of actively eliminating toxic substance [6] such as 2-ME. In addition to both organs, reproductive organ was also found to have response towards toxic substance. This response was more prominent in female, as female animal was found to have higher sensitivity towards substance toxicity compared to male, at which same dose already induced physical symptom in female but yet to cause change in male animal [7]. Based on previous elaboration, this study was designed to evaluate damage in cells of liver, kidney, and ovary of mice after exposure to 2-methoxyethanol.

2. Materials and methods

As much as 3-4 months old 20 female mice strain Balb/C weighed 25-30 g were used as animal model in this study. Mice was acclimatized for three weeks before divided into 4 treatment groups consisted of 5 mice each; N (normal control), G1 (given 200 mg/kg bw 2-ME), G2 (given 250 mg/kg bw 2-ME), and G3 (given 300 mg/kg bw 2-ME). 2-methoxyethanol was dissolved into distilled water and injected intraperitoneally. Injection of 2-ME at various doses for each treatment group was performed daily for 5 consecutive days. In the end of treatment, mice were sacrificed then liver, kidney, and ovary were fixed. Sample was processed into histologic slides using paraffin method, sectioned in 4 µm thicknesses, and stained with hematoxylin-eosin. Slides were evaluated for tissue damages. Data collected was analyzed statistically (α=0.05).

3. Result and Discussion

Result of evaluation from histological slides of liver from each treatment group was presented in Figure 1. Section of liver from each treatment group was presented in Figure 2. Normal hepatocyte cell count was highest from normal control, amounted 54.84 cell/cm² (Fig. 1A), while highest necrotic cell number was found in G3 at 33 cell/cm² (Figure 1(B)). Highest number of swollen hepatocyte was also found in G3 at 16.22 cell/cm². From data analyzed, the higher 2-ME dose injected to mice, hepatocyte damage level in the form of necrotic and swollen cell was also found to be significantly increased.

Evaluation of damage in histological section of renal tubules from each treatment group was presented in Figure 3, including normal, necrotic, edema, and swollen cell count, while histological image from each group was presented in Figure 4. Highest count of normal tubule cells was found from normal control (72.5 cell/cm²), while highest count of necrotic, edema, and swollen tubule cells was found from G3 (62.2 cell/cm², 41.16 cell/cm², and 33.32 cell/cm² respectively) which injected with highest dose of 2-ME compared to other treatment group. Normal cell count was found to decrease significantly along with increase of dose, while necrotic, edema, and swollen cell count increased significantly along with increasing of 2-ME dose injected to mice.
Figure 1. Graphic showing number of (A) Normal, (B) Necrotic, and (C) Swollen hepatocyte cells after 2-ME injection. N: Normal group, G1: Group 1 given 200 mg/kg BW 2-ME, G2: Group 2 given 250 mg/kg BW 2-ME, G3: Group 3 given 300 mg/kg BW 2-ME.

Figure 2. Histological section of liver of (A) Normal group, (B) Group 1 (200 mg/kg BW 2-ME), (C) Group 2 (250 mg/kg BW 2-ME), and (D) Group 3 (300 mg/kg BW 2-ME). VC: central vein, S: sinusoid, green arrow: swollen cell, red arrow: necrotic cell.
Figure 3. Graphic showing number of (A) Normal, (B) Necrotic, (C) Edema, and (D) swollen tubule cell count after 2-ME treatment from N (Normal control), G1 (200 mg/kg BW 2-ME), G2 (250 mg/kg BW 2-ME), and G3 (300 mg/kg BW 2-ME).

Figure 4. Histological section of kidney from each treatment group. (A) Normal control, (B) Group 1 (200 mg/kg BW 2-ME), (C) Group 2 (250 mg/kg BW 2-ME), (d) Group 3 (300 mg/kg BW 2-ME). black arrow: glomerulus, green arrow: edema, red arrow: necrotic cell, blue arrow: swollen cell.
Evaluation of 2-ME effect in ovary histological section including normal and atresia secondary and tertiary follicle was presented in Figure 5, while histological section of ovary from each treatment group was presented in Figure 6. The number of normal and atresia secondary and tertiary follicle in normal control was found to be statistically different from any of treatment group injected with various 2-ME doses. Highest number of normal secondary and tertiary follicle was found from normal control (11.72 and 9.1 respectively), while highest number of atresia secondary and tertiary follicle was found from G3 (9.3 and 7.8 respectively). The count of atresia follicle increased, while normal follicle reduced significantly along with 2-ME dose given to mice, indicated that 2-ME injection was able to induce damage in accord with level of dose.

Figure 5. Graphic showing count of (A) Normal and (B) Atresia secondary follicle and (C) Normal and (D) Atresia tertiary follicle per ovary from each treatment group. N: Normal group, G1: Group 1 (200 mg/kg BW 2-ME), G2: Group 2 (250 mg/kg BW 2-ME), G3: Group 3 (300 mg/kg BW 2-ME).

Necrotic hepatocyte due to negative effect of 2-ME-induced ROS was affected by oxidative stress due to imbalance of free radicals with existed antioxidant. Based on Lu, liver frequently became target of toxic substance. Most of toxicant entered the body via gastrointestinal system which would then circulate within the blood into the liver via hepatic portal vein. In this study, the higher 2-ME dose injected, the higher number of necrotic hepatocytes found. Cell death was commonly indicated by change of nucleus. Nucleus of dead cell would shrink, had no clear margin, and colored dark, called pyknosis. In addition, nucleus could also be destructed, leaving remains of chromatin in the cell called karyorrhexis. This would leave cell lost its ability to be stained, termed as karyolysis [8]. Death of cell occurred together with disintegration of cell membrane [5].
Figure 6. Histological section of mice ovary from each treatment group. (1) Normal group: (1a) normal secondary follicle, (1b) normal tertiary follicle, (2) Group 1; (2a) atresia secondary follicle, (2b) atresia tertiary follicle, (3) Group 2; (3a) normal secondary follicle, (3b) normal tertiary follicle, (4) Group 3 (4a) atresia secondary follicle, (4b) atresia tertiary follicle. Blue arrow: nucleus, red arrow: granulose, black arrow: cytoplasm.

Based on evaluation result, necrotic cells elevated along with increase of dose injected, indicating that 2-ME was able to increase ROS level, which induced oxidation stress and lipid peroxidation that caused damage in plasma membrane. High level of Reactive Oxygen Species (ROS) occurred due to high level of toxin, led to loss of hepatocyte membrane integrity. Lipid peroxidation was one of the processes caused by free radicals, which would disrupt Ca^{2+} homeostasis and induce damage on hepatocyte structural integrity and cell necrosis [9].

In addition to liver, kidney was also affected by toxic effect of 2-ME, as kidney had high volume of blood circulation, concentrated toxicant in its filtrate, and brought along toxicant via tubule cells, and also was able to activate certain toxicant. These properties correlated to kidney function for filtering blood. After filtration process in proximal tubules, a part of filtrate would be reabsorbed, thus toxicant concentration in renal tubules would be higher and finally induce damage to its cells. Damage found in mice renal cells after 2-ME exposure including necrosis, swelling, and shrinking.

Result of statistical analysis showed that normal cell count was different significantly in N from G1, G2, and G3, indicated that 2-ME injection at various dose affected the number of normal and damaged cells. This mainly showed in the higher count of tubule cells that were found to be necrotic, edema, and swollen.

Main target of ROS was protein, unsaturated fatty acid, and lipoprotein. Unsaturated fatty acid was most fragile against ROS presence [10]. High level of unsaturated fatty acid in phospholipid of cell membrane made it a main target to be oxidized by various free radicals [11]. 2-Methoxyethanol induced oxidative stress after it entered the body, indicated by rising ROS concentration at level higher than immune system able to eliminate.

Unsaturated fatty acid was critical element of cell membrane. Damage occurred in cell membrane would disrupt Na^+ and K^+ ion pump processes it the cell, leading to homeostasis disruption. This would cause ion saturation within the cell, making it hypotonic to its external surrounding. Thus extracellular
fluid would enter the cell, leading to pathological effect called hydrophilic degeneration. In general, cell degeneration due to Na\(^+\) ion pump disruption would lead to swelling of cell [12].

Swelling of proximal tubule cells was an early manifestation of injury caused by metabolism of 2-ME in the kidney. In the histological slides, it could be seen that proximal tubule cells were swollen with granular cytoplasm due to extracellular fluid movement into intracellular. This was caused by 2-ME-induced free radicals that altered electrical charge in the surface of tubule cell, active transport of various ions and organic acids, and also disrupted the kidney ability to concentrate filtrate, leading to damage of renal tubules, disruption of urinary vesicle flow, elevation of intratubule pressure, and decrease of glomerular filtration rate [13].

Necrosis was localized cell death occurred in certain tissue. Necrosis in renal cell due to lipid peroxidation or free radicals could occurs locally, centrally, peripherally, or massively [14]. Excessive free radicals level would induce oxidative stress, leading to abnormality of physiological function and renal metabolism that followed by cell damage [15]. Free radicals compound such as 2-ME was able to disrupt cell integrity due to its ability to react with cellular components, either structural such as membrane or functional such as enzyme.

Another organ that also affected by 2-ME exposure was reproductive organ [16]. In female individual, ovary was important sexual organ, as its role was to produce ovum. After exposed to 2-ME at various dose, ovarian follicle was also found to be affected. The damage occurred to follicle could be due to its cytotoxic property and/or hormonal disruption that was affected by enzymatic antioxidant activity occurred inside the body. Anti-oxidant was produced as a response to excessive oxidative agent level, such as free radicals, to inhibit further cellular damage. Excessive level of antioxidant produced as response of 2-ME exposure could also elevate ROS level that in turn would also affect follicle in the ovary, indicated by the formation of atresia follicles.

4. Conclusion
Based on current study, it could be concluded that 2-methoxyethanol exposure to female mice induced cellular damage to hepatocyte cells in liver, renal tubule cells in kidney, and secondary and tertiary follicles in ovary. Damage level occurred in respective organ was increasing along with increase of 2-ME dose injected.

References
[1] Sax, NI and Lewis RJ 1989 Journal of Environmental and Sustainable Energy 10 A7
[2] Rumanta M, Tien WS and Sri S 2001 Pengaruh asam metoksiasetat terhadap organ reproduksi mencit Proseding Institut Teknologi Bandung, Bandung, Indonesia
[3] Johanson G 2000 Toxicity Review of Monomethyl Ether and its Acetate Ester Critical Review in Toxicology 30 307
[4] Moslen MT, Kaphalia L, Balasubramanian H, Yin Y.-M, William WA 1995 Toxicol 96: 217-224.
[5] Lu FC 1995 Toksikologi dasar, asas, organ sasaran, dan penilaian resiko edisi ke-2 (Jakarta: UI Press)
[6] Grant DM 1991 Journal of Inherited and Metabolism Disorder 14 421
[7] OECD Guideline for Testing of Chemicals, Acute Oral Toxicity-Up and Down Procedure No. 423
[8] Anderson, Elisabeth T 2000 Buku ajar keperawatan komunitas: teori dan praktek (Jakarta: EGC)
[9] Panjaitan, dkk 2007 makara, kesehatan 11 11
[10] Hery W 2007 Antioksidan alami dan radikal (Yogyakarta: Kanisius)
[11] Pratt DA, Tallman KA and Porter NA 2011 Accounts of Chemical Research 44 458
[12] Krisnasari D, Diah K, Hidayat S, Viva RBA 2014 Efek propolis terhadap fungsi dan perlemakan hepar tikus putih (Rattus Norvegicus) model hiperkolesterolemia (Purwokerto: Universitas Jendral Soedirman)
[13] Wijaya I dan Miranti IP 2005 Patologi ginjal & saluran kemih. ed 3 (Semarang: Badan Penerbit Fakultas Kedokteran Universitas Diponegoro)
[14] Robbins et al 2007 Buku ajar patologi volume 2 edisi 7 (Jakarta: Penerbit buku Kedokteran EGC).
[15] Langseth L 1995 Oxidant, antioxidant, and disease prevention (Belgium: International Life Science Institute press)
[16] Nagano K, Nakayama E, Oobayashi H, Yamada T, Adachi H, Nichizawa T, Ozawa H, Nakaichi M, Okuda H, Minami K and Yamazaki K Toxicology 20 335