

Systematics Review: Effect Ethanol To Dna Methylation

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ABSTRACT

Aims: The purpose of writing a literature review is to determine the relationship between the effects of ethanol exposure on DNA methylation in embryos. **Methods:** (1) The inclusion criteria used were free full text, without comparison, Outcomes: DNA Methylation, research articles, published between 2012-2022, and using English. While the exclusion criteria used were abstract, there were comparisons, not research articles, and did not use English. (2) The data source comes from PubMed, writing this literature review begins on May 16, 2022. **Results:** Total data based on searches using the keywords "Ethanol" AND "DNA Methylation" (n=278), focusing on embryos (n=13), research articles and free full text (n=8), and inclusion criteria (n=5). **Discussion:** (1) There is a relationship between the effect of ethanol exposure on DNA methylation in embryos. (2) There is limited evidence so that original research research on the subject is needed. **Conclusions:** (1) There is a relationship between the effect of ethanol exposure on DNA methylation in embryos. (2) Exposure to ethanol affects gene expression changes in the hippocampus, bone marrow, and major olfactory epithelium. (3) The effects of ethanol can be reduced by taking betaine supplements.

Keywords: Ethanol, DNA Methylation, Embryo, Betaine, Pregnancy, Secure Work

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INTRODUCTION

Ethanol is a colorless liquid that is volatile and has a characteristic aroma. Ethanol is also known as ethyl alcohol. The chemical formula of ethanol is C_2H_5OH or CH_3CH_2OH with a boiling point of $78.4^{\circ}C$. Based on its physical properties, ethanol is easily influenced by the presence of hydroxyl groups and the short carbon chain of ethanol. Meanwhile, based on its chemical properties, ethanol is included in primary alcohol. In everyday life, ethanol is commonly used as a solvent, antiseptic, food, and beverage. The use of alcohol as a drink is currently increasing among the community, including workers. Alcohol is one of the most abused substances. Alcohol metabolism can cause interactions with other compounds in the body that can produce toxic compounds such as highly reactive oxygen-contained molecules, such as Reactive Oxygen Species (ROS). The ROS produced can react with cell molecules and can cause complex damage.

Every chemical must have Threshold Limit Value (TLV) in the environment. Indonesia's threshold value for chemicals is adopted from other countries or institutions in the world such as ACGIH and OSHA. It is known that the threshold limit value for ethanol is $1,880 \text{ mg/m}^3$. If the ethanol content exceeds the NAV, then ethanol can have an impact on the health of workers.

Ethanol or ethyl alcohol consumed in excess can have an effect on DNA methylation. According to Nuraeny Nanan (2019), the DNA methylation process is the addition of a methyl group ($-CH_3$) which binds to Cytosine-Phosphate-Guanine (CpG) in DNA. DNA methylation generally occurs at the fifth carbon position of cytosine in the CpG site by the DNMT (DNA

Methyl Transferase) enzyme (Suksmarini Ni Made Pramita Widya, 2018). There are about 5- 10% CpG sites scattered in human genes and reach 70-80% methylation outside the CpG island. CpG island is an area with at least 200 base pairs with a guanine and cytosine percentage of more than 50%. In general, this region is not methylated and the base is located on the gene promoter. To establish DNA methylation, DNMT relocates the methyl group taken from S-adenosylmethionine (SAM) and covalently binds to the fifth carbon of the pyrimidine ring of cytosine to form 5-methyl cytosine (5-mC).

DNMTs are enzymes that play a role in forming and maintaining cells with methylated DNA and it is known that there are three active catalysts of DNMT, namely DNMT1, DNMT3A, DNMT3B, and DNMT2. DNMT1 acts as a methyltransferase that maintains the methylation pattern in DNA. DNMT3A and DNMT3B play a role in initiating de novo methylation by forming a methylation pattern. While DNMT2 does not yet have a clear function, its strength in binding DNA is thought to play a role in determining the specific sequence of genes. DNA methylation plays a role in inhibiting gene expression (gene silencing), regulating gene expression, growth, aging, and the occurrence of a disease. DNA methylation can also prevent transcription directly and cause changes in chromatin structure that inhibit the access of transcription factors to promoter genes.

According to several studies, it is stated that the effects of excessive alcohol consumption on DNA methylation are very diverse. According to Rumgay Harriet (2021), about 4% of cancers worldwide are caused by alcohol consumption. This

equates to more than 740,000 cancer cases globally in 2020. The impact of alcohol consumption on the cancer burden varies according to the type of cancer. Most alcoholics suffer from cancer of the esophagus, liver, lung, and breast. According to Niinep Kerly (2021), excessive alcohol consumption can also lead to complex psychiatric disorders. The disorder is caused by excessive alcohol consumption, resulting in dependence and can recur after a long stop consuming alcohol. Besides that, Alcohol dependence can also affect many parts of the brain and exert its effects through different neurotransmitter systems.

Excessive

alcohol consumption can lower a person's consciousness. Thus, it can increase the risk of work accidents. According to Maharja Rizky (2018), work safety is influenced by three factors, namely psychological or individual factors, work factors, and situational factors. In addition to lowering workers' awareness, excessive alcohol consumption can also affect fetal development. According to Marjonen Heidi (2018), alcohol consumption in pregnant women can affect the development of the embryo and cause various health problems that cause birth defects and nervous system disorders in the fetus. Therefore, workers who are pregnant are expected to reduce their consumption or exposure to alcohol.

Based on the results of the research above, there are still not many researchers who discuss the effect of ethyl alcohol or ethanol on DNA methylation in embryos. Therefore, it is necessary to do a literature search through a literature review in order to obtain a library with a fairly good number and quality. The purpose of writing this literature review is to analyze the

effect of ethyl alcohol on DNA methylation in embryos.

METHOD

The writing of this article uses a qualitative method with data collection techniques from literature studies from several related articles. Literature studies according to Syaibani (2012) are all efforts made by researchers to collect information that is relevant to the topic or problem that will be or is being studied. The information was obtained online from a literature search of PubMed. As illustrated in Figure 1, the writing of this systematic review uses the PRISMA-P (Preferred Reporting Item for Systematic Review and Meta-Analysis Protocols) method. All references used in this systematic review are articles written in English with the terms of publication from 2012-2022. The references in each article found have gone through a process of initial search or identification, investigation, and selection of relevant articles.

Identification

Studies were identified using the search terms alcohol, effect, and DNA methylation combined with the Boolean "AND" linker.

1. Person: human and mouse
2. Exposure: ethanol
3. Comparison: -
4. Outcome: DNA Methylation

Screening

Prior to the screening process, all non-article references and articles published before 2012 were removed.

Eligibility

The eligibility criteria applied in this systematic review are as follows:

1. Published between 2012 and 2022

2. Human and mouse research subjects
3. Involves the effect of ethyl alcohol on DNA methylation
3. Yield measures (such as the effect of ethyl alcohol on DNA methylation)

Included

All selected references have gone through all the PRISMA-P processes. based on this research, the important information obtained consists of:

1. General information (such as author, country, year of publication)
2. Research design and methodology

RESULTS

Based on the PubMed search method, five articles were obtained that discussed the effect of ethyl alcohol on DNA methylation in embryos. Below is a flow chart of PRISMA-P data search results using the PICOS method along with an explanation.

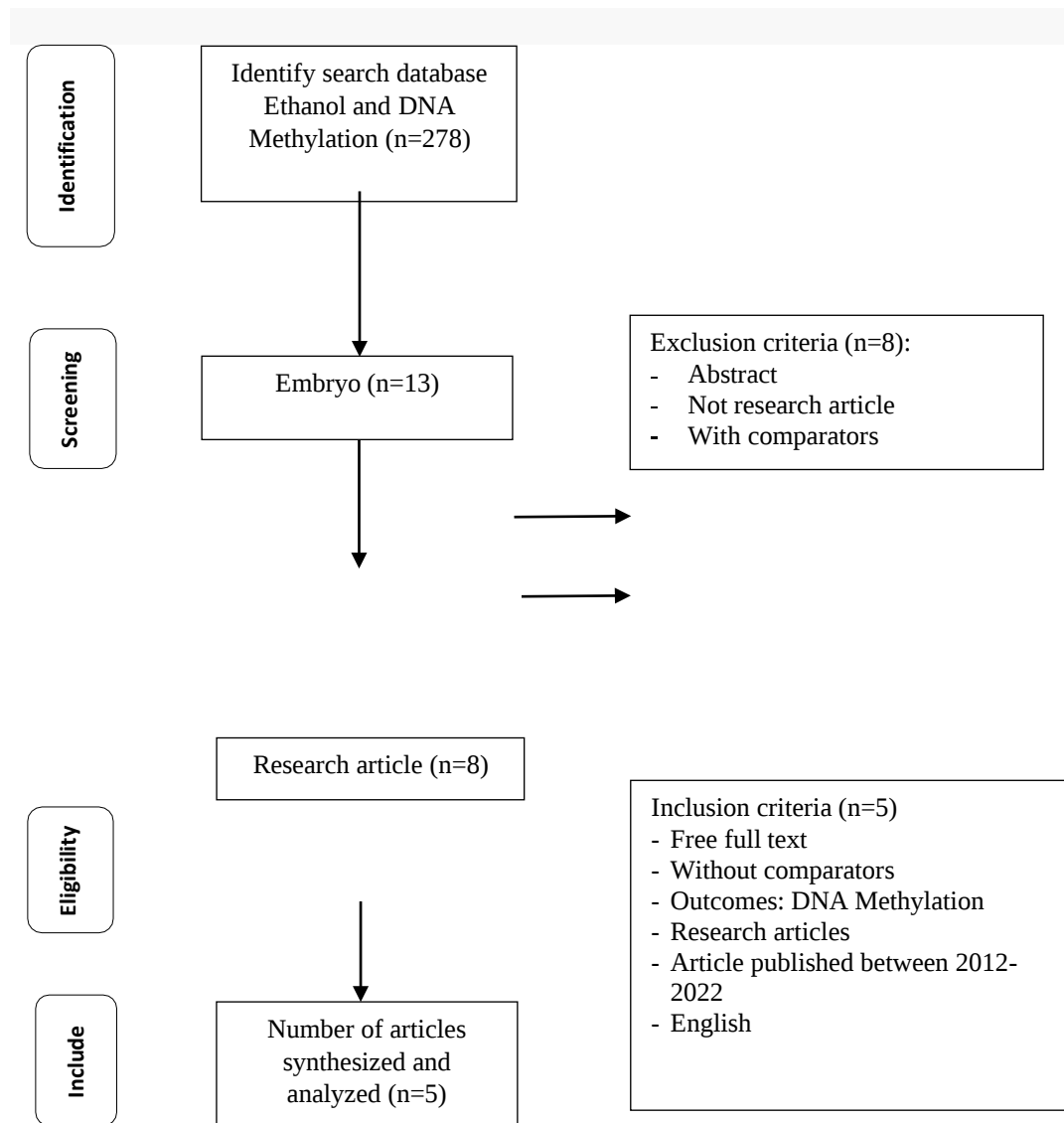


Image 1: Diagram of the data search process for the effect of ethanol on DNA methylation in embryos. Total traceability data based on “Ethanol” AND “DNA Methylation” AND “Embryo” (n=13). Then the data is focused on “Embryonic” (n=13). Then search by type of research article (n=8). Furthermore, the eight articles were analyzed based on the PICOS inclusion criteria (Population, Intervention, Comparison, Results, Study Design, Year of Publication, and Language) as follows: 1) Free full text 2) No comparison 3) Outcome: DNA Methylation, nervous system

A total of 5 international articles met the criteria and were sampled for synthesis and analysis. The inclusion

disorders, birth defects 4) Research articles 5) Publications in 2012-2022 6) English. Each article was studied and analyzed according to the inclusion criteria. There were many articles that were eliminated because they did not meet the criteria, including: there were comparisons, not research articles, the results section does not describe the specified variables, and the research year is not between the years 2012-2022. The analysis of the articles was obtained after carrying out the stages of identification, screening, and determining criteria, so that there were 5 articles that were analyzed.

criteria "outcome" was determined according to the purpose of writing a literature review, variables were included in the PICOS method so

that they could be explored widely.
There are 5 articles that meet the
criteria, these articles are then

analyzed and synthesized in table 1
below:

No.	Title	Sample Design and Measurement	Analysis Techniques	Results
1	Alcohol Exposure Promotes DNA Methyltransferase DNMT3A Upregulation Through Reactive Oxygen Species-Dependent Mechanisms	Quantitative analysis	Two-way ANOVA model using post hoc Bonferroni test	DNMT3A accumulates on alcohol exposure by paving the way for mechanisms that may mediate the protective or detrimental effects of ethanol, via epigenome modification.
2	Supplement of Betaine into Embryo Culture Medium Can Rescue Injury Effect of Ethanol on Mouse Embryo Development	Quantitative analysis	One-way ANOVA model	Betaine supplementation into the medium can effectively enhance the embryonic development of ethanol-treated mice including blastocyst formation, implantation, and post implantation development, as well as restore the global DNA methylation level in ethanol-treated blastocysts to normal status.
3	Alcohol Modulates Expression of DNA Methyltransferases and Methyl CpG-/CpG Domain-	Quantitative analysis	One-way ANOVA model	Ethanol reduces the expression of proteins important for regulating DNA methylation in ubiquitous

	Binding in Embryonic Fibroblasts	Proteins Murine			proteasome- dependent manner
4	Early Alcohol Consumption Hippocampal Methylation, Gene Expression and Volume in a Mouse Model	Maternal Alters DNA Gene Expression	Quantitative analysis	One-sided student t- test	Exposure to ethanol in early pregnancy can cause changes in DNA methylation, gene expression, and embryonic brain structure
5	Early Alcohol Exposure Alters Gene Expression in Placenta and Embryo in a Mouse Model	Prenatal Exposure Imprinted Expression	Quantitative analysis	Shapiro- Wilk test, multivariate Hotelling T2 test, two-way ANOVA test, and two-way student t- test	There was decreased expression of growth promoter Igf2 in alcohol-exposed placentas, increased expression of negative growth controller H19 in alcohol- exposed embryos, and was consistent with the growth- restricted offspring phenotype in this model.

Based on the results of a literature review, in the first article it was found that alcohol exposure can increase DNMT3A transcript and protein levels in neuronal precursor cells 1C11 and MEF, there is an upregulation of DNMT mRNA levels, some other epigenetics are not connected to the HFS pathway, and the accumulation of DNMT3A protein depends on ROS. It can be concluded that EtOH exposure outside the modulation of the DNMT3A transcript by an as yet unidentified mechanism favors the accumulation of DNMT3A protein in 1C11 cells, with a process dependent on the production of ROS by NADPH oxidase.

In the second article, it was stated that the effect of ethanol on embryonic development depends on its concentration in the culture

medium. The article states that when the concentration of ethanol in the medium rises to 0.5% and above, ethanol can inhibit the early development of the embryo at each stage. Thus, it can be concluded that ethanol has a negative impact on the embryo during the pregnancy process. However, this article mentions that betaine supplementation administered into culture media can effectively save the development of ethanol-damaged mouse embryos as well as reduce the global increased level of DNA methylation in blastocyst cells.

In the third article, it was mentioned that ethanol (200 mM) could significantly reduce global DNA methylation in MEF cells by 5.8%. In addition, ethanol exposure can significantly reduce 5-methylcytosine immunostaining and can reduce DNMT-1 activity and expression at both mRNA and protein

levels in MEF cells. Thus, it can be concluded that ethanol reduces the expression of proteins important for regulating DNA methylation in a ubiquitous proteasome-dependent manner.

In the fourth article, it was mentioned that early gestational ethanol exposure causes changes in gene expression in the hippocampus, bone marrow, and major olfactory epithelium. In addition, the article also describes the occurrence of structural brain abnormalities in ethanol-exposed offspring. Based on the results of the study conducted, a slight decrease in total brain volume was found (approximately $d=-0.29$) after exposure to ethanol. The ethanol-exposed mice also had a large increase in left hippocampal volume ($d=0.84$), a slight increase in lateral ventricular volume ($d=0.65$), as well as a large decrease in OB ($-d=0.85$).

Meanwhile, in the fifth article, it was stated that alcohol exposure can cause specific methylation changes in alleles in the printed area. Moderate alcohol exposure causes highly variable phenotypes for the offspring. While the initial exposure to alcohol does not affect or is not able to change the specific epigenetics. In this study, it was also stated that there was a decrease in the expression of the growth promoter Igf2 in alcohol-exposed placentas, an increase in the expression of negative growth control H19 in alcohol-exposed embryos, and consistent with the growth-restricted

offspring phenotype.

DISCUSSION

This literature review focuses on the impact or effect that ethanol has on the embryo, because ethanol or alcohol can affect the embryo from

various pathways. Based on the results of a literature review, alcohol exposure can inhibit embryo development in early pregnancy. According to Miozzo Federico (2018), this is because alcohol exposure can increase DNMT3A transcript and protein levels in neuronal precursor cells 1C11 and MEF, there is an upregulation of DNMT mRNA levels, some other epigenetics are not connected to the HFS pathway, and the accumulation of DNMT3A protein depends on ROS. Another study conducted by Mukhopadhyay Partha (2013) stated that ethanol can reduce global DNA methylation in MEF cells by 5, 8% and reduced 5-methyl-cytosine immunostaining and could reduce DNMT-1 activity and expression at both mRNA and protein levels in MEF cells. This will have an impact on specific methylation changes in alleles in the imprinted region and cause highly variable phenotypes for the offspring (Marjonen Heidi, 2018). Another impact that may arise is changes in gene expression in the hippocampus, bone marrow, and the main olfactory epithelium (Marjonen Heidi, 2015).

However, research conducted by Marjonen Heidi (2018) shows that early exposure to alcohol does not affect or is unable to change specific epigenetics. This effect of ethanol also depends on the concentration of ethanol consumed. According to Zhang Di (2018), when the concentration of ethanol in the medium rises to 0.5% and above, ethanol can inhibit the early development of the embryo at each stage. Zhang Di (2018) also mentions that this effect can be reduced with betaine supplements. betaine supplementation administered into the culture medium can effectively save the development of ethanol-damaged mouse embryos as well as reduce the

global increased level of DNA methylation in blastocyst cells.

CONCLUSION

Conclusion from the results this literature review is:

1. There is a relationship between the effect of ethanol exposure on DNA methylation in embryos
2. Exposure to ethanol affects gene expression changes in the hippocampus, bone marrow, and major olfactory epithelium
3. The effects of ethanol can be reduced by taking betaine supplements
4. The results of this literature review can increase the knowledge of workers who are during pregnancy so that they can avoid or reduce their exposure to alcohol
5. There is limited evidence, so research is needed to prove the relationship between the effects of ethanol exposure on DNA methylation in embryos

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