Mung Bean Sprouts (*Vigna radiata*) Ethanol Extract on Alanine Aminotransferase (ALT) Activity and Malondialdehyde (MDA) Levels in Toluene-Induced Rats

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Abstract

Objective –This study aimed to evaluate the effect ofethanol extract from mung bean sprouts on alanine aminotransferase (ALT) activity and malondialdehyde levels in toluene-induced white rats.

Methods–This research is an experimental laboratory with a research design that is post-test only control group design using 40 rats (Rattus norvegicus) which were divided randomly into 5 groups, G1 (control without toluene), G2 (900 mg/kg toluene), G3 (mung bean sprouts extract 250 mg/kg + 900 mg/kg toluene), G4 (mung bean sprouts extract 500 mg/kg + 900 mg/kg toluene), G5 (mung bean sprouts extract 1000 mg/kg + 900 mg/kg toluene). ALT measurement using spectrophotometer method. Measurement of MDA using the ELISA method. The data collected will be statistically tested with the help of SPSS with a significant ($p < 0.05$).

Results–The results of the measurement of ALT activity ($p=0.972$) and MDA levels ($p=0.190$) in each group had no significant effect ($p>0.05$) in each treatment.

Conclusion–It can be concluded that there was no significant effect of ethanol extract of sprouts on alanine aminotransferase (ALT) activity and malondialdehyde levels in white rats *(Rattus norvegicus*) induced by oral toluene.

Keywords: *Alanine aminotransferase, Mung bean sprouts, malondialdehyde, toluene*.

Introduction

The painting industry requires toluene solvent as a paint thinner[1]. However, its use has side effects on health problems because toluene is volatile and inhaled[2]. Toluene can pass the blood-brain barrier and cause neurotoxicity, nephrotoxicity[3], headaches, fatigue, nausea, and loss of consciousness[4]. Toluene will be converted by cytochrome p450 into benzyl alcohol[5. T]his reaction also produces anionsuperoxide radicals[6].Increased production of anions superoxide can cause an imbalance between free radicals and antioxidants, known as oxidative stress.It can damage cell[7], increase the malondialdehyde (MDA), and increasethe excretion of Alanine aminotransferase (ALT) enzyme into the blood circulation[8]. Oxidative

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stress conditions can trigger degenerative diseases such as diabetes mellitus, hypertension, anemia, vitiligo, Alzheimer's disease, Parkinson's disease, bipolar disorder disease, cancer, and schizophrenia [9].

The body has endogenous antioxidants (superoxide dismutase, glutathione peroxidase, and catalase) that reduce free radicals[10]. The imbalance of free radicals with antioxidants causes oxidative stress conditions[11]. Mung bean sprouts come from green bean seed plants that have germinated. During germination, there may be an increase in compounds that can act as antioxidants and affect antioxidants such as flavonoids, vitamin C, quercetin[12]. Therefore, the germination time of mung bean sprouts can increase the nutritional content of bean sprouts and improve the nutrition that can be used as an additional antioxidant to see the free radicals formed[13].

On this basis, this study aimed to evaluate the ethanol extract of mung bean sprouts against the activity of alanine aminotransferase (ALT) and malondialdehyde (MDA) in white rats (*Rattus norvegicus*) induced by oral toluene. This research hypothesizes that bean sprouts extract can reduce MDA and ALT activity.

Materials and Methods

Experiment design

This study is a laboratory experiment with a research design that is post test only control group design using 40 male rats (Wistar novergicus strains) weighing 150-230 grams which are divided into 5 groups, G1 (n=8, control without toluene), G2 (n=8,900mg/kg toluene) G3 (n=8,250 mg/kg bean sprout ethanol extract + 900mg/kg toluene), G4 (n=8,500 mg/kg bean sprout ethanol extract + 900 mg/kg toluene), and G4 (n= 8, 1000mg/kg bean sprout extract + 900 mg/kg toluene). All procedures have been approved by the Ethics Research Committee of the Faculty of Veterinary Medicine, Airlangga University (2.KE.008.01.2021)

Mung bean sprout ethanol extract

Mung bean sprouts (*Vigna Radiata*) were bean sprouts with an age of 4 days; the extract was made with ethanol as a solvent. First,Mung bean sprouts were air-dried, mashed and the extraction process was carried out by dissolving it with ethanol as a solvent. Then, the ethanol extract of Mung bean sprouts mixed with ethanol was evaporated until the remaining ethanol mung bean sprouts extract. Finally, bean sprout extract was given to groups of rats G3 (250 mg/kg), G4 (500 mg/kg), and G5 (1000 mg/kg) in the morning.

Toluene

Toluene is given orally with a hefty dose of 900 mg/kg/BW; toluene is administered using a glass syringe. Toluene (EMSURE, GERMANY, 1,08325,2500) was used at a concentration of 98%.

Blood samples and blood analysis

Blood samples were taken from the heart of rats. After being taken, the blood samples were put into a vacutainer tube and centrifuge for 15 minutes at a speed of 3000 rpm.Measurement of alanine aminotransferase (ALT) activity using a spectrophotometer with a wavelength of 340 nm. Measurement of malondialdehyde (MDA) using the ELISA method (using an ELISA reader) with an absorbance spectrophotometer of 450 Nm. The MDA sensitivity level is 0.023 nmol/mL.

Statistical Analysis

Data analysis techniques used statistical software packet for social science (SPSS) version 25 (Chicago, IL, USA). The normality test used the Shapiro-Wilk test. The homogeneity test used the Levene test. Data that were normally distributed and had homogeneous variants were tested using One-way

ANOVA and continued with the post hoc Least Significant Difference (LSD) test. All datapresented as mean±SD. All statistical analyzes used a significant level (p<0.05).

Results

The results of measurements of alanine aminotransferase (ALT) and malondialdehyde (MDA) can be seen in Table 1.

Table 1. Results of ALT and MDA measurements.

Variable	Group					ANOVA p-value
	G1 (n=8)	G2 (n=8)	G3 (n=8)	G4 (n=8)	G5(n=8)	
ALT (U/L)	68,40±4,77	69,60±2,60	69,40±18,20	69,40±13,70	73,00±10,33	0,972
MDA (nmol/mL)	3,60±1,18	4,07±0,55	3,56±1,58	4,67±2,16	5,44±0,58	0,190

G1 (control without toluene), G2 (900mg/kg toluene), G1 (mung bean extract 250 mg/kg + 900 mg/kg toluene), G4 (mung bean extract 500 mg/kg + 900 mg/kg toluene), G5 (mung bean extract 1000 mg/kg + 900 mg/kg toluene). One way-ANOVA. Data are presented as mean±SD.

Based on Table 1, the results of the One Way-ANOVA test, the average ALT and MDA are not significant (p>0.05). The results of ALT measurements can be seen in Fig.1.

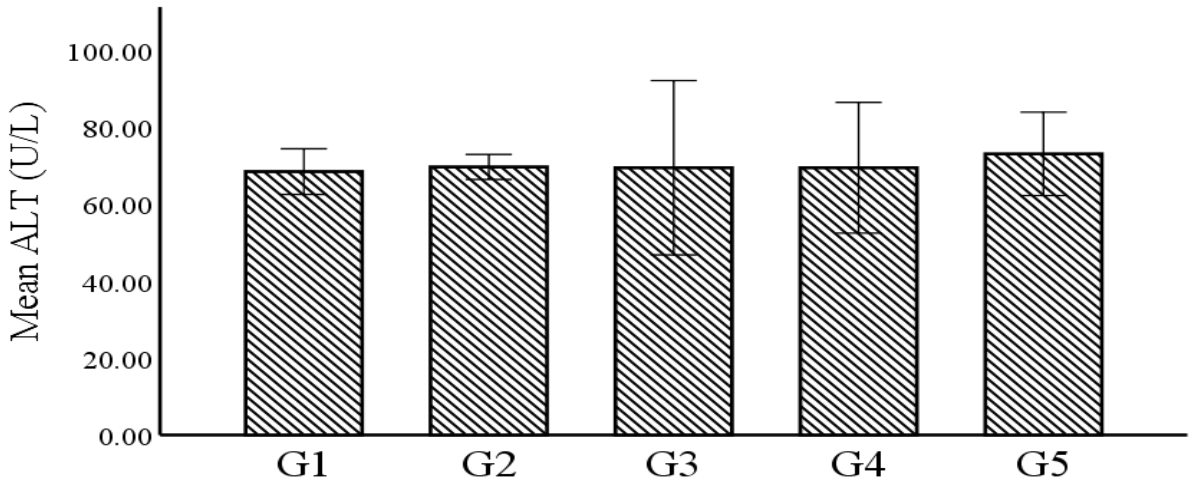

Fig. 1 The Average ofALT in Each Group.G1 (control without toluene), G2 (900mg/kg toluene), G1 (mung bean extract 250 mg/kg + 900 mg/kg toluene), G4 (mung bean extract 500 mg/kg + 900 mg/kg toluene), G5 (mung bean extract 1000 mg/kg + 900 mg/kg toluene). One way-ANOVA. Data are presented as mean±SD.

Based on Fig.1, the results of the one-way ANOVA test on ALT activity did not indicatea significant difference (p>0.05) where there is no difference between G1 and G2, G3, G4, and G5.

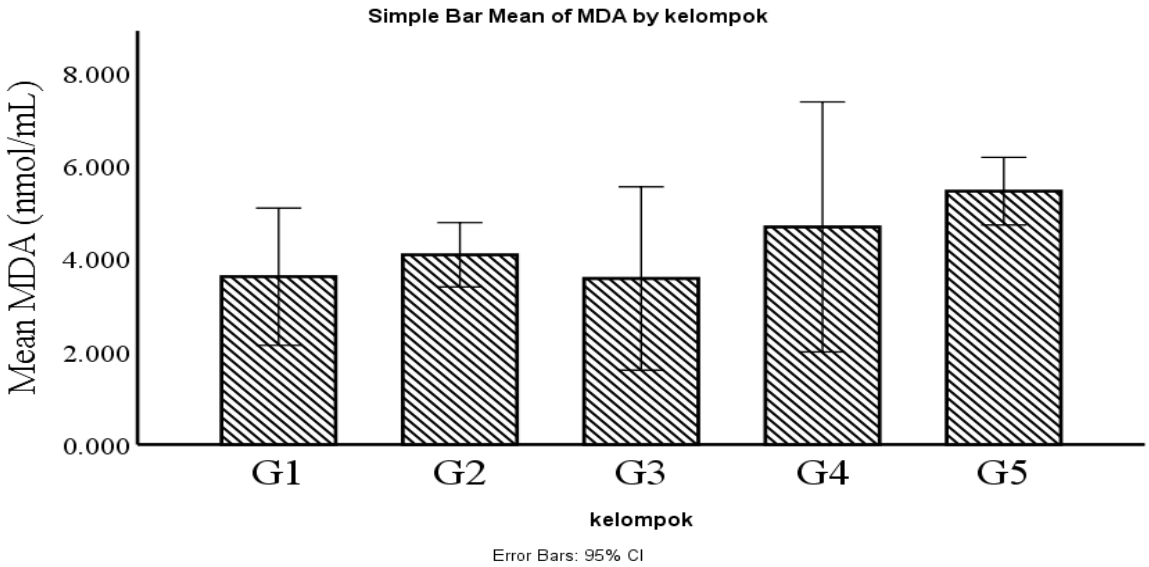

Fig. 2 The Average of MDA Levels in Each Group. G1 (control without toluene), G2 (900mg/kg toluene), G1 (mung bean extract 250 mg/kg + 900mg/kg toluene), G4 (mung bean extract 500 mg/kg + 900mg/kg toluene), G5 (mung bean extract 1000 mg/kg + 900mg/kg toluene). One way-ANOVA. Data are presented as mean±SD.

Based on Fig.2, the results of the ANOVA test on the examination of MDA activity showed no significant difference ($p>0.05$). There was an increase between G1 (3.60±1.18) and G2 (4.07±0.55), but the increase was not significant ($p>0.05$).

Discussion

The results of this study indicate that the measurement of alanine aminotransferase (ALT) between G2 (68.40±4.77) is higher than G1 (69.60±2.60). The results of One-Way ANOVA showed no significant difference between each group. These results follow the study of Tas, Ogeturk14, which stated an increase in ALT in white rats (*Rattus norvegicus*) with inhalation of toluene exposure[15]. This difference may occur because previous researchers used a larger dose of inhalation exposure[16]. There are antioxidant mechanisms in the body that can still prevent free radicals formed due to toluene[17]. Several studies have also stated that ALT activity can be used as a parameter to see liver damage[18]. ALT can be caused by the mechanism of the inability of antioxidants to reduce free radicals so that ROS are formed, which can cause damage to cell components and cause an increase in the excretion of ALT enzymes into blood vessels[18,19].

Another important finding is that the body20 can metabolize toluene, toluene binds to cytochrome p450[21]. It causes an increase in the formation of superoxide anion radicals in monooxygenase metabolism[16, 22], an increase in superoxide anion radicals will be converted by the enzyme superoxide dismutase (SOD) into hydrogen peroxide[23]. When reacted with metal ions, hydrogen peroxide can cause Fenton and Haber Weiss reactions and cause more dangerous hydroxyl radicals[24]. In addition, the increase in free radicals causes oxidative stress conditions that will cause necrosisf[25]. Necrosis conditions can cause ALT excretion out into the blood vessels and increase ALT activity in blood vessels[26].

The current study found that the administration of bean sprouts ethanol extract at G3 (69.40±18.20), G4 (69.40±13.70), and G5 (73.00±10.33) had no

significant effect (p> 0.05) on the ANOVA test. These results explain the administration of bean sprout extract has not been able to affect ALT activity. Mung bean sprouts contain flavonoid compounds that can be used as free radicals formed and prevent cell damage caused by free radicals[27].

Results Measuring the average level of malondialdehyde (MDA) found that G2 (4.07±0.55) was higher than G1 (3.60±1.18). The results of the One-Way ANOVA test showed no significant differences in each group (p>0.05). These results follow the research of Iqbal, Mansyur[28,] which states that toluene can increase MDA levels but will be different from the study of Ayu, Tualeka[29,]which says that exposure to toluene requires large doses to cause an effect on MDA. Toluene can increase freeradicals. Free radicals can react with lipids to cause lipid peroxidation and produce MDA[30]. Exposure to toluene can cause an increase in free radicals and cause oxidative stress conditions. Research Coskun, Oter[31,] who researched the induction of 3000 ppm toluene, stated that oxidative stress levels significantly affect the increase in MDA levels. Examination of MDA levels is often with oxidative stress levels[30,32].

The current study found that the administration of mung bean sprout extract in G3 was lower than in G2. Therefore, giving bean sprouts extract to G3 can reduce MDA levels. The results of the One-Way ANOVA test stated that there was no significant difference (p>0.05) between G3 and G2. Bean sprouts contain flavonoid compounds that can reduce free radicals[33]. Research by El-Newry, Shaffie[34] proves that flavonoids can make free radical scavengers to prevent the increase of free radicals in alcohol-induced rats.

Conclusion

Overall, this study showed that ethanol extract from mung bean sprouts could not reduce ALT and MDA in toluene-induced white rats.Endogenous antioxidant status in rats plays a vital role in increasing ALT and MDA in toluene treatment. This endogenous antioxidant can reduce free radicals caused by toluene induction.

Conflict of Interest : The authors declared no conflict of interest. Furthermore, all the authors agreed that the manuscript is submitted to the Indian Journal of Forensic Medicine and Toxicology.

Ethical Clearance: : The Ethics Research Committee approved this study of the Faculty of Veterinary Medicine, Airlangga University (2.KE.008.01.2021)

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References

1. Kuranchie FA, Angnunavuri PN, Attiogbe F and Nerquaye-Tetteh EN. Occupational exposure of benzene, toluene, ethylbenzene, and xylene (BTEX) to pump attendants in Ghana: Implications for policy guidance. *Cogent Environmental Science*. 2019; 5: 1603418.
2. Wispriyono B and Handoyo E. Risiko Kesehatan Pajanan Benzena, Toluena Dan Xylena Petugas Pintu Tol. *KEMAS: Jurnal Kesehatan Masyarakat*. 2016; 11: 188-94.
3. Meydan S, Nacar A, Oztürk HO, et al. The protective effects of caffeic acid phenethyl ester against toluene-induced nephrotoxicity in rats. *Toxicology and Industrial Health*. 2016; 32: 15-21.
4. Ekaputri S and Oginawati K. Hubungan Paparan Toluen Dengan Kadar Asam Hipurat Urin Pekerja Pengecatan Mobil (Studi pada bengkel Mobil Informal di Karasak, Kota bandung).

Fakul-tasTeknikSipildanLingkungan ITB. 2012.

5. Ding X, Murray G, Lewis D, et al. *Cytochromes P450: Role in the metabolism and toxicity of drugs and other xenobiotics*. Royal Society of Chemistry, 2008.
6. Peet A. *Marks' basic medical biochemistry*. Lippincott Williams & Wilkins, 2012.
7. Carocho M, Ferreira IC, Morales P, and Soković M. Antioxidants and prooxidants: effects on health and aging. Hindawi, 2018.
8. Yousefi M, Adineh H, Reverter M, et al. Protective effects of black seed (Nigella sativa) diet supplementation in common carp (Cyprinus carpio) against immune depression, oxidative stress and metabolism dysfunction induced by glyphosate. *Fish & Shellfish Immunology*. 2021; 109: 12-9.
9. Nandi A, Yan L-J, Jana CK and Das N. Role of catalase in oxidative stress-and age-associated degenerative diseases. *Oxidative medicine and cellular longevity*. 2019; 2019.
10. Ighodaro O and Akinloye O. First line defense antioxidants-superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defense grid. *Alexandria journal of medicine*. 2018; 54: 287-93.
11. R. Dias T, Martin-Hidalgo D, M. Silva B, F. Oliveira P, and G. Alves M. Endogenous and exogenous antioxidants as a tool to facilitate male infertility induced by reactive oxygen species. *Antioxidants & redox signaling*. 2020; 33: 767-85.
12. Kim TY, Leem E, Lee JM and Kim SR. Control of Reactive Oxygen Species for the Prevention of Parkinson's Disease: The Possible Application of Flavonoids. *Antioxidants*. 2020; 9: 583.
13. Guo X, Li T, Tang K and Liu RH. Effect of germination on phytochemical profiles and antioxidant activity of mung bean sprouts (Vigna radiata). *Journal of Agricultural and Food Chemistry*. 2012; 60: 11050-5.
14. Tas U, Ogeturk M, Meydan S, et al. Hepatotoxic activity of toluene inhalation and protective role of melatonin. *Toxicology and Industrial Health*. 2011; 27: 465-73.
15. Amien MSM, Suwondo A and Jayanti S. Hubungan Paparan Toluene dengan Gangguan Fungsi Hati pada Pekerja Bagian Pengecatan sebuah Industri Karoseri di Magelang. *Jurnal Kesehatan Masyarakat (Undip)*. 2015; 3: 395-404.
16. Montes S, Yee-Rios Y, and Páez-Martínez N. Environmental enrichment restore oxidative balance in animals chronically exposed to toluene: Comparison with melatonin. *Brain research bulletin*. 2019; 144: 58-67.
17. Guru A, Lite C, Freddy AJ, et al. Intracellular ROS scavenging and antioxidant regulation of WL15 from cysteine and glycine-rich protein two demonstrated in zebrafish in vivo model. *Developmental & Comparative Immunology*. 2021; 114: 103863.
18. Lehmann-Werman R, Magenheim J, Moss J, et al. Monitoring liver damage using hepatocyte-specific methylation markers in cell-free circulating DNA. *JCI insight*. 2018; 3.
19. Liu J, Zhao Y, Xia J, and Qiu M. Matrine induces toxicity in mouse liver cells through a ROS-dependent mechanism. *Research in Veterinary Science*. 2020; 132: 308-11.
20. Dick AL, Zhao Q, Crossin R, et al. Adolescent chronic intermittent toluene inhalation dynamically regulates the transcriptome and neuronal methylome within the rat medial prefrontal cortex. *Addiction Biology*. 2021; 26: e12937.
21. Ramu R, Wanna WH, Janmanchi D, et al. Mechanistic study for the selective oxidation of benzene and toluene catalyzed by Fe (ClO4) 2 H2O2-H2O-CH3CN system. *Molecular Catalysis*. 2017; 441: 114-21.

22. Laio T-Y, Chen C-C, Tsou H-H, Liu T-Y, and Wang H-T. Acute and Chronic Exposure of Toluene Induces Genotoxicity in Different Regions of the Brain in Normal and Allergic Mouse Models. *Neurotoxicity research*. 2019; 36: 669-78.
23. Zhang C, Zhang G, Liao Y, and Gong D. Myricetin inhibits the generation of superoxide anion by reduced form of xanthine oxidase. *Food chemistry*. 2017; 221: 1569-77.
24. Toyosaki T and Sakane Y. Effect of medium-chain triacylglycerols on reactive oxygen species in light irradiation-induced Maillard reaction with glucose-lysine systems. *African Journal of Food Science*. 2020; 14: 167-73.
25. Ayala A, Muñoz MF, and Argüelles S. Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nominal. *Oxidative medicine and cellular longevity*. 2014; 2014.
26. Zhao S, Jiang J, Jin Y, et al. The concentration of tumor necrosis factor-α determines its protective or damaging effect on liver injury by regulating Yap activity. *Cell death & disease*. 2020; 11: 1-13.
27. Ohyama Y, Ito J, Kitano VJ, Shimada J and Hakeda Y. The polyethoxy flavonoid sudachitin suppresses inflammatory bone destruction by directly inhibiting osteoclastogenesis due to reduced ROS production and MAPK activation in osteoclast precursors. *PloS one*. 2018; 13: e0191192.
28. Iqbal MI, Mansyur M, Sari P and Suryandari DA. The Effect of Exposure Process of Toluene To The Spermatogonia Cell a Rattus Strain Wistar. *Journal Of The Indonesian Medical Association*. 2021; 71: 11-7.
29. Ayu PS, Tualeka AR, Arini SY, et al. Relationship between Toluene Concentration, Malondialdehyde (MDA) Level and Health Complaints in Workers of Surabaya Printing Industry. *Indian Journal of Forensic Medicine & Toxicology*. 2020; 14.
30. Gęgotek A and Skrzydlewska E. Biological effect of protein modifications by lipid peroxidation products. *Chemistry and physics of lipids*. 2019; 221: 46-52.
31. Coskun O, Other S, Korkmaz A, Armutcu F, and Kanter M. The oxidative and morphological effects of high concentration chronic toluene exposure on rat sciatic nerves. *Neurochemical research*. 2005; 30: 33-8.
32. Cui X, Gong J, Han H, et al. Relationship between free and total malondialdehyde, a well-established marker of oxidative stress, in various types of human biospecimens. *Journal of thoracic disease*. 2018; 10: 3088.
33. Xiang Q, Liu X, Liu S, Ma Y, Xu C and Bai Y. Effect of plasma-activated water on mung bean sprouts' microbial quality and physicochemical characteristics. *Innovative food science & emerging technologies*. 2019; 52: 49-56.
34. El-Newary SA, Shaffie NM, and Omer E. The protection of Thymus vulgaris leaves alcoholic extract against hepatotoxicity of alcohol in rats. *Asian Pacific journal of tropical medicine*. 2017; 10: 361-71.