

Abstrak

ANALISIS BIOMARKER POTENSIAL PLASMA TIKUS YANG DIINDUKSI DIETILEN GLIKOL MENGGUNAKAN LC-HRMS MELALUI PENDEKATAN *NON-TARGETED METABOLOMIC*

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Latar belakang: Dietilen glikol (DEG) merupakan senyawa organik yang dapat digunakan sebagai pelarut pada produk kimia dan sediaan farmasi. DEG dapat menjadi kontaminan yang bersifat toksik apabila terpapar melebihi ambang batas yaitu lebih dari 0,5 mg/kgBB per hari. Efek toksik yang tidak segera ditangani dapat menyebabkan kematian. Penyebab kematian akibat keracunan DEG masih belum bisa diketahui secara pasti dan penegakan diagnosis pasien akibat keracunan DEG masih terbilang lambat. Penelitian ini bertujuan menganalisis penyebab ketoksikan DEG sehingga dapat memberikan alternatif dalam penegakan diagnosis keracunan DEG dengan mencari biomarker potensial menggunakan metode analisis metabolomik non-target.

Metodologi: Hewan uji tikus sebanyak 32 ekor dibagi menjadi 2 kelompok yaitu kelompok tikus yang tidak diinduksi DEG dan yang diinduksi DEG sebanyak 3g/kgBB selama 3 hari dengan masing-masing kelompok sebanyak 16 tikus. Darah diambil melalui bagian sinus orbital mata menggunakan mikrohematokrit dan ditampung dalam tabung *vacutainer* EDTA lalu disentrifugasi pada putaran 4000 rpm selama 15 menit pada suhu 4°C. Preparasi sampel dilakukan dengan cara plasma darah diambil lalu ditambahkan asetonitril dengan perbandingan 1:1 dan divortex selama 10 detik kemudian disentrifugasi 1000 rpm selama 10 menit pada suhu 4°C untuk selanjutnya dianalisis menggunakan LC-HRMS fase gerak air dengan penambahan ammonium format 5 mM dan asetonitril dengan penambahan asam format 0,05% dengan sistem elusi gradien dan fase diam kolom C18. Hasil analisis LC-HRMS kemudian dianalisis menggunakan software data processing MS-DIAL versi 4.9.2.

Hasil Penelitian: Proses analisis dengan menggunakan MS-DIAL berhasil mendapatkan 34 dan 25 *putative metabolite* secara berturut-turut pada plasma tikus yang diinduksi DEG dan tidak diinduksi DEG. Selain itu, analisis penentuan biomarker terdapat 9 metabolit dugaan yang berhasil diidentifikasi menjadi biomarker potensial ($p > 0,05$ dan $FC > 1,2$) dengan *score* VIP > 1. Satu diantaranya terdapat di kedua kelompok pada diagram venn yaitu *choline* dengan *total score* identifikasi sebesar 72,8%.

Kesimpulan: Pada keracunan DEG ditemukan 9 metabolit dugaan yang berhasil diidentifikasi menjadi biomarker potensial keracunan DEG dengan *choline* sebagai metabolit utama.

Kata Kunci: Dietilen glikol, biomarker potensial, metabolomik non-target, LC-HRMS

Abstract

ANALYSIS OF POTENTIAL PLASMA BIOMARKERS INDUCED BY DIETHYLENE GLYCOL IN RATS USING LC-HRMS THROUGH A NON-TARGETED METABOLOMIC APPROACH

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Background: Diethylene glycol (DEG) is an organic compound that can be used as a solvent in chemical products and pharmaceutical preparations. DEG can become a toxic contaminant when exposed beyond the threshold limit, which is more than 0.5 mg/kgBW per day. Untreated toxic effects can lead to death. The exact cause of death due to DEG poisoning is still uncertain, and the diagnosis of patients affected by DEG poisoning is relatively slow. This study aims to analyze the toxicological causes of DEG to provide alternatives in diagnosing DEG poisoning by searching for potential biomarkers using non-targeted metabolomic analysis methods.

Methodology: Thirty-two test rats were divided into two groups: a group not induced with DEG and a group induced with DEG at a dose of 3g/kg body weight for 3 days, each group consisting of 16 rats. Blood was collected through the orbital sinus of the eye using a microhematocrit capillary and collected in EDTA vacutainer tubes, then centrifuged at 4000 rpm for 15 minutes at 4°C. Sample preparation involved taking plasma from the blood, adding acetonitrile in a 1:1 ratio, vortexing for 10 seconds, and then centrifuging at 1000 rpm for 10 minutes at 4°C. The prepared samples were then analyzed using LC-HRMS with a mobile phase of water with the addition of 5 mM ammonium formate and acetonitrile with the addition of 0.05% formic acid, employing a C18 column and a gradient elution system. The LC-HRMS analysis results were subsequently processed using MS-DIAL version 4.9.2 data processing software.

Result: The analysis process using MS-DIAL has successfully identified 34 and 25 putative metabolites consecutively in the plasma of rats induced with DEG and those not induced with DEG. Furthermore, in the biomarker determination analysis, there are 9 suspected metabolites that have been successfully identified as potential biomarkers ($p > 0,05$ and $FC > 1,2$) with a VIP score > 1 . Interestingly, one of them is found in both groups in the Venn diagram, namely choline, with a total identification score of 72,8%.

Conclusion: In cases of DEG poisoning, 9 suspected metabolites have been recognized as potential biomarkers. Choline, specifically, was identified at the intersection of the Venn diagram, appearing in both the volcano plot and meeting the criteria of a VIP score > 1 . This underscores the significance of choline as a potential biomarker for DEG poisoning, suggesting its role in the metabolic response to DEG exposure.

Keyword: Diethylene glycol, potential biomarkers, non-targeted metabolomics, LC-HRMS