The Effect of Black Cumin (*Nigella sativa*) Oil Supplementation on Hematotoxicity Induced by Benzene in Gasoline Vapors from Gas Stations in Male Wistar Rats

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Abstract

This study aimed to prove the effect of black cumin oil supplementation through analysis of levels of CYP2E1, MDA, GSH, and hematological profiles in experimental animals, Wistar rats, which were exposed to benzene in gasoline vapors from gas stations. Sixty-four experimental Wistar rats were divided into eight groups with eight rats each, two control groups (C1 and C2), and six experimental groups (T1, T2, T3, T4, T5, and T6). Blood samples of experimental rats were taken through intracardiac with ketamine-xylazine-acepromazine anesthesia on day 15 for group C1, C2, T1, T3, and T5; and day 29 for group T2, T4, and T6. The ELISA method measured the levels of CYP2E1, GSH, and MDA in this study, while the hematological profile used a hematology analyzer. This study indicates that benzene in gasoline vapors exposure caused significant hematotoxicity ($P < 0.05$). Supplementation of black cumin seed oil at a dose of 2.5 ml/kg, 5 ml/kg, and 10 ml/kg, all of which have the potential to provide a protective effect against the hematotoxicity of benzene in gasoline vapors through increased internal antioxidants (GSH). The GSH can play a role in the mechanism of vapor metabolism of substances in gasoline vapors in the body through decreased CYP2E1 levels and decreased MDA levels. Further research is still needed with a more extended treatment duration to ascertain the chronic effects of benzene hematotoxicity on the genomic pathway to assess black cumin's role, whether in the form of oil, powder, or other forms of extraction.

Keywords: *Benzene, gasoline vapors from gas stations, black cumin, CYP2E1, GSH, MDA, hematological profiles*

Introduction

Benzene has been known to cause hematotoxicity and blood disorders in humans[1,2]. Blood disorders or also known as hematological disorders are all conditions that affect the ability of the blood (cell quantity and function) in one or more of its parts (erythrocytes, leukocytes, or platelets) to function correctly, for example, anemia, severe bleeding, leukopenia, infection, leukemia, myeloma, etc[3,4,5].

One of Indonesia's industrial sectors that uses benzene is the Oil and Gas and Geothermal Industry, the Downstream Oil and Gas Industry, the Management Sector for Gas Stations (SPBU). Until now, benzene is still used as a constituent to increase the octane number of gasoline. Gas station workers are a working population who have a high-risk level of benzene exposure because they are regularly and continuously exposed. Benzene in gas stations can enter gas station workers' body through inhalation of the lungs, digestive tract, and skin[1]. Benzene metabolism in the liver is mediated

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by cytochrome P-450 monooxygenase, namely the cytochrome P450 isoform 2E1 (CYP2E1) enzyme that produces metabolites. Most of the benzene metabolites will be excreted from the body through urine[6] in the form of phenolic compounds (phenol, hydroquinone, catechol, and trihydroxy benzene), trans, trans-muconic acid (ttMA), and S-phenylmercapturic acid (S-PMA), as well as benzene–unmetabolized[7].

One of the natural ingredients that can be used is black cumin (*Nigella sativa*). *Nigella sativa*, known in Indonesia as *jintan hitam*, is a native herbal plant originating from Southwest Asia which has therapeutic benefits that have been shown in many studies from the active ingredients contained in it[8]. The antioxidant and anti-inflammatory effects of black cumin have been reported in various disease models, including encephalomyelitis, diabetes, asthma, and carcinogenesis. Also, thymoquinone can act as an anti-free radical and superoxide and increase the activity of various antioxidant enzymes such as catalase, glutathione peroxidase, and glutathione-S-transferase. The anticancer effects of thymoquinone are mediated through various action models, including antiproliferation, apoptosis induction, cell cycle arrest, ROS generation, and anti-metastasis/antiangiogenesis[9]. Therefore, this study aimed to prove the effect of black cumin oil supplementation through analysis of levels of CYP2E1, MDA, GSH, and hematological profiles in experimental animals, Wistar rats, which were exposed to benzene of gasoline vapors.

Materials and Methods

Research Design

This study employed a true experimental research design with a randomized post-test-controlled group design.

Experimental Design

The experimental animals used in this study were healthy male rats, type *Rattus norvegicus* Albinus Wistar, aged 12-14 weeks with a weight of 200-300 grams prepared by the Laboratory of the Center for Research and Development of Stem Cell, Universitas Airlangga. The experimental rats were placed into standard cages provided and adapted for seven days before the study in a well-ventilated laboratory animal room with a constant temperature of 26±2 °C and a 12-hour cycle of light and dark cycles. During the adaptation and research experiment period, the experimental rats were given food and drink daily with *ad libitum* standard feed.

The experimental rats were divided into eight groups with eight rats each, two control groups (C1, C2) and six experimental groups (T1, T2, T3, T4, T5, and T6):

i. The control group (C1): the normal control group that was not exposed to benzene and black cumin seed oil, was only given standard feed.

ii. The control group (C2): the experimental control group given exposure to gasoline vapors with an average concentration of benzene in the air-exposure box of 152 ppm for 6 hours per day for two weeks (14 days), without giving black cumin seed oil, only given standard feed.

iii. Experimental groups T1, T3, and T5: the experimental animal groups given gasoline vapors exposure, were given black cumin seed oil orally at a dose of 2.5 ml/kg (T1), 5 ml/kg (T3), and 10 ml/kg (T5) before the exposure took place.

iv. Experimental groups T2, T4, and T6: the experimental animal groups given gasoline vapors exposure, were given black cumin seed oil orally at a dose of 2.5 ml/kg (T1), 5 ml/kg (T3), and 10 ml/kg (T5) after 14 days of exposure, which started on day-15 to day-28.

On day 15 and day 29, experimental rats' blood samples were taken through intracardiac with ketamine-xylazine-acepromazine anesthetic to measure CYP2E1, GSH, MDA levels, and their hematological profile. After that, each rat was then sacrificed by cutting the aortic blood vessel and then inserting it into the incinerator.

Measurement of CYP2E1, GSH and MDA levels

The levels of CYP2E1, GSH, and MDA in this study were measured using ELISA method. Samples taken to be assessed were blood samples of Wistar rats, taken using serum separator tube. The analytical procedure was carried out with a commercially available kit based on reference through experiments. The process of

measuring CYP2E1, GSH, and MDA levels was carried out at the Laboratory of the Research and Development Center for Stem Cells, Universitas Airlangga.

Measurement of Hematological Profiles

Measurements of the hematological profile in this study included the levels of erythrocytes, Hb, leukocytes, platelets, lymphocytes, monocytes, the number of eosinophils, basophils, neutrophils, reticulocytes, hematocrit, MCV, MCH, and MCHC from experimental rats using a hematology analyzer and expressed in units of each hematological profile. The process of measuring the hematology profile was carried out at the Center for Health Laboratory of the Ministry of Health, Surabaya.

Statistical Analysis

Data were analyzed statistically using the SPSS program. The normality test used the Kolmogorov-Smirnov test, and the homogeneity test used the Levene test ($P > 0.05$). Furthermore, the analysis was continued with ANOVA to determine the differences between groups and carried out a post hoc LSD comparison test to see the differences between groups ($P < 0.05$).

Results and Discussion

The effect of black cumin oil supplementation on changes in levels of CYP2E1, GSH, and MDA induced by benzene in gasoline vapors

Table 1 shows the results of CYP2E1, GSH, and MDA levels in each group. Exposure to benzene in gasoline vapors to experimental rats caused a significant increase in CYP2E1 levels and a decrease in GSH levels ($P < 0.05$) but did not significantly increase MDA levels ($P > 0.05$) when compared to the control group. Supplementation of black cumin oil before exposure to benzene in gasoline vapors (groups T1, T3, and T5) and after exposure to benzene in gasoline vapors (groups T2, T4, and T6) at a dose of 2.5 ml/kg, 5 ml/kg, and 10 ml/kg caused a significant reduction in CYP2E1 levels when compared to the benzene (C2) group ($P < 0.05$).

Table 1. The effect of *Nigella sativa* oil (NSO) supplementation on levels of CYP2E1, GSH, and MDA in a group of experimental animals exposed to benzene in gasoline vapors.

Group	Treatment	Mean±SD		
		CYP2E1 (ng/ml)	GSH (ng/ml)	MDA (ng/ml)
C1	Control	10.38±6.18a*	10.03±1.64a*	65.38±59.04b,c*
C2	Benzene of gasoline vapors	34.62±16.65a,b,c*	6.91±1.19a,c*	32.69±4.05b,c*
T1	NSO 2.5 ml/kg+benzene of gasoline vapors	13.50±7.25b*	7.63±1.31NS	85.86±155.69
T3	NSO 5 ml/kg+benzene of gasoline vapors	20.35±7.70b*	7.36±2.31NS	19.75±3.90b*
T5	NSO 10 ml/kg+benzene of gasoline vapors	21.45±5.89b*	8.09±1.03NS	24.13±11.87b*
T2	Benzene of gasoline vapors+NSO 2.5 ml/kg	17.57±6.88c*	9.02±1.97c*	24.84±14.27c*
T4	Benzene of gasoline vapors+NSO 5 ml/kg	12.20±3.75c*	9.13±1.43c*	39.90±29.75NS
T6	Benzene of gasoline vapors+NSO 10 ml/kg	17.50±6.71c*	10.06±3.11c*	65.56±62.92NS

[a,b,c*]: Different superscript within each column indicates significant difference between the means (P <0.05). NS: difference not significant.

The effect of black cumin oil supplementation on changes in the hematological profile induced by benzene of gasoline vapors

Table 2 shows that the lowest erythrocyte levels were in the experimental group (T3), and the highest was in the control group (C1). The mean value of erythrocytes in the experimental groups (T1, T2, and T6) was higher than in the benzene group (C2), but all three were lower than the control group (C1). Meanwhile, the mean value of erythrocytes for the experimental groups (T3, T4, and T5) was lower than the control group (C1) and the benzene group (C2). Supplementation of black cumin oil before exposure to benzene of gasoline vapors (T1, T3, and T5 groups) at doses of 2.5 ml/kg, 5 ml/kg, and 10 ml/kg showed significant differences in erythrocyte levels when compared to the control group (C1) and the benzene (C2) group (P <0.05). While giving black cumin oil supplementation after exposure to benzene of gasoline vapors (T2, T4, and T6 groups) was only at a dose of 10 ml/kg, but not at a dose of 2.5 ml/kg and 5 ml/kg, which showed significant differences in erythrocyte levels when compared to the control group (C1) and the benzene group (C2) (P <0.05).

Table 2. The Effect of Benzene of Gasoline Vapors and *Nigella sativa* oil (NSO) on the levels of the Hematological Profile in the Experimental Animals

Paramater	Group (Mean±SD)							
	C1	C2	T1	T3	T5	T2	T4	T6
Erythrocytes (106/µL)	7.88a,b±0.42	7.23a,*±0.64	7.36a*±0.40	6.59a*±0.98	7.21a*±0.50	7.34NS±0.89	7.14NS±1.14	7.33b*±0.41
Hb (g/dL)	13.05a,b*±0.42	11.78a*±1.02	11.70a*±0.59	10.95a*±1.19	11.78a*±0.60	10.83b*±3.40	11.38b*±1.20	11.78b*±0.64
Reticulocytes (%)	4.08a*±1.44	3.11a*±0.42	6.34a*±1.97	7.79a*±3.29	4.54a*±1.26	3.41NS±1.10	5.64b*±3.22	4.46NS±1.82
Hematocrit (%)	40.63a*±1.51	36.25a*±2.43	37.00a*±1.41	35.50a*±3.34	36.50a*±1.93	37.25b*±4.83	37.13b*±3.83	37.38b*±1.77
MCV (fL)	51.25NS±1.83	50.38NS±2.20	50.38NS±3.29	54.50NS±5.26	50.75NS±2.71	51.13NS±2.17	52.63NS± 4.66	51.13NS±1.25
MCH (pg)	16.50NS±0.76	16.38NS±0.52	16.13NS±1.13	16.88NS±1.13	16.38NS±0.52	14.75NS±3.28	16.25NS±1.17	16.25NS±0.46
MCHC (g/dL)	32.25a*±0.71	32.50a*±1.31	31.63a*±0.74	30.75a*±0.71	32.25a*±0.71	28.13b*±6.89	30.75b*±0.71	31.50b*±0.93
Platelets (103/µL)	937.13NS±285.57	893.00NS±144.65	917.50NS±108.09	711.63NS± 295.92	962.25NS± 128.82	646.50NS±379.58	874.00NS± 176.88	995.00NS±121.12
Leukocytes (103/µL)	6.88NS±1.83	8.33NS±2.49	8.63NS±2.77	7.09NS±1.15	7.81NS±1.11	6.53NS±2.05	5.63NS±1.43	7.43NS±2.47
Lymphocytes (%)	65.13NS±7.74	59.38NS±10.46	68.75NS±9.65	69.50NS±6.00	71.88NS±8.69	60.25b*±5.12	66.38b*±8.19	69.00b*±5.88
Monocytes (%)	4.75NS±2.82	5.00NS±4.11	8.38NS±3.58	7.63NS±4.03	6.88NS±4.05	7.75NS±1.58	7.88NS±3.31	6.88NS±7.68
Neutrophils (%)	26.13a*±6.42	33.25a*±8.94	21.38a*±7.41	21.25a*±3.01	19.88a*±7.66	30.38b*±4.37	24.50b*±8.18	22.50b*±8.96
Eosinophils (103/µL)	294.50a*±245.73	180.00a*±85.69	118.75a*±69.99	108.75a*± 85.93	113.75a*±53.44	108.75b*±16.42	82.50b*±53.12	110.00b*±80.18

[a,b*]: Different superscript within each column indicates significant difference between the means (P<0.05). Abbreviation: Hb= haemoglobin; MCV= Mean corpuscular volume; MCH=Mean corpuscular haemoglobin; MCHC= Mean corpuscular haemoglobin concentration; NS: difference not significant.

This study used local Indonesian cold-pressed black cumin (*Nigella sativa*) seed oil as a treatment material. Black cumin is a type of flower plant that produces seeds as its fruit. The fruit is big, and each fruit contains three to seven groups of seeds, with each seed group containing very many seeds. Furthermore, these seeds are used as ingredients for herbal therapy.

Black cumin seeds contain various compositions, including moisture, yellowish volatile oil, fixed oil, protein, amino acids, alkaloids, organic acids, tannins, resins, toxic glucosides, metarbin, glycogen saponins, crude fiber, minerals, and vitamins[10]. Some of its important active compounds include TQ (30-48%), thymohidroquinone, dithymoquinone, p-cymene (7-15%), carvacrol (6-12%), 4-terpineol (2-7%), t-anethol (1-4%), longifolene sesquiterpene (1-8%), a-piene, and thymol[11,12,13].

Black cumin seeds also contain several micronutrients and macronutrients needed by the body. Macronutrients contained in black cumin include protein (26.7%), fat (28.5%), and carbohydrates (24.9%). Meanwhile, the micronutrients in it are in the form of vitamins and minerals such as vitamins C, Cu, P, Zn, and Fe. Fatty acids are also reported to be present in black cumin seeds, both saturated and unsaturated. The unsaturated fatty acids contained include linolenic acid (50% -60%), oleic acid (20%), eicosadienoic acid (3%), and dihomolinoleic acid (10%). While the saturated fatty acids contained, include stearic acid and palmitic acid[14-18].

These results indicate that black cumin seed oil supplementation can protect against the hematotoxicity of benzene from gasoline vapors. The protective effect of black cumin seed oil against the toxicity of benzene of gasoline vapors can be attributed to its diverse nutritional contents and its active antioxidant properties. The results obtained in this study are not in line and at the same time consistent with the results of previous studies regarding the effect of continuous dichlorvos (DDVP) exposure on renal function and hematological parameters (erythrocyte count, packed cell volume, leukocyte count, MCV, MCH, MCHC, Hb, neutrophils, and lymphocytes) and possible antidote activity of *Nigella sativa* oil. In this study, the results were inconsistent; namely, the administration of black cumin oil (*Nigella sativa*) had a significant effect by maintaining optimal erythrocyte count, hemoglobin, and solid cell volume (PCV), and the number of leukocytes. Meanwhile, what is in line is that the administration of black seed oil (*Nigella sativa*) does not significantly affect the number of MCV, MCV, and MCHC. Thus, it can be concluded that black cumin oil also can provide a protective effect against toxicity for exposure to the organophosphate pesticide, namely DDVP[19].

Conclusion

In summary, this study's results indicate that the hematotoxicity induced by the benzene exposure of gasoline vapors can be associated with the benzene metabolic pathway and oxidative stress. Supplementation of local Indonesian cold-pressed black cumin seed oil can protect against the hematotoxicity of benzene of gasoline vapors by increasing internal antioxidants (GSH), decreasing CYP2E1 levels, and decreasing MDA levels. Furthermore, black cumin seed oil's protective effect against the toxicity of benzene of gasoline vapors can be attributed to its various nutritional content and its active antioxidant properties.

Conflict of Interest: The author declare that they have no conflict of interest.

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